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ABBREVIATIONS

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Conference Organizers

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AIDS Vaccine 2012 is proud to introduce this year's New Investigator Awardees.

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Sunday, 9 September

13:00 – 20:00	**Registration**	BCEC Level 3, Ballroom Foyer
14:00 – 19:00	**Speaker Check-in**	BCEC Level 2, Room 251

16:30 – 18:30 Opening Session — BCEC Level 3, Ballroom East

Chairs: Dan Barouch, Galit Alter, Bruce Walker, and Lindsey Baden

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18:30 – 20:00 Welcome Reception — BCEC Level 3, Ballroom Foyer

Monday, 10 September

07:30 – 18:00	**Registration**	BCEC Level 3, Ballroom Foyer
07:30 – 17:30	**Speaker Check-in**	BCEC Level 2, Room 251

08:30 – 10:30	**Plenary Session 01:** HIV Vaccines in the Broader HIV Prevention Landscape	BCEC Level 3, Ballroom East
	Chairs: Michel Nussenzweig and Dan Barouch	
08:30 – 08:45	**Welcome** Deval L. Patrick, Governor of Massachusetts	
08:45 – 09:15	**Special Lecture** **An HIV Vaccine: A Critical Component of a Comprehensive HIV Prevention Strategy** *A Fauci*	PL01.01
09:15 – 09:40	**The Current State of Microbicides as Prevention** *S Abdool Karim*	PL01.02
09:40 – 10:05	**Oral PrEP for HIV Prevention: Next Steps** *J Baeten*	PL01.03
10:05 – 10:30	**ART for Prevention of HIV: Glimmers of Success** *M Cohen*	PL01.04
10:30 – 11:00	**Tea and Coffee Break**	BCEC Level 3, Ballroom Foyer

11:00 – 12:30	**Oral Abstract Session 01:** Animal Models	BCEC Level 3, Room 253 ABC
	Chairs: Genoveffa Franchini and Rama Rao Amara	
11:00 – 11:15	**Significant Protection from Infection and AIDS Progression After Gastrointestinal and Oral Vaccinations, Respectively, with a SIV DNA/rMVA Vaccine** *A Aldovini, M Manrique, A Cobo-Molinos, P Kozlowski, A Carvlle*	OA01.01
11:15 – 11:30	**Antibodies to the Envelope Protein Protect Macaques from SIVmac251 Acquisition in an Immunization Regimen That Mimics the RV-144 Thai Trial** *G Franchini, P Pegu, S Gordon, B Keele, M Doster, Y Guan, G Ferrari, R Pal, MG Ferrari, S Whitney, L Hudacik, E Billings, M Rao, D Montefiori, D Venzon, C Fenizia, J Lifson, D Stablein, J Tartaglia, N Michael, J Kim*	OA01.02
11:30 – 11:45	**Immune Control of an SIV Challenge by a Heterolgous and Direct Mucosal Vaccination Regimen in Rhesus Monkeys** *C Sun, Z Chen, X Tang, Y Zhang, L Feng, Y Du, L Xiao, L Liu, W Zhu, L Chen, L Zhang*	OA01.03

11:45 – 12:00	**A Recombinant Attenuated Mycobacterium Tuberculosis-SIV Combination Vaccine Is Safe and Immunogenic in Immunocompromised, SIV-infected Infant Macaques** *K Jensen, UD Ranganathan, P Kozlowski, K Van Rompay, D Canfield, R Ravindran, I Khan, P Luciw, G Fennelly, M Larsen, K Abel*	OA01.04
12:00 – 12:15	**MAb PGT121 Protects Against Mucosal SHIV Challenge in Macaques at Concentrations Corresponding to Its Highly Potent In Vitro Neutralization Capacity** *B Moldt, EG Rakasz, N Schultz, P Chan-Hui, K Swiderek, DI Watkins, DR Burton, P Poignard*	OA01.05
12:15 – 12:30	**Epitope Specificity Appears to Be an Important Determinant of In Vivo Killing Ability of Simian Immunodeficiency Virus (SIV)-specific CD8+ T Cells** *L Pozzi, A Carville, V Varner, J Sen, D Knipe, A Kaur*	OA01.06

11:00 – 12:30 Oral Abstract Session 02: Vaccine Concepts–Protein Immunogens

BCEC Level 2, Ballroom East

Chairs: Richard Wyatt and Tim Cardozo

11:00 – 11:15	**Identification of a Clade A HIV Envelope Immunogen from Protocol G That Elicits Neutralizing Antibodies to Tier 2 Viruses** *S Hoffenberg, S Kosakovsky Pond, A Carpov, D Wagner, A Wilson, R Powell, R Lindsay, H Arendt, J DeStefano, P Poignard, M Simek, S Fling, S Phogat, C Labranche, D Montefiori, D Burton, C Parks, C King, W Koff, M Caulfield*	OA02.01
11:15 – 11:30	**Prime-boost Regimen Potency and Efficacy with Alphavirus Replicons (SIV Antigen) in Non-human Primates Challenged with Low-dose Intra-rectal SIVsmE660** *K Banerjee, S Balsitis, T Jamil, C Jones, A Dey, J Flandez, L Brito, Y Cu, C Beard, S Santra, R Pal, N Miller, NM Valiante, P Mason, SW Barnett, GR Otten*	OA02.02
11:30 – 11:45	**Minimally Invasive and Surface Electroporation Delivery of DNA Vaccines for the Induction of Robust Humoral Immune Responses Against HIV Antigens** *NY Sardesai, AS Khan, J Mc Coy, F Lin, JM Mendoza, M Yang, J Yan, N Hutnick, K Muthumani, DB Weiner, KE Broderick*	OA02.03
11:45 – 12:00	**Design of Lipid Nanoparticle Delivery Agents for Multivalent Display of Recombinant Env Trimers in HIV Vaccination** *S Pejawar-Gaddy, J Kovacs, D Barouch, B Chen, D Irvine*	OA02.04
12:00 – 12:15	**Eliciting Neutralizing Antibodies with gp120 Outer Domain** *Y Qin, DP Han, K Takamoto, MW Cho*	OA02.05
12:15 – 12:30	**Oral Immunization With a Recombinant Lactococcus Lactis Expressing HIV-1 Gag on the Tip of the Pilus Induces Strong Mucosal Immune Responses** *V Chamcha, JR Scott, R Amara*	OA02.06 LB

11:00 – 12:30	**Oral Abstract Session 03:** Innate Immunity	BCEC Level 2, Room 258 ABC

Chairs: Alexandra Trkola and Keith Reeves

11:00 – 11:15	**SIVΔnef Vaccination Mobilizes Systemic and Mucosal Natural Killer Cells in Mamu A*01+ Macaques** *R Reeves, T Evans, J Gillis, M Connole, F Wong, Y Yu, R Johnson*	OA03.01
11:15 – 11:30	**RV144-like Trial in Macaques Using ALVAC-SIV & gp120, Induces Innate Immunity and Increases the Frequency of NK22 & NKG2A+ Cells in Mucosal Tissues** *NP Liyanage, P Pegu, S Gordon, M Cameron, K Foulds, M Doster, M Vaccari, R Koup, M Roederer, R Sékaly, G Franchini*	OA03.02
11:30 – 11:45	**Adenovirus Vectors from Various Serotypes Induce Distinct Cytokine Profiles** *JE Teigler, M Iampietro, DH Barouch*	OA03.03
11:45 – 12:00	**Infiltration of Dendritic Cells and Antigen Uptake in the Muscle After Injection of HIV-1 Env gp120 in Adjuvant** *F Liang, KJ Sandgren, H Fausther-Bovendo, D O'hagan, A Seubert, E De Gregorio, S Rao, N Sullivan, RA Seder, RA Koup, K Loré*	OA03.04
12:00 – 12:15	**Early Pro-inflammatory Host Response to Recombinant HSV-SIV Vaccination in Sooty Mangabeys** *N Rout, S Yu, V Varner, C Kasala-Hallinan, K Rogers, J Sen, D Knipe, F Villinger, A Kaur*	OA03.05
12:15 – 12:30	**Vaccination with MVA/HIV Induces Differential Recruitment of Monocyte Subsets into the Circulation and Monocyte-specific Transcriptional Programs** *E Andersen-Nissen, DE Zak, TR Hensley, DJ Adams, X Hu, A Sato, M Elizaga, PA Goepfert, HL Robinson, A Aderem, MJ McElrath, the NIAID HIV Vaccine Trials Network*	OA03.06 LB

12:30 – 13:30	**Lunch**	BCEC Level 2, Rooms 252 AB / 254 AB / 257 AB

12:30 – 13:30	**Networking Lunch**	BCEC Level 2, Room 259 AB

Join us in BCEC Level 2, Room 259 AB where lunch tables have been reserved for young and early-career investigators and community representatives to network with senior researchers.

13:30 – 14:45 Oral Abstract Session 04: Mucosal Immunity

BCEC Level 3, Room 258 ABC

Chairs: Daniel Douek and Jo-Ann Passmore

13:30 – 13:45 **Immune Complexes Can Dampen Inflammatory Signaling at the Mucosal Surface During Protective SIV Vaccination** OA04.01
AJ Smith, SW Wietgrefe, CS Reilly, PJ Southern, L Duan, KE Perkey, L Shang, R Johnson, AT Haase

13:45 – 14:00 **Vaccine-elicited Systemic and Mucosal Humoral Responses of Lactating Rhesus Monkeys Vaccinated with the Transmitted/Founder HIV Envelope 1086C** OA04.02
G Fouda, J Amos, AB Wilks, A Chand, D Montefiori, B Haynes, N Letvin, D Pickup, H Liao, SR Permar

14:00 – 14:15 **Expanded Memory CD4+ T Cells in the Fetal and the Infant Gut; a Mucosal Route for Mother-to-Child-Transmission of HIV-1** OA04.03
MJ Bunders, C van der Loos, PL Klarenbeek, J van Hamme, J Wilde, N de Vries, RA van Lier, N Kootstra, ST Pals, TW Kuijpers

14:15 – 14:30 **Cell Free HIV-1 Virus Can Infect Inner and Outer Foreskin Polarized Explants** OA04.04
MP Lemos, ML Perez, J Sanchez, J Lama, S Montano, J McElrath

14:30 – 14:45 **Inflammation in the Male Genital Tract: Implications for HIV Acquisition and Transmission** OA04.05
AJ Olivier, L Roberts, D Coetzee, A Williamson, JS Passmore, WA Burgers

13:30 – 14:45 Oral Abstract Session 05: Social/Ethical Issues

BCEC Level 2, Room 253 ABC

Chairs: Barney Graham and Jonathan Fuchs

13:30 – 13:45 **New Tools to Measure Community and Stakeholder Engagement and Its Impact on Outcomes of Clinical Research** OA05.01
P Bahati, S Hannah, S Seidel, S Aggett, R Siskind, R Campbell, S Williams, A Downie, S Rosas

13:45 – 14:00 **An Assessment of Good Participatory Practice Guidelines at HIV Prevention Research Clinical Centers in Eastern and Southern Africa** OA05.02
BP Ngongo, S Hannah, J Mbogua, M Seyoum, M Warren, E Baas, F Priddy

14:00 – 14:15 **Social and Ethical Considerations in Engaging American Indian and Alaska Native Communities in HIV Clinical Research** OA05.03
J Velcoff, DL Humes, R Foley, M Ignacio

14:15 – 14:30 **Knowledge/Attitude/Practices of HPV & Cervical Cancer, Willingness to Participate in Vaccine Trial in Preparation for HIV & HPV Vaccine Trials in Mali** OA05.04
D Poole, K Tracy, L Levitz, S Yekta, E Kossow, T Huang, M Rochas, K Sangare, K Tounkara, B Aboubacar, O Koita, F Siby Diallo, S Sow, I Téguété, A Dolo, F Bougoudogo, M Lurie, AS De Groot

14:30 – 14:45 **Referral and Access to Care of HIV Prevalent Cases; Experience from the Early Capture HIV Cohort Study in Kampala** OA05.05
LN Mutengu, H Kibuuka, M Millard, A Sekiziyivu, S Wakabi, J Nanyondo, G Kawooya, M Robb, N Michael

13:30 – 14:45 **Oral Abstract Session 06:** V1/V2 Antibody Responses — BCEC Level 2, Ballroom East

Chairs: Susan Zolla-Pazner and Georgia Tomaras

13:30 – 13:45 **A Short Segment in the HIV-1 gp120 V1/V2 Region Is a Major Determinant of Neutralization Resistance to PG9-like Antibodies** — OA06.01
NA Doria-Rose, I Georgiev, RP Staupe, S O'Dell, G Chuang, J Gorman, JS McLellan, M Pancera, M Bonsignori, BF Haynes, DR Burton, WC Koff, PD Kwong, JR Mascola

13:45 – 14:00 **Characterization of V1V2-Specific Antibodies Present in Broadly Neutralizing Plasma Isolated from HIV-1 Infected Individuals** — OA06.02
C Krachmarov, K Revesz, R Prattipati, C Reichman, Z Lai, W Honnen, B Li, C Derdeyn, A Pinter

14:00 – 14:15 **Design of an HIV Env Antigen That Binds with High Affinity to Antibodies Against Linear, Conformational and Broadly Neutralizing Epitopes Within V1/V2** — OA06.03
L Liao, M Bonsignori, K Hwang, AM Moody, R Park, S Crawford, H Chen, TL Jeffries, M Cooper, X Lu, R De, N Karasavvas, S Rerks-Ngarm, S Nitayaphan, J Kaewkungwal, S Tovanabutra, P Pitisuttithum, J Tartaglia, F Sinangil, J Kim, NL Michael, GD Tomaras, Z Yang, K Dai, M Pancera, GJ Nabel, JR Mascola, PD Kwong, A Pinter, S Zolla-Pazner9, MS Alam, BF Haynes

14:15 – 14:30 **Evidence for Env-V2 Sieve Effect in Breakthrough SIVMAC251Infections in Rhesus Monkeys Vaccinated with Ad26/MVA and MVA/Ad26 Constructs** — OA06.04
S Sina, S Tovanabutra, E Sanders-Buell, A Bates, M Bose, S Howell, G Ibitamuno, M Lazzaro, A O'Sullivan, J Lee, T Cervenka, J Kuroiwa, K Baldwin, DH Barouch, M Robb, R O'Connell, NL Michael, JH Kim, M Rolland

14:30 – 14:45 **Structural Basis for Germline Gene Usage of a Potent Class of Antibodies Targeting the CD4 Binding Site of HIV-1 gp120** — OA06.05 LB
AP West, Jr., R Diskin, MC Nussenzweig, PJ Bjorkman

14:45 – 16:00 **Poster Session 1 with Tea and Coffee Break** — BCEC Level 3, Ballroom West
See inside back cover for the poster presentation schedule.

Poster Discussion 1a: B Cell Immunology and Antibody Function — BCEC Level 3, Ballroom West

Chair: Mattia Bonsignori

15:00 – 15:07 **V1/V2-Directed Antibodies Elicited in RV144 Vaccinees Bind to a Structurally Polymorphic Site** — P03.64 LB
JS McLellan, J Gorman, M Bonsignori, K Hwang, H Liao, S Rerks-Ngarm, S Nitayaphan, NL Michael, JH Kim, BF Haynes, PD Kwong

15:07 – 15:14 **Structural Definition for a New Modality of Broad and Potent Antibody Neutralization at the CD4-binding Site on HIV-1 gp120** — P03.14
T Zhou, S Moquin, R Lynch, X Wu, J Zhu, Y Yang, B Zhang, JR Mascola, PD Kwong

15:14 – 15:21 **The Breadth of Maternal HIV-1 Specific Neutralizing Antibodies Is Not Associated with a Lower Risk of Mother-to-Infant Transmission** P03.01
A Chaillon, T Wack, M Braibant, L Mandelbrot, S Blanche, J Warszawski, F Barin

15:21 – 15:28 **Structural Comparison of Somatically Related PG9 and PG16 in Complex with Their Epitope Reveals Differences in Glycan Recognition** P03.31
M Pancera, JS McLellan, S Shahzad-ul-Hussan, N Doria-Rose, B Zhang, Y Yang, DR Burton, WC Koff, CA Bewley, PD Kwong

15:28 – 15:35 **Antibody Subclass Skewing Predicts Enhanced ADCC Activity in Both Natural Infection and Vaccination** P03.39
A Dugast, A Chung, Y Chang, A Licht, M Ackerman, G Alter

15:35 – 15:42 **Hyperglycosylated gp120 Mutants Elicit Improved CD4-binding Site Directed Antibodies in a Heterologous Prime:Boost Regimen** P03.30
FK Ahmed, BE Clark, R Pantophlet

Poster Discussion 1b:
HIV Transmission and Viral Diversity — BCEC Level 3, Ballroom West

Chair: Carolyn Williamson

15:00 – 15:07 **Dynamics and Frequency of Gag Transmitted Polymorphisms in Zambia** P05.19
M Schaefer, J Carlson, D Claiborne, J Prince, W Kilembe, J Tang, P Farmer, R Shapiro, T Ndung'u, J Frater, P Goulder, R Kaslow, S Allen, P Goepfert, D Heckerman, E Hunter

15:07 – 15:14 **Deep Sequencing Reveals an Association Between HIV-1 Subtype C Mutations in gp41 MPER Epitopes and Mother-to-Child Transmission** P05.17
L Yin, Y Cai, K Chang, BP Gardner, W Hou, K Nakamura, M Sinkala, C Kankasa, DM Thea, L Kuhn, GM Aldrovandi, MM Goodenow

15:14 – 15:21 **Postnatally-transmitted HIV-1 Variants Are Efficient at Dendritic Cell Trans-infection and Sensitive to Autologous and Heterologous Neutralization** P05.11
GG Fouda, T Mahlokozera, K Rizzolo, J Salazar-Gonzalez, M Salazar, G Learn, S Barotra, M Sekaran, E Russell, F Jaeger, F Cai, F Gao, B Hahn, R Swanstrom, S Meshnick, V Mwapasa, L Kalilani, S Fiscus, D Montefiori, B Haynes, J Kwiek, M Alam, S Permar

15:21 – 15:28 **A New Transmission Map of HIV-1 CRF07_BC in China: Analysis of Sequences from 12 Provinces over a Decade** P05.02
M Zhefeng, L Jingyun, Z Ping, X Jianqing, Z Xiaoyan

15:28 – 15:35 **Limited Evidence for Alterations in Gag-mediated HIV Replication Capacity Over the Course of the North American Epidemic (1979-Present)** P05.20
L Cotton, D Chopera, K Penney, J Carlson, E Martin, A Le, T Kuang, B Walker, J Fuchs, S Buchbinder, T Wagner, M John, S Mallal, B Koblin, K Mayer, A Poon, M Brockman, Z Brumme

15:35 – 15:42 **Evolutionary Dynamics of HIV-1 Subtype C Accessory and Regulatory Genes in Primary Infection** P05.05
R Rossenkhan, V Novitsky, TK Sebunya, R Musonda, BA Gashe, M Essex

16:00 – 18:00	**Symposium 01:** Exploiting the Innate Immune Response	BCEC Level 3, Room 258 ABC
	Chairs: Christiane Moog and Galit Alter	
16:00 – 16:25	**Development of Dendritic Cell-based prophylactic and Therapeutic HIV Vaccine** *Y Levy, A Palucka*	S01.01
16:25 – 16:50	**Adjuvants Influence the Magnitude, Quality and Durability of HIV Env Specific Humoral Immunity in Non-human Primates** *JR Francica, G Tomaras, M Alam, G Ferrari, J Mascola, DT O'Hagan, R Seder*	S01.02
16:50 – 17:15	**Systems Analysis of HIV Vaccine Triggered Innate Immune Responses** *D Zak*	S01.03
17:15 – 17:40	**Profiling Humoral Immunity: Determining Antibody Innate Immune Recruiting Capacity** *M Ackerman*	S01.04
17:40 – 17:55	**Natural Killer (NK) Cell Responses at Female Genital Mucosa to SIV Vaginal Challenge** *L Shang, J Duan, A Smith, S Wietgrete, M Zupancic, A Haase*	S01.05 OA

16:00 – 18:00	**Symposium 02:** Lessons from Clinical Trials	BCEC Level 2, Room 253 ABC
	Chairs: Nina Russell and Lindsey Baden	
16:00 – 16:25	**RV144 Update: Insights from Correlates of Risk, Breakthrough Infections, and Animal Studies in the Context of Future Vaccine Development** *J Kim*	S02.01
16:25 – 16:50	**Comparative Immunogenicity of HIV Vaccine Candidates in the HVTN** *N Frahm, G Tomaras, D Montefiori, P Gilbert, L Corey, JM McElrath*	S02.02
16:50 – 17:15	**Clinical Studies with Adenovirus 26 Vectored Candidate HIV-1 Vaccines** *R Dolin, LR Baden, MS Seaman, DH Barouch*	S02.03
17:15 – 17:40	**HIV Immunity and Reservoir Following Interventions in Acute HIV Infection: Implication for Functional Cure** *J Ananworanich*	S02.04
17:40 – 17:55	**Vector Induced Skewing of Antibody Fc-Effector Functions** *A Chung, A Dugast, H Robinson, Y Chan, ME Ackerman, J Cox, W Koff, D Barouch, S Rerks-Ngarm, N Michael, J Kim, G Alter*	S02.05 OA

16:00 – 18:00	**Symposium 03:** New Env Immunogens	BCEC Level 2, Ballroom East
	Chairs: Joseph Sodroski and Rogier Sanders	
16:00 – 16:25	**Structural basis for HIV-1 Trimeric Env Immunogen** *Y Mao*	S03.01
16:25 – 16:50	**Pure Native Env Trimers on VLP Surfaces and in Soluble Form** *JM Binley*	S03.02
16:50 – 17:15	**Envelope Immunogen: Stable and Homogeneous HIV-1 gp140 Trimers** *B Chen*	S03.03
17:15 – 17:40	**Epitope-focused Vaccines** *W Schief*	S03.04
17:40 – 17:55	**Eliciting Broadly Neutralizing Antibodies Against HIV-1 That Target gp41 MPER** *D Han, H Habte, Y Qin, K Takamoto, C LaBranche, D Montefiori, MW Cho*	S03.05 OA
18:30 – 23:00	**Banquet at the Seaport World Trade Center**	

Just a short 5-minute walk from the hotel you will find the site for our 2012 banquet. All delegates are invited to join us for an outdoor reception offering views of Boston, champagne and a great opportunity to connect with colleagues. The banquet will offer dinner, a dessert buffet and dancing to the best band in Boston, Flipside.

Tuesday, 11 September

07:30 – 17:30	**Speaker Check-in**	BCEC Level 2, Room 251
08:00 – 18:00	**Registration**	BCEC Level 3, Ballroom Foyer

08:30 – 10:00	**Plenary 02:** A New Look at the Transmission Event	BCEC Level 3, Ballroom East
	Chairs: Bruce Walker and Margie McGlynn	
08:30 – 09:00	**Barriers to Mucosal Transmission: Challenges and Opportunities** *JD Estes*	PL02.01
09:00 – 09:30	**Viral Dynamics and Immune Response in Acute Infection and Their Impact on Viral Set-point** *M Robb*	PL02.02
09:30 – 10:00	**Viral Evolution in Early HIV Infection, and the Implications for Vaccines** *B Korber*	PL02.03
10:00 – 10:30	**Tea and Coffee Break**	BCEC Level 3, Ballroom Foyer

10:30 – 12:30	**Oral Abstract Session 07:** B Cell Responses	BCEC Level 3, Ballroom East
	Chairs: Leo Stamatatos and Georgia Tomaras	
10:30 – 10:45	**Structure-guided Modification and Optimization of Antibody VRC07** *Y Kwon, I Georgiev, S O'Dell, W Shi, G Chuang, Y Yang, B Zhang, J Zhu, GJ Nabel, JR Mascola, PD Kwong*	OA07.01
10:45 – 11:00	**Isolation of a Clonal Lineage of IgA Broadly Neutralizing Antibodies from a Chronically Infected Tanzanian Subject** *M Moody, MS Drinker, TC Gurley, JD Amos, JA Eudailey, LC Armand, R Parks, ES Gray, L Morris, A Finzi, X Yang, J Sodroski, H Liao, GD Tomaras, DC Montefiori, BF Haynes*	OA07.02
11:00 – 11:15	**Deep Sequencing with Longitudinal Sampling of a VRC01-like Antibody Response in a Chronically Infected Individual** *Z Zhang, X Wu, N Longo, B Zhang, J Zhu, C Nisc, JC Mullikin, L Wu, GJ Nabel, M Connors, PD Kwong, JR Mascola, L Shapiro*	OA07.03
11:15 – 11:30	**Antibody Effector Function Is Regulated by a Combination of Adaptive and Innate Signals** *A Mahan, K Dionne, J Eusebio, G Alter*	OA07.04

11:30 – 11:45	**Non-neutralizing IgG Anti-PID Antibodies Decreased Viral Load Following High Dose Vaginal Challenge of Non-human Primates** *C Moog, N Dereuddre-Bosquet, M Biedma, S Schmidt, T Decoville, I Mangeot, S Zolla-Pazner, B Vcelar, D Katinger, V Holl, R Le Grand*	OA07.05
11:45 – 12:00	**Vaccine-induced ADCC-mediating Antibodies Target Unique and Overlapping Envelope Epitopes** *J Pollara, M Bonsignori, M Moody, M Alam, H Liao, K Hwang, J Pickeral, J Kappes, C Ochsenbauer, K Soderberg, TC Gurley, DM Kozink, DJ Marshall, JF Whitesides, D Montefiori, JE Robinson, J Kaewkungwal, S Nitayaphan, P Pitisuttithum, S Rerks-Ngarm, J Kim, N Michael, G Tomaras, BF Haynes, G Ferrari*	OA07.06
12:00 – 12:15	**454 Pyrosequencing and Bioinformatics Analysis of the Lineage of the Broad and Potent gp41-directed Antibody 10e8** *G Ofek, J Zhu, Y Yang, B Zhang, K Mckee, M Louder, J Huang, L Laub, M Connors, J Mascola, PD Kwong*	OA07.07
12:15 – 12:30	**Engineered Mice and B Cell Lines Expressing Broadly Beutralizing Antibodies and Their Unmutated Precursors: Tools for HIV Vaccinology** *D Nemazee, C Doyle-Cooper, T Ota, AB Cooper, M Huber, E Falkowska, K Doores, L Hangartner, K Le, D Sok, J Jardine, J Lifson, X Wu, JR Mascola, P Poignard, JM Binley, BK Chakrabarti, WR Schief, RT Wyatt, DR Burton*	OA07.08 LB

10:30 – 12:30 Oral Abstract Session 08: T Cell Responses

BCEC Level 2, Room 258 ABC

Chairs: Julie McElrath and Julian Schulze zur Wiesch

10:30 – 10:45	**Cellular Immune Responses and Changes in VL After a Dendritic Cells (DC)-based Therapeutic Vaccine in cART Treated Chronic HIV-infected Patients with CD4 T Cells Above 450/mm** *F García, AC Guardo, M Maleno, L Papagno, M Bargalló, N Climent, B Autran, J Gatell, T Gallart, M Plana*	OA08.01
10:45 – 11:00	**Alterations in Function and Distribution of Regulatory T Cells (Tregs) May Blunt Vaccine Induced Immune Responses in HIV Infection** *J van Lunzen, I Toth, P Hartjen, J Schulze zur Wiesch*	OA08.02
11:00 – 11:15	**Identification of CD8+ T Cell Host Factors Involved in HIV Control** *G Gaiha, K McKim, M Woods, M Lichterfeld, A Brass, B Wallker*	OA08.03
11:15 – 11:30	**PD-1, IL-10, IFN-γ and IL-12 Form a Network to Regulate HIV-1-specific CD4 T Cell and Antigen-presenting Cell Function** *F Porichis, L Barblu, DS Kwon, M Hart, J Zupkosky, GJ Freeman, DG Kavanagh, DE Kaufmann*	OA08.04
11:30 – 11:45	**Distinct Gene Expression Profiles Associated with the Susceptibility of Pathogen-specific CD4+ T Cells to HIV-1 Infection** *H Hu, M Nau, P Ehrenberg, A Chenine, Z Daye, Z Wei, N Michael, M Vahey, J Kim, M Marovich, S Ratto-Kim*	OA08.05

11:45 – 12:00 **HIV Control Through a Single Nucleotide on the HLA-I Locus** OA08.06
H Kløverpris, M Harndahl, J Carlson, A Leslie, M van der Stok, G Huang, F Chen, L Riddell, D Steyn, D Goedhals, C van Vuuren, J Frater, B Walker, T Ndung'u', S Buus, P Goulder

12:00 – 12:15 **Accelerated Heterologous Prime-boost Adenovirus Vector-based SIV Vaccine in Neonatal Rhesus Monkeys** OA08.07
J Liu, H Li, M Iampietro, DH Barouch

12:15 – 12:30 **Intra-dermal Immunisation with SIV Gag-based Vaccines Alone Inhibits Acquisition of SIVmac251** OA08.08
N Almond, R Stebbings, M Page, B Li, N Berry, C Ham, D Ferguson, N Rose, E Mee, C Stahl-Hennig, G Dickson, T Athanasapoulos, A Benlahrech, S Herath, A Meiser, S Patterson

10:30 – 12:30 Oral Abstract Session 09: Clinical Trials
BCEC Level 2, Room 253 ABC

Chairs: Yiming Shao and Sandhya Vasan

10:30 – 10:45 **Safety and Immunogenicity of a Randomized Phase I Prime-boost Trial with ALVAC-HIV (vCP205) and gp160 MN/LAI-2 Adjuvanted in Alum or Polyphosphazene** OA09.01
RJ O'Connell, VR Polonis, S Ratto-Kim, J Cox, LL Jagodzinski, J Malia, NL Michael, J Excler, ML Robb, JH Kim

10:45 – 11:00 **In Vivo Targeting of HIV Gag to Dendritic Cells in Combination with Poly ICLC Is Safe and Immunogenic in Healthy Volunteers** OA09.02
M Caskey, C Trumpfheller, S Pollak, L Sinnenberg, A Hurley, J Pring, I Shimeliovich, B Yipp, N Anandasabapathy, S Mehandru, P Sarma, R Koup, R Bailer, G Tomaras, A Sato, T Keler, R Steinman, S Schlesinger

11:00 – 11:15 **First-in-Human Phase 1 Trial of the Safety and Immunogenicity of a Recombinant Adenovirus Serotype 5 HVR48 (rAd5HVR48) HIV-1 Vaccine** OA09.03
SR Walsh, MS Seaman, JA Johnson, RP Tucker, KH Krause, M Weijtens, MG Pau, J Goudsmit, R Dolin, DH Barouch, LR Baden

11:15 – 11:30 **Antibody-mediated Inhibition of HIV-1 Elicited by HIV-I DNA Priming and Boosting with Heterologous HIV-1 Recombinant MVA in Healthy Tanzanian Adults** OA09.04
A Joachim, C Nilsson, S Aboud, M Bakari, EF Lyamuya, M Robb, M Marovich, C Ochsenbauer, B Wahren, F Mhalu, E Sandström, G Biberfeld, G Ferrari, VR Polonis

11:30 – 11:45 **A First-in-Man, Double Blind, Placebo Controlled Study of the Candidate Therapeutic Vaccine Opal-HIV-Gag(c) in HIV Infected Patients Receiving HAART** OA09.05
AG Jackson, HN Kløverpris, A Handley, P Hayes, J Gilmour, M Atkins, B Walker, J Ackland, M Sullivan, P Goulder

11:45 – 12:00 **Multiple Antibody Specificities (gp41, V1V2, and V3) Elicited in the Phase II Multiclade (A, B, C) HIV-1 DNA Prime, rAd5 Boost Vaccine Trial** OA09.06 LB
WB Williams, K Jones, A Krambrink, D Grove, P Liu, NL Yates, MA Moody, G Ferrari, J Pollara, Z Moodie, CA Morgan, H Liao, DC Montefiori, C Ochsenbauer, J Kappes, S Hammer, J Mascola, R Koup, L Corey, G Nabel, P Gilbert, G Churchyard, M Keefer, BS Graham, BF Haynes, GD Tomaras

12:00 – 12:15	**rAd5 Prime/NYVAC-B Boost Regimen is Superior to NYVAC-B Prime/rAd5 Boost Regimen for Both Response Rates and Magnitude of CD4 and CD8 T-cell Responses** *P Bart, Y Huang, N Frahm, S Karuna, M Allen, NK Kochar, S Chappuis, J Gaillard, B Graham, G Pantaleo*	OA9.07 LB
12:15 – 12:30	**Phase 2a Safety and Immunogenicity Testing of DNA and Recombinant Modified Vaccinia Ankara Virus Vaccines Expressing Virus-like Particles** *P Goepfert, M Elizaga, D Montefiori, J Hural, S DeRosa, G Tomaras, K Seaton, A Sato, L Ouedraogo, Y Donastorg, M Cardinali, J Lama, L Baden, M Keefer, J McElrath, S Kalams, H Robinson*	OA09.08 LB
12:30 – 13:30	**Lunch**	BCEC Level 2, Rooms 252 AB / 254 AB / 257 AB
12:30 – 13:30	**Networking Lunch**	BCEC Level 2, Room 259 AB

Join us in BCEC Level 2, Room 259 AB where lunch tables have been reserved for young and early-career investigators and community representatives to network with senior researchers.

13:30 – 14:45 Oral Abstract Session 10: HIV Transmission / Diversity

BCEC Level 3, Room 258 ABC

Chairs: Bette Korber and Zabrina Brumme

13:30 – 13:45	**Impact of Transmitted CTL Escape Mutations on Replicative Capacity and HIV Pathogenesis in Early Infection** *J Prince, D Claiborne, D Heckerman, J Carlson, H Prentice, M Schaefer, L Yue, J Mulenga, J Tang, P Goepfert, P Farmer, R Kaslow, S Allen, E Hunter*	OA10.01
13:45 – 14:00	**A Deeper View of Transmitted/Founder Viruses Using 454 Whole Genome Deep Sequencing** *DC Tully, KA Power, H Bedard, CL Boutwell, P Charlebois, EM Ryan, NJ Lennon, M Altfeld, MR Henn, TM Allen*	OA10.02
14:00 – 14:15	**Modeling Virus Exposure During Male to Female Transmission of HIV-1** *A Carias, M McRaven, M Anderson, RS Veazey, TJ Hope*	OA10.03
14:15 – 14:30	**Beneficial HLA-mediated Viral Polymorphisms on the Transmitted Virus Additively Influence Disease Progression in HIV-1, Subtype C Infection** *RS Ntale, DR Chopera, NK Ngandu, M Abrahams, A Debra, M Mlotswa, L Werner, Z Woodman, K Mlisana, S Abdool Karim, CM Gray, C Williamson, CAPRISA 002 AI Study Team*	OA10.04
14:30 – 14:45	**T-cell Based Sieve Analysis Ties HLA A*02 to Vaccine Efficacy and IgA-C1 Immune Correlate in RV144 Thai Trial** *T Hertz, A Gartland, H Janes, S Li, Y Fong, GD Tomaras, D Morris, D Geraghty, GH Kijak, PT Edlefsen, M Rolland, BB Larsen, S Tovanabutra, E Sanders-Buell, AC DeCamp, CA Magaret, H Ahmed, S Nariya, K Wong, H Zhao, W Deng, BS Maust, M Bose, S Howell, M Lazzaro, A Bates, E Lei, A Bradfield, G Ibitamuno, V Assawadarachai, RJ O'Connel, MS deSouza, S Nitayaphan, S Rerks-Ngarm, ML Robb, MJ McElrath, BF Haynes, NL Michael, PB Gilbert, JI Mullins, JH Kim*	OA10.05 LB

13:30 – 14:45 **Oral Abstract Session 11:**
Vaccine Immunogens / Delivery
BCEC Level 2, Ballroom East

Chairs: Harriet Robinson and Sallie Permar

13:30 – 13:45 **HIV-1 Envelope Trimer Elicits Higher Neutralizing Antibody Responses Than Monomeric gp120** OA11.01
JM Kovacs, JP Nkolola, H Peng, A Cheung, J Perry, CA Miller, MS Seaman, D Barouch, B Chen

13:45 – 14:00 **Shaping Humoral Responses Against Mini-libraries of HIV Env Antigens via Lipid Nanoparticle Vaccine Delivery** OA11.02
MC Hanson, J Mata-Fink, W Abraham, KD Wittrup, DJ Irvine

14:00 – 14:15 **Vaccine Responses to Conserved Regions of the HIV-1 Proteome Are Associated with an Increased Capacity to Inhibit Multiple Virus Isolates Ex Vivo** OA11.03
A Ashraf, J Kopycinski, H Cheeseman, F Lala, J Czyzewska-Khan, A Spentzou, DK Gill, M Keefer, J Excler, P Fast, P Hayes, JH Cox, J Gilmour

14:15 – 14:30 **Antigen-specific T Lymphocyte Responses Elicited by a DNA – MVA HIV CN54gp140 Immunization Regime Are Significantly Altered by the TLR4 Adjuvant GLA** OA11.04
PF McKay, AV Cope, J Swales, S Joseph, M Esteban, R Tatoud, D Carter, J Weber, RJ Shattock

14:30 – 14:45 **Quality of T-cell Responses Versus Reduction in Viral Load: Results from an Exploratory Phase II Clinical Study of Vacc-4x, a Therapeutic HIV Vaccine** OA11.05 LB
K Ellefsen-Lavoie, J Rockstroh, R Pollard, G Pantaleo, D Podzamczer, D Asmuth, J van Lunzen, K Arastéh, D Schürmann, B Peters, B Clotet, D Hardy, A Lazzarin, J Gatell, MA Sommerfelt, I Baksaas, V Wendel-Hansen, B Sørensen

13:30 – 14:45 **Oral Abstract Session 12:**
Vaccine Concepts – Vectors and Inserts
BCEC Level 2, Room 253 ABC

Chairs: Punnee Pitisuttithum and Karin Lore

13:30 – 13:45 **DNA Vaccines Expressing Conserved Elements Provide Potent and Broad Immune Responses** OA12.01
GN Pavlakis, V Kulkarni, A Valentin, M Rosati, NY Sardesai, B Mothe, C Brander, S LeGall, DB Weiner, M Rolland, JI Mullins, BK Felber

13:45 – 14:00 **Reinventing the Nucleic Acid Vaccine with Self-amplifying RNA** OA12.02
AJ Geall, GR Otten, A Hekele, W Bogers, H Oostermeijer, P Mooij, K Gerrit, E Verschoor, K Banerjee, Y Cu, CW Beard, LA Brito, JB Ulmer, CW Mandl, SW Barnett

14:00 – 14:15 **Adenovirus Serotype 26 Utilizes CD46 as Primary Cellular Receptor and Only Transiently Activates T Lymphocytes Following Vaccination of Rhesus Monkeys** OA12.03
H Li, EG Rhee, K Masek-Hammerman, JE Teigler, P Abbink, DH Barouch

14:15 – 14:30	**Full-length HIV-1 Immunogens Induce Greater T Lymphocyte Responses to Conserved Epitopes Than Conserved-Region-Only HIV-1 Immunogens in Monkeys** *KE Stephenson, A SanMiguel, NL Simmons, K Smith, JJ Szinger, BT Korber, DH Barouch*	OA12.04
14:30 – 14:45	**Rational Immunogen Design to Target Specific Germline B Cell Receptors** *J Jardine, O Kalyuzhniy, T Ota, A McGuire, S Menis, J Julien, E Falkowska, S MacPherson, M Jones, DR Burton, IA Wilson, L Stamatatos, D Nemazee, WR Schief*	OA12.05 LB
14:45 – 16:00	**Poster Session 2 with Tea and Coffee Break** *See inside back cover for the poster presentation schedule.*	BCEC Level 3, Ballroom West

Poster Discussion 2a:
Innate Immunity

BCEC Level 3, Ballroom West

Chair: Dan Zak

15:00 – 15:07	**HLA-Cw*0102-Restricted HIV-1 p24 Epitope Variants Can Modulate the Binding of the Inhibitory KIR2DL2 Receptor and Primary NK Cell Function** *C Koerner, L Fadda, S Kumar, N van Teijlingen, A Piechocka-Trocha, M Carrington, M Altfeld*	P07.09
15:07 – 15:14	**Frequent and Strong Antibody-mediated NK Cell Activation to HIV-1 Env in Individuals with Chronic HIV-1 Infection** *C Thobakgale, L Fadda, K Lane, I Toth, F Pereyra, S Bazner, T Ndung'u, BD Walker, E Rosenberg, G Alter, M Carrington, T Allen, M Altfeld*	P07.03
15:14 – 15:21	**HIV Triggers Immunoregulatory Dendritic Cells And Regulatory T Cells Through The Non-canonical NF-kB Pathway** *O Manches, MV Fernandez, J Plumas, L Chaperot, N Bhardwaj*	P07.16 LB
15:21 – 15:28	**Biodistribution of Neutralizing Monoclonal Antibodies IgG1 b12 and LALA in Mucosal and Lymphatic Tissues of Rhesus Macaques** *AJ Hessell, E Epson, B Moldt, E Rakasz, S Pandey, WF Sutton, Z Brower, VM Hirsch, DR Burton, NL Haigwood*	P08.17
15:28 – 15:35	**Critical Role for Monocytes in Mediating HIV-specific Antibody-dependent Cellular Cytotoxicity** *M Kramski, GF Lichtfuss, A Schorcht, AP Johnston, R De Rose, R Center, A Jawarowski, S Kent*	P03.08
15:35 – 15:42	**KIR/HLA Genotype Combinations Are Determinants of Natural Killer (NK) Cell Mediated Antibody-dependent Cellular Cytotoxicity (ADCC) Potency** *MS Parsons, P Zanoni, B Tallon, S Miconiatis, N Shoukry, J Bruneau, CM Tsoukas, NF Bernard*	P07.19 LB

Poster Discussion 2b:
Clinical and Pre-Clinical Vaccine Trials

BCEC Level 3, Ballroom West

Chair: Kathryn Stephenson

15:00 – 15:07 **Rapid Development Of Cross-clade Neutralizing Antibody Responses After Clade B Gp120/Gp140 Protein Priming And Clade C Gp140 Protein Boosting** P04.30 LB
P Spearman, G Tomaras, D Montefiori, Y Huang, H Ahmed, M Elizaga, J Hural, J McElrath, L Ouedraogo, M Pensiero, C Butler, S Kalams, ET Overton, S Barnett, N Group

15:07 – 15:14 **Uptake and Tolerability of Repeated Mucosal Specimen Collection in Two Phase 1 AIDS Preventive Vaccine Trials in Kenya** P04.15
G Mutua, G Omosa-Manyonyi, H Park, P Bergin, D Laufer, PN Amornkul, J Lehrman, P Fast, J Gilmour, O Anzala, B Farah

15:14 – 15:21 **Immunogenicity of a Universal HIV-1 Vaccine Vectored by DNA, MVA and CHADV-63 in a Phase I/IIA Clinical Trial** P04.11
NJ Borthwick, T Ahmed, A Rose, U Ebrahimsa, A Black, E Hayton, H Yang, G Hancock, S Campion, N Frahm, S Colloca, A Nicosia, A McMichael, L Dorrell, T Hanke

15:21 – 15:28 **A Dose-escalation Clinical Trial to Evaluate the Safety and Immunogenicity of a Replication-defective HIV-1 Vaccine-HIVAX** P04.20
F Tung, J Tung, M Fischl

15:28 – 15:35 **Priming with a "Simplified Regimen" of HIV-1 DNA Vaccine Is as Good as a "Standard Regimen" When Boosted with Heterologous HIV-1 MVA Vaccine** P04.01
P Munseri, A Kroidl, C Nilsson, C Moshiro, S Aboud, A Joachim, C Geldmacher, E Aris, D Buma, E Lyamuya, F Gotch, K Godoy-Ramirez, K Pallangyo, L Maboko, M Marovich, M Robb, M Hoelscher, M Janabi, P Mann, S Joseph, S Mfinanga, W Stoehr, F Mhalu, B Wahren, G Biberfeld, S McCormack, E Sandstrom, M Bakari

15:35 – 15:42 **Increased Mucosal CD4+ T-cell Activation Following Vaccination with an Adenoviral Vector in Rhesus Macaques** P11.25
I Bukh, R Calcedo, S Roy, DG Carnathan, R Grant, SJ Ratcliffe, JM Wilson, MR Betts

16:00 – 18:00	**Symposium 04:** Cross-cutting Issues in Clinical Trial Design	BCEC Level 3, Room 253 ABC
	Chairs: Margaret McCluskey and Chidi Nweneka	
16:00 – 16:15	**PrEP and Vaccines: When and How to Respond to Positive Data** *M Warren*	S04.01
16:15 - 16:30	**Evolving Standards of Prevention and Implications for Future Trials** *DR Wassenaar*	S04.02
16:30 - 16:45	**Combination Prevention: Scaling Up Delivery While Accelerating Discovery, Lessons From Medical Male Circumcision in Kenya** *K Agot, E Omanga*	S04.03
16:45 - 17:00	**Combination Prevention: Designing and Implementing a New Era of Trials** *RV Barnabas*	S04.04
17:00 – 17:15	**Combination Prevention: A Community Perspective on Complex Trial Results and Designs** *M Rose*	S04.05
17:15 – 18:00	**Moderated Panel Discussion**	

16:00 – 18:00	**Symposium 05:** Mucosal Immunology	BCEC Level 2, Room 258 ABC
	Chairs: Francoise Barré-Sinoussi and Doug Kwon	
16:00 – 16:25	**Vaccine-microbicide Combination Studies in a Rhesus Macaque Vaginal Transmission Model** *JP Moore*	S05.01
16:25 – 16:50	**Genital Tract Inflammation and Susceptibility to HIV Infection in Women from the CAPRISA 004 Microbicide Trial of Tenofovir Gel** *JS Passmore, L Masson, L Werner, S Karim*	S05.02
16:50 – 17:15	**The Current State of Mucosal Immunity Elicited by Vaccines** *R Shattock*	S05.03
17:15 – 17:40	**Persistence Pays Off: Stringent Control and Potential Clearance of AIDS Virus Infection by Persistent Vaccine-induced Effector Memory T Cells** *L Picker*	S05.04
17:40 – 17:55	**HIV Interactions and the Perils of Epithelial Thinning in the Female Reproductive Tract** *A Carias, M McRaven, M Anderson, T Henning, E Kersh, J Smith, K Butler, S Vishwanathan, JM McNicholl, RM Hendry, R Veazey, T Hope*	S05.05 OA

16:00 – 18:00 **Symposium 06:** Preclinical Studies and Vaccine Platforms — BCEC Level 2, Ballroom East

Chairs: Nelson Michael and Bonaventura Clotet

16:00 – 16:25 **Novel HIV Vaccine Strategies** — S06.01
JC Sadoff, H Schuitmaker, L Baden, L Digilio, M Grazia Pau, M Marovitch, M Robb, N Michael, J Goudsmit, D Barouch, R Dolin

16:25 – 16:50 **Poxvirus-based Vectors as Immunization Vehicle: Past, Present and Future** — S06.02
J Tartaglia

16:50 – 17:15 **Pre-clinical Studies and Vaccine Platforms using Chimp Adenovirus Vectors** — S06.03
T Hanke

17:15 – 17:40 **Passive Immunization for HIV Prevention** — S06.04
F McCutchan, F Randazzo

17:40 – 17:55 **E-DNA IM or ID Delivery Prime Enhances Antibody and T cell Responses Following Recombinant gp120 Env Boost** — S06.05 OA
NA Hutnick, M Karuppiah, J Pollara, J Yan, DJ Myles, K Broderick, M Morrow, N Sardasai, D Montefiori, S Barnett, G Ferrari, DB Weiner

18:00 – 20:00 **Poster Session 3 and Reception** — BCEC Level 3, Ballroom West
See inside back cover for the poster presentation schedule.

Poster Discussion 3a: T Cell Immunity — BCEC Level 3, Ballroom West

Chair: Zaza Ndhlovu

18:30 – 18:37 **Different Memory T Cell Phenotypes Are Elicited by Ad5 and Rare Adenoviruses** — P11.04
P Penaloza, E Borducchi, A McNally, N Simmons, J Teigler, N Provine, W Tan, R Ahmed, DH Barouch

18:37 – 18:44 **A High-dimensional Immune Monitoring Model of HIV-1-specific CD8 T Cell Responses Accurately Identifies Subjects Achieving Spontaneous Viral Control** — P11.38
ZM Ndhlovu, L Chibnik, J Proudfoot, S Vine, A McMullen, K Cesa, F Porichis, D Alvino, A Piechocka-Trocha, P De Jager, BD Walker, D Kaufmann

18:44 – 18:51 **Maturation of Protective Immunity Induced by SIVΔnef Correlates with Differential Expression of Transcription Factors in SIV-specific CD8+ T Cells** — P02.10
JM Billingsley, PA Rajakumar, NC Salisch, YV Kuzmichev, HS Hong, MA Connole, RK Reeves, H Kang, W Li, RP Johnson

18:51 – 18:58 **Preferential Targeting Of Co-evolving Gag Residues in Long-term Non Progressors** — P11.36
J Sunshine, K Shekhar, D Heckerman, AK Chakraborty, N Frahm

18:58 – 19:05 **Early Changes in the CD8 T Cell Immunodominance Hierarchy in Primary HIV Infection Prior to Seroconversion** — P11.02
N Keane, C Almeida, A Chopra, D Cooper, E Demaine, S Mallal, M John

19:05 – 19:12 **The Early Th17/Treg Ratio Predicts The Immune Activation Set Point In Patients With Primary HIV Infection** — P11.43 LB
MF Chevalier, G Petitjean, C Didier, P Girard, L Meyer, F Barré-Sinoussi, D Scott-Algara, L Weiss

Poster Discussion 3b:
Vaccine Concepts and Design

BCEC Level 3, Ballroom West

Chair: Eric Sandström

18:30 – 18:37 **Targeting HIV-1 Envelope Glycoprotein Trimers to B Cells Using APRIL Improves Antibody Responses** P12.11
M Melchers, I Bontjer, T Tong, N Chung, P Klasse, D Eggink, M Gentile, A Cerutti, D Montefiori, W Olson, B Berkhout, J Binley, J Moore, R Sanders

18:37 – 18:44 **Extensive Glycoform Heterogeneity in the gp120 Envelope Proteins Used in the RV144 Trial** P12.07
B Yu, JF Morales, SM O'Rourke, GP Tatsuno, PW Berman

18:44 – 18:51 **Native Envelope-based Immunogens Derived from Critical Timepoints in the Development of Breadth Elicit Rapid Neutralizing Antibodies in Rabbits** P12.56
DC Malherbe, AJ Hessell, ND Sather, B Guo, S Pandey, F Pissani, H Robins, S Kalams, L Stamatatos, NL Haigwood

18:51 – 18:58 **A Minimal T-cell Immunogen Designed to Cover HIV-1 Specificities Associated with Control Is Immunogenic in Mice and Breaks CTL Immunodominance** P12.16
B Mothe, A Llano, M Rosati, S Perez-Alvarez, V Kulkarni, B Chowdhury, C Alicea, RK Beach, NY Sardesai, GN Pavlakis, BK Felber, C Brander

18:58 – 19:05 **Preferential Targeting of Conserved Gag Regions After Vaccination with a Heterologous DNA Prime Modified Vaccinia Ankara Boost HIV Vaccine Regime** P12.41
A Bauer, L Podola, A Haule, L Sudi, C Nilsson, P Mann, M Missanga, B Kaluwa, L Maboko, C Lueer, M Mwakatima, S Aboud, M Bakari, J Currier, M Robb, S Joseph, S McCormack, E Lyamuya, B Wahren, E Sandström, G Biberfeld, M Hoelscher, A Kroidl, C Geldmacher

19:05 – 19:12 **CN54gp140: Product Characteristics, Preclinical and Clinical Use - Recombinant Glycoprotein for HIV Immunization** P12.62 LB
D Katinger, S Jeffs, F Altmann, A Cope, P McKay, N Almond, E Sandström, B Hejdeman, G Biberfeld, C Nilsson, D Hallengärd, B Wahren, T Lehner, M Singh, DJ Lewis, C Lacey, R Shattock

Wednesday, 12 September

07:30 – 12:30	**Speaker Check-in**	BCEC Level 2, Room 251
08:00 – 14:00	**Registration**	BCEC Level 3, Ballroom Foyer

08:30 – 10:30	**Symposium 07:** Priming Effective B Cell Responses	BCEC Level 3, Ballroom East
	Chairs: Lynn Morris and Ralph Pantophlet	
08:30 – 08:55	**The Unique T Cell Receptor Expressed by Each Naive Helper T Cell Instructs Effector Cell Fate** *MK Jenkins*	S07.01
08:55 – 09:20	**T Follicular Helper Cells in HIV Infection** *H Streeck*	S07.02
09:20 – 09:45	**Evolution of Broadly Cross-neutralizing Antibodies During HIV-1 Infection** *PL Moore*	S07.03
09:45 – 10:10	**Genetic and Structural Basis for Development of High Affinity Antibodies** *J Crowe*	S07.04
10:10 – 10:25	**CD40L Adjuvant for DNA/MVA Vaccine: Enhanced Protection from Acquisition of Neutralization Sensitive & Neutralization Resistant Mucosal SIV Infections** *S Kwa, S Sadagopal, J Hong, S Gangadhara, R Basu, L Lai, S Iyer, K Araki, PL Earl, L Wyatt, F Villinger, B Moss, R Ahmed, RR Amara*	S07.05 OA

08:30 – 10:30	**Symposium 08:** Therapeutic Vaccination and Strategies for the Cure	BCEC Level 2, Room 253 ABC
	Chairs: Janet Siliciano and Xu Yu	
08:30 – 08:55	**Therapeutic Approaches to Curing HIV Infection** *S Deeks*	S08.01
08:55 – 9:20	**Therapeutic Vaccines in Strategies for Functional Cure of HIV** *B Autran*	S08.02
09:20 – 09:45	**Barriers to HIV-1 Eradication** *R Siliciano*	S08.03
09:45 – 10:10	**Persistence of Latently Infected CD4+ T Cells During HAART: Keeping Memory, Keeping HIV** *N Chomont*	S08.04
10:10 – 10:30	**Assembling the Tools to Clear HIV Infection: Contributions from Virology and Immunology** *D Margolis*	S08.05

10:30 – 11:00	**Tea and Coffee Break**	BCEC Level 3, Ballroom Foyer

11:00 – 12:30 Plenary 03: New Concepts in Immune Induction

BCEC Level 3, Ballroom East

Chairs: Larry Corey and Dennis Burton

11:00 – 11:30 **Follicular T Helper Cells in Vaccination and Lentivirus Pathogenesis** PL03.01
RA Koup

11:30 – 12:00 **Maturation Pathways of Broad Neutralizing Antibodies: Blueprints for Vaccine Design** PL03.02
B Haynes

12:00 – 12:30 **Experimental HIV Vaccines Induce Immunological Memory in Murine and Human NK Cells** PL03.03
S Paust

12:30 – 13:30 Rapporteurs

BCEC Level 3, Ballroom East

Chairs: Galit Alter and Dan Barouch

12:30 – 12:45 **The Target: How Do We Hit the Virus Where it Hurts?**
Morgane Rolland
MHRP, HJF, USA

12:45 – 13:00 **People: Where Do We Stand with Moving Towards Efficacy Trials?**
Magdalena Sobieszczyk
Columbia University, USA

13:00 – 13:15 **Vaccimmunology: Advances in Our Understanding of Immune Correlates**
Julie Boucau
The Ragon Institute of MGH, MIT and Harvard, USA

13:15 – 13:30 **Animal Models: What Can Our Furry Friends Teach Us?**
Nichole Klatt
National Institute of Health, USA

13:30 – 14:00 Closing Session and Handover

BCEC Level 3, Ballroom East

Chairs: Galit Alter and Dan Barouch

13:30 – 13:35 Bill Snow
Global HIV Vaccine Enterprise

13:35 – 13:50 Galit Alter and Dan Barouch
2012 Conference Chairs

13:50 – 14:00 José Gatell and Bonaventura Clotet
2013 Conference Chairs

Plenary Session 01: HIV Vaccines in the Broader HIV Prevention Landscape

PL01.01

An HIV Vaccine: A Critical Component of a Comprehensive HIV Prevention Strategy

A Fauci[1]

[1]National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA

Traditionally, preventive vaccines have been stand-alone components of public health campaigns. Repeatedly, they have demonstrated extraordinary value as tools to prevent infectious diseases, stop epidemics, and save millions of lives, with rates of effectiveness as high as 80-95 percent. With regard to HIV infection, we are facing a relatively unique situation where this time-honored concept of a vaccine as a stand-alone prevention modality is unlikely. Rather, in global efforts to end the AIDS pandemic, an HIV vaccine would be a critical component of a comprehensive combination prevention strategy. In designing and testing candidate HIV vaccines, we must operate within a new paradigm that considers the additive as well as potentially synergistic effects of delivering an effective HIV vaccine in the context of implementing other scientifically proven HIV/AIDS treatment and prevention interventions. These interventions include condoms, education and counseling, and harm-reduction programs; treating infected individuals with antiretroviral therapy (ART) to prevent the spread of infection (treatment as prevention); preventing mother-to-child transmission; pre-exposure prophylaxis with oral ART or microbicides containing ART; and medically supervised male circumcision. An HIV vaccine could be a major factor in achieving an AIDS-free generation if added to a toolkit of other scientifically proven interventions that when used in combination and adhered to, could form a highly effective, integrated HIV prevention strategy that equals or surpasses the effectiveness of vaccines for other infectious diseases.

PL01.02

The Current State of Microbicides as Prevention

S. Abdool Karim[1]

[1]Centre for the AIDS Programme of Research in South Africa, Durban, South Africa

AIDS is particularly severe in Africa, where women bear a disproportionate burden of the epidemic. Worldwide, just over half of all people living with HIV are women and between 70-90% of all HIV infections among women are due to heterosexual intercourse. Although the majority of new HIV diagnoses in the US are through male-to-male sexual contact, heterosexual contact accounts for 84% of new infections among women. Despite the greater vulnerability of women, they have few options to reduce their risk of acquiring HIV infection and new technologies to protect women from sexual transmission of HIV, are urgently needed. Topical microbicides are products designed to prevent HIV and other sexually transmitted infections. Potentially, they can be applied vaginally or rectally to prevent HIV transmission. Despite numerous disappointing efficacy trial results over the past 20 years, substantial progress is now being made in microbicide development after the release of the CAPRISA 004 trial, which provided proof-of-concept that topical antiretroviral microbicides can prevent sexual transmission of HIV and herpes simplex type-2 infection. Currently, research on microbicides is dominated by antiretroviral agents. The candidate microbicide in the most advanced stages of effectiveness testing is tenofovir gel. In addition to the clinical trials of tenofovir, trials are also assessing other antiretroviral drugs, dapivirine (TMC-120) and maraviroc, formulated as a vaginal gel and a vaginal ring, and some early human studies on Amphora™ gel, a barrier and vaginal defense enhancer, and VivaGel™, an entry and fusion inhibitor, are ongoing.The development of microbicides for rectal use has gathered momentum recently but remains several years away. In addition to new formulations and delivery devices, future microbicide development is likely to focus on a combination of antiretroviral drugs and combinations of antiretroviral agents with contraceptives.

PL01.03

Oral PrEP for HIV Prevention: Next Steps

J. Baeten[1]

[1]University of Washington, Seattle, WA, USA

For HIV uninfected persons with repeated and ongoing sexual exposure to HIV, pre-exposure prophylaxis (PrEP) using oral antiretroviral medications offers a possible primary prevention intervention. Proof-of-concept that PrEP protects against sexual HIV acquisition has been demonstrated in three clinical trials, among men who have sex with men and heterosexual men and women. These trials used the antiretroviral medication tenofovir disoproxil fumarate, alone or co-formulated with emtricitabine, and the degree of HIV protection in these trials was strongly related to the level of adherence to PrEP. Two trials among African women did not demonstrate HIV protection with PrEP. Behavioral and biologic hypotheses have been proposed to explain the divergent findings across PrEP trials, with low adherence to daily use of PrEP the leading hypothesis to explain lack of efficacy in two trials and the range of efficacy across the other studies. The demonstration that PrEP works for HIV prevention provides new challenges and opportunities for HIV prevention, including how to deliver PrEP to those who could benefit most from this intervention, how to evaluate new HIV prevention strategies against a background in which some individuals at higher risk for HIV may choose to use PrEP, and how integrate PrEP into potent combination HIV prevention interventions to achieve maximal population benefits. Next steps in the field include rigorous evaluation of uptake and adherence to PrEP in implementation settings and research into next-generation PrEP agents with longer half-life and less user-dependence. The goals of this talk will be to evaluate the evidence for PrEP for HIV prevention, consider what can be learned to guide implementation of PrEP in non-clinical trial settings, and discuss PrEP within the context of the continued need to deliver and evaluate new HIV prevention strategies, particularly an effective prophylactic vaccine.

PL01.04

ART for Prevention of HIV: Glimmers of Success

M. Cohen[1]

[1]University of NC at Chapel Hill, Chapel Hill, NC, USA

Antiretroviral agents (ART) penetrate and sometimes concentrate in the male and female genital tract. The suppression of replication of HIV can be expected to reduce the sexual transmission of the virus. In several observational studies, people treated with ART appeared to be rendered less contagious. The HPTN 052 study, a prospective randomized trial of early (median CD4 count 446 cell/cubic mm blood) vs. delayed ART enrolled 1763 HIV discordant couples at 13 sites in 9 countries. Suppression of HIV replication over 2 years in the early treatment group led to more than 96 per cent reduction of the sexual transmission of HIV. Subjects treated early also had improved personal health. These results led to rapid changes in PEPFAR, WHO and several country treatment guidelines. However, the individual benefits observed for ART may or may not translate into a population level benefit. Successful use of ART to prevent the spread of ART must depend on early identification of people with HIV infection, linkage to care, and durable suppression of viremia. In some setting it may also be necessary to detect and treat people with acute HIV infection because of the disproportionate contribution of people in this stage of disease to the spread of HIV. Some (but not all) ecological studies suggest that the benefits of ART may already be realized in several communities. However, to better understand this potential effect of ART prospective community randomized trials of earlier and broader ART have been launched in Tanzania, Botswana, Zambia and South Africa. At this point earlier and broader use of ART seems inevitable and desirable. With the right stewardship it seems likely that ART will help to mitigate the AIDS pandemic.

Plenary 02: A New Look at the Transmission Event

PL02.01

Barriers to Mucosal Transmission: Challenges and Opportunities

J.D. Estes[1]

[1]SAIC-Frederick, Frederick National Laboratory, Frederick, MD, USA

During HIV-1 mucosal transmission, the viral diversity of the infecting virus is dramatically reduced as the virus transverses the various host barriers in order to establish a new infection. While the stringency of the mucosal blockade is now well documented, there is a paucity of understanding on where the bottleneck occurs including the contributions of various host components in determining the nature of transmitted founder viruses. A detailed understanding of the process of lentiviral transmission will likely be critical to guide the design of efficacious vaccine approaches that capitalize on opportunities to interdict productive infection of the host. This talk will discuss the development of new tools that can be used to elucidate the biological and immunological barriers surrounding mucosal transmission, the timing and process of establishment of infection, and progression to systemic infection. Using nonhuman primate models of mucosal transmission allows for extensive tissue analyses to define the early stages and host responses involved in the establishment and spread of virus in the first days following mucosal inoculation, and to determine the tissues, cells and pathways that are involved in transmission through systemic dissemination in order to better guide the development of preventative strategies.

PL02.02

Viral Dynamics and Immune Response in Acute Infection and Their Impact on Viral Set-Point

M. Robb[1]

[1]Henry M. Jackson Foundation, Bethesda, MD, USA

Understanding early viral and immune events during acute HIV infection is critical to inform prevention and treatment efforts. We characterized plasma viral load, peripheral blood mononuclear cell populations and cytokine/chemokine profiles at baseline and during early stages of acute infections from East Africa and Thailand in the prospective RV217 high-risk cohort. Individuals at high risk for HIV-1 were prospectively followed with twice weekly small blood volumes collected and tested for HIV-1 RNA (NAT) to detect acute infection prior to the advent of antibody. The relationship of events observed prior to and at peak viremia will be discussed in relation to the determination of viral load set-point and immune activation.

Plenary 02: A New Look at the Transmission Event

PL02.03

Viral Evolution in Early HIV Infection, and the Implications for Vaccines

B. Korber[1]

[1]Los Alamos National Laboratory, Los Alamos, NM, USA

Over the past few years, our understanding of events surrounding HIV transmission, early viral expansion, and the role of early T cell immunity in viral control and the evolution of immune escape, has become increasingly well resolved. Many aspects of acute human infection by HIV and infection of macaques with SIV are similar, and animal heterologous viral challenge models provide a good mimic of natural infection for exploring vaccine candidates, with some inevitable limitations due to differences in the host and viral biology. Here I will first review some of the aspects of acute HIV infection, focusing on aspects that relate to viral evolution and selection. Transmitted/founder viruses that establish new infections have some distinctive characteristics, and in the last part of this talk I will describe how we are using these viruses to inform strategies for vaccine design.

Plenary 03: New Concepts in Immune Induction

PL03.01

Follicular T Helper Cells in Vaccination and Lentivirus Pathogenesis

R.A. Koup[1]

[1]Vaccine Research Center, Bethesda, MD, USA

Follicular CD4 T helper (TFH) cells promote the survival, isotype switching and selection of high affinity memory B cells and plasma cells. We investigated the phenotype, function, localization and molecular profile of TFH in rhesus macaques. Similar to human TFH, rhesus macaque TFH are characterized by a CCR7lowPD-1highCTLA-4highICOShighCXCR4high phenotype and represent a heterogeneous population with regard to cytokine function. SIV infection leads to their expansion in lymphoid tissues, which is associated with increased frequency of activated germinal center B cells and SIV-specific antibodies. We have tested several adjuvant preparations (in association with HIV Env vaccination) to determine their potency in stimulating TFH cells, and any impact upon the magnitude and quality of the antibody response. We have also studied SHIV-infected monkeys and HIV-infected humans to determine the levels and duration of lentivirus antigen persistence that are associated with the induction of TFH and broadly neutralizing antibody responses.

PL03.02

Maturation Pathways of Broad Neutralizing Antibodies: Blueprints for Vaccine Design

B. Haynes[1]

[1]Duke University Medical Center, Durham, NC, USA

Most broad neutralizing antibodies (bnAbs) share one of several unusual characteristics: long heavy chain complementarity determining regions, high levels of somatic mutations, polyreactivity and VH restricted usage. All of these antibody characteristics reflect traits that disfavor bnAb induction. Antibody clonal lineages are being elucidated from chronically HIV-1 infected subjects who make bnAbs to define bnAb maturation pathways, and to design new prime, boost vaccine regimens based on bnAb lineages. For vaccine induction of bnAbs that are controlled by immune tolerance, deletional mechanisms must be incomplete, and the remaining bnAb B cells must be able to be activated by immunogens to make clinically relevant levels of plasma antibodies. This talk will discuss lessons learned from the study of bnAb clonal lineages and report results of immunization strategies to stimulate anergic bnAb B cells.

Plenary 03: New Concepts in Immune Induction

PL03.03

Experimental HIV-Vaccines Induce Immunological Memory in Murine and Human NK Cells

S. Paust[1]

[1]Harvard Medical School, Boston, MA, USA

Using mice and humanized mice, we found that a single vaccination with replication-deficient experimental HIV-1 vaccines results in vaccination-dependent NK cell mediated recall responses to HIV-1 group antigen and envelope.

Symposium 01: Exploiting the Innate Immune Response

S01.01

Development of Dendritic Cell-based Prophylactic and Therapeutic HIV Vaccine

Y. Levy[1], A. Palucka[2]

[1]CHU Henri Mondor, Creteil, France; [2]Baylor Institute for Immunology Research, Dallas, TX, USA

Dendritic cells (DCs), as the most potent antigen-presenting cells, have a pivotal role in the initiation, regulation and maintenance of immune responses against cancers and pathogens. They form a unique link between the innate and adaptive immune system. DC subsets display different receptors and express different sets of cytokines/chemokines, leading to distinct immunological outcomes. For example, CD14+ dermal DCs preferentially control humoral immunity while epidermal DCs (Langerhans cells) preferentially induce cellular immunity. Several studies have tested ex vivo generated DC loaded with inactivated HIV or HIV peptides. We report the results of the DALIA trial testing autologous DCs generated ex vivo from monocytes cultured with GM-CSF/IFN-alpha and loaded with five lipidated HIV antigens (LIPO5). Vaccination elicited polyfunctional HIV-specific responses associated with a reduced peak of viral rebound upon HAART interruption. Targeting antigens directly to DCs via anti-DC receptor monoclonal antibody-antigen fusion proteins (FP) is another promising approach to HIV vaccine development. We are developing anti-DC FP with the objective to target Langerin, CD40, DCIR and Lox-1 receptors. In vitro, CD40 and DCIR-HIV FP expanded a broad repertoire of Ag specific and multifunctional CD4+ and CD8+ T cells from HIV-infected patients. The final goal is to combine FPs targeting HIV Env and HIV T cell epitopes to different populations of DC with the aim to elicit B and T cell responses. These FP are now being tested in NHP in prime boost strategies in combination with MVA and Pox vectors.

S01.02

Adjuvants Influence the Magnitude, Quality and Durability of HIV Env Specific Humoral Immunity in Non-human Primates

J.R. Francica[3], G. Tomaras[1], M. Alam[1], G. Ferrari[1], J. Mascola[3], D.T. O'Hagan[2], R. Seder[3]

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An effective preventive vaccine against HIV will require potent and durable HIV Env specific antibody responses. Accordingly, immune adjuvants will be critical for optimizing HIV Env immunity with protein-based vaccines. We performed a comparative analysis of how adjuvants influence HIV Env specific immunity in non-human primates (NHP) when administered with a trimeric HIV Clade C Env protein. The adjuvants chosen were based either on their extensive clinical use (alum, MF 59) or their ability to mediate adaptive immunity through distinct toll-like receptor dependent or other innate pathways. NHP were immunized at week 0, 4, 12 and 24 with HIV Env protein and Alum or MF59 with or without TLR 4 (MPL) or TLR 7 ligands or Poly ICLC or ISCOMs. A comprehensive analysis of HIV binding titer, neutralization against Tier 1 and 2 viruses, CD4BS, ADCC, avidity and serum and mucosal IgG and IgA were performed. Moreover, HIV Env specific B cells were sorted and the Ig loci analyzed using high-throughput deep sequencing to determine the gene family usage, and levels of somatic mutation. Overall, the data presented highlight how adjuvants alter both the magnitude and quality of HIV Env specific antibody responses.

S01.03

Systems Analysis of HIV Vaccine Triggered Innate Immune Responses

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An efficacious HIV vaccine has the greatest potential to halt the HIV pandemic. Systems vaccinology – the application of systems biology analysis tools to vaccine trials – facilitates HIV vaccine development in four major ways. (1) It enables the identification of correlates of immunogenicity and protection; (2) it reveals the cellular regulatory networks that control host immune responses; (3) it can guide the re-engineering of vaccine regimens to enhance desired responses; and (4) it allows comprehensive analysis of failed candidate vaccines to identify what went wrong, opening new avenues of investigation. In this presentation, I describe our work applying systems vaccinology to clinical and preclinical trials of a broad range of candidate HIV vaccines - from novel viral vectors to Toll-like receptor (TLR) adjuvants in combination with HIV/SIV proteins. Our strategy derives from previous work demonstrating how innate immune responses, which develop very early after vaccination, shape the magnitude and quality of vaccine-induced adaptive immunity. There are two components of the approach. We first employ blood-level transcriptional profiling to extensively characterize systemic innate immune responses induced hours or days after vaccination. This analysis allows comparison between vaccines in terms of innate immunogenicity – the extent to which known and novel innate inflammatory pathways are triggered specifically or generically by different vectors or adjuvants. We then computationally integrate the systemic innate immune responses with vaccine induced adaptive immune responses and/or efficacy, which are measured months or years after vaccination. In this manner, we define innate response signatures that are predictive of desirable vaccine responses. These signatures provide insights into why certain vaccine regimens are more efficacious than others and help prioritize molecules and pathways to be targeted in the next generation of enhanced vaccines.

S01.04

Profiling Humoral Immunity: Determining Antibody Innate Immune Recruiting Capacity

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In vivo, antibody responses to vaccination or natural infection are highly polyclonal, with multiple somatic variants directed to multiple epitopes on multiple antigens. This diversity of variable domain recognition characteristics is further complemented by diversity in constant domain subclass and ability to interact with innate immune receptors such as FcgR. Antibody based protection is thus derived from the sum of specificities and activities of this polyclonal humoral milieu. Here we demonstrate biophysical characterization of the innate immune recruiting capacity of polyclonal, antigen-specific antibodies capable of parsing the complex humoral milieu into components that can be associated with relevant clinical, genetic, or functional characteristics. Thousands of measurements of the humoral immune response can be determined, approaching the number of parameters achieved in genotyping and gene expression analyses--providing a comprehensive landscape of antibody activity and better understanding of the characteristics and development of both protective and pathological humoral immunity that may be critical to understanding vaccine-mediated protection.

S01.05 OA

Natural Killer (NK) Cell Responses at Female Genital Mucosa to SIV Vaginal Challenge

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Background: Previous studies of SIV transmission at rhesus genital mucosa have identified a vulnerable window of viral replication within the first 3 days post vaginal inoculation. This provides a potential opportunity for host mucosal innate immune responses to control viral infection.

Methods: In the current work, we examined NK cell (NKG2A+CD3-) responses to SIV vaginal challenge in vaginal and cervical mucosa using IHC and ISH.

Results: NK cells are maintained at a low baseline level in the genital tissue of naïve animals. In the first week post infection, a small number of NK cells (up to 1500 cells/cm sq) infiltrated into genital mucosa. The initial recruiting signal didn't require viral replication as shown by equal NK influx in animals challenged with either WT or AT-2 inactivated viruses. The majority of infiltrating NK cells was Granzyme H+ and 40-60% was IFN-gamma+. However, viral inoculation also enhanced the expression of HLA-E in genital tissues, generating an inhibitory environment to NK functionality. In addition, mucosal NK cells appeared to be IL2Rbeta negative, indicating impaired IL-2 and IL-15 signaling. Therefore, the initial influx of NK cells was not able to eliminate early viral infection at genital mucosa. Surprisingly, in the second week, the number of mucosal NK cells rapidly decreased, which is inversely associated with the number of infected cells in genital tissues and is consistent with an increase in the level of sera IP10 and IL18. Interestingly, at the end of the fourth week (the VL set point), the number of NK cells in genital mucosa was partially recovered, probably because of less viral replication due to CD4 depletion and a reduction of sera IP10 and IL18.

Conclusion: In this study, we found that densities of cervical NK cells are inversely correlated with those of infected cells in animals at Day 5-10 post infection (n=7, p=0.0187).

Symposium 02: Lessons from Clinical Trials

S02.01

RV144 Update: Insights From Correlates of Risk, Breakthrough Infections, and Animal Studies In The Context of Future Vaccine Development

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Of RV144 vaccine-induced immune responses measured at peak immunogenicity, 2 weeks post final vaccination, two were correlated with infection risk. IgA to HIV envelope correlated directly and IgG to gp120 V2 was inversely correlated with infection. Analysis of breakthrough viruses further identified two sites within V2 that showed, positions 169 and 181, that contained distinct genetic signatures comparing vaccine to placebo. A post-hoc analysis suggested that there might be an "early effect" – consistent with an inducible but transient antibody response. Further work defining the correlates of risk over time is ongoing. It is not known whether anti-V2 responses will serve as a correlate for other vaccines. A previous low-dose intrarectal challenge NHP study looking at Ad26 and MVA-based SIV vaccines highlighted anti-V2 (SIV) responses as a potential correlate, and preliminary sequencing of breakthrough SIV from that study demonstrates genetic signatures within the V2 region. Taken together these data suggest a hypothesis that anti-V2 responses may have a more general role in protection, and this concept can be addressed in future clinical trials. The impact of these developments on current and planned studies of ALVAC and gp120 will be discussed.

S02.02

Comparative Immunogenicity of HIV Vaccine Candidates In The HVTN

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The partial success of the ALVAC/AIDSVAX vaccine in reducing HIV acquisition in RV144 led to an increased focus on pox/protein vaccine regimens for the prevention of HIV infection, but few data exist in humans on the immunogenicity of the newer generation of poxvirus vectors, and no new GMP-grade proteins have entered the vaccine pipeline for several years. At the same time, several new vaccine strategies – vectors, combinations, adjuvants and delivery technologies – are in phase I studies in the HVTN, some with dramatic effects on immunogenicity. In the absence of correlates of protection from HIV acquisition, the HVTN laboratories aim at providing a broad understanding of vaccine-induced humoral and cellular immune responses, to allow for rational decisions on future product development in light of the recently proposed correlates of risk in RV144.

S02.03

Clinical Studies With Adenovirus 26 Vectored Candidate HIV-1 Vaccines

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Adenovirus serotype 26 (Ad26) vectors have the potential advantages of relatively low seroprevalence, and have substantial biological differences from the more extensively studied adenovirus 5 vectors. These include differences in receptor usage, tissue tropism, innate immune profile and adaptive immune phenotype. In addition, Ad26 vectors have shown significant protection against acquisition of infection in the SIV macaque model. We conducted phase I studies of a prototype Ad26.EnvA vaccine candidate in healthy volunteers at doses of 109-1011 vp administered IM at 0, 1, and 6 months. The vaccine was generally well tolerated and immunogenic at all doses tested, and stimulated EnvA specific ELISA titers and EnvA specific ELISPOT responses. Colorectal biopsies from vaccinees demonstrated that Ad26.EnvA also stimulated EnvA specific mucosal responses, in addition to those detected in peripheral blood samples.

In collaboration with IAVI, HVTN, and the Ragon Institute, we have also undertaken a study to compare homologous and heterologous immunization regimens utilizing Ad26.EnvA and an Ad35.EnvA candidate vaccine. Ad26 vectors with mosaic Env, Gag, and Pol inserts are under manufacture for testing in humans and should be considered as part of immunization regimens for larger clinical trials.

S02.04

HIV Immunity and Reservoir Following Interventions In Acute HIV Infection: Implication for Functional Cure

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The acute HIV infection period, particularly during the first month of infection, represents a unique window of opportunity to intervene to mitigate massive immune destruction and rapid seeding of the reservoir. So far, with a few exceptions, antiretroviral therapy instituted during early infection has not altered the HIV disease course nor resulted in viremic control in the absence of antiretrovirals. The studies varied by stages of acute HIV infection, onset and types of interventions and endpoints. Many studied interventions in recent HIV seroconverters, likely missing the early critical window for an optimal effect. This presentation will provide a critical review of the published literature of clinical trials of interventions in acute HIV infection and its implication for functional cure. It will also include the latest data of systemic and mucosal immunity and reservoir from ongoing clinical trials including the RV254/SEARCH 010 study that investigates the use of multi-targeted antiretrovirals in the earliest stages of infection. The presentation will offer a perspective on examining interventions in acute HIV infection that may contribute to a functional cure of HIV.

S02.05 OA

Vector Induced Skewing Of Antibody Fc-Effector Functions

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Background: The RV144 vaccine showed a moderate efficacy of protection from HIV infection. The major immune response induced by RV144 was non-neutralizing HIV-specific antibodies (Abs), that may have potentially mediated Ab Dependent Cellular Cytotoxicity (ADCC) and/or Ab dependent Cellular Phagocytosis (ADCP). However little is known about the potential role of different vaccine regimens on inducing these types of humoral immune responses, and whether particular vaccine approaches may preferentially induce robust innate immune recruiting antibody activity that could confer more protection against infection. We therefore aimed to characterize the antibody-effector functional profiles of antibodies elicited by a number of different vaccine approaches including those induced in the: VAX003 trial (bivalent rgp120 clade B/E), RV144 (ALVAC vCP1521 + rgp120 B/E), IPCAVD001 (rAd26.ENVA.01), IAVI-C002 (MVA), IAVI-P002 (DNA + MVA) and IAVI-V001 (DNA + rAd5).

Methods: Abs were purified from the plasma or serum of vaccinees. IgGs were then assayed for ADCC, ADCP, NK degranulation and cytokine production, antibody isotype selection, and Ab affinity for Fc-receptors (FcγRIIa, FcγRIIb and FcγRIIIa).

Results: IAVI-C002 and IAVI-P002 vaccination induced negligible Fc-mediated innate immune responses, while IAVI-V001 was able to induce ADCP in 33% of vaccines. IPCAVD001 was also able to induce strong ADCP in 90% of subjects, but only weak ADCC, NK degranulation or cytokine release. Interestingly, only RV144 and VAX003 vaccination induced strong ADCC, ADCP, NK degranulation and cytokine responses. Furthermore, Abs induced by RV144 and IPCAVD exhibited a more polyfunctional profile compared to VAX003, associated with a skewed isotype distribution of HIV-specific Abs and selective Fc-receptor affinity binding profile.

Conclusion: These data suggest for the first time that distinct vaccine regimens, and vaccine vectors, may selectively induce antibodies with Fc-enhanced functional profiles able to elicit polyfunctional antibody responses, that may provide improved protection from infection.

Symposium 03: New Env Immunogens

S03.01

Structural Basis For HIV-1 Trimeric Env Immunogen

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The trimeric envelope glycoprotein complex of human immunodeficiency virus (HIV-1) is a membrane-fusing machine that mediates virus entry into host cells. Binding of the gp120 exterior envelope glycoprotein to CD4 and the chemokine receptor on target cells triggers conformational changes that allow the gp41 transmembrane envelope glycoprotein to fuse the viral and cell membranes. We determined an atomic structure of the fully glycosylated HIV-1 envelope trimer in its unliganded, pre-fusion state, including the complete exterior and transmembrane regions, by cryo-electron microscopy. The atomic model reveals a dramatic conformational transition of gp120 between its unliganded and CD4-bound states, a torus-like fold of gp41 entirely different from its post-fusion conformation, and a conserved topology of the glycan shield. The quaternary structure of the trimer exhibits tensegrity that stores the free energy fuelling virus entry. The structure provides insights into virus-host interactions and represents an atomic reference for vaccine immunogen design.

S03.02

Pure Native Env Trimers On VLP Surfaces and in Soluble Form

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We hypothesize that as the target of nAbs, the native Env trimer is the most logical basis for a nAb vaccine. Two possible formats to present native trimer are in situ in lipid membranes (e.g. VLPs) or as a soluble recombinant. Different problems have limited progress in both areas: 1) Although VLPs and other particle based approaches present native trimer, they invariably also express non-functional forms of Env. The problem here is that non-functional Env is immunodominant and may interfere with responses against the more antibody-resistant native trimer. 2) Attempts to produce soluble trimers involve truncating gp160 before the transmembrane spanning domain (i.e. gp140). The problem here has been inefficient gp120/gp41 maturation and a failure of gp140 to assemble into stable trimers. Although various innovative mutations have helped address these issues, none have so far have resulted in trimers that fully recapitulate the native complex. We have been working to solve these two problems. Regarding the first problem, we have developed pure native “trimer VLPs” in which junk Env is eliminated using protease digests together with specific mutations that improve trimer folding and stability. Ongoing immunogenicity trials in rabbits will be presented. Regarding the second problem, we have shown that soluble SOS mutant-based trimers but not WT trimers can be extracted from lipid membranes and are stable at 37°C for extended periods in certain buffers. These trimers retain an ability to bind to PG9 and PG16 and other broad nAbs, but do not bind to non-nAbs. We have generated a producer cell line and are currently developing purification procedures to yield mg quantities of trimer for structural and immunogenicity purposes. These new pure trimer platforms provide new tools for other research areas, including mapping broad HIV+ serum neutralization and for accessing new neutralizing mAbs from infected donors.

S03.03

Envelope Immunogen: Stable and Homogeneous HIV-1 gp140 Trimers

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HIV-1 envelope glycoprotein is the primary target for HIV-1-specific antibodies. The native HIV-1 envelope spike on the virion surface is a trimer, but trimeric gp140 and monomeric gp120 are currently believed to induce comparable immune responses. We show that suitably prepared envelope trimers have nearly all the antigenic properties expected for native viral spikes. These stable, rigorously homogenous trimers have markedly different antigenic properties than do monomeric gp120s derived from the same sequences, and they induce potent neutralizing antibody responses for a cross-clade set of tier 1 and tier 2 viruses with titers substantially higher than those elicited by the corresponding gp120 monomers. The implications for HIV-1 vaccine development will be discussed.

S03.04

Epitope-focused vaccines

W Schief[1]

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Conserved epitopes targeted by highly potent and broadly neutralizing antibodies provide starting points for reverse vaccine engineering. We will give an update on our structure-based immunogen design efforts employing some of these epitopes, and also describe a study that provides proof-of-principle for epitope-focused vaccines.

S03.05 OA

Eliciting Broadly Neutralizing Antibodies Against HIV-1 that Target gp41 MPER

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Background: The membrane-proximal external region (MPER) of HIV-1 gp41 is highly conserved and is targeted by broadly neutralizing antibodies (bnAbs). Thus, it is an attractive target for AIDS vaccine development. Here, we describe a mini-protein that can induce bnAbs in rabbits.

Methods: We generated a mini-protein that is structurally rigid, yet efficiently recognized by 2F5, 4E10 and Z13e1. It contains the C-terminal 54 a.a. of gp41 ectodomain (gp41-54Q), which includes the HR2 and the MPER. A 6xHis tag at the C-terminus was used to attach gp41-54Q to Zn-chitosan, which served as an antigen carrier/adjuvant. Rabbits were immunized subcutaneously, 4 times, using two different schedules (wks 0, 4, 9 and 16 vs. 0, 8, 16 and 24). A total of 9 animals were immunized with gp41-54Q in 3 independent experiments. Antibody responses were evaluated by ELISA and in neutralization assays using both TZM-bl and A3R5.7 cells.

Results: Eight of nine rabbits mounted bnAbs (89%). Neutralizing activity was observed against all but two of 44 viruses evaluated to date, including 27 Tier 2 viruses from clades A, B, C, D, AE, and CRF02_AG. Although Nabs could be detected after three immunizations, a fourth immunization was necessary for maximum neutralizing activity. The slower immunization regimen induced higher Nab titers, suggesting that longer rest periods improve affinity maturation. Neutralization inhibition analyses using various peptides identified one neutralizing epitope (N671, W672, F673 and D674) that overlaps with those recognized by Z13e1, 4E10 and 10E8 mAbs. Based on antibody absorption assays, there might be other non-linear epitopes. We are in the process of generating rabbit mAbs for more detailed analyses.

Conclusion: We have successfully demonstrated that we can reproducibly induce bnAbs in rabbits using a mini-protein containing gp41 MPER. These results suggest that gp41 can be a promising vaccine immunogen.

Symposium 04: Cross-cutting Issues in Clinical Trial Design

S04.01

PrEP and Vaccines: When and How to Respond to Positive Data

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Good news can bring tough questions. As biomedical prevention trials demonstrate effectiveness, there is intense attention on the gap between the end of the trial and the next step to access—especially necessary implementation research and access for participants in the original study that provided positive results. In particular, recent positive results from the HPTN 052 "treatment as prevention" trial and a range of pre-exposure prophylaxis trials (PrEP) have transformed the HIV prevention field. In addition to possible public health implications of delivery, these results also raise important questions about the implications for how these results will influence HIV prevention trials in the future.

This presentation will review the current state of the field; present some of the critical issues that need to be considered by researchers, advocates, trial designers, program implementers and policy makers; and explore how the AIDS vaccine field might adapt to emerging results from PrEP and other biomedical prevention trials and responding to positive data with new ideas for trial design and combination prevention. In particular, this presentation will outline some of the critical issues related to the recent US FDA approval of PrEP, WHO guidance and how ongoing and future trials might respond to it.

Vaccine researchers and advocates need to explain and rally support for the continued search for a preventive vaccine, within the context of these other scientific advances. As PrEP and other prevention strategies are introduced, the niche that a partially effective vaccine might fill can become better defined in terms of geography, route of exposure, background combination package and so on, but the AIDS vaccine field will need to take the initiative in bringing this into focus.

S04.02

Evolving Standards of Prevention and Implications for Future Trials

D.R. Wassenaar[1]

[1]SARETI, UKZN, Pietermaritzburg, South Africa

This presentation will discuss selected ethical issues charcterising the current HIV prevention landscape with particular reference to implications for standard of prevention in current and future HIV prevention trials. These issues will be discussed against a background of other general ethical issues in HIV prevention research.

Symposium 04: Cross-cutting Issues in Clinical Trial Design

S04.03

Combination Prevention: Scaling Up Delivery While Accelerating Discovery, Lessons From Medical Male Circumcision in Kenya

K Agot[1], *E Omanga*[1]

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The presentation will focus on combination HIV prevention programs being implemented in Kenya, the complementary role each is playing individually and collectively and the synergies arising therefrom, as well as the challenges faced in scaling them up. Using the rollout of voluntary medical male circumcision (VMMC) in Kenya as a case study, the presentation will delve into how the positive results of VMMC was just the beginning of a series of new discoveries that have helped to strengthen the scale up to its current heights of inching towards the half-million mark by the close of 2012. Scaling up VMMC service delivery while accelerating further discovery underscore the point that the breakthroughs in the HIV prevention field in the last three years are refreshing news but are by no means a call to complacency. With each new discovery in HIV prevention should be a fresh call to design even more studies, look for newer/better answers, and improve the delivery and uptake of what we have available.

S04.04

Combination Prevention: Designing and Implementing a New Era of Trials

R. Barnabast[1]

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How to measure the impact of HIV combination prevention programs is a central question for public health efforts to control HIV epidemic spread. Implicit in this question is how intensive do prevention programs need to be to reduce HIV incidence? To answer these questions, three large community randomized trials are planned to evaluate HIV combination prevention approaches in South Africa, Zambia, Tanzania and Botswana. All propose to use intensified methods of HIV testing (two are using home-based testing) to increase testing coverage, knowledge of status and linkage to care including antiretroviral therapy. The main outcome for the studies is the change in HIV incidence in the intervention communities compared to the control communities. Ongoing monitoring, evaluation and modeling analyses could help studies reach their goals and adapt their design to achieve their primary objectives. Also, the data from these studies will provide insight into implementation strategies moving forward.

Symposium 04: Cross-cutting Issues in Clinical Trial Design

S04.05

Combination Prevention: A Community Perspective on Complex Trial Results and Designs

M. Rose[1]

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This will be a discussion key consideration when thinking about complex trial results and design with the community. Discussing the role of the community from the beginning of the process through the course of the trial. What are key take messages and lessons for helping to keep the community engaged in the process and in sharing the message. As trials become more complex in their design and undertaking how does the community weave into the larger narrative.

Symposium 05: Mucosal Immunology

S05.01

Vaccine-Microbicide Combination Studies in a Rhesus Macaque Vaginal Transmission Model

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In two separate experiments, four groups of rhesus macaques were vaccinated with a T cell based adenovirus vectored vaccine aimed at reducing post-infection viral loads, and/or a partially effective dose of a vaginal microbicide that impedes transmission of a high dose of challenge virus. In the first study, the only two protected animals were in the group that received Ad26/Ad5HVR48 vaccine vectors combined with the fusion inhibitor T-1249 as the microbicide before SIVmac251 challenge. In the second study, vaccination with Ad35/Ad26 vectors combined with the CCR5 inhibitor Maraviroc as the microbicide led to significant reductions of both acquisition of infection and post-infection viral loads following SHIV-162P3 challenge. As expected, the vaccine by itself reduced viral loads but had no acquisition effect, whereas the microbicide had a partial acquisition effect but minimal impact on viremia. For both measures of protective efficacy, the vaccine-microbicide combination differed more from controls than did either separate intervention. Vaccines and microbicides are complementary HIV-1 prevention techniques that may protect better when used together than separately.

S05.02

Genital Tract Inflammation and Susceptibility to HIV Infection in Women from the CAPRISA 004 Microbicide Trial of Tenofovir Gel

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Tenofovir gel, a vaginal microbicide, was found to reduce male-to-female transmission of HIV by 39% overall and by 54% in those who used the gel consistently. We investigated the role of genital inflammation in increasing the risk of HIV infection and as a potential factor undermining the effectiveness of tenofovir gel. The concentrations of 12 cytokines were measured by Luminex in cervicovaginal lavage (CVL) samples collected during the CAPRISA 004 tenofovir gel trial from 62 women who later became HIV-infected (26 assigned to tenofovir gel) and 104 women who remained HIV-uninfected during the study (49 assigned to tenofovir gel). CVL cytokine concentrations did not differ between women using tenofovir and placebo gel. Elevated cervicovaginal concentrations of IL-1α, IL-1β, IL-8, MIP-1α, MIP-1β and GM-CSF were associated with increased susceptibility to HIV infection, irrespective of gel assignment. Additionally, pre-infection inflammation was found to be sustained over time, with the CVL concentrations of 7/12 cytokines similar in individual women at two separate time-points in this study (8-104 weeks apart). Elevated genital inflammatory cytokine concentrations may increase the number of activated CD4+ T cell targets in the genital tract, thereby enhancing the risk of HIV infection following exposure. If so, improving the efficacy of the tenofovir microbicide gel will either require better management of genital inflammation or augmentation of the next generation of microbicides with agents to modulate inflammation.

S05.03

The Current State of Mucosal Immunity Elicited By Vaccines

R. Shattock[1]

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The development of a successful vaccine against HIV is likely to require induction of strong and long-lasting humoral and cellular immune responses at the mucosal portal of virus entry. Mucosal antibodies able to prevent initial target cell infection, reinforced by cellular responses that can control or eliminate the initial foci of infected cells may offer the best potential for robust sterilizing protection against HIV acquisition. While the design of vaccine strategies able to induce such responses may be crucial to development of effective vaccines, little has been done to define possible mucosal correlates of protection against vaginal or rectal exposure. Indeed, to date, most human clinical HIV vaccine trials have omitted to evaluate mucosal immune responses and have focused only on parenterally administered candidates. The observation that the modest protective efficacy of the RV144 trial did not correlate with neutralization but V1/V2 binding antibodies has generated speculation that non-neutralizing antibodies may have had some impact on HIV acquisition, most likely at the mucosal level. Indeed a range of other effector functions such as viral aggregation, mucus trapping, inhibition of transcytosis, antibody dependent cellular cytotoxicity (ADCC), and Fc-mediated inhibition of infection may all enhance mucosal protection by antibody. However there is a lack of definitive data to establish that localized responses offer additional benefit over and above effector functions mediated by mucosal expression of systemic induced responses. Drawing on the wider field of vaccinology this presentation will review current understanding of how adjuvants, delivery strategies and routes of immunization modify mucosal responses to vaccination in humans and non-human primates and will assess the relative strengths and weakness of different approaches as they relate to mucosal protection against HIV acquisition.

S05.04

Persistence Pays Off: Stringent Control And Potential Clearance of AIDS Virus Infection by Persistent Vaccine-Induced Effector Memory T Cells

L. Picker[1]

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This presentation will discuss recent advances in understanding the efficacy of CMV/SIV vaccine vectors in protection against highly pathogenic SIV challenge.

S05.05 OA

HIV Interactions and the Perils of Epithelial Thinning in the Female Reproductive Tract

A. Carias[1], M. McRaven[1], M. Anderson[1], T. Henning[2], E. Kersh[2], J. Smith[2], K. Butler[2], S. Vishwanathan[2], J.M. McNicholl[2], R.M. Hendry[2], R. Veazey[3], T. Hope[1]

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Background: Currently, there is much debate on whether epithelial thinning from hormonal contraceptives can increase HIV acquisition. Previously, we illustrated that HIV can penetrate to depths in squamous epithelium where it can interact with target cells, such as CD4+ T-cells and macrophages. Using a similar approach, we show that epithelial thinning affects virus penetration, along with target cell and cellular junction distribution.

Methods: To investigate how genital epithelial thickness may affect HIV penetration, ten female rhesus macaques were pretreated with 30mg depo-medroxyprogesterone acetate (Depoprovera®) 4-5 weeks prior to vaginal photoactivatable (PA-GFP) HIV exposure. Additionally, eight female pigtail macaques were exposed to PA-GFP HIV at various menstrual cycle stages. Genital tracts were removed 4 hours post-exposure and immediately dissected and snap frozen in optimal cutting temperature (OCT) compound. Comparison of pre- and post-photoactivation image z-stacks revealed the presence of virus, accounting for background.

Results: Within 4 hours, PA-GFP virions were observed between squamous epithelial cells penetrating up to depths of 50µm. This is within the reach of target cell populations. Furthermore, current analysis illustrated epithelial thickness to be inversely proportional to the number of penetrating virions and target cells, independent of thinning mechanism. Also, cellular junction distribution in pigtail macaques with thinned squamous epithelia mirrored those results of progesterone-treated rhesus macaques.

Conclusion: Our current results suggest that HIV acquisition in women may be influenced by menstrual cycle and hormonal contraceptives. CD4+ T-cells and CD68+ macrophage distribution and virus penetration were dependent on epithelial thickness, suggesting HIV interactions with female genital epithelia may differ in the luteal and follicular stages of the menstrual cycle. Our results also suggest that progestin-based contraceptives may alter the barrier properties of the stratified squamous epithelium, possibly increasing the risk of HIV acquisition in women.

Symposium 06: Preclinical Studies and Vaccine Platforms

S06.01

Novel HIV Vaccine Strategies

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Alternative serotype adenovirus (Ad) vectors such as Ad26 and Ad35 are biologically substantially different than Ad5 vectors. We have advanced prototype Ad26 vectors expressing HIV-1 Env into phase 1 clinical trials, Ad35 expressing TB antigens in Phase II trials in adults and infants and Ad35 and Ad26 vectors expressing Malaria CS in human challenge trials. These vectors have proven safe and immunogenic in humans at doses of 109 vp, 1010 vp, and 1011 vp. We have evaluated Ad26, Ad35, and MVA vectors expressing SIV antigens in immunogenicity and challenge studies in rhesus monkeys, and we have shown that Ad35/Ad26 as well as Ad26/MVA prime-boost regimens elicit partial protection against acquisition of infection and partial virologic control following fully heterologous, intrarectal SIVmac251 challenges. Interestingly, blocking acquisition of infection correlated with env specific humoral immunity whereas control of viral replication was associated with gag specific cellular immunity. For our HIV vaccine candidate that will move into clinical trials shortly, we will use computationally optimized "mosaic" HIV-1 Gag/Pol/Env antigens that give rise to substantially expanded cellular immune breadth and depth and induce noninferior antibody responses as compared with consensus or natural sequence antigens in rhesus monkeys.To further improve quality of the humoral immune response, and thereby increase the chances of protection against acquisition of infection, we intend to also boost with a trimeric gp120 envelope protein.

S06.02

Poxvirus-based Vectors as Immunization Vehicle: Past, Present and Future

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An effective prophylactic HIV vaccine has eluded us for more than 30 years, with 33 million infected and 7000 new infections daily. The HIV vaccines field has taken an iterative vaccine design and development approach and have tested multiple HIV vaccine candidates, including peptides, proteins, nucleic acid, viral vectors and prime-boost combination regimens. Towards a globally relevant vaccine, multiple approaches will continued to be tested to elicit broadly reactive T and B-cells and generation of broadly reactive/neutralizing antibodies. The canary pox virus vector, ALVAC, was linked to modest efficacy in RV144 and will need further evaluation. Other poxvirus-based vectors i.e. Vaccinia virus strains (Tian Tian, MVA, NYVAC and replication competent NYVAC) and Avipox (ALVAC, Fowl pox) are under development as HIV vaccine candidates. In this regard, historical perspective as well as current status of multiple poxvirus -based vectors as HIV vaccine candidates will be discussed.

S06.03

Pre-Clinical Studies And Vaccine Platforms Using Chimp Adenovirus Vectors

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The frequency and quality of vaccine-induced immune responses are in part determined by intrinsic immunogenicity of HIV-1-derived immunogens, but the most significant influence comes from the way these are presented to the immune system. The specificity of vaccine-induced adaptive responses is also critical because of the HIV-1 variability and escape. The most impressive protection in the macaque-SIV challenge model to date has been achieved by life-attenuated SIV and vaccines vectored by persisting rhesus CMV, however, both approaches face safety issues. Thus, the currently most studied platforms employ non-replicating vectors. Of these, adenoviruses are the most immunogenic, however, vaccine carriers based on common human adenovirus serotypes have several major disadvantages, which were highlighted in the proof-of-concept phase IIb STEP study. These include strong pre-existing immunity associated with potential safety issues and dampened induction of immune responses specific for the transgene product, and insufficient immunogenicity as stand-alone vaccines. Therefore, either rare human serotypes, chimeric, or animal adenoviruses are being explored for potential human use combined in heterologous prime-boost regimens with other vaccine modalities. Recently outstanding regimens incorporate chimp adenovirus prime and poxvirus boost. The current pre-clinical state of the ChAdV vaccines will be discussed keeping in mind that these results are trumped by human data.

S06.04

Passive Immunization for HIV Prevention

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Passive immunization with serum antibodies has long been used for temporary protection against infectious agents and toxins. At the current time, there is only one monoclonal antibody (mAb) product in clinical use for passive immunization against an infectious disease, and a single course of treatment over a few months costs thousands of dollars in the US. The broader application of mAbs for passive protection against an infectious disease in developing countries would require nothing short of a technical revolution, coupled with a critical public health need.

The development of an HIV vaccine is such a public health need. The history of vaccines suggests that the elicitation of broadly neutralizing antibodies may be required for consistent, durable, high level protection with an HIV vaccine. Although some humans develop broad, potent HIV-neutralizing antibodies (bNabs) 2-3 years after infection, the current candidate vaccines don't seem to elicit them, despite sustained, focused effort on the problem. The first safe, highly effective, and durable HIV vaccine appears to be at least a decade away. At the same time, the discovery and optimization of natural HIV bNabs, isolated from HIV-infected individuals, has gathered momentum, with many new mAbs of increased potency and impressive breadth described just in the last 2 years. The available natural bNabs represent prototypes, with insufficient potency and/or breadth, in their current form, for widespread human clinical use.

Meanwhile, outside of the field of infectious diseases, impressive gains in potency of monoclonal antibodies for treatment have been made, in part by engineering them for bi-specificity, and lower-cost manufacturing approaches, and innovative delivery approaches with gene transfer, are being developed. Here we consider the gap between what mAbs cost today and what could be described as an affordable product for HIV prevention, and identify areas where major, rather than incremental, gains may be needed.

S06.05 OA

E-DNA IM or ID Delivery Prime Enhances Antibody and T Cell Responses Following Recombinant gp120 env Boost

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Background: The results of the RV144 trial support the ability of vaccine induced Antibodies to protect from HIV acquisition. The STEP trial also appears to have impacted viral load in individuals who produced strong T cell immunity. The HVTN has recently reported that our enhanced DNA (Pennvax) can drive T cell immunity as robust as Adenoviral and Pox platforms in an accelerated format in humans. We have now focused on further driving T and B cell immunity by utilizing prime boost

Methods: Groups of rabbits or Indian Rhesus macaques were vaccinated with enhanced DNA vaccine plasmids encoding pGAG, pPOL + combinations of 3-5 consensus or COT designed envelopes that were either gp140 or gp160 constructs. Both IM as well as a novel minimally invasive skin delivery EP arrays (MID) were studied. DNA vaccinated animals were boosted two months following the final DNA immunization with SF162 gp120 in MF59.

Results: In rabbits, nAb titers against a broad panel of Tier 1 viruses was observed by the combination immunization approaches. Titers in the hundreds were induced by DNA and these titers were enhanced almost a log by a protein boost. Similar data was also observed when these studies were extended to the non-human primate model. Importantly, DNA-prime protein-boost had detectable ADCC activity as measured by the GTL and luciferase assay. A bias for improved antibody responses driven by the MID delivery array is also observed.

Conclusion: Building on the success observed in HVTN 080 we report that further enhancement of DNA constructs in a prime boost setting (E-DNA prime + protein Boost) is capable of eliciting high binding, broad antibody responses and neutralization titers against a panel of tier 1 viruses. Combining improved E-DNA with other strategies shown to enhance antibody responses such new gene adjuvants are being investigated.

Symposium 07: Priming Effective B Cell Responses

S07.01

The Unique T Cell Receptor Expressed by Each Naive Helper T Cell Instructs Effector Cell Fate

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Polyclonal naïve CD4+ T cells with microbial peptide-specific T cell antigen receptors (TCR) produce two types of effector cells during infection, ones that activate macrophages and others that help B cells. This split in T cell differentiation is influenced by extrinsic factors from antigen-presenting cells such as cytokines and costimulatory signals. We used a single cell adoptive transfer method to determine if effector cell differentiation is also influenced by an intrinsic property of each naïve T cell. Remarkably, some single naïve T cells produced predominantly macrophage-activating effector cells; others produced mostly B cell helpers; while others produced both types. These differences were the result of the TCR since monoclonal cells with different TCRs had different patterns of effector cell generation. The strength of signal transduced by the TCR likely determined the effector cell pattern since increasing the amount of peptide increased the fraction of B cell helpers produced by a given naïve clone. These results suggest that the type of effector cell progeny produced by an individual naïve is influenced the strength of signals transduced by its unique TCR. An implication of this work is that antigen dose is a determinant of the quality of the helper T cell response.

S07.02

T Follicular Helper Cells In HIV Infection

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Nearly all successful human vaccines induce significant, durable titers of high-affinity neutralizing antibodies. The generation of such antibodies is critically dependent on CD4 T cell help - in particular the signals mediated by T follicular helper (TFH) cells. Indeed, virtually all licensed vaccines elicit substantial levels of CD4 T cell responses. However, there is only little understanding of how and when TFH cells shape the humoral immune response. Given the preferential infection and depletion of HIV-specific CD4 T cells, their role in the control of viral replication and the induction of neutralizing antibody responses has not been a topic of intensive HIV vaccine research. Interestingly, gp120-specific CD4 T cell responses are significantly expanded in subjects with high viremia, but are almost completely absent in individuals who control viral replication. This expansion is also reflected as a significant enlargement of TFH cell pool in the lymph nodes of chronically infected individuals. However, these TFH cells show signs of a unique functional dysregulation which is associated with a perturbed B cell compartment, including prematurely expanded germinal center B cells and plasma cells. A better understanding of the specific TFH signals involved in the development of neutralizing antibodies will be critical in guiding the identification of correlates of viral protection and control in future HIV vaccine design efforts.

S07.03

Evolution of Broadly Cross-Neutralizing Antibodies During HIV-1 Infection

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Recent cohort studies have shown that broadly cross-neutralizing (BCN) antibodies develop during the natural course of infection in about a fifth of HIV-infected people. Mapping the specificities of these antibodies has enabled the identification of novel vaccine targets. However the pathway to the development of BCN antibodies, and the interplay between strain-specific antibodies, BCN antibodies and autologous viral evolution is not clear. We have characterized the longitudinal development of BCN antibodies in the CAPRISA cohorts. BCN antibodies developed in 10/74 (14%) of infected women by 3 years of infection. We describe changes in neutralizing specificity associated with the onset of breadth, and sequential waves of BCN antibodies targeting distinct epitopes, both likely reflecting affinity maturation and driven by the changing autologous viral populations. We show that in some individuals, the BCN epitope was not present on the transmitted/founder virus, but evolved within 6 months of infection, often as a consequence of viral escape from an earlier strain-specific antibody targeting the same region. Overall these studies aim to determine whether maturation of early strain-specific binding or neutralizing antibodies gives rise to later BCN responses, or whether breadth is mediated by specificities targeting the same region that arise de novo in chronic infection.

S07.04

Genetic And Structural Basis For Development Of High Affinity Antibodies

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Several of the most broadly neutralizing HIV antibodies (bnAbs) contain unique genetic or structural elements, including long heavy chain complementarity determining region 3 (HCDR3) loops and large numbers of somatic mutations. The processes of generating long HCDR3s and of inducing large numbers of somatic mutations appear to be independent mechanisms of increasing antibody diversity. Induction of long HCDR3 antibodies may be critical to the design of an effective vaccine strategy for HIV, however it is unclear at present how to induce such antibodies. There has been speculation that antibodies with long HCDR3s are generated primarily through the accumulation of somatic hypermutation-associated insertions. These short insertion events are rare, and design of an immunogen that efficiently induces multiple insertions in a single antibody sequence is likely to be extremely difficult. Through the use of high-throughput antibody sequencing, we identified genetic evidence that antibodies with long HCDR3s typically are formed at the original recombination event, not through accumulation of somatic hypermutation-induced insertions. Antibodies with long HCDR3s are found in all healthy individuals regardless of HIV status, and long HCDR3 antibodies typically use a restricted subset of D and J gene segments, resulting in the incorporation of highly conserved genetic elements in the majority of such antibody sequences. This work provides an important step toward the design of a vaccine that efficiently induces HIV bnAbs with long HCDR3s by identifying a conserved genetic target through which B cells encoding long HCDR3 antibodies may be induced. In contrast, HIV-specific neutralizing antibodies with shorter HCDR3s appear to achieve potency and breadth through extensive mutation.

S07.05 OA

CD40L Adjuvant For DNA/MVA Vaccine: Enhanced Protection From Acquisition of Neutralization Sensitive & Neutralization Resistant Mucosal SIV Infections

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Background: Generating highly functional antibodies against HIV-1 is critical to prevent infection. Here we evaluated the ability of CD40L (a co-stimulatory molecule for B cells and dendritic cells) as an adjuvant to prevent mucosal infection from neutralization-susceptible (SIVE660) and neutralization-resistant (SIV251) SIVs.

Methods: Groups of rhesus macaques (10-15 animals/group) were immunized intramuscularly at 0 and 8 weeks with 3mg of DNA/SIV or DNA/SIV-CD40L, and boosted with 10^8 pfu of MVA/SIV at 16 and 24 weeks. In addition, animals challenged with SIV251 received 10^6 pfu of MVA/CD40L premixed with MVA/SIV immunizations. Both the DNA and MVA immunogens expressed SIV239 Gag, Pol and Env. At about 24 weeks after final vaccination, animals were challenged intrarectally with either SIV251 (8 weekly doses) or SIVE660 (12 weekly doses) until all unvaccinated controls became infected.

Results: Adjuvanting DNA with CD40L enhanced ($p<0.05$) the titer and avidity of antibodies against SIV239 and SIVE660 Envs.Adjuvanting both DNA and MVA with CD40L enhanced ($p<0.05$) the frequency of B cell follicles with germinal centers (sites of antibody affinity maturation) in the lymph nodes. Following challenge, animals vaccinated with CD40L adjuvant showed markedly enhanced protection from virus acquisition against both SIVE660 (number of challenges for 50% infection was 2 in controls, 5 in DNA/MVA and 12 in DNA/MVA with CD40L; $p=0.003$, 76% vaccine efficacy) and SIV251 (number of challenges for 50% infection was 2 in controls, 2 in DNA/MVA and 6 in DNA/MVA with CD40L; $p=0.04$, 55% vaccine efficacy). The enhanced protection against SIVE660 correlated directly with increased avidity of anti-SIVE660 Env antibody and correlation analyses for protection against SIV251 are ongoing. CD40L-adjuvanted animals also showed 100-fold reduction in peak virus replication in both infection models.

Conclusions: These results demonstrate that CD40L enhances the functional quality of anti-Env antibody and protection from acquisition of neutralization sensitive and resistant mucosal SIV infections.

Symposium 08: Therapeutic Vaccination and Strategies for the Cure

S08.01

Therapeutic Approaches to Curing HIV Infection

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The current therapeutic strategy for established HIV infection is to control viral replication with combination antiretroviral drugs. This approach has limitations as it does not restore health and as many individuals have trouble accessing and/or adhering to these drugs on indefinite manner. Recognizing the need for a therapy that is short-term and scalable, there is intense interesting in potentially curative approaches. There are now a number of potential cure strategies being tested in pre-clinical animal models, with several now advancing into phase I human clinical trials. The status of these approaches will be summarized.

S08.02

Therapeutic Vaccines in Strategies for Functional Cure of HIV

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Symposium 08: Therapeutic Vaccination and Strategies for the Cure

S08.03

Barriers to HIV-1 Eradication

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This talk will summarize barriers to HIV eradication in patients on HAART, with a particular emphasis on the potential need for therapeutic HIV vaccines as part of eradication strategies.

S08.04

Persistence of Latently Infected CD4+ T Cells During HAART: Keeping Memory, Keeping HIV

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The initiation of highly active antiretroviral therapy (HAART) in HIV-infected individuals infected results in a rapid drop in plasma viral load and in a substantial reduction in the number of cells carrying proviral DNA in both blood and tissues. After prolonged viral suppression, a low frequency of latently infected cells is still detectable and although several cellular reservoirs may contribute to HIV persistence, the vast majority of the proviral DNA is detected in memory CD4+ T-cells. This cellular reservoir decays very slowly with a half-life of 40 to 44 months, indicating than more than 70 years of intensive therapy would be required for its elimination. Interestingly, the lifespan of long-lived memory CD4+ T-cells is comparable to this duration, suggesting that the mechanisms involved in the maintenance of antigen (Ag)-specific memory CD4+ T-cells also ensure HIV persistence under HAART. These observations suggest that strategies aimed at reducing the pool of latently infected cells should interfere with the survival pathways responsible for the long-term maintenance of memory CD4+ T-cells. Because memory CD4+ T-cells are critical for appropriate immune defense, targeted approaches are needed to interfere only with the long-term survival of discrete fractions of memory T-cells carrying proviral DNA.

S08.05

Assembling the Tools to Clear HIV Infection: Contributions From Virology and Immunology

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Despite antiretroviral therapy, proviral latency of human immunodeficiency virus type 1 (HIV-1) remains a principal obstacle to curing the infection. Inducing the expression of latent genomes within resting CD4+ T cells is the primary strategy to clear this reservoir. We have recently shown that the histone deacetylase inhibitor suberoylanilide hydroxamic acid (also known as vorinostat, VOR) can disrupt HIV-1 latency in vivo. A single dose of VOR increased both biomarkers of cellular acetylation, and simultaneously induced an increase in HIV RNA expression in resting CD4+ cells. This demonstrates that a molecular mechanism known to enforce HIV latency can be therapeutically targeted in humans. However in HIV-infected patients, the frequency of circulating HIV-specific CTL declines during prolonged successful ART. If expression of latent HIV is induced by agents such as Vorinostat (SAHA), CTL response may temporally lag, preventing effective clearance of infected cells. Additionally, some patients with a history of advanced disease may have lost the ability to mount an HIV-specific CTL response relevant for the viral clones archived within their resting CD4+ T cell reservoir. In addition to developing purging strategies to clear latent reservoirs, therapeutic immunization or similar approaches may be needed to eradicated HIV infection.

Oral Abstract Session 01: Animal Models

OA01.01

Significant Protection from Infection and AIDS Progression After Gastrointestinal and Oral Vaccinations, Respectively, with a SIV DNA/rMVA Vaccine

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Background: Nasal SIV vaccination can significantly protect from AIDS progression.

Methods: We compared four mucosal routes of vaccination in four groups of seven female Rhesus Macaques (RM) each, immunized in the oral cavity (O), gastrointestinally (GI), nasally (N) and vaginally (V) with mutated proviral SIV, IL-2 and IL-15 DNAs and SIV rMVA. Vaccinated and control animals were challenged vaginally with repeated low-dose of SIVmac251.

Results: Only N vaccination induced a significant increase in plasma SIV-IgG titers. Significantly higher systemic, rectal and vaginal SIV-specific T-cell responses were detected in the oral group during the immunization. The median number of challenges required to become infected was significantly higher in the GI group (32; 16 for O, 12 for V, 9 for N, 11 for controls). Repeated SIV exposure expanded vaginal anti-SIV T-cells in some of the animals. Seven vaccinated RM (3 in the O, 3 in the N and 1 in the GI group) suppressed the viremia after the initial infection peak and maintained it undetectable over the course of the trial. Immunized, infected animals had significantly lower levels of systemic T-cell immune activation, better preservation of CD4+ central memory and α4β7high+ CD4+ T-cells, with consequent better protection from AIDS. However a lower protection from AIDS progression was observed in the GI group compared to the other vaccinated RM, with a median survival of 24 weeks. A significantly higher loss of CD4+ CM T-cells, detected early on in this group, correctly predicted its poor long-term outcome.

Conclusion: Protection from infection in The GI group correlated with higher anti-SIV CD8+ T cells responses in vaginal T-cells on the day of first challenge. More than 50% of the O and N vaccinated RM were still disease-free 72 weeks after infection, and protection correlated with levels of systemic anti-SIV IFN-g+/CD8+ T-cells on the day of first challenge.

OA01.02

Antibodies to the Envelope Protein Protect Macaques from SIVmac251 Acquisition in an Immunization Regimen That Mimics the RV-144 Thai Trial

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Background: The canarypox vector ALVAC-HIV, together with the HIV gp120 envelope, has protected 31.2% of Thai heterosexual individuals from HIV acquisition in the RV144 HIV vaccine trial. This outcome was unexpected, given the limited ability of the ALVAC-HIV vaccine component to induce CD8+T-cell responses, and of the HIVgp120 envelope to elicit broad neutralizing antibodies.

Methods: We vaccinated macaques with an immunization regimen that mimics the RV144 trial and exposed them to a mucosal dose of SIV_{mac251} that transmits few virus variants, similar to HIV transmission to humans.

Results: Vaccination induced anti-envelope antibodies, modest CD4+ and CD8+ T-cell responses. One third of the vaccinated macaques were protected from SIV_{mac251} acquisition, whereas the remaining infected vaccinees progressed to disease. Vaccine induced SIV_{mac251} specific T-and B-cell responses were not different in protected or infected animals. The sera of the animals protected had higher avidity antibodies to the gp120 envelope protein, recognized the variable envelope region V2, and reduced SIV_{mac251} infectivity in cells that express high level of α4β7, suggesting a functional role to antibodies to V2.

Conclusion: The SIV_{mac251} infection macaque faithfully reproduces results in humans, and is instrumental in the development of more efficacious vaccines for HIV.

OA01.03

Immune Control of an SIV Challenge by a Heterolgous and Direct Mucosal Vaccination Regimen in Rhesus Monkeys

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Background: The mucosal surface is the major route for HIV-1 transmission, yet a safe and effective AIDS vaccine through direct mucosal immunization remains elusive.

Methods: Here, we report a novel vaccination regimen consisting of a mucosal prime with replication-competent vaccinia Tiantan rMVTTSIVgpe and an intramuscular boost with non-replicating rAd5SIVgpe expressing SIV Gag, Pol and Env. Twenty Chinese rhesus macaques were used to evaluate its safety, immunogenicity and protective potential.

Results: Compared with three control groups, the rMVTTSIVgpe-rAd5SIVgpe regimen elicited robust cellular immune responses with enhanced magnitude, sustainability and polyfunctionality, and higher titers of neutralizing antibodies against SIVmac1A11. Moreover, one rMVTTSIVgpe-rAd5SIVgpe vaccinated animal was fully protected, while the rest demonstrated 1.74-log and 1.2-log reductions in peak and set-point viral loads upon intrarectal challenge with a high dose (5x105 TCID50/animal) of a pathogenic and neutralization-resistant SIVmac239. Importantly, the rMVTTSIVgpe-rAd5SIVgpe vaccinated animals remained healthy up to 850 days post-challenge, while the majority (~75%) of controls progressed to simian AIDS. The protective effect was found to correlate with SIV-specific CD8+ T cell ELIspot responses against Gag and Pol, but not Env.

Conclusion: Our findings indicate that vaccine strategy engaging the mucosal surface from the beginning of vaccination may provide protective immunity against HIV-1 infection in humans.

OA01.04

A Recombinant Attenuated Mycobacterium Tuberculosis-SIV Combination Vaccine Is Safe and Immunogenic in Immunocompromised, SIV-Infected Infant Macaques

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Background: HIV and Tuberculosis show high co-prevalence and cause high morbidity and mortality, especially in infants. There is no HIV vaccine, and the only licensed TB vaccine, BCG, can cause disseminated disease in HIV-infected infants. We propose to develop a pediatric combination HIV-TB vaccine hypothesizing that a highly attenuated strain of M. tuberculosis (AMtb) (i) would result in improved safety, but similar immunogenicity compared to BCG, (ii) could be modified to co-express HIV genes (rAMtb-HIV), and (iii) therefore induce HIV and Mtb specific immunity. Towards this goal, we tested distinct H37Rv Mtb mutants that differed in their attenuation for replication and immune evasion in infant rhesus macaques

Methods: Six infants that were SIV infected at birth, and 20 healthy infants received rAMtb-SIV orally at one week of age, and were followed for 3-6 months. Vaccine safety was assessed by clinical monitoring, histopathology, and Mtb detection using multiple culture methods. SIV and Mtb-specific T cell responses were measured by flow cytometry. Plasma, saliva and stool were tested for SIV and Mtb antibody responses.

Results: Among three vaccine tested, the rAMtb-SIV strain mc6435 with deletions in panCD, LeuCD, and secA2 was determined to be safe in healthy and SIV-infected infant macaques. Lungs and other tissues were free of TB pathology, and viable mycobacilli could not be recovered from any tissues despite severe immune suppression by SIV.

Immunized animals showed increased dendritic cell activation, elicited polyfunctional SIV- and TB-specific CD4+ and CD8+ T cell responses in blood and tissues, and developed plasma IgG antibodies specific for Mtb and for SIV. The effectiveness of oral immunization was confirmed by detection of mucosal IgA responses to SIV in saliva and stool.

Conclusion: A rAMtb-SIV vaccine is a safe alternative to BCG, is immunogenic in infants, and could be developed as a combination vaccine against pediatric HIV and Mtb infections.

Oral Abstract Session 01: Animal Models

OA01.05

MAb PGT121 Protects Against Mucosal SHIV Challenge in Macaques at Concentrations Corresponding to Its Highly Potent In Vitro Neutralization Capacity

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Background: We recently characterized several new broadly neutralizing monoclonal antibodies (bnMAbs) remarkable for their in vitro potency. A number of these bnMAbs are almost 10 fold more potent than previously isolated bnMAbs such as PG9 and VRC01 and hundred fold more potent than older prototype bnMAbs such as 2G12 and b12. Of the new antibodies, PGT121 is one of the most broad and potent, and recognizes a glycan-dependent epitope. Based on in vitro neutralization potency, we speculated that PGT121 may be protective in vivo at serum concentrations below those previously determined for other broadly neutralizing anti-HIV antibodies.

Methods: The neutralization potency of PGT121 against SHIV $_{SF162P3}$ was evaluated using a TZMbl-based neutralization assay. To evaluate the protective potency of PGT121 in vivo, we then designed a protection study in rhesus macaques. Animals were administered intravenously with 3 different doses of antibody (5 mg/kg, 1 mg/kg or 0.2 mg/kg) 24 hours before being vaginally challenged with a single high dose of SHIV $_{SF162P3}$ (300 TCID50). Serum levels of antibody and viral loads were determined throughout the study.

Results: PGT121 potently neutralizes SHIV $_{SF162P3}$ with an IC50 of about 0.005 µg/ml. Preliminary data suggest that an administered dose of PGT121 at 1 mg/kg is sufficient to induce sterilizing immunity against a single high dose vaginal challenge of SHIV $_{SF162P3}$. A lower 0.2 mg/kg dose appears to be partially protective.

Conclusion: PGT121 is one of the most broad and potent neutralizing antibodies isolated to date. Here, we provide evidence that in vivo protection by PGT121 correlates with the high in vitro potency of the antibody. PGT121 can thus mediate sterilizing immunity at concentrations that are significantly lower than previously observed from passive protection studies with bnMAbs and may be readily achievable through vaccination.

OA01.06

Epitope Specificity Appears to Be an Important Determinant of In Vivo Killing Ability of Simian Immunodeficiency Virus (SIV)-Specific CD8+ T Cells

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Background: CD8+ cytotoxic T lymphocytes (CTL) are a critical component of antiviral immunity and play an important role in the control of lentiviral infection.

Methods: We have developed an in vivo CTL assay to directly measure the killing capacity of MHC-restricted SIV-specific CTL in rhesus macaques (RM). In order to evaluate the in vivo efficacy of different epitope-specific CTL, we compared the in vivo killing capacity of Mamu-A*02-restricted Nef YY9 and Gag GY9 CD8+ T lymphocytes in three RM vaccinated with a recombinant HSV prime/ DNA boost SIV vaccine regimen.

Results: Tetramer frequencies of Mamu-A*02-restricted Nef YY9-specific CD8+ T-cells were at least 10-fold higher than Mamu-A*02-restricted Gag GY9-specific CD8+ T-cells in individual animals both pre- and post-challenge. Prior to SIV challenge, both CTL populations showed poor and incomplete killing (22-35%) of target cells over 18 hours. Seven weeks post-challenge, there was a marked increase in the CTL killing capacity of both Nef YY9 and Gag GY9-specific CTL with 26-38% killing occurring over the first two hours and up to 100% killing over 18 hours. Surprisingly, Gag GY9-specific CTL consistently showed equivalent or greater in vivo killing when compared to Nef YY9-specific CTL even though the percentages of IFN-γ secreting and degranulating cells upon peptide stimulation were comparable.

Conclusion: These data suggest that epitope specificity rather than tetramer frequency determines the ability of SIV-specific CTL to kill infected cells. Taken together, these data may have important implications for the development of a successful HIV vaccine.

Oral Abstract Session 02: Vaccine Concepts – Protein Immunogens

OA02.01

Identification of a Clade A HIV Envelope Immunogen from Protocol G That Elicits Neutralizing Antibodies to Tier 2 Viruses

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Background: Broadly neutralizing antibodies PG9 and PG16 have been isolated from the B cells of one clade A-infected individual from IAVI Protocol G. PG16 is relatively trimer-specific whereas PG9 binds trimer preferentially, but can bind monomeric gp120 from several viral isolates. Both antibodies are potent neutralizers that recognize greater than 70% of tier 2 pseudoviruses in the TZM-bl assay. We sought to begin immunogen design efforts based on sequences from the Protocol G donor, however all viruses isolated from the donor were resistant to neutralization by PG9 and PG16. We used a bioinformatics approach to infer the most recent common ancestor (MRCA) sequence for the viral envelope (Env) to identify closely related viruses sensitive to PG9/16.

Methods: Alignment of the MRCA sequence with 99 subtype A gp160 sequences from the Los Alamos HIV database identified BG505 as the virus with the highest degree of homology (73%) to the MRCA sequence.

Results: Pseudoviruses prepared with this Env are sensitive to neutralization with a broad panel of bNAbs, including PG9 and PG16, indicating that BG505 has an antigen profile desirable in a vaccine candidate. When expressed as a soluble gp120 monomer from 293T cells, BG505 displayed a unique antigenicity profile – it bound well to both PG9 and PG16. We further show that a point mutation enables production of stable gp120 monomers that preserves the major neutralization epitopes on Env. Finally, we show that an adjuvanted formulation of this gp120 protein elicited neutralizing antibodies in rabbits (following a gp120 DNA vaccine prime) and that the resulting antisera compete with the bNAbs from 3 non-overlapping epitope classes for binding to gp120.

Conclusion: The results indicate that BG505 Env warrants further investigation as an HIV vaccine candidate either as a protein or in a viral vector platform.

OA02.02

Prime-Boost Regimen Potency and Efficacy with Alphavirus Replicons (SIV Antigen) in Non-human Primates Challenged with Low-Dose Intra-rectal SIVsmE660

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Background: Self-amplifying RNAs (replicons) of positive-strand viruses such as alphaviruses are potentially safe and useful vectors for delivering vaccine antigens. Recombinant alphavirus replicon particles (VRP), carrying the self-amplifying RNA, protects rhesus macaques against SHIVSF162P4 challenge when used in a prime-boost regimen.

Methods: Novartis VRPs are being further tested using a current state-of-the art physiologically relevant low-dose SIV virus swarm challenge. To meet the need for the large numbers of VRP an alphavirus packaging cell line (PCL) was used for VRP production. We manufactured, characterized, stability and small animal potency tested VRPs expressing SIVmac239 envelope (env) and gag/pol fusion proteins (VRP Env, VRP Gag/pol, respectively) for a large macaque vaccine study. Macaques were co-immunized with both VRPs thrice followed by two boosts with an MF59-adjuvanted CHO cell-derived SIVmac239 trimeric env protein.

Results: Here we show that three VRP priming immunizations induce both env- and gag-specific IgG and T-cell responses, robustly. Binding env-specific IgG titers were demonstrable in 100% of animals with titers ranging from ~10000-400000. T-cell responses to env and gag developed in 80% of macaques (env-specific range ~100-1200, gag-specific range ~100-700 SFC/106 PBMCs) when assayed using an IFNγ T-cell ELISpot. The MF59-adjuvanted Env protein by itself was also robustly immunogenic with Env-specific IgG titers and T-cell responses ranging from ~100000-1700000 (100% response) and ~100-1000 SFC/106 PBMCs (90% response), respectively. No adverse events were reported upon immunization of either the VRP or the MF59-adjuvanted env vaccines.

Conclusion: We provide further details on the currently ongoing efficacy evaluations of these safe and immunogenic vaccines in a prime-boost regimen using repeated low-dose heterologous SIVsmE660 intra-rectal challenges. NIH Grant N01-AI-50007.

OA02.03

Minimally Invasive and Surface Electroporation Delivery of DNA Vaccines for the Induction of Robust Humoral Immune Responses Against HIV Antigens

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Background: Clinical data from the HVTN-080 study demonstrated that intramuscular electroporation (EP) delivery of PENNVAX®-B DNA vaccine and the plasmid adjuvant IL-12 generated strong antigen specific cellular immune responses in humans with nearly 90% response rate. We have now developed minimally invasive EP delivery technologies (MID-EP) to target dermal tissue and demonstrate their ability to generate strong antibody (Ab) responses in animal models with DNA antigens – including small pox, influenza, dengue – and have shown protection from viremia and lethality following challenge.

Methods: We demonstrate MID-EP delivery of consensus HIV gp140 antigens and show the generation of cross-clade neutralizing responses in guinea pigs and rabbits. These EP enhanced humoral responses were significantly broader and higher than naked DNA delivery alone or with a protein antigen. We demonstrated NAb titers against a broad panel of 15 Tier-1 HIV viruses from Clades A-D in the range of 20 -200 measured in the Tzm-Bl neutralization assay. The magnitude but not the breadth of the responses was boosted to 20-1000 range using a MID-EP DNA prime-protein boost regimen.

Results: We further developed a surface EP device (SEP) for the simultaneous, but spatially segregated, delivery of multi-component HIV vaccines. The SEP device operates under substantially lower voltage parameters than conventional EP devices resulting in significant improvements in tolerability. The separation of multi-component HIV vaccines avoids potential issues with plasmid interference at the transcriptional or translational levels. SEP produces Ab responses comparable to the penetrating DNAEP devices.

Conclusion: Our results suggest that MID/SEP electroporation devices offer safe, tolerable and potent methods to administer HIV DNA vaccinations in a prophylactic clinical setting. Combined with the design of novel HIV consensus based Env antigens these DNA-EP combination vaccines are suitable for further HIV vaccine product development.

OA02.04

Design of Lipid Nanoparticle Delivery Agents for Multivalent Display of Recombinant Env Trimers in HIV Vaccination

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Background: Immunization strategies that elicit antibodies capable of neutralizing diverse strains of the virus will likely be an important part of a successful vaccine against HIV. The envelope trimer is the only neutralizing target on the virus, and strategies to promote durable, high avidity antibody responses against the native intact trimer structure are lacking. We recently developed chemically-crosslinked lipid nanocapsules as carriers of molecular adjuvants and encapsulated or surface-displayed antigens, which promote follicular helper T-cell responses and elicited high-avidity, durable antibody responses to a candidate malaria antigen (Moon et al. Nat. Mater. 10 243 (2011); Moon et al. PNAS 109 1080 (2012)).

Methods: To apply this system to the delivery of HIV antigens, we developed a strategy to anchor recombinant envelope trimers to the surfaces of these particles under conditions preserving the antigenic integrity of the trimers, allowing multivalent display of these immunogens for immunization. To anchor trimers in their native orientation, gp140 trimers with terminal his-tags were anchored to the surface of lipid nanocapsules via Ni-NTA-functionalized lipids.

Results: Owing to their significant size (409 kDa) and heavy glycosylation, we found that liquid-ordered and/or gel-phase lipid compositions were required to stably anchor trimers to particle membranes. Trimer-loaded nanocapsules carrying monophosphoryl lipid A elicited durable antibody responses with titers comparable to a Complete Freund's Adjuvant (CFA)-like emulsion in mice, without the toxic inflammation associated with the latter adjuvant. Further, nanocapsules elicited strong helper T-cell responses associated with a steadily increasing avidity of trimer-binding antibody over 90 days, which was not replicated by other adjuvants.

Conclusion: These results suggest that nanoparticles displaying HIV trimers in an oriented, multivalent presentation can promote key aspects of the humoral response against Env immunogens.

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OA02.05

Eliciting Neutralizing Antibodies with gp120 Outer Domain

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Background: Although gp120 elicits strong antibody responses, it fails to induce broadly neutralizing antibodies (bnAbs). One strategy being evaluated is using immunogens based on gp120 outer domain (gp120-OD). A number of gp120-OD constructs have been reported. However, none of them have been shown to induce potent nAbs. Here, we describe gp120-OD-based immunogens that can induce potent nAbs.

Methods: We constructed gp120, gp120-OD, and a trimeric form of gp120-OD (ODx3) based on an M group consensus sequence. Proteins were expressed in 293 cells, and their antigenic properties were evaluated by immunoprecipitation using gp120 bnAbs (b12, 2G12 and 447-52D) and by surface plasmon resonance (SPR). Rabbits were immunized and antibody responses were characterized by ELISA and neutralization assays.

Results: All three proteins were recognized by bnAbs b12, 2G12 and 447-52D. SPR analyses indicated that b12 has lower affinity to gp120-OD compared to gp120 or ODx3, largely due to a faster dissociation rate. All immunogens induced potent nAbs against Tier 1 viruses from clades B, C and AE. Neutralizing activity against Tier 2 viruses was weaker and sporadic. The induction kinetic of nAbs by gp120-OD was slower than that for gp120 and ODx3. Although the V3 loop was a major target of nAbs, results suggested other epitopes are also targeted. A panel of about 100 rabbit mAbs was generated, two of which exhibited neutralizing activity. One of them was molecularly cloned and sequenced. It exhibited a similar neutralization profile as the immune serum. Work is in progress to identify its epitope.

Conclusion: We have successfully generated OD-based immunogens that can induce nAbs. Although they were effective primarily against Tier 1 viruses, the breadth of neutralizing activity achieved is highly significant. Our trimeric ODx3 construct is novel and is a highly promising immunogen for further development of OD-based immunogen.

OA02.06 LB

Oral Immunization With A Recombinant Lactococcus Lactis Expressing HIV-1 Gag on the Tip of the Pilus Induces Strong Mucosal Immune Responses

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Background: The induction of a potent humoral and cellular immune response both in the peripheral and mucosal tissues is important for the development of an effective HIV vaccine. The present study explores the ability of a Lactococcus lactis (L. lactis), a probiotic organism, based oral vaccine to elicit HIV-specific immune responses in the mucosal and systemic compartments of mice and rhesus.

Methods: To express the HIV-1, Gag (p24) on the tip of the pilus, we made a fusion construct of Gag and Cpa protein of the type-3 pilus found in Streptococcus pyogenes and expressed in L. lactis (rLL-Gag). Four monthly intragastric immunizations were given to the mice and macaques. In mice, we studied the humoral, cellular, and innate immune responses in mucosal and systemic compartments. In macaques, we completed analyses only on the cellular immunity.

Results: In mice, we observed a strong Gag specific IgG and IgA in serum, feces, and vaginal secretions following rLL-Gag oral immunizations. However, the Gag-specific CD8 T cell responses in the blood were at or below our detection limit. Following an intramuscular MVA/Gag boost, we observed a strong Gag-specific CD8 T cell responses both in systemic and mucosal tissue including IEL/LP of small intestine, peyer's patches, and mesenteric lymph node. Consistent with immunogenicity, rLL-Gag induced a strong activation of CD8α+, CD11b+ DC in the peyer's patches at 8 hours after oral immunization. Interestingly, the DC activation was not observed with L. lactis without the pilus. In rhesus macaques, vaccination with rLL-Gag elicited a strong Gag-specific CD4 T cell response both in the blood and rectum producing IL-2 and TNFα and moderate levels of IFNγ and IL-17.

Conclusion: These results demonstrate that L. lactis expressing antigen on the tip of the pilus can serve as an excellent priming vector to induce a strong mucosal antibody and cellular immunity.

Oral Abstract Session 03: Innate Immunity

OA03.01

SIVΔnef Vaccination Mobilizes Systemic and Mucosal Natural Killer Cells in Mamu A*01+ Macaques

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Background: Although vaccination with live attenuated SIV is the most effective means of inducing protection against lentiviruses, the immunologic mechanisms responsible remain unclear. Previous studies have yielded conflicting data regarding the role of adaptive immune responses in mediating protection, suggesting that innate immune responses, including natural killer (NK) cells, may play a role.

Methods: Rhesus macaques were vaccinated with SIVΔnef and challenged with SIVmac239. Cellular dynamics were measured by polychromatic flow cytometry and absolute counts of lymphocytes in blood and rectal biopsies were determined by a bead-based, flow cytometry assay.

Results: Following SIVΔnef vaccination, circulating NK cells increased 8-fold, peaking at 2 weeks post-vaccination and preceding SIV-specific T cell responses. Furthermore, the gut-homing marker α4β7 was upregulated on circulating NK cells, coinciding with a 2.5-fold increase in NK cells in colorectal tissue. Ki67 expression was upregulated 2- to 5-fold in circulating, lymphoid, and mucosal NK cells. NK cell expansion was also stratified by MHC genotype — Mamu A*01+ macaques showed significant and sustained expansion of NK cells, but not Mamu A*02+ or Mamu B*17+ macaques. SIVΔnef also induced a significant expansion of KIR+ and cytotoxic NK cells in the colorectal mucosa. Interestingly, in vaccinated macaques challenged with wild-type SIV, α4β7 and Ki67 were both upregulated in circulating and mucosal NK cells, even in protected animals and in the absence of an obvious anamnestic response.

Conclusion: Although current lentiviral vaccines stress the importance of induction of SIV-specific T and B cell responses, NK cells could contribute to protection by inhibiting initial rounds of wild-type virus replication in the mucosa. Our data indicate SIVΔnef induces a robust and sustained expansion of NK cells that traffic to the gut mucosa, and that the protective SIVΔnef NK responses may be modulated, in part, by interaction of Mamu A*01 with an as of yet unidentified KIR.

OA03.02

RV144-like Trial in Macaques Using ALVAC-SIV & gp120, Induces Innate Immunity and Increases the Frequency of NK22 & NKG2A+ Cells in Mucosal Tissues

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Background: NK cells play a pivotal role in the innate immunity and patrol various tissues, including mucosal sites, the portal of entry of HIV. We recently reproduced in the SIV $_{mac251-}$ rhesus macaque (RM) model the limited protection reported in the RV144 HIV vaccine trial in humans (32% protection from HIV acquisition), using similar vaccines (ALVAC-SIV & gp120).

Methods: In here, we immunized, by the intramuscular route, 6 RM with 10^8 PFU of ALVAC-SIV at weeks, 0 (V0), 4 (V2), and with both ALVAC SIV and the SIVgp120 envelope proteins, adjuvanted with ALUM at week 12 (V3) and 24 (V4) and analyzed the profile of gene expression at 24 hours after each vaccination. In parallel, we studied the phenotypical and functional changes in the NK cell subsets after vaccination by multi parametric flow cytometry.

Results: Microarray analysis in the PBMC after V1 and V2 showed the up regulation of anti viral (MX1, HERC-5) and IFN responsive genes and a down regulation of pro-inflammatory genes. Significant alteration in the gene expression profile was also observed after the gp120/Alum boost (V3). Interestingly, NK cells associated genes were up regulated after V3 vaccination. These finding paralleled our results observed by FACS analysis that demonstrated an increased frequency of NK22 cells at mucosal sites. These NK22 cells expressed CCR6 (a gut homing marker) and are thought to play a role in mucosal immunity. Similarly, vaccination also increased the frequency of NKG2A+ cells that were either cytotoxic (CD107a+) or cytokine producing (IFNg+).

Conclusion: Thus, the ALVAC SIV/gp120 vaccine regimen induces a significant activation of NK cells and other innate immune responses. Given that this vaccine platform has conferred some degree of protection from HIV infection in humans, understanding the role of innate immunity in protection from SIVmac251 may guide our effort in the development of novel vaccine strategies against HIV.

OA03.03

Adenovirus Vectors from Various Serotypes Induce Distinct Cytokine Profiles

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Background: Adenovirus (Ad) vectors from various serotypes which differ markedly in their basic biology are being pursued as candidate HIV vaccines. However, the innate immune responses elicited by different Ad vectors remain poorly characterized. We therefore evaluated cytokine responses to Ad vector stimulation both in vitro in human PBMC and in vivo following vaccination of rhesus monkeys.

Methods: Human PBMC were stimulated in vitro with 10^3 vp/cell Ad5, Ad35, Ad26, Ad48, or Ad5/35 chimeric vectors. Rhesus monkeys were immunized with $3x10^{10}$ vp Ad5, Ad35, Ad26, Ad48, or Ad5HVR48. Cytokines in culture supernatant and serum from vaccinated monkeys were measured by luminex assays and ELISA.

Results: Ad35 and Ad26 induced higher levels of antiviral and proinflammatory cytokines (e.g. IFNα2, IFNγ, IL-1β) compared to Ad5 in human PBMC ($p<0.01$, Kruskal-Wallis test; Dunn's correction). Replacement of Ad5 fiber with that of Ad35 (Ad5f35) increased cytokine induction, while Ad35f5 displayed decreased stimulation, indicating the importance of fiber-receptor interactions for innate immune stimulation. Similarly, monkeys vaccinated with Ad35 or Ad26 also displayed markedly higher levels of antiviral and proinflammatory cytokines compared to Ad5 on day 1 post-vaccination ($p<0.05$, Mann-Whitney U test).

Conclusion: These data demonstrate that CD46-uilizing Ad35 and Ad26 vectors induce profoundly different innate immune responses as compared to CAR-utilizing Ad5 vectors both in vitro and in vivo. These findings confirm that major biologic differences exist among Ad vectors and may help explain their different adaptive immune phenotypes.

OA03.04

Infiltration of Dendritic Cells and Antigen Uptake in the Muscle After Injection of HIV-1 Env gp120 in Adjuvant

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Background: Most vaccines are delivered into the muscle although it contains very few potent antigen presenting cells, such as dendritic cells (DCs) that are critical for driving adaptive immune responses. Understanding the early mechanisms that dictate vaccine responses and why some adjuvants like the oil-in-water emulsion MF59 are shown to be more potent than alum is important for the design of new vaccines. Here, we investigated the recruitment of immune cells to the vaccine injection site and uptake of a clinically relevant HIV-1 envelope glycoprotein (Env) and MF59 in a non-human primate (NHP) model.

Methods: Rhesus macaques received intramuscular injections of either fluorescently-labeled Env gp120 alone or together with MF59 in the deltoid and quadriceps muscles. Donor-matched injections of PBS and MF59 alone served as controls. At 24-72 hrs, blood, muscle and lymph nodes were sampled for flow cytometry and confocal microscopy.

Results: There was a robust infiltration into the muscle of multiple immune cells by MF59+/-Env. CD66abce+ neutrophils were most frequent followed by CD14+ monocytes and CD11c+ myeloid DCs. CD123+ plasmacytoid DCs which do not normally reside in muscle, were also recruited by MF59. Internalization of Env and MF59 was readily detectable in all DC subsets both in the muscle and in the draining lymph nodes. Although injection of Env alone did not lead to cell infiltration, the few resident DCs showed efficient Env uptake. Groups receiving Env together with distinctly different adjuvants (alum and TLR7 ligand) are underway.

Conclusion: MF59 as an adjuvant leads to significant influx of cells that efficiently engulf vaccine protein antigen. Antigen/adjuvant carrying DC subsets appear early in the lymph nodes draining the injection site. As NHP DC subsets are similar to humans, this offers a powerful model that can yield data to be translated into optimizing future vaccine formulations and delivery strategies.

OA03.05

Early Pro-inflammatory Host Response to Recombinant HSV-SIV Vaccination in Sooty Mangabeys

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Background: Naturally infected sooty mangabeys (SM) do not progress to AIDS unlike SIV-infected non-natural hosts including rhesus macaques (RM). Differences in the innate immune response to SIV may be critical in avoidance of disease progression in SIV-infected SM. In this study we investigated innate and adaptive immune responses to recombinant HSV vectors expressing SIV Gag, Env, and a Rev-Tat-Nef fusion protein in six SIV-negative SM and four RM following single intramuscular inoculation.

Methods: Plasma samples were analyzed using Luminex 28-plex assay. Innate immune cells and SIV-specific cellular immune responses were investigated by flow cytometry and IFN-γ ELISPOT assay.

Results: A significant transient decline of circulating Natural Killer T (NKT) cells, pDCs, and NK cells was observed in SM on day one post vaccination, with a return to baseline levels by day 3-7. Also an increased activation of SM NK cells was observed that returned to baseline by day 9. In contrast, RM displayed no significant change in circulating NKT cell and NK cell frequencies. Both RM and SM exhibited similar transient increases in plasma levels of several inflammatory cytokines/chemokines including IL-1RA, IFN-γ, IL-6, IL-8, macrophage migration inhibitory factor (MIF), monocyte chemotactic protein 1 (MCP-1), vascular endothelial growth factor (VEGF), and interferon-inducible T cell alpha-chemoattractant (I-TAC) by day one, suggesting activation of multiple immune cells including macrophages and DCs. By day 14, plasma levels of analytes such as IL-6 and Eotaxin persisted at elevated levels in RM, but not SM, suggesting delayed resolution of immune activation in RM. SIV-specific cellular immune responses were detected in both species.

Conclusion: These data confirm that SM mount a robust innate immune response and do not have blunted immune activation. Further investigation of the mechanism by which SM resolve the transient but higher immune activation would be helpful in improving vaccine strategy.

OA03.06 LB

Vaccination with MVA/HIV Induces Differential Recruitment of Monocyte Subsets Into The Circulation And Monocyte-Specific Transcriptional Programs

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Background: Monocyte subpopulations are recruited from marrow to blood in response to infection and migrate into inflamed tissues to initiate adaptive immune responses. We sought to understand the effect of an MVA-vectored HIV vaccine on peripheral blood monocytes within the first week post-vaccination.

Methods: Volunteers were vaccinated with MVA/HIV62 gag/pol/env (protocol HVTN 205/908); blood was collected pre-vaccination and 1, 3, and 7 days post-vaccination. Serum cytokine analysis was performed (n=13). Monocytes were phenotyped by flow cytometry (n=14) and microarray transcriptional profiling was performed on freshly-sorted monocytes (n=7).

Results: MVA/HIV induced serum elevations of the monocyte chemoattractant CCL2, and seven proinflammatory cytokines at 24 hours post-vaccination ($p<0.001$). Two waves of monocyte population changes occurred: concomitant with the rise in CCL2, $CD14^{++}$ $CD16^{-}$ 'classical' monocyte concentrations increased at 24 hours post-vaccination ($p=0.002$). In contrast, $CD14^{++}CD16^{+}$ 'intermediate' and $CD14^{+}CD16^{++}$ 'patrolling' monocyte concentrations did not increase until 72 hours post-vaccination ($p< 0.001$). Vaccination induced expression of the activation markers CD86 and HLA-DR at the 72 hour timepoint on all subpopulations examined ($p<0.01$), indicating a phenotypic shift towards increased antigen presentation. Microarray analysis of sorted $CD14^{+}$ monocytes (encompassing all three subpopulations) revealed that the majority of gene expression changes occurred at 24 hours (494 up- and 53 down-regulated genes; FDR≤10% and average absolute $\log_2$ (fold-change) ≥0.5, including antigen-processing genes (PSMA2, 3, 5), IL-6, and TLR-pathway genes (TLR1, LY96). Only 39 genes were up-regulated at 72 hours post-vaccination. 351/547 genes regulated at 24 hours were not observed in bulk PBMC profiles from the same individuals, indicating the value of early profiling of monocyte subpopulations.

Conclusion: These data provide the most detailed profile of the human in vivo monocyte response to an MVA poxviral vector to date. Analyses of transcriptional signatures that predict T-cell and antibody responses will enable the identification of innate immune response genes to target with future HIV vaccines.

Oral Abstract Session 04: Mucosal Immunity

OA04.01

Immune Complexes Can Dampen Inflammatory Signaling at the Mucosal Surface During Protective SIV Vaccination

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Background: The long-term goal for an efficacious HIV vaccine is to provide sterilizing protection from HIV infection. Thus far, this scenario has only been achieved experimentally using live-attenuated SIV vaccines. As such, great interest lies in identifying correlates of protection from a successful host response to pathogenic SIV. To this end, we have used a global genomics approach, tissue analysis, and explant cultures to identify immune complex (IC) signaling as an important component of a protective host response in the female reproductive tract (FRT) of animals vaccinated with the live-attenuated virus known as SIV_{mac239} ΔNef.

Methods: RNA from cervical tissue was purified for microarray analysis. Significant genes were functionally classified and protein expression determined using single-cell analytical procedures for tissue sections. FRT tissue was removed from healthy, uninfected, adult Rhesus macaques and cervix isolated/dissected into small tissue pieces for ex vivo culturing. WT SIV_{mac251} 32H alone or SIV-specific ICs were added drop-wise to mucosal surfaces of explants and incubated for 24 hr at 37°C / 5% CO_2.

Results: A genome-wide transcriptomics analysis revealed selective enrichment of an anti-inflammatory program upon virus exposure in SIV_{mac239}-ΔNef-vaccinated animals, with localized expression of these anti-inflammatory mediators in the mucosal epithelium of the FRT, coinciding with dampened inflammation, limited $CD4^+$ T cell infiltration, and stunted virus replication. Explant cultures derived from the FRT of Rhesus macaques were used as a physiological platform to identify the inhibitory Fc receptor for IgG, FcγRIIB, and carbohydrates in the Fc portion of SIV-specific ICs as centrally important in mediating this anti-inflammatory signaling program in mucosal epithelial cells.

Conclusion: These results highlight an unappreciated, non-neutralizing role for antiviral antibodies at mucosal surfaces and implicates the mucosal epithelial cell as an important host sensor that integrates external signals to elicit a host program that either promotes (inflammatory) or suppresses (anti-inflammatory) immunodeficiency virus infection.

OA04.02

Vaccine-Elicited Systemic and Mucosal Humoral Responses of Lactating Rhesus Monkeys Vaccinated with the Transmitted/Founder HIV Envelope 1086C

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Background: We previously demonstrated that vaccination of lactating rhesus monkeys with a DNA prime/ vector boost strategy induces strong SIV-specific cellular immune responses, but limited Envelope-specific humoral responses in breast milk. Therefore, we sought to improve vaccine-elicited Envelope-specific antibody responses in the milk compartment by using a transmitted/founder (T/F) HIV Envelope immunogen in a prime-boost strategy modeled after that of the moderately-successful RV144 HIV vaccine trial.

Methods: Eight female, hormone-induced lactating rhesus monkeys were intramuscularly primed with either recombinant DNA (n = 4) or MVA pox virus vector (n = 4) expressing the T/F clade C HIV Envelope 1086C. All animals were intramuscularly boosted twice with the 1086C gp120 protein and the adjuvant MF59. Milk, vaginal, rectal and plasma samples were assessed for HIV Envelope-binding IgG and IgA responses. Anti-V1V2 antibodies and neutralization responses were also measured in milk and plasma.

Results: Envelope 1086C-binding IgG responses were detected in plasma, milk, and vaginal samples of all vaccinated animals and two of four rectal samples from MVA-vaccinated animals. Moreover, anti-V1V2 IgG antibodies were detected in all plasma, but only one milk sample. Low magnitude Envelope 1086C-specific IgA responses were detected in milk of two of four DNA-primed and three of four MVA-primed animals, but in none of the rectal samples. In contrast, all vaginal samples from MVA-primed, but none from DNA-primed, animals had detectable Envelope-specific IgA. Remarkably, strong tier 1 and low to moderate tier 2 neutralization was detected in plasma and milk of each group. The plasma neutralization titers against MW965 (clade C tier 1, p=0.03) and CAP45 (clade C tier 2, p=0.03) were significantly higher in MVA-primed than DNA-primed animals.

Conclusion: MVA prime/ T/F Envelope protein boost strategy appears to induce stronger systemic and mucosal binding and neutralizing antibody responses than the DNA prime/protein boost regimen in lactating rhesus monkeys.

OA04.03

Expanded Memory CD4+ T Cells in the Fetal and the Infant Gut; a Mucosal Route for Mother-to-Child-Transmission of HIV-1

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Background: Cord blood-derived CD4+ T cells have a naïve phenotype and do not express CCR5, the mandatory co-receptor for transmitted HIV-1 R5 strains in infants. This leaves the question unanswered: what are the target cells for MTCT of HIV-1 and where do they reside? We hypothesized that in infant mucosal tissues, CD4+CCR5+ T cells may be present to facilitate mucosal transmission of HIV-1.

Methods: Using multicolor immuno-histochemistry, flowcytometry and next-generation sequencing of the T cell receptor, we analyzed various human fetal and infant tissues to identify memory CD4+ T cells as targets for HIV-1.

Results: Here, we demonstrate the previously unrecognized abundance of memory CD4+CCR5+ T cells in the human fetal and infant gut mucosa. CD4+ T cells from mesenteric lymph node were mostly naïve, similar to blood. T helper differentiation profiles as determined by transcription factors differed by tissue, with T-bet and ROR_t predominantly expressed by memory T cells in the gut mucosa. Next-generation sequencing for high-resolution screening of the T-cell receptor _-chain repertoire of clonal T cells as a hallmark of memory cells, identified expanded T cell clones in the gut mucosa (30%) and not in lymph node or cord blood. The gut mucosal fetal and infant CD4+ T cells were extremely susceptible to HIV-1 without any prestimulation; pol proviral DNA levels were similar to infected PHA stimulated adult PBMCs.

Conclusion: In conclusion, we show that extensive adaptive immunity, with a tissue-depended distribution is present before birth, resulting in the gut mucosa as the preferential site for memory CD4+ T cells. These memory CD4+CCR5+T cells provide a large pool of susceptible cells for ingested HIV-1 at birth and during breastfeeding, indicating a mucosal route of MTCT of HIV-1, which can be targeted in future prevention strategies.

OA04.04

Cell Free HIV-1 Virus Can Infect Inner and Outer Foreskin Polarized Explants

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Background: In sexually insertive men, HIV is predominantly transmitted at the penile surface. We report two mayor advances in the study of HIV infections at the foreskin.

Methods: First, we have developed cryopreservation methods that allow infection of foreskin tissue explants after thawing (654 +/- 178.3 pg of p24/gr of tissue) and provide comparable infection rates to fresh samples(716.5 +/- 446pg of p24/gr of tissue). Second, we have developed an ex vivo assay that uses human foreskin explants to replicate the polarized viral entry. After 12h of polarized exposure, HIV entering the explant is amplified for 6 days of culture with activated PBMCs and measured using p24 ELISA.

Results: Preserving the epithelial barriers, we show that polarized infections permit the entry and expansion of less virus (68.28 +/- 10.65pg of p24/gr of tissue) than non-polarized infections (650.4 +/- 205.9pg of p24/gr of tissue) where the virus can enter the CD4 T cell rich epidermal-dermal interface (p=0.04). Using 10000 TCID50 of HIV-1Bal per explants, we can detect infection in 100% of the non-polarized assays and 69% of the polarized explants. Lastly, comparing foreskin tissue from 4 donors, we demonstrate that the inner and outer foreskin are both equally able to support cell-free HIV infection in polarized assays (inner 77.67 +/- 64.7 vs. outer 92.82 +/- 66.81pg p24/gr of tissue p=0.369) and in non-polarized assays (inner 730.9 +/- 323.7 vs. outer 625 +/ 301.2pg p24/gr of tissue p=0.41).

Conclusion: We hope that this approach could be used efficiently as a model to evaluate the efficacy of prevention strategies.

OA04.05

Inflammation in the Male Genital Tract: Implications for HIV Acquisition and Transmission

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Background: Elevated plasma levels of pro-inflammatory mediators such as TNFα, IL-1β, IL-6 and IL-8, MIP-1α, MIP-1β and RANTES have been demonstrated in HIV-infected individuals and HIV induces higher levels of pro-inflammatory cytokines in the female genital tract. We characterized levels of inflammation in semen, to gain an understanding of factors influencing transmission and acquisition in the male genital tract. Our hypothesis was that infected men would exhibit higher levels of inflammation in semen than uninfected men.

Methods: We investigated concentrations of 20 pro-inflammatory and other mediators in the semen and blood of 38 HIV-infected and 42 uninfected men forming part of an HIV-discordant heterosexual couples study. We measured plasma and seminal viral loads to examine the relationship between viral replication and inflammation.

Results: We found that the majority of cytokines/chemokines were at higher concentrations in semen than blood, both in HIV-infected and uninfected men. There were no significant differences between any cytokines/chemokines in the semen of HIV-infected vs uninfected men. We found that TNFα ($p=0.013$; $r=0.55$), G-CSF ($p=0.0057$; $r=0.61$), IL-10 ($p=0.006$; $r=0.61$) and IFNγ ($p=0.01$; $r=0.57$) seminal levels were significantly associated with increases in seminal viral load. Furthermore, subsequent to controlling for the effect of plasma viral load in a multivariate regression analysis, we found that both seminal IL-10 and IFNγ levels were associated with a significant rise in seminal viral load.

Conclusion: Taken together, the data demonstrate that the immune milieu of the genital tract differs substantially from blood, with the majority of cytokines/chemokines tested elevated in semen. However, there were no differences in the levels of pro-inflammatory mediators in the semen of HIV-infected and uninfected men, or HIV-infected men on suppressive ART. Thus, even in the absence of HIV infection, the male genital tract appears to maintain a state of inflammation, which may have been the result of undetected and untreated co-infections.

Oral Abstract Session 05: Social/Ethical Issues

OA05.01

New Tools to Measure Community and Stakeholder Engagement and Its Impact on Outcomes of Clinical Research

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Background: Community and stakeholder engagement have increasingly been acknowledged as best practice in the design and implementation of global clinical research, results dissemination and strategies for access to new health products. Existing guidelines and best practices, however, provide little insight into the expected outcomes of engagement activities, indicators of success, or useful monitoring and evaluation (M&E) tools for assessing impact on research and communities.

Methods: To fill this gap, a consortium of organizations (AVAC, HANC, International HIV/AIDS Alliance, IAVI, NIAID, TB Alliance and Wellcome Trust), developed and field tested a user-friendly M&E Toolkit for engagement programs in clinical research settings in developing countries. From a broad review across clinical trial settings, the toolkit builds on existing practices and introduces new methods for M&E, indicators for impact and guidelines for effective use. The toolkit was piloted with TB and/or HIV research sites in Africa and Asia that have a history of incorporating engagement strategies in research.

Results: New indicators for measuring the degree of engagement and its impact on clinical research were identified. Participants in pilot-testing utilized existing and new methods of evaluation, and assessed the relationship of these strategies on targeted outcomes of clinical research. Feedback and lessons learned are being incorporated into a final version of the M&E Toolkit.

Conclusion: Understanding the impact of stakeholder engagement on clinical research is critical for the development and implementation of effective strategies, and for planning and optimizing clinical trials and their outcomes in developing countries. Through the use of the toolkit site and sponsor staff can better evaluate strategies and activities, demonstrate benefit, reallocate resources to activities that were most productive, and make the case for engaging stakeholders in research.

OA05.02

An Assessment of Good Participatory Practice Guidelines at HIV Prevention Research Clinical Centers in Eastern and Southern Africa

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Background: Compliance with ethical, regulatory, and scientific guidelines does not always guarantee consideration of external stakeholders' concerns in research. In 2007, UNAIDS and AVAC: Global Advocacy for HIV Prevention developed the Good Participatory Practice (GPP) Guidelines to ensure community and stakeholder engagement in biomedical HIV prevention research. In 2010-2011, the International AIDS Vaccine Initiative (IAVI) and AVAC conducted the first comprehensive evaluation of perceptions, practices and recommendations for improvement of participatory practice at eight IAVI-sponsored research centers (RCs) in Eastern and Southern Africa.

Methods: A total of 234 pre- and post-workshop questionnaires, 20 focus group discussions (FGD), and nine interviews with research staff, community advisory board members and other stakeholders were administered by AVAC staff primarily, to eliminate potential bias. STATA11 and ATLAS.ti were used to analyze the quantitative and qualitative data respectively.

Results: Quantitative analysis showed a high level of baseline support regarding the relevance of 11 of 16 GPP focus areas. Lower relevance was placed at baseline on engagement in: protocol development, standards of HIV prevention, policies on non-HIV related harms, and trial accrual and follow-up. Support increased for all 16 GPP areas post-workshop with statistical significance levels varying between $p<0.05$ – $p<0.01$. Preliminary findings from qualitative data revealed that stakeholder engagement in formative research, advisory mechanisms, and informed consent processes were considered effective at most RCs. Areas of needed improvement included communication and transparency of stakeholder engagement activities, formalized documentation of GPP practices, and funding for GPP activities at RCs.

Conclusion: Findings suggest that evaluation workshops significantly increased RC buy-in of GPP relevance to research. Some GPP areas may require more emphasis than others in future evaluations and technical assistance. On-site participatory GPP evaluations were highly effective in identifying strengths and gaps, increasing ownership, and facilitating ease of GPP implementation for research centers.

OA05.03

Social and Ethical Considerations in Engaging American Indian and Alaska Native Communities in HIV Clinical Research

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Background: American Indians and Alaska Natives (AI/AN) have the 4th highest rate of new HIV diagnoses among racial/ethnic groups and the highest mortality rate after an AIDS diagnosis (CDC, 2012), yet continue to be underrepresented in HIV clinical research trials due to historical and cultural factors. This presentation will highlight social and ethical experiences that contribute to mistrust of Western research and medicine and low involvement in HIV clinical research trials, and provide effective strategies for respectfully engaging Native communities.

The presentation will also describe the Native American Engagement in HIV Clinical Research (NAEHCR) project, a pilot aimed at increasing awareness and engagement in HIV clinical research with urban Indigenous communities using a participatory framework. NAEHCR was developed in partnership between the National Native American AIDS Prevention Center and the Legacy Project. NAEHCR is currently being conducted with DAIDS-funded research sites in Seattle, USA (HVTU, ACTU) and Denver, USA (HVTU, INSIGHT).

Methods: Formative research assessed awareness, barriers, and facilitators of involvement in HIV clinical research using qualitative and quantitative methods. Focus groups were held with Native advisory boards to explore perceptions and experiences with clinical research. Individual interviews were conducted with clinical research site staff to assess perceptions and experiences with AI/AN communities. Surveys used to assess AI/AN community members' awareness and experiences around clinical research.

Results: Initial results indicate high levels of interest in the project among AI/AN community members and clinical research staff. Results also indicate low levels of awareness and engagement in HIV clinical research among the AI/AN community and a disconnect between the AI/AN community and HIV clinical research sites.

Conclusion: Using a multi-method participatory approach offers a holistic depiction of barriers, opportunities for engagement, and important considerations when engaging AI/AN communities in HIV clinical research given the social and ethical legacy of research conducted in AI/AN communities.

OA05.04

Knowledge/Attitude/Practices of HPV & Cervical Cancer, Willingness to Participate in Vaccine Trial in Preparation For HIV & HPV Vaccine Trials in Mali

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Background: The GAIA Vaccine Foundation (GAIA VF) has been collaborating with the Malian regional DOH, local HIV clinicians, and scientists in Bamako to prepare a site for Phase I-III HIV vaccine trials. We recently performed two studies to evaluate HIV and HPV knowledge and willingness to participate (WTP) in an HIV or HPV vaccination trial.

Methods: Knowledge, Attitudes, and Practices (KAP) studies were performed in 2008 and 2011 to assess KAP related to HIV, HIV transmission, HIV prevention, HPV, cervical cancer, and WTP in vaccine trials. The 2008 KAP study examined HIV KAP and WTP (399 subjects), while the 2011 pilot study examined HPV KAP and WTP for 51 subjects in the same region of Bamako. Results from a more extensive HPV KAP (300 participants) are pending.

Results: HIV knowledge was high: over 73% of participants in the 2008 study were knowledgeable about modes of HIV transmission. 78% said they would participate in an HIV vaccine trial, 65% in a malaria vaccine trial, and 61% in a tuberculosis vaccine trial. In contrast, in 2011, less than 1% of individuals had heard of HPV. Yet 98% of participants were WTP in an HPV vaccine trial with the aim of obtaining approval of the vaccine in Mali.

Conclusion: WTP in vaccine trials is high among participants in these West African surveys. In previous African KAP and WTP studies, WTP ranged from 20% to 77% (average 47%). Even though participants were highly willing to participate in an HPV vaccine trial, levels of knowledge were very low. There is a significant need for expanded public education about the link between viruses and infection in West Africa. This study demonstrates challenges in implementing ethical clinical trials and highlights the need for a significant investment in health education if truly informed consent is to be obtained.

OA05.05

Referral and Access to Care of HIV Prevalent Cases; Experience from the Early Capture HIV Cohort Study in Kampala

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Background: Trial sponsors and implementers are ethically obligated to refer HIV infected Individuals identified in a research study at screening for HIV care and treatment. Makerere University Walter Reed Project is conducting HIV surveillance among high risk uninfected female sex workers. We describe patterns in participants' receipt of HIV results and response to referral for HIV care and treatment.

Methods: Subsequent to informed consent, risk eligibility is determined using Audio Computer Assisted Self Interview (ACASI). Medical history, physical exam and blood draw are done to determine HIV sero-status and further eligibility. Participants determined HIV positive by ELISA/Western Blot require confirmatory testing before being screened out and referred for care.

Results: HIV prevalence was 35% (221/631) at screening. Out of the 221 prevalent cases, only 96 participants (43%) received HIV confirmatory results and were referred for care, while 9(4%) declined referral. The majority did not return for either their initial or confirmatory HIV result; while a few declined a blood re-draw. Of the 96 participants referred, 58% are currently in care, 14% did not report for care predominately citing indecisiveness while 28% could not be tracked. Majority of acutely infected participants (6/8) are in care.

Conclusion: Although trial implementers may fulfil their obligation in referring study participants for HIV care, participants have a key role to play in facilitating this process. The large number of HIV prevalent female sex workers who did not return for their HIV results and may not be aware of their status could be a potential driver of the epidemic in Uganda.

Oral Abstract Session 06: V1/V2 Antibody Responses

OA06.01

A Short Segment in the HIV-1 gp120 V1/V2 Region Is a Major Determinant of Neutralization Resistance to PG9-Like Antibodies

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Background: Antibody PG9 is a prototypical member of a class of V1/V2-directed antibodies that effectively neutralizes diverse strains of HIV-1. The crystal structure of PG9 bound to scaffolded V1/V2 has provided insight into its mode of recognition. We sought to gain a more complete understanding of the interaction of PG9 with the functional viral spike, and to extend our understanding to other antibodies of this class.

Methods: We analyzed amino acid frequencies in the V2 region of PG9 sensitive and resistant strains to identify potentially important residues. We also used the crystal structure of PG9 with scaffolded V1/V2 to identify potential contact sites. Based on these analyses we designed mutations in PG9 resistant strains with the goal of "knocking in" sensitivity. Parent and mutant Envs were used to make pseudoviruses. The potency of PG9 as well as V1/V2 antibodies PG16, CH01, CH04, PGT141, and PGT145 against the pseudoviruses was assessed by TZM-bl neutralization assay.

Results: For 20/20 of resistant strains, mutations in a short segment of V1/V2 resulted in gain of sensitivity to at least one antibody of the PG9 class, and 13/20 showed gain of sensitivity to 3 or more. Mutations in V2 strand C, particularly the addition or substitution of lysine at positions 168, 169, or 171, had the greatest effects.

Conclusion: These results highlight the importance of strand C contacts for neutralization by V1/V2 antibodies, provide functional confirmation of the crystal structure, and suggest a general mechanism of resistance to V1/V2-directed broadly neutralizing antibodies.

OA06.02

Characterization of V1V2-Specific Antibodies Present in Broadly Neutralizing Plasma Isolated from HIV-1 Infected Individuals

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Background: Recent studies of antibodies in human plasma from infected and immunized individuals revealed an important role of the V1V2 region of the gp120 protein in immune response. The function of V1V2-specific antibodies and their potential in blocking the HIV-1 infection are still not well established. In this study we present data about the appearance of such antibodies in plasma from hundreds of patients from North America and Africa, about their clade specificity, development and neutralization activity.

Methods: Human plasma was isolated from infected individuals and screened for anti V1V2 ELISA binding activity with gp70/V1V2 fusion glycoproteins, representing clades B, C and A/E sequences. All samples were tested in virus neutralization assay for activity versus panel B, panel C, and other Tier 2, pseudoviruses. Immunoaffinity chromatography of selected plasma, on gp70/V1V2 protein columns, was used to isolate V1V2-specific antibodies.

Results: Most of the HIV-1 infected subjects (above 80%) have robust levels of V1V2 binding activity versus the three antigens. This activity was commonly detected in chronic HIV-1 infection. Interestingly, the development of V1V2-reactive antibodies tracked with the development of autologous neutralizing antibodies. Immunoaffinity purified V1V2-specific antibodies from selected broadly neutralizing plasma samples possessed broad neutralization activity with IC50's generally in the 1 -20 µg/ml range.

Conclusion: Highly cross-reactive V1V2-specific Abs are present in almost all broadly neutralizing human plasmas. These antibodies are in large amounts and can be linear, conformational or quaternary epitope dependent. Such antibodies are induced in humans (Thailand trial) and because of that the region may be considered as immunogenic. In addition, isolated anti-V1V2 antibodies show neutralization activity toward Tier 2 viruses. The above characteristics make the V1V2 region an important target for candidate HIV-1 vaccines.

Oral Abstract Session 06: V1/V2 Antibody Responses

OA06.03

Design of an HIV Env Antigen That Binds with High Affinity to Antibodies Against Linear, Conformational and Broadly Neutralizing Epitopes Within V1/V2

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Background: The RV144 HIV-1 vaccine trial showed protection from HIV-1 acquisition with vaccine efficacy of 31.2%. Study of the immune correlates demonstrated an inverse association of V1/V2 antibodies with infection risk. A key task for HIV-1 vaccine development is to improve the level of efficacy seen in the RV144 trial with subsequent vaccine designs.

Methods: E.A244 V1/V2 Env tags contains an N-terminal Ig leader sequence and C-terminal Avi- and His6-tags linked to the V1/V2 domain, was expressed in 293F cells and purified by nickel column. Binding of Tier 1 neutralizing mAb CH58 from RV144 vaccinees, V2 conformational mAb 697D and broadly neutralizing antibodies (bnAb) CH01 and PG9/PG16 to 33 HIV-1 gp140/gp120s and 12 HIV-1 V1/V2 scaffold Envs was tested by ELISA and surface plasmon resonance.

Results: Among 45 HIV-1/SIV Envs tested, E.A244 V1/V2 tags and E.A244 gp120Δ11 Env were the only Env antigens recognized by all three types of mAbs: CH58, 697D, and bnAbs CH01, and PG9/PG16. E.A244 V1/V2 tag bound CH58 with a Kd of 0.33 nM and 697D with a Kd of 117 nM. Although PG9 preferentially recognizes trimers, PG9 bound well to both E.A244 gp120Δ11 (Kd = 47.3) and E.A244 V1/V2 tags (Kd = 83.3 nM). BnAb CH01 bound V1/V2 tags as well (Kd = 334 nM). E.A244 V1/V2 Env tags was also recognized by the unmutated ancestor antibodies (UAs) of CH58 with ELISA EC50 = 4.9 nM and CH01 with EC50 = ~1μM. E.A244 V1/V2 tags and AE.gp70 V1/V2 scaffold were the best recombinant Envs for detection of plasma V1/V2 antibodies in RV144 vaccinees.

Conclusion: Recombinant E.A244 V1/V2 Env tags Env expresses linear as well as conformational determinants recognized by V1/V2 mAbs and some of their UAs. This V1/V2 construct is a candidate immunogen to target RUAs and intermediate ancestors of V1/V2 antibodies to drive their induction.

OA06.04

Evidence for Env-V2 Sieve Effect in Breakthrough SIV$_{MAC251}$ Infections in Rhesus Monkeys Vaccinated with Ad26/MVA and MVA/Ad26 Constructs

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Background: We had previously shown that rhesus monkeys receiving Ad26/MVA and MVA/Ad26 vaccines expressing SIV$_{SME543}$ were protected against SIV$_{MAC251}$ challenge (doi:10.1038/nature10766). Protection was associated with Env-specific binding ELISA antibody responses, including V2-specific antibodies.

Methods: We amplified 66 sequences from the SIV$_{MAC251}$ challenge stock, and 409 near-full length genomes from 13 vaccine and 13 control monkeys. A series of pre-specified phylogenetic and statistical tests for sieve effects was performed.

Results: The mean pairwise AA diversity among the 66 SIV$_{MAC251}$ Env sequences was 0.38%, and they differed from the vaccine strain SIV$_{SME543}$ (Env) by 21.94%. The repeated low-dose challenge resulted in infections with an average of 1.7 founder variants - with no evidence that the vaccine restricted the number of variants ($p = 0.813$). We explored whether the vaccine induced a sieve effect, i.e. whether breakthrough viruses differed between the vaccine and control groups. There was no difference for full-length Env sequences. Focusing on Env segments preferentially recognized by vaccinated monkeys in antibody arrays, we identified a sieve effect in the Env-V2 segment AA163-193: sequences from vaccinated animals were more divergent from the vaccine SIV$_{SME543}$ or from the challenge stock SIV$_{MAC251}$ than sequences in control animals ($p \leq 0.002$).

Conclusion: The sieve effect in Env-V2, combined with Env-V2-specific binding antibodies identified as a correlate of protection against SIV$_{MAC251}$ acquisition in the study, provide evidence supporting the importance of protective responses directed against the Env-V2 region.

OA06.05 LB

Structural Basis for Germline Gene Usage of a Potent Class of Antibodies Targeting the CD4 Binding Site of HIV-1 gp120

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Background: A large number of anti-HIV-1 antibodies targeting the CD4 binding site (CD4bs) on gp120 have recently been reported. These antibodies, typified by VRC01, are remarkable for both their breadth and potency. Crystal structures have revealed a common mode of binding for several of these antibodies; however, the precise relationship among CD4bs antibodies remains to be defined.

Methods: We analyze existing structural and sequence data, propose a set of signature features for potent VRC01-like (PVL) antibodies, and test the importance of these features by mutagenesis.

Results: A group of highly potent CD4bs antibodies, previously isolated from 5 different individuals, all derive from the human VH1-2 gene segment and share a set of characteristic residues, including W50, N58, R71, and W100B. Mutagenesis studies on a half-germline version of a VRC01-like antibody confirm that these signature residues are critical for gp120 binding. Neutralization assays with viruses mutated at sites that contact these critical antibody residues also confirm the significance of these interactions.

Conclusion: The signature features explain why PVL antibodies derive from a single germ-line human VH gene segment and why certain gp120 sequences are associated with antibody resistance. This analysis also suggests that immunization experiments to elicit VRC01-like antibodies may be problematic in mice or rabbits since they lack germ-line VH genes with all the critical residues. Our results bear on vaccine development and structure-based design to improve the potency and breadth of anti-CD4bs antibodies.

Oral Abstract Session 07: B Cell Responses

OA07.01

Structure-Guided Modification and Optimization of Antibody VRC07

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Background: Eliciting a neutralizing human antibody against HIV-1 still remains to be elusive. Nevertheless, a number of studies have reported isolation of potent and broadly reactive antibodies against HIV-1 from HIV-1 infected patient serum. Antibody VRC01 is one of these kinds that binds to the CD4 binding site of gp120 and neutralizes the viruses. Recently, we identified antibody VRC07, which is more potent and broadly reactive anti-HIV-1 antibody than its derivative, VRC01.

Methods: In this study, we determined the crystal structure of gp120 in complex with VRC07 and utilized mechanistic insights and structure-guided modification to increase potency and breadth.

Results: A four amino acid insertion in the CDR H3 loop of VRC07 provided for more extensive contacts with gp120 than with VRC01. The structure also revealed that residue Gly54 of VRC07 could be replaced with amino acids with bulky side chains to mimic residue Phe43 of CD4. Indeed, all VRC07 variants, in which Gly54 was replaced with Arg, Leu, Phe, Trp, or Tyr, showed enhanced affinity to a panel of different HIV-1 gp120s. Furthermore, most of these Gly54 alterations showed enhanced potency and breadth against a panel of clade B and C viruses in TZM-bl cell-based neutralization assay. Crystal structures of gp120 in complexes with these VRC07 Gly54 variants confirmed that their side chains mimicked Phe43 of CD4. Computational analysis of the VRC07-gp120 interface in the crystal structure identified residues Ile30 and Ser58 as likely targets for improvement (with Gln and Asn, respectively). These changes introduced additional hydrogen bonds to the VRC07-gp120 interfaces and further enhanced VRC07 potency.

Conclusion: Thus, our optimization of antibody VRC07 demonstrated that a structure-guided approach can be used to increase both antibody potency and breath. The optimized VRC07 developed here can be used as the basis for further structure-guided improvement or for optimization via in vitro selection.

OA07.02

Isolation of a Clonal Lineage of IgA Broadly Neutralizing Antibodies from a Chronically Infected Tanzanian Subject

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Background: Only ~20% of HIV-1-infected subjects develop broadly neutralizing antibodies (bnAbs) and the origins of such antibodies remains obscure. To date, all isolated bnAbs have been of the IgG isotype.

Methods: Memory B cells from a chronically infected Tanzanian subject with plasma broad neutralizing activity were labeled with a consensus C envelope (Env) and Env+ cells sorted as single cells. Immunoglobulin heavy and light chain genes were amplified by PCR and analyzed for gene usage and isotype, and then were expressed as recombinant monoclonal antibodies (mAbs). MAbs were assayed for binding to Env proteins and for neutralization of multiple HIV-1 strains.

Results: We isolated 13 mAbs that bound Env proteins; of these, 11 were members of three clonal lineages, each of which spanned two time points. Six mAbs in one lineage were all IgG1, used V_H1~69, had an average mutation frequency of 12%, mapped to the CD4 contact region, and had modest neutralization activity. In contrast, three mAbs in a second lineage had two members that were IgA2, used V_H3~66, had an average mutation frequency of 16%, and neutralized 11/27 (41%) of tier 2 pseudoviruses tested.

Conclusion: We have isolated the first natural IgA mAbs with broad neutralizing activity. These data demonstrate that class switching to IgA can occur in the generation of bnAbs, an event that is essential for the generation of neutralizing IgA antibodies at mucosal surfaces.

OA07.03

Deep Sequencing with Longitudinal Sampling of a VRC01-Like-Antibody Response in a Chronically Infected Individual

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Background: VRC01-like antibodies use heavy chain mimicry of the CD4-receptor to achieve effective neutralization of HIV-1. The VRC01-like antibodies that have been observed in a number of HIV-1-infected individuals (i) display extensive somatic changes (70-100 nucleotide changes in VH-gene), (ii) can be detected only after several years of infection, (iii) derive from VH1-2, and (iv) are compatible with several different heavy J chains and different light chains.

Methods: To understand the persistence, evolution, and lineage of VRC01-like antibodies, we sampled PBMCs from donor 45, the source of VRC01 and VRC03 antibodies, at approximately yearly intervals over a 15-year period, and performed deep sequencing on the heavy and light chain variable portions of expressed antibodies. Anti-idiotypic antibodies were used to correlate mRNA levels of antibodies identified by the deep sequencing with expressed levels of these antibodies in serum.

Results: High expression levels of VRC01-like antibody sequences persisted over the entire 15-year period. Multiple lineages of VRC01-like antibodies were detected at each time point, and some of these, in particular the lineages that include VRC01 and VRC03, persisted over multiple time points, and displayed extensive branching in their evolution.

Conclusion: Deep sequencing provides a means to define the genetic record of the lineage and maturation of antibodies effective at neutralizing HIV-1. Precise definition of the natural ontogeny of broadly neutralizing antibodies may be essential in defining appropriate strategies to elicit such antibodies in vaccine settings.

OA07.04

Antibody Effector Function Is Regulated by a Combination of Adaptive and Innate Signals

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Background: The Fc region of IgG contains a single N-glycosylation site, which is known to be important for effecting immune activation through interaction with Fc receptors and complement molecules. Changes to the structure of these sugars can thus be very influential in determining the specific effector function of individual antibodies. Whereas therapeutic antibodies can be produced with particular effector functions in vitro, little is known regarding how glycosylation is regulated in primary B cells.

Methods: Purified B cells were stimulated with a variety of synthetic TLR ligands alone or in combination with soluble CD40L, anti-IgG, anti-IgM, or supernatants derived from stimulated antigen presenting cells. After 16 hours, quantitative RT-PCR was used to determine expression of genes known to be specifically involved in IgG N-glycan synthesis.

Results: We found that the expression of glycosylation genes is significantly impacted both by specific TLR stimulation alone and in combination with adaptive signals received either through the B cell receptor or CD40. Specifically, virus-derived stimuli that activate TLR 7, 8 or 9 can significantly decrease the expression of galactose adding enzymes, whereas TLR 9 stimulation in combination with CD40 stimulation decreases the expression of both sialic acid– or GlcNac-adding enzymes. These changes in expression result in the production of antibodies with Fc glycan structures with increased NK cell– and monocyte-recruiting capabilities.

Conclusion: Overall, these results are the first to show that the production of antibodies with specific effector functions can be regulated by external stimuli, including both innate and adaptive immune signals, suggesting that antibodies with specific, strong effector functions can be induced in vivo following vaccination.

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OA07.05

Non-neutralizing IgG Anti-PID Antibodies Decreased Viral Load Following High Dose Vaginal Challenge of Non-human Primates

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Background: Fc-mediated inhibitory activity of neutralizing antibodies has been shown to participate in HIV protection (Hessell et al. 2007). In addition, a non-neutralizing antibody F240 was found to partially protect macaques from SHIV vaginal transmission (Moore et al., 2011). However, mechanisms involved in this protection need further investigations. In this study, two non-neutralizing antibodies have been selected on the basis of their Fc-mediated inhibitory functions in vitro for further analysis of their protective role on vaginal challenge in non-human primate (NHP).

Methods: We have assessed and scored various in vitro HIV-inhibitory activities of non-neutralizing antibodies: Fc-mediated inhibition of macrophages through phagocytosis of immunecomplexes, ADCC in primary infected CD4+ T lymphocytes by autologous NK cells, capture of native primary virus particles. Efficacy of two anti-PID antibodies with high in vitro functional scores has been tested in NHP. The combination of two antibodies formulated in 1.6% HEC gel has been topically applied in the vagina of macaques (n=6), 1 hour before vaginal challenge with high dose (10 AID50) of SHIVSF162P3.Infection was followed by assessing viral load in the plasma.

Results: Although unable to block virus entry at mucosal site as all treated animals became persistently infected, the antibody treated macaques have significant decrease of plasma viral load (day 7, p=0.0479; day 14, p=0.0351, day 42: p=0.0370).

Conclusion: Decrease in viral load following antibody treatment strongly suggests that non-neutralizing inhibitory antibodies could interfere with early viral replication and dissemination through Fc-mediated inhibitory functions. Additional studies will be required to optimize this inhibition, and combined strategies should be developed to assess the potential synergy between neutralizing and non-neutralizing inhibitory antibodies.

OA07.06

Vaccine-Induced ADCC-Mediating Antibodies Target Unique and Overlapping Envelope Epitopes

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Background: Antibody Dependent Cellular Cytotoxicity (ADCC) may be a contributing factor of immune responses controlling HIV-1 replication. Understanding the epitopes recognized by ADCC-mediating antibodies is likely to be important for the development of an effective AIDS vaccine. We characterized the epitope specificity and breadth of the ADCC-mediating antibody response elicited by the RV144 vaccine regimen.

Methods: Twenty-three monoclonal antibodies (mAbs) were isolated from 6 vaccine recipients either from IgG^+ memory B cells cultured at near clonal dilution for 14 days (n=115,200) followed by sequential screenings of culture supernatants for HIV-1 gp120 Env binding, or from memory B cell (n=206,745) sorting for HIV-1 group M consensus gp140Con.S Env binding. Target cells infected with infectious molecular clones expressing Clade A/E (CM235), B (BaL), and C (DU422 and DU151) env were used to characterize the specificity and breadth of the 23 mAbs that display ADCC activity. We defined the epitope specificity of the isolated mAbs by mapping with B.MN and/or AE.92TH023 linear peptides in ELISA and with Fab-competition in ADCC assays.

Results: Linear mapping revealed that 2 mAbs recognized the V2, and 1 mAb the V3 regions of the gp120. Nineteen (19) mAbs recognized conformational epitopes overlapping the C1 A32 epitope; one mAb (CH20) recognized a conformational epitope that was not blocked by any of the Fabs (A32, 19B, 17b) utilized in our assay. Fourteen of the 20 mAbs directed against conformational epitopes mediated ADCC against the clade B BaL Env; 4 recognized the clade C DU151 Env and 1 recognized the clade C DU422 Env.

Conclusion: The RV144 vaccine regimen induced broadly-reactive ADCC Abs that recognized both unique and overlapping regions of gp120. The mAbs with the greatest breadth may be useful for passive protection trials in rhesus macaques. If protective in non-human primates, the epitopes recognized by these mAbs may inform immunogen design.

OA07.07

454 Pyrosequencing and Bioinformatics Analysis of the Lineage of the Broad and Potent gp41-Directed Antibody 10e8

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Background: The 10e8 antibody is a novel MPER-specific antibody that is both broad and potent, and lacks detectable autoreactivity. 454 pyrosequencing has recently been shown to allow for a bioinformatics analysis of the genetic record of a broadly neutralizing antibody, once its sequence is known.

Methods: 454 pyrosequencing of B cells from donor N152, from whom 10e8 was isolated, was undertaken to characterize additional variants of 10e8 and to define its lineage. PCR reactions using 10e8 germline-specific heavy and light chain primers were performed and 454 pyrosequencing results analyzed using a multi-step in-house bioinformatics pipeline.

Results: 37,669 IGHV3-15 heavy chain sequences and 91,951 IGLV3-19 light chain sequences were obtained and the distribution of these sequences analyzed by two key parameters – divergence from germline and sequence identity to 10e8. Grid-based clustering allowed for the selection of 61 heavy chain sequences and 48 light chain sequences. These were reconstituted with the appropriate 10e8 light or heavy chain partner, expressed and tested for MPER recognition. 12 heavy chain variants and 27 light chain variants were found to bind MPER, and several of the reconstituted antibodies neutralized a panel of HIV-1 isolates better than the original 10e8. Phylogenetic analyses identified clonally-related sequences, and at least three subgroups of 10e8-like heavy chains were observed. 10e8 light chains meanwhile also showed a few subgroups, though with less sequence variation.

Conclusion: 454 pyrosequencing was thus able to identify clonal variants of 10e8 with substantially improved neutralization potency. Corresponding phylogenetic analyses are providing insight into the ontogeny of 10e8, and structural and biophysical studies are underway to functionally characterize this lineage.

*These authors contributed equally to this work.

OA07.08 LB

Engineered Mice and B Cell Lines Expressing Broadly Neutralizing Antibodies and Their Unmutated Precursors: Tools for HIV Vaccinology

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Background: Eliciting broadly neutralizing antibodies (bNAbs) to HIV Env through immunization has been problematic.

Methods: To better understand the requirements for activation of B cells producing bNAbs, we generated a series of cell lines and transgenic mice expressing such antibodies or selected germline-reverted versions as B cell surface receptors. The bNAb cell lines included those that recognize the CD4 binding site (b12, VRC01, PGV04, PGV19, NIH45-46), the MPER of gp41 (4E10), and additional glycan-dependent sites on the trimer (PG9, PG16, PGT145, 2G12, PGT128, PGT135, PGT121). Different Env-containing antigens and virions were tested for the ability to stimulate bNAb cell lines.

Mouse strains expressing germline or mutated forms of 4E10 and b12 bNAb Ig genes were generated by gene targeting to the physiological loci. These “knock-in” mice were studied for their B cell development, and responses to HIV immunogens.

Results: Many HIV Env antigen preparations, notably including infection-competent pseudovirions, were poorly recognized by high affinity bNAb-expressing cells, as measured by calcium flux assay. However, other antigen forms were highly stimulatory: in particular, soluble gp140 foldon trimers and a multimerized, scaffolded epitope protein.

4E10, but not b12 knock-in mice showed signs of abortive B cell development. b12 H mice had gp120-binding cells and responded well in vivo to gp140 trimers.

Conclusion: Analysis of bNAb cell line activation suggested that HIV is difficult to recognize by B cells, probably because of the low density of surface proteins. Based on these results, soluble gp140 trimers or epitope scaffolds might offer more promise as vaccine candidates. In knock-in mice, primary 4E10 B cell precursors appeared to be negatively selected, whereas b12 B cells were normal and readily stimulated with gp140 trimers.

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OA08.01

Cellular Immune Responses and Changes in VL After a Dendritic Cells (DC)-Based Therapeutic Vaccine in cART Treated Chronic HIV-Infected Patients with CD4 T Cells Above 450/mm

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Background: We have performed a blinded placebo-controlled study immunizing antiretroviral (cART) treated chronically HIV-1 infected patients with autologous Myeloid-Derived Dendritic Cells (MD-DCs) pulsed with heat inactivated autologous HIV-1.

Methods: 36 patients with CD4+ >450 cells/mm3 were randomized (2:1) to receive 3 immunizations every 2 weeks with DC pulsed with autologous heat-inactivated HIV-1 (Cases, n=24) or with non-pulsed DC (Controls, n=12). Changes in viral load (VL) as well as changes in CD4 cell counts have been evaluated. Additionally HIV specific responses were measured in PBMC samples from different time-points by LPR and by IFN-_-Elispot against gag, nef and gp41 HIV overlapping peptide pools, respectively.

Results: VL rebounded to detectable level in all the patients. At week 12 and 24, a decrease of VL >= 1 log was observed in 12/22 (55%) vs 1/11(9%) and in 7/20(35%) vs 0/10(0%) in cases and controls, respectively (p=0.02, p=0.03). CD4 drop to baseline value before any cART without differences between groups. Although only transient positive responses to HIV p24 were observed, the median change in LPR to HIV p24 at week 24 from baseline was 0.96 vs -0.50 (p=0.02) in cases vs controls, respectively. Baseline median values of the total sum of HIV specific responses against HIV peptide pools were similar in both arms (2625 versus 2283 SFC/106 PBMC, p=0.462). After vaccination, the median change of total sum of SFC/106 PBMC at week 24 was 3567 vs 838 SFC/106 PBMC (p=0.0459) in cases and controls, respectively. This difference was more evident when analyzing responses against gag p17 and Nef peptide pools (p=0.0288 and p=0.03615, respectively). No statistically significant correlations between immune responses and VL were found.

Conclusion: These results indicate that HIV-1 specific immune responses elicited by therapeutic DC vaccines could significantly change pVL set-point after cART interruption in chronic HIV-1 infected patients.

OA08.02

Alterations in Function and Distribution of Regulatory T Cells (Tregs) May Blunt Vaccine Induced Immune Responses in HIV Infection

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Background: Conflicting data exist about the frequency and role of regulatory T cells (Tregs) during the course of HIV infection. Tregs may substantially contribute to T cell homeostasis and fine regulation of T cell mediated immune responses.

Methods: Peripheral blood and individual lymph nodes of a large cohort of HIV+ patients (n=131) at different disease stages, including 15 long-term nonprogressors and 21 elite controllers, was analyzed to determine the frequency, phenotype and function of Tregs.

Results: A significantly increased relative frequency of Tregs within the CD4+ compartment of HIV+ patients in comparison with healthy controls (p<0.0001) was observed. The relative frequency of Tregs directly correlated with HIV viral load and inversely with CD4+ counts and was higher in corresponding lymph nodes. However, absolute Treg number was reduced in HIV infected patients vs. healthy controls (p<0.0001), but not in elite controllers (p>0.05). Loss of absolute Treg numbers was associated with increased markers of immune activation (HLA-DR, CD38 Ki-67)(p<0.0006). Initiation of antiviral therapy significantly increased absolute Treg numbers (p<0.0031). Moreover, we find that the expression of CD39 and CD73, newly defined ectonucleotidases involved in ATP degradation, correlated with progressive HIV disease, VL and immune activation. Of note, the capacity to suppress T cell proliferation in vitro was limited to the CD4+CD25highCD39+ T cell subset. Depletion of this distinct Treg subset in vitro resulted in a restoration of HIV specific T cell responses. Tregs of elite controllers exhibited the highest expression of CCR5, CTLA-4 and ICOS and the lowest level of CD39 indicating the functional importance of this ATP modulating enzymatic reaction.

Conclusion: These data reconcile the seemingly contradictory results of previous studies on Tregs in HIV and highlight the complexity of Treg mediated immunoregulation. Blocking of ATP modulating molecules which are highly expressed on Tregs may restore HIV specific immune responses.

OA08.03

Identification of CD8+ T Cell Host Factors Involved in HIV Control

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Background: The goal of the is project was to further define our understanding of the critical antiviral effector molecules and cell signaling pathways involved in CD8 T cell mediated control of HIV infection.

Methods: To accomplish this, we performed whole genome transcriptional profiling on sorted epitope-specific CD8 T cells, in the presence and absence of cognate peptide, from a total of 8 B*2705 elite controllers and progressors. Epitope-specific CTLs were isolated using HLA class I tetramers and used to prepare mRNA for evaluation by gene expression profiles using whole genome Illumina bead arrays. Bulk CD8 T cells were used as an internal control for each patient.

Results: Statistically significant genes enriched by our analysis include those involved in cellular cytotoxicity, cytokine signaling, T-cell receptor signaling, cell-cell adhesion molecules, and T cell homeostasis. Further analysis of the gene set identified 137 unique genes that were both induced by peptide stimulation in epitope-specific CD8 T cells, and significantly different between peptide-stimulated controller and progressor CD8 T cells. Further refinement of this list of 137 genes using a protein-protein network analysis revealed that 13 of these genes are statistically significant in driving the network connectivity. We have now focused our efforts on one of these genes, Caspase 8.

Conclusion: This study defines a novel set of 137 genes in epitope-specific CD8 T cells that are upregulated upon peptide stimulation and different between patients that control and have progressive infection. Further refinement of this list defines 13 unique candidates that drive network connectivity, of which one was Caspase 8. Our initial studies also reveal that Caspase 8 mRNA expression, which has a known role in T cell homeostasis, is upregulated in CD8 T cells in controllers, suggesting its potential role as a novel T cell correlate of control.

OA08.04

PD-1, IL-10, IFN-γ and IL-12 Form a Network to Regulate HIV-1-Specific CD4 T Cell and Antigen-Presenting Cell Function

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Background: PD-1 and IL-10 blockade can restore antigen-specific T cell functions in chronic infections and cancer. However, not all subjects respond to inhibition of either pathway, the potential differences in functions restored by these interventions are unknown, and mechanistic interactions between these pathways are poorly understood.

Methods: We investigated 45 subjects with HIV-1 infection with different disease status. We used CFSE assays to measure proliferation of HIV-1-specific CD4 T cells and Luminex arrays to analyze IFN-γ(Th1), IL-2(Th0), IL-13(Th2) and IL-12 secretion in supernatants of CD8-depleted PBMC stimulated for 48h with Gag peptide pools in the presence of isotype control antibody, anti-PD-L1 and/or anti-IL-10Ra, anti-IFN-γ or anti-IL-12. We used flow cytometry to evaluate the role of IFN-γ in regulating PD-L1, HLA-DR, HLA-ABC and CD86 expression by monocytes.

Results: Whereas PD-L1 blockade had a balanced impact on proliferation and cytokine secretion by HIV-1-specific CD4 T cells, anti-IL-10Rα preferentially restored IFN-γ production. Combined blockade resulted in a dramatic 9.8-median-fold increase of IFN-γ, contrasting with the moderate effect of single blockade (2.5 median-fold). Antigenic stimulation of HIV-1-specific CD4 T cells upregulated PD-L1, HLA-DR and HLA-ABC on monocytes through an IFN-γ-dependent mechanism. Combined PD-L1/IL-10Rα induced a striking increase in IL-12 production by antigen-presenting cells(APCs) that was governed by IFN-γ derived from the Thelper cells. Neutralization of IL-12 reduced the dramatic effect of combined blockade on IFN-γ, demonstrating a positive feedback loop between IFN-γ produced by HIV-1-specific CD4 T cells and IL-12 produced by APCs.

Conclusion: These data provide important evidence on the therapeutic potential of combined interventions on the PD-1 and IL-10 pathways to restore HIV-1-specific CD4 T cell and antigen-presenting cell function. We provide mechanistic insight on the mode of action of dual blockade by showing that IFN-γ produced by HIV-1-specific CD4 T-cells and IL-12 secreted by APCs regulate each other in a positive feedback loop

Oral Abstract Session 08: T Cell Responses

OA08.05

Distinct Gene Expression Profiles Associated with the Susceptibility of Pathogen-Specific CD4+ T Cells to HIV-1 Infection

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Background: HIV infection causes the progressive depletion of CD4+ T cells. Contrary to the early loss of CD4 response to opportunistic pathogens like Candida albicans, cytomegalovirus (CMV)-specific CD4 response is persistent when total CD4+ T cell number is low. The mechanism is less clear. Despite considerable knowledge for the impact of HIV infection on total CD4+ T cells and their subsets, little is known about HIV infection of CD4+ T cells of different pathogen/antigen (Ag) specificity.

Methods: PBMC from HIV-negative donors were CFSE-labeled and stimulated ex vivo with pathogen-specific antigens including viral (CMV), bacterial (Tetanus Toxoid: TT) and fungal (Candida albicans) antigens. HIV infection of Ag-specific CD4+ T cells was determined by intracellular p24 production in CFSE-low population.

Results: While TT- and Candida-specific CD4+ T cells were permissive, CMV-specific CD4+ T cells are highly resistant to both X4 and R5 HIV independent of coreceptor useage. Quantification of HIV DNA in sorted, antigen-specific CD4+ T cells demonstrated a reduction of both strong-stop and full-length HIV DNA in CMV-specific CD4+ T cells. β-chemokine neutralization enhanced HIV entry and viral replication in TT- and Candida-specific CD4+ T cells, whereas HIV infection in CMV-specific CD4+ T cells remained low despite increased HIV entry by β-chemokine neutralization, suggesting both entry and post-entry HIV restriction in CMV-specific cells. Microarray analysis revealed distinct gene expression profiles that involved selective upregulation of a broad array of antiviral genes in CMV-specific CD4+ T cells, whereas TT- and Candida-specific CD4+ T cells mainly upregulated a Th17 inflammatory response.

Conclusion: Our data suggest a mechanism for the persistence of CMV-specific CD4 response and the earlier loss of mucosal Th17-associated TT- and Candida-specific CD4 response in AIDS patients. The model described is useful in HIV vaccine studies by evaluating the susceptibility of vaccine-specific CD4 responses to HIV infection.

OA08.06

HIV Control Through a Single Nucleotide on the HLA-I Locus

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Background: In correlative studies HLA class I type is consistently found to have the strongest impact on HIV disease progression. However, the exact mechanism involved is complicated by several factors; many alleles are ligands for NK cells as well as CD8 T-cells, and strong linkage disequilibrium between Class I alleles makes it difficult to distinguish the effect of individual alleles from other HLAs or from other important loci found on the HLA haplotype, such as the recently described -35 SNP.

Methods: Here we study two recently diverged HLA alleles, B*4201 and B*4202, which only differ by a single amino acid. Crucially, they occur primarily on identical Class I haplotypes and do not act as NK cell ligands. Therefore, they represent a unique opportunity to study the impact of a single HLA allele on HIV immune control not confounded by other genetic factors in a large outbred cohort (n=2,093) of C-clade infected individuals.

Results: Here we show that the amino acid change in position 9 of the HLA-B molecule, is critical for peptide binding and significantly alters the Gag CTL epitopes targeted ($P=2x10^{-10}$), measured both directly ex-vivo by ELISPOT and indirectly through CTL escape mutation ($P=2x10^{-8}$). Strikingly, HLA-B*4201 is associated with significantly lower viral load setpoint than HLA-B*4202 (P=0.02).

Conclusion: This naturally controlled experiment represents perhaps the clearest demonstration of the direct impact of particular HIV Gag specific CTL on disease control.

OA08.07

Accelerated Heterologous Prime-Boost Adenovirus Vector-Based SIV Vaccine in Neonatal Rhesus Monkeys

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Background: A pediatric HIV-1 vaccine is required to protect infants against HIV-1 transmission from breastfeeding. Such a vaccine would need to induce protective immunity at mucosal surfaces in neonates as soon as possible after birth. Recombinant adenovirus (rAd) vectors have been shown to elicit potent systemic and mucosal virus-specific immune responses in adult non-human primates and humans but have not previously been studied in detail in infants.

Methods: Newborn rhesus monkeys were injected intramuscular (i.m.) with 10^11 viral particles of rAd serotype 26 or 35 vectors expressing SIVmac239 Gag. Peripheral blood was collected to determine systemic Gag-specific cellular and humoral immune responses. At week 52, peripheral lymph nodes, bronchoalveolar lavage (BAL) and pinch biopsies of colorectal, duodenal and oral cavity mucosa were collected to evaluate mucosal Gag-specific T lymphocyte responses.

Results: A single injection of rAd26 encoding SIVmac239 Gag in rhesus monkeys on the day of birth elicited detectable SIV-specific cellular immune responses, but these responses were transient and waned quickly. In contrast, an accelerated heterologous prime-boost regimen involving administration of rAd35 at birth and rAd26 at 4 weeks of life elicited potent and durable Gag-specific cellular and humoral immune responses in neonatal rhesus monkeys, including mucosal responses that remained detectable at one year of age.

Conclusion: These results suggest the potential of an accelerated heterologous rAd prime-boost regimen as a candidate neonatal HIV-1 vaccine in newborns.

OA08.08

Intra-dermal Immunisation with SIV Gag-Based Vaccines Alone Inhibits Acquisition of SIVmac251

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Background: It is increasingly recognised that effective anti-viral cell mediated immunity depends not only on the frequency of antigen specific T cells, but also on the quality of these T cells. We have evaluated a vaccine protocol involving DNA prime and Adenoviral vector boost to deliver SIV gag in MHC typed Mauritian derived Macaca fascicularis.

Methods: Groups of 8 macaques were immunised 3 times on weeks 0, 4, 8 with 100µg purified plasmid DNA designed to express SIVmac239 derived gag under the control of the CMV immediate early promoter. Three different SIV gag vaccines were compared. The DNA plasmids expressed native SIVmac239gag, ubiquinated SIVmac239 gag or fragmented, ubiquinated SIVmac239 gag derived peptides. On week 19, the groups were boosted with 10e7 infectious units recombinant Ad 5 expressing the same SIV Gag antigens. Cell mediated immunity was assessed after each immunisation and after virus challenge. At week 23, all vaccinated macaques, along with a group of naive challenge controls began 10 weekly, atraumatic challenges via the rectal mucosal with 150TCD_{50} SIVmac251.

Results: Delivery of this vaccine via the intra-dermal route elicited CD8 and CD4 T cell responses. Moreover, approximately 50% of antigen specific CD4+ T cells expressed the mucosal homing marker alpha4 beta7. When vaccinated macaques were exposed to a stock of uncloned SIVmac251 that had been propagated on simian PBMC's, by repeated low dose challenge via the intra-rectal route, a significant delay (Wilcoxon test; p=0.015) in acquisition of SIV was obtained amongst vaccinated macaques compared with naive controls challenged in a similar manner. Furthermore, peak viral loads amongst vaccinated macaques were significantly lower than challenge controls (Kruskal-Wallis test; p=0.010).

Conclusion: We are investigating whether the route of immunisation was crucial to the vaccine's success.

Oral Abstract Session 09: Clinical Trials

OA09.01

Safety and Immunogenicity of a Randomized Phase I Prime-Boost Trial with ALVAC-HIV (vCP205) and gp160 MN/LAI-2 Adjuvanted in Alum or Polyphosphazene

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Background: ALVAC-HIV prime/HIV-1 Env protein boost regimens have shown HIV-specific neutralizing antibody (NAb) and cell-mediated immune responses, but the impact of protein subunit schedule and adjuvant requires further definition.

Methods: A Phase 1 trial was conducted in two parts. In Part A, (open-label) 19 volunteers received oligomeric gp160 MN/LAI-2 (ogp160) with a dose escalation (25, 50, 100 µg). In Part B, 72 volunteers (60 active, 12 placebo) received placebo or recombinant canarypox expressing HIV-1 antigens, (ALVAC-HIV, vCP205) prime with different doses and schedules of ogp160MN/LAI-2 in alum or polyphosphazene (PCPP).

Results: The vaccines were safe and well tolerated with no vaccine-related serious adverse events. Cumulative chromium release CTL frequency was 37%, and 54% of volunteers showed proliferative responses to HIV antigens. Lymphoproliferative CD4+, HIV-specific responses were seen in 53% of ogp160 only and 57% of prime-boost recipients, respectively. Induced binding antibody to ogp160 was dose-dependent. NAb responses to vaccine homologous Tier 1 HIV-1 MN were seen in 99% of vaccine recipients. While NAb to the heterologous Tier 2 US-1 (R5, clade B) pseudovirus was negative in all volunteers tested using TZM-bl cells, in a PBMC-based assay, US-1 primary isolate Nab was induced in 2/19 (10.5%) recipients of ogp160 protein alone and in 5/30 (16.7%) prime-boost volunteers who received ogp160 in PCPP. Primary isolate neutralization was observed more frequently overall in recipients of ogp160 in PCPP, as compared with alum (p=0.027). Using an intracellular p24 flow-cytometry assay, sera from an ALVAC-HIV/ogp160 recipient demonstrated 94% neutralization of US-1.

Conclusion: A small percentage of vaccine recipients developed Nab to heterologous primary isolates, responses that to our knowledge have not been previously described. These results constitute proof of concept that Tier 2 NAb can be elicited by vaccination in humans, and underscore the importance of further optimization of prime-boost vaccination and adjuvanting strategies for HIV-1 prevention.

OA09.02

In Vivo Targeting of HIV Gag to Dendritic Cells in Combination with Poly ICLC Is Safe and Immunogenic in Healthy Volunteers

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Background: In vivo delivery of HIV antigens within α-DEC 205 antibodies to maturing dendritic cells (DCs) in combination with maturation stimuli is a potential new vaccine platform. This phase-I study evaluates the safety and immunogenicity of DEC-targeting of HIV gag p24 in combination with poly ICLC in healthy volunteers.

Methods: 45 volunteers aged 18-60 were enrolled. 9 volunteers per dosage group (low: 0.3mg; mid: 1.0mg; high: 3.0mg) received α-DEC205-HIVp24 mAb plus a fixed dose of poly ICLC s.c., 3 volunteers received poly ICLC only, and 3 volunteers received saline, in a randomized double-blinded dose escalation design. Volunteers were vaccinated at weeks 0, 4, 12 and followed for 12 months.

Results: Study remains blinded. Transient local and systemic reactogenicity occurred, without vaccine-related serious adverse events to date. Gag p24-specific IgG was induced in 9/15 (60%, 9 received vaccine plus adjuvant) volunteers in both low dose and mid dose groups at weeks 4, 8, 12, and 16. IgG titers were higher in the mid dose group and responses persisted for at least 6 months after last low dose immunization. Gag-specific CD4+ T cells were also detected following immunizations. IL-2 and TNF-α were the predominant cytokines. For CD4+ cells producing IL-2 or TNF-α, the response rates ranged from 33 to 46% (5-7/15 volunteers, 9 received vaccine plus adjuvant) and 8 to 40% (1-6/15 volunteers) post-vaccination in the low and mid dose groups respectively. Among positive responders, the median magnitude across visits ranged from 0.09% to 0.23% in the low dose group and from 0.06% to 0.17% in the mid dose group.

Conclusion: This novel DC-targeted protein HIV vaccine in combination with poly ICLC is safe and immunogenic in humans. Cellular and humoral immune responses are induced. Antibody responses are durable, with antibody titers unchanged at 6 months following last immunization.

OA09.03

First-in-Human Phase 1 Trial of the Safety and Immunogenicity of a Recombinant Adenovirus Serotype 5 HVR48 (rAd5HVR48) HIV-1 Vaccine

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Background: Adenovirus serotype 5 (Ad5) is a potent vector, but widespread seroprevalence may limit its potential use. Replacement of the hexon variable regions (HVR) of Ad5 with the HVR of the less prevalent Ad48 may result in a potent vector which bypasses pre-existing vector immunity.

Methods: Recombinant Ad5 with seven HVRs derived from Ad48 and expressing the VRC EnvA test antigen (rAd5HVR48. ENVA) was made. 48 healthy volunteers who were seronegative to Ad5, Ad48, HIV-1, and HIV-2 were enrolled in a randomized, double-blind, placebo-controlled, dose-escalation phase 1 study. The first three groups of 12 subjects received doses of 10^9, 10^{10}, or 10^{11} vp of rAd5HVR48.ENVA vector (n=10/group) or placebo (n=2/group) at weeks 0, 4, and 24 and the fourth group received a single injection of 10^{10} vp or placebo. We performed pre-specified blinded immunogenicity analyses at day 56 and day 196 after the first immunization.

Results: 31/48 (65%) of subjects were female; median age at enrollment was 24 (range: 18-50). Vaccination was generally well tolerated: mild to moderate local and systemic reactogenicity was observed after the initial immunization, more commonly in the highest dose group, but typically resolved within 24h. No vaccine-associated SAEs occurred. In all four dose groups, 10 subjects per group developed positive EnvA-specific ELISA titers and EnvA-specific interferon-gamma ELISPOT responses following vaccination. Immune responses were seen two weeks following inoculation in the majority of subjects. Two subjects per group exhibited no vector- or insert-specific immune responses at any timepoint and are presumed placebo recipients.

Conclusion: The rAd5HVR48 vector is generally safe and immunogenic in humans at all three doses. Immune responses against EnvA could be detected two weeks following the first inoculation. Ad5HVR48 is a promising new chimeric vector to evaluate novel inserts in further clinical trials.

OA09.04

Antibody-Mediated Inhibition of HIV-1 Elicited by HIV-I DNA Priming and Boosting with Heterologous HIV-1 Recombinant MVA in Healthy Tanzanian Adults

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Background: We evaluated HIV antibody (Ab) responses elicited by immunization, in a phase I/II placebo-controlled double blind trial using multiclade, multigene HIV-1-DNA prime boosted with HIV-MVA conducted among healthy volunteers in Tanzania (HIVIS03).

Methods: Sixty HIV-uninfected volunteers, randomized into groups of 20 received placebo or 1 mg HIV-DNA intradermally (id) or 3.8 mg intramuscularly (im). DNA plasmids containing HIV-1 gp160 subtypes A, B, C; rev B; p17/p24 gag A, B and RTmut B were given at months 0, 1 and 3 using a needle-free Biojector device. HIV-MVA expressing CRF01_AE HIV-1 env, gag, pol was administered im by needle at months 9 and 21. Sera were tested at baseline, two months post-first and four weeks post-second HIV-MVA boosting. HIV Ab responses were tested using pseudoviruses and TZM-bl cells as well as luciferase-expressing infectious molecular clones (IMC-LucR) in PBMC-based assays. ADCC responses were tested using the flow cytometry GranToxiLux-based assay.

Results: Neutralizing Ab activity was demonstrated only in the PBMC assay, and after the second MVA boost in 24 (83%) of 29 vaccinees against the clade CRF01_AE CM235 IMC and in 21 (72%) of 29 vaccinees against clade B SF162-IMC. NK cell depletion from PBMC targets resulted in a significant loss of HIV inhibition by vaccinee sera, indicating a role of Ab-mediated Fcγ-receptor function. Vaccine-induced ADCC responses were detected in 21 (75%) of 28 vaccinees after the second HIV-MVA boost. ADCC Ab titers did not differ significantly between id- (median 840, range 300-5400) and im-primed (median 880, range 400-3600) vaccinees (p=0.45).

Conclusion: HIV-DNA priming followed by two HIV-MVA boosts elicited HIV-specific inhibitory and/or ADCC-mediating antibody responses in a high proportion of Tanzanian adults.

OA09.05

A First-in-Man, Double Blind, Placebo Controlled Study of the Candidate Therapeutic Vaccine Opal-HIV-Gag(c) in HIV Infected Patients Receiving HAART

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Background: Preclinical studies of overlapping 15mer peptides spanning SIV, SHIV or HIV pulsed autologously ex vivo have demonstrated high level, virus-specific T cells responses and viral load suppression in Macaca nemestrina. The objective of this study was to evaluate the safety and preliminary immunogenicity of Clade C consensus peptides administered ex vivo to HIV positive adults.

Methods: Synthetic 15mer peptides (n=123, Opal-HIV-Gag(c)) spanning Clade C, consensus Gag were manufactured to current good manufacturing practice and evaluated in a good laboratory practice toxicology study in Macaca mulatta. A first-in-human, single centre, placebo-controlled, double-blind, dose escalation study was conducted. Twenty three people with well controlled HIV (CD4+ > 350cells/mm3 and a HIV < 400 copies/mL), stratified by clade, were enrolled in four groups: 12mg (n=6), 24mg (n=7), 48mg (n=2) or matching placebo (n=8). Treatment was administered intravenously bedside (closed system) by enrichment of 120mL of whole blood for WBCs using a Sepax S-100 device, ex vivo mixing the peptides (or diluent alone) and incubation at 37°C for one hour prior to reinfusion. Subjects received 4 administrations at 4 weekly intervals followed by a 12 week post-treatment follow up. Immunogencity was assessed by ELIspot.

Results: Opal-HIV-Gag(c) was generally well tolerated at doses of 12 and 24mg. There was an increased incidence of temporally associated pyrexia, chills, rigor, and transient/self-limiting lymphopenia in Opal-HIV-Gag(c) recipients compared to placebo. Only 2 subjects were recruited to the 48mg cohort. A serious adverse event of anuria, hypotension and tachycardia secondary to diarrhoea occurred following a single dose of vaccine at 48mg. No difference in ex vivo IFN-γ ELISpot response was observed at any time.

Conclusion: An infectious cause for the event could not be identified, leaving the possibility of immunologically-mediated reaction to the vaccine thus leading to early termination of the study.

OA09.06 LB

Multiple Antibody Specificities (gp41, V1V2, and V3) Elicited in the Phase II Multiclade (A, B, C) HIV-1 DNA Prime, rAd5 Boost Vaccine Trial

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Background: The phase II DNA prime, rAd5 boost vaccine (HVTN 204) exhibited sufficient safety and immunogenicity to advance into a phase IIb efficacy trial (HVTN 505) in Ad5 seronegative volunteers in the US. In the RV144 ALVAC prime, A/E gp120 protein boost trial, levels of V1V2 IgG antibodies significantly correlated with decreased risk of infection.

Methods: Sera from 203 vaccinees receiving VRC-HIVDNA016-00-VP DNA (m 0,1,2), and VRC-HIVADV014-00-VP rAd5 (m 6), at two weeks post rAd5 boost, and 208 placebos were examined for binding gp140 and gp120 recombinant proteins, gp41, and an antigen panel of 16 V1V2 IgG scaffolds representing clades A, B and C V1V2 sequences. A subset of vaccinees and placebo was screened for binding CD4BS antigens. Monoclonal antibodies were generated from antigen specific memory B cell sorts and tested for binding, neutralization, ADCC and virion capture.

Results: Clade A and B V1V2 IgG antibodies were elicited in 38.4% and 19.2% of vaccinees, respectively. A clonal lineage of 3 gp41 mAbs (CH69, CH70, CH71), and a V3-specific gp120 mAb (CH73) were generated from vaccinees. The gp41 mAbs captured infectious HIV-1 transmitted/founder viruses, while CH73 mediated ADCC activity against subtypes B and C infected cells, neutralized subtypes B and C tier 1A viruses, and bound multiple Envs of subtypes A, B and C.

Conclusion: The phase II multi-clade DNA prime, rAd5 boost vaccine regimen elicited antibody responses to multiple epitope specificities, including V1V2, V3, and gp41. CH69-CH71 and CH73 mAbs represent the initial human mAbs from HVTN 204 vaccine recipients, and reflect the functional profile of vaccine-elicited antibodies. These data suggest that the HVTN 505 Phase IIb efficacy study using this same vaccine regimen may provide an opportunity to examine a diversity of antibody specificities that have been hypothesized as a correlate of HIV-1 infection risk.

OA9.07 LB

rAd5 prime/NYVAC-B Boost Regimen is Superior to NYVAC-B prime/rAd5 Boost Regimen for Both Response Rates and Magnitude of CD4 and CD8 T-Cell Responses

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Background: HVTN 078 is a randomized, double blind phase 1b clinical trial to evaluate the safety and immunogenicity of heterologous prime/boost vaccine regimens (NYVAC-B/rAd5 vs. rAd5/NYVAC-B) in healthy, HIV-1 uninfected, Ad5 seronegative adult participants.

Methods: The rAd5 vaccine expressed clade B Gag-Pol and the gp140 of HIV-1 92RW020 (clade A), HxB2/Bal-V3/ΔV1V2 (clade B) and 97ZA012 (clade C). The NYVAC-B vaccine expressed clade B Gag-Pol-Nef and the gp120 of Bx08 (clade B). 80 healthy, HIV-1 uninfected, Ad5 seronegative volunteers, aged 18 to 45 years, were randomized to the placebo arm (n=5) or one of 4 treatment (T) arms: T1 (n=30), 2xNYVAC-B/1xrAd5 (10E10); T2 (n=15), 1xrAd5 (10E8)/2xNYVAC-B; T3 (n=15), 1xrAd5 (10E9)/2xNYVAC-B; T4 (n=15), 1xrAd5 (10E10)/2xNYVAC-B.

Intracellular cytokine staining responses (percent of CD4+ and CD8+ T cells producing IFN-γ and/or IL-2 in response to stimulation with global PTE peptides) were assessed two weeks after the final vaccination.

Results: For CD4+ T cells, the overall response rates for IFN-γ and/or IL-2 among the vaccinees were 42.9%, 93.3%, 92.3%, and 85.7% for T1-T4, respectively; and the median response magnitudes for positive responders were 0.26%, 0.76%, 0.40%, and 0.76% for T1- T4, respectively. Both response rates ($p<0.01$) and magnitudes ($p<0.03$) of CD4+ T-cell responses were significantly lower in T1 compared to the other three treatment groups. For CD8+ T cells, the overall response rates were 65.5%, 73.3%, 76.9% and 85.7% for T1-T4, respectively; and median response magnitudes for positive responders were 0.32%, 0.99%, 1.86%, and 1.65%, respectively. Response rates were not significantly different between groups; however, response magnitudes were significantly lower in T1 compared to the other three arms ($p<0.04$).

Conclusion: Priming with rAd5 followed by NYVAC-B boost is superior to priming with NYVAC-B followed by rAd5 boost for both response rates and the magnitude of CD4+ and CD8+ T-cell responses.

OA09.08 LB

Phase 2a Safety and Immunogenicity Testing of DNA and Recombinant Modified Vaccinia Ankara Virus Vaccines Expressing Virus-Like Particles

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Background: The Phase 2a HVTN 205 trial was undertaken to further compare full-dose regimens of DNA priming with MVA boosting and MVA priming and boosting.

Methods: 150 vaccinia-naïve participants were inoculated i.m. via needle and syringe with 3 mg of pGA2/JS7 DNA at months 0 and 2, and 1x108 TCID50 of MVA/HIV62B at months 4 and 6 (DDMM regimen). 75 participants received 1x108 TCID50 of MVA/HIV62B at months 0, 2, and 6 (MMM regimen) and 75 received placebo. While the safety data are still blinded, the vaccine regimens appeared safe and well tolerated. Immune studies were performed at 2 weeks following the final vaccination.

Results: Similar to Phase 1 testing, the DDMM regimen induced higher rates of T cell responses whereas the MMM regimen induced higher rates of antibody responses. CD4 T cell responses were elicited in 65% of the DDMM and 43% of the MMM recipients ($p=0.01$) whereas CD8 T cells were induced in 22% and 16%, respectively. The majority of T cells were directed against Gag with fewer against Env and only occasional responses to Pol. gp120 IgG antibodies were demonstrated in 45% and 68% of the DDMM and MMM recipients, respectively ($p=0.001$). gp41 IgG antibodies were seen in over 90% of both groups. The magnitudes of serum IgG responses exceeded the magnitudes of serum IgA responses by >10 fold with higher IgG to IgA responses being present in the MMM group ($p=0.03$). The antibody avidity index to the gp41 immunodominant epitope, a preclinical correlate of protection against infection demonstrated levels of affinity maturation comparable to preclinical studies. Sporadic weak neutralizing activity against Tier 1 and Tier 2 viruses was seen in both groups and was greater for MVA alone.

Conclusion: The vaccine safety data and immune responses seen here are supportive of further testing.

Oral Abstract Session 10: HIV Transmission / Diversity

OA10.01

Impact of Transmitted CTL Escape Mutations on Replicative Capacity and HIV Pathogenesis in Early Infection

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Background: Multiple HLA class I alleles have been shown to influence both HIV-1 transmission and viral load. In transmission pairs, viral loads of acutely infected partners correlate with viral loads (VL) of their chronically infected donors. This correlation becomes highly significant after adjustment for host factors known to modulate viral load. In addition, we have previously demonstrated that transmission of a virus containing multiple HLA-I associated polymorphisms resulted in a lower set point VL in Zambian linked recipients. These studies imply that transmitted viral characteristics play a role in defining early HIV-1 pathogenesis, and it will be important for vaccine development to understand which viral characteristics are responsible for this.

Methods: We investigated the role that the in vitro replicative capacity (RC) of the transmitted Gag plays in defining HIV-1 clinical parameters, by cloning gag genes from the founder virus in newly infected partners of 149 epidemiologically linked transmission pairs into the subtype C, R5 tropic MJ4 provirus.

Results: We observed a statistically significant positive correlation between the RC of Gag-MJ4 chimeras and set point VL in seroconverters (P=0.013). The RC of the transmitted Gag also correlated (P=0.025) to the viral load in the chronically infected donor, pointing to RC as the major viral characteristic responsible for the link between donor and linked recipient viral loads. The long term clinical benefit associated with the transmission of attenuated viruses was investigated by performing a Kaplan Meier analysis of time to CD4+ count less than 350. Individuals infected with attenuated gag sequences (RC< 1) were delayed in their progression to CD4+ counts < 350 compared to high (RC >2) replicating viruses (P = 0.034).

Conclusion: Interestingly, this phenomenon seemed to be independent of viral load perhaps highlighting the role that early viral replication may play in defining HIV-1 pathogenesis.

OA10.02

A Deeper View of Transmitted/Founder Viruses Using 454 Whole Genome Deep Sequencing

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Background: Understanding the composition of transmitted/founder viruses that represent the primary target for vaccine-elicited responses is highly relevant to the design of an effective HIV-1 vaccine. The identification of transmitted and early founder viruses in primary infection has remained remarkably opaque, but recent advances in sequencing technologies have enabled a more comprehensive and sensitive assessment of the properties of transmitted/founder viruses.

Methods: We applied full-length HIV-1 genome 454 deep sequencing of plasma virus to 22 clade B subjects from a cohort of men who have sex with men (MSM) identified during the earliest phase of acute HIV-1 infection (Fiebig stage II to III). Using novel assembly and variant detection algorithms coupled with a mathematical model we were able to comprehensively evaluate the viral diversity of transmitted/founder viruses in these individuals.

Results: Using intra-host codon diversity frequencies, coupled with a mathematical model of random virus evolution during acute HIV-1 infection, we were able to unambiguously identify that 30% of subjects exhibited convincing evidence for multiple transmitted/founder viruses in line with prior reports on MSM transmission. Surprisingly, deep sequencing outside of the Env region identified additional low frequency variants possibly reflective of evidence of multiple transmitted/founder viruses in an additional 20% of subjects.

Conclusion: Our study reveals that, in line with prior reports, approximately 30% of MSM subjects exhibit multiple transmitted/founder viruses, with deep sequencing possibly identifying additional cases of multiple founder viruses which will require further validation. In summary, this data highlights the potential that whole deep sequencing has to uncover additional footprints originating from multiple transmitted/founder viruses. These findings, coupled with the knowledge that dual infections are associated with accelerated disease progression, demonstrate that the higher risk of virus acquisition in MSM could be a greater barrier for vaccine control which may have to contend with multiple transmitted variants.

OA10.03

Modeling Virus Exposure During Male to Female Transmission of HIV-1

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Background: Previously, we illustrated that HIV is able to penetrate the intact female genital mucosal barrier. We found that HIV penetrates to depths where target cells reside. Using data obtained from tissues of more than 40 donors, we are able to generate an average for the ability of HIV to penetrate the mucosal. Using these averages we can extrapolate the number of HIV particles penetrating the mucosa per coital act for male to female HIV transmission for both acute and chronic infections.

Methods: Human cervical explants and macaque genital tracts were exposed to photoactivatable (PA-GFP) HIV. Rhesus macaques were inoculated intravaginally and genital tissues were removed 4 hours post-inoculation and dissected into relevant tissue specimens. Samples were snap frozen, sectioned, stained accordingly, and imaged for virus penetration.

Results: We observed HIV penetration of the stratified squamous epithelium per image field to be 1.21 penetrating virions/scan when applied at 500ng/ml p24. Virus entered the tissue by diffusion/adsorption as revealed by fluid phase markers. Using these data, coupled with published determinations of average vaginal surface area and semen viral loads, we estimate that up to 14,465 virions penetrate the mucosal barrier during transmission during acute phase infection and approximately 18 virions during HIV chronic infection. We estimate that approximately 1-2 cm2 of the endocervix is exposed without a thick mucus barrier allowing 1-2 virions to penetrate.

Conclusion: This model is consistent with known characteristics of transmission. Having a very low number of virions with the ability to initiate transmission is consistent with the low transmission rate seen under conditions of chronic infection in monogamous heterosexual couples. Data is also consistent with the knowledge that typically, only 1 virion establishes systemic infection after male to female HIV transmission. These results provide a biological description of the functional virus dose of HIV during transmission.

OA10.04

Beneficial HLA-Mediated Viral Polymorphisms on the Transmitted Virus Additively Influence Disease Progression in HIV-1, Subtype C Infection

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Background: Transmitted viral factors have been shown to affect disease progression but whether infection with viruses carrying beneficial HLA-mediated escape polymorphisms affects disease progression in HLA-mismatched participants remains controversial.

Methods: Gag was sequenced from 56 participants with acute HIV infection from the CAPRISA 002 cohort. For the newly identified mutation, replication fitness of viruses in PBMCs was compared in a subtype C infectious molecular clone. Disease progression was compared in participants infected with viruses carrying polymorphisms associated with beneficial HLA- B*57/58:01 and B7 supertype (B*39:10/81:01) allelic selective pressure in HLA mismatched participants.

Results: In HLA-B*57/58:01-negative participants, 51% (25/49) were infected with viruses carrying at least one of the B*57/58:01 fitness cost-associated escape mutations in ISW9 (A146P:19/49), KF11 (A163G: 10/49) or TW10 (T242N/S: 11/49) epitope. In HLA-B7 negative participants, only 9.4% (3/32) had viruses carrying mutations in the HLA-B7 immunodominant TL9 epitope. Furthermore, we identified in viruses from HLA-B7 participants and included in this analysis a novel mutation in Gag p17, Q65H; found in 9.4% (3/32) of B7 negative participants, and with mutant viruses replicating 11% slower than wild-type in tissue culture. Unlike a previous study in this cohort, infection with viruses carrying mutations in HLA-B*57/58:01 restricted epitopes alone did not impact on viral load setpoint in HLA-mismatched participants, although there was a transient trend towards higher CD4 counts at 3 months post infection (p=0.0551). However, HLA-mismatched participants infected with viruses carrying 3 or more HLA footprints associated with fitness cost in Gag had significantly lower viral load and higher CD4 counts at 3 and 12 months post-infection (p=0.028 and p=0.0264, and p=0.0025 and 0.0689

Conclusion: These results suggest that multiple mutations generated when viruses are passaged through individuals with beneficial HLAs are needed to attenuate the virus, supporting vaccination methodologies that aim to render the virus less fit.

OA10.05 LB

T-Cell Based Sieve Analysis Ties HLA A*02 to Vaccine Efficacy and IgA-C1 Immune Correlate in RV144 Thai Trial

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Background: The RV144 trial showed an estimated 31% vaccine efficacy (VE) against HIV-1 infection. Two immunological correlates of risk were found in vaccine recipients: Envelope V1/V2 antibody titers and IgA binding to Envelope (Haynes et al., 2012).

Methods: We conducted a $CD8^+$ T-cell based sieve analysis of the V1/V2 region testing for differential escape from vaccine induced epitopes as predicted using computational methods. Breakthrough peptides that differed from predicted epitopes in the vaccine insert and had reduced HLA binding affinity were considered escapes.

Results: Three of twelve epitopes in V1/V2 of the MN protein boost showed evidence of escape (start-positions 147, 163, 168 restricted by A*11 and A*02), with more escapes in vaccine recipients (p=0.018). We hypothesized that if escape was indicative of anamnestic responses, recipient's HLA alleles should not modify VE. While VE was not different in A*11(+/-) subgroups (p=0.45), it was higher in A*02(+) versus A*02(-) participants (VE=54% vs. 3%, interaction p-value=0.05). Previous analysis showed that HIV variants matching the vaccine insert at site 169 (K169) (in V2, implicated in antibody binding) were preferentially excluded from infections in vaccine recipients (VE against K169=48%, p=0.0036). We found significant VE against K169 in only the A*02(+) subgroup (74%, p=0.001; p-value for difference A*02(+/-)=0.01). Reanalyzing the immune correlates within A*02(+/-) subgroups, we found a direct correlation between IgA-C1 titers and infection rate in A*02(-) participants (OR=2.07, p=0.0002), but not in the A*02(+) participants (OR=1.12, p=0.71; A*02(+/-) interaction p-value=0.05).

Conclusion: Our exploratory analysis, driven by a T-cell based sieve effect in envelope V1/V2, revealed an association between an HLA class I allele and VE, suggesting that VE was restricted to A*02(+) participants and that IgA-C1 antibodies inhibited protective effects of other responses in A*02(-) participants. This highlights the importance of considering the effects of host genetics on VE in future HIV vaccine trials.

Oral Abstract Session 11: Vaccine Immunogens / Delivery

OA11.01

HIV-1 Envelope Trimer Elicits Higher Neutralizing Antibody Responses Than Monomeric gp120

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Background: HIV-1 envelope glycoprotein is the primary target for HIV-1-specific antibodies. The native HIV-1 envelope spike on the virion surface is a trimer, but trimeric gp140 and monomeric gp120 are currently believed to induce comparable immune responses. Indeed, most studies on the immunogenicity of HIV-1 envelope oligomers have revealed only marginal improvement over monomers. We report here that stable and homogenous envelope trimers with characteristics expected for the native viral spikes are substantially superior at eliciting neutralizing antibodies in guinea pigs.

Methods: Stable envelope gp140 trimer derived from clinical isolate sequences were stabilized with the T4-fibritin C-terminal trimerization tag and produced in stablely transfected 293T cells. Characterization of Env trimers was performed by Western blotting, size-exclusion chromatography (SEC), analytical-ultra centrifugation (AUC), multi-angle light scattering (MALS) and surface plasmon resonance (SPR). Guinea pigs were immunized six times with 100 µg of protein trimer or monomer in CpG/Emulsigen adjuvants. Antibody responses were determined by ELISA and TZM.bl neutralizing antibody assays.

Results: Homogeneous trimer and monomer preparations exhibited high purity as measured by SEC and SDS-PAGE. AUC and MALS analyses revealed expected molecular weight for both trimer and monomer. SPR analyses revealed expected binding with CD4 and multiple broadly neutralizing antibodies. These trimers have markedly different antigenic properties than those of monomeric gp120s derived from the same sequences. They induce potent, cross-clade neutralizing antibody responses with titers substantially higher than those elicited by the corresponding gp120 monomers for a diverse set of both tier 1 and tier 2 viruses.

Conclusion: We have demonstrated the importance of generating high-quality envelope trimers for antigenic and immunogenic studies; furthermore these results highlight the immunologic differences between monomers and high-quality envelope trimers, illustrating important implications for HIV-1 vaccine development and immunogen selection in large clinical trials.

OA11.02

Shaping Humoral Responses Against Mini-libraries of HIV Env Antigens via Lipid Nanoparticle Vaccine Delivery

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Background: Humoral immune responses elicited by an HIV vaccine would ideally be comprised of durable high titers of broadly neutralizing antibodies. Importantly, recent studies of broadly neutralizing antibodies isolated from infected patients have suggested that high degrees of somatic hypermutation (SHM) are a common feature of antibodies with high potency and good breadth. Thus, a successful vaccine will likely require both immunogens capable of focusing the humoral response against conserved neutralizing epitopes and appropriate adjuvants/delivery systems capable of promoting elevated SHM and lasting responses against these epitopes.

Methods: We generated a small library of gp120 mutants engineered to have diverse surface compositions but a conserved CD4 binding pocket recognized by the broadly neutralizing antibody VRC01. These gp120 mutants were linked to "stealth" liposomes via 2KDa PEG linkers. These lipid nanoparticles were simultaneously loaded with immunostimulatory adjuvant molecules such as MPLA (TLR4 agonist) or CpG (TLR9 agonist) to support differentiation of helper T-cells and promote avidity maturation of the antibody response. Mice were immunized repeatedly with stealth liposomes carrying unique gp120 mutants in each boost.

Results: Stealth liposomes carrying TLR4 agonists (TLRa) promoted long-lived humoral responses against env antigens superior to traditional adjuvants such as alum, montanide, or soluble protein mixed with TLRa. Studies of liposome/antigen trafficking in vivo suggest these enhanced responses reflect efficient trafficking of these nanoparticle vectors to lymph nodes. Notably, this heterologous immunization strategy elicited anti-gp120 sera focused almost exclusively on the CD4 binding site and that competed with VRC01 for binding to gp120.

Conclusion: This approach of combined immunogen design with effective multivalent, nanoparticle-based antigen delivery may provide a strategy to promote strong and long-lived neutralizing antibody responses against HIV.

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Oral Abstract Session 11: Vaccine Immunogens / Delivery

OA11.03

Vaccine Responses to Conserved Regions of the HIV-1 Proteome Are Associated with an Increased Capacity to Inhibit Multiple Virus Isolates Ex Vivo

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Background: The majority of assays currently used to assess HIV-1 vaccine candidate immunogenicity in humans fail to predict protection against HIV-1 acquisition or control of viraemia. However, a correlation between in vivo and ex vivo control mediated by CD8+ T-cell populations has been described using an ex vivo virus inhibition assay (VIA) in chronically infected individuals and vaccinated non-human primates. Here we attempt to relate the specificity of vaccine-induced virus-specific CD8 responses to the inhibition of HIV-1 ex vivo.

Methods: Using peptide epitope mapping, we assessed the breadth and specificity of CD8 T-cell responses induced by vaccination using two adenovirus serotype 35 (Ad35) vectors containing gag, reverse transcriptase, integrase and nef (Ad35-GRIN) and env (Ad35-ENV), respectively, derived from HIV-1 subtype A isolates. The conserved regions targeted by these 25 subjects were related to the capacity of vaccine-induced CD8 T-cells to inhibit replication of a cross-clade panel of HIV-1 isolates using the VIA.

Results: A median of 4 peptides were recognised in vaccinated individuals (range 1-9). When related to the log reduction of p24 production as measured in the VIA, mapping data suggest that targeting immunodominant responses towards highly conserved regions of the HIV-1 proteome tended towards an increased ability to inhibit multiple clades of HIV-1 ex vivo.

Conclusion: These data support the plausibility of inducing conserved CD8+ T cell responses using a consensus HIV-1 subtype A sequence in an adenovirus-based vector.

OA11.04

Antigen-Specific T Lymphocyte Responses Elicited by a DNA – MVA HIV CN54gp140 Immunization Regime Are Significantly Altered by the TLR4 Adjuvant GLA

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Background: We assessed the antigen-specific CD4 and CD8 T lymphocyte responses elicited by a unique vaccine antigen matched and single Clade C DNA-poxvirus-protein regimen in mice and determined the immunomodulatory effect of the novel micellar formulation of a synthetic TLR4 ligand, GLA-AF (GLA) administered either sequentially or simultaneously with the MVA and protein.

Methods: Groups of 10 BALB/c mice were primed with plasmid DNA encoding CN54 env/gag-pol-nef and then boosted with MVA-C (env-gag-pol-nef) and CN54gp140 protein with or without GLA-AF. The MVA and protein were either given sequentially at 3 weekly intervals or simultaneously in different legs at 3 and 6 weeks. Cellular responses were assessed at necropsy three weeks after the final immunization. Splenocytes were harvested and analysed for antigen-specific T cell responses using peptide pools spanning the Env and Gag proteins by intracellular cytokine staining and CFSE labelling. Multiparametric flow profiles were analysed using FlowJo and data sets were organized and charted using the SPICE software.

Results: GLA adjuvanted CN54gp140 substantially influenced the antigen-specific T lymphocyte cytokine expression profiles and proliferative responses in animals primed with DNA and boosted by MVA or those immunized with MVA alone. We observed adjuvant-induced changes in the polyfunctional IFN-γ, TNF-α and IL-2 expression profiles of both the CD4 and CD8 T lymphocyte populations and differential responses to separate peptide pools.

Conclusion: We have shown that the GLA adjuvant alters both the degree and the nature of the antigen-specific T lymphocyte responses and that these immunomodulatory changes are critically dependent upon the timing of application. These effects are likely due to either the presence of systemic GLA providing a general immunostimulatory environment or enhanced T cell priming in the local draining lymph nodes. This ability to tailor CN54gp140-specific T cell immunity is a valuable tool to be exploited in vaccine design.

OA11.05 LB

Quality of T-Cell Responses Versus Reduction In Viral Load: Results From An Exploratory Phase II Clinical Study Of Vacc-4x, A Therapeutic HIV Vaccine

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Background: Immunization with Vacc-4x, a peptide-based therapeutic vaccine for HIV-1, has shown a statistically significant reduction in viral load set point compared to placebo during treatment interruption in an exploratory phase II clinical study enrolling 135 subjects (NCT00659789). This vaccine aims to induce sustained cell-mediated immune responses to conserved domains on HIV p24.

Methods: After 6 immunizations on ART over 28 weeks, treatment was interrupted for up to 24 weeks (Vacc-4x n=88; placebo n=38). Immunological analyses (ELISPOT, proliferation, intracellular cytokine staining (ICS)) to HIV p24 were carried out at central laboratories. The HLA class I profile (Vacc-4x n=73, placebo n=32) was also determined.

Results: For subjects that remained off ART until week 52 (Vacc-4x n=56, placebo n=25), there was a log 0.44 reduction in viral load set point between the Vacc-4x and placebo groups (p=0.0397). There was a similar distribution of HLA class I alleles in the two treatment arms, with the exception of the B35 allele (27% of Vacc-4x subjects versus 8% placebo subjects). The viral load of ELISPOT positive Vacc-4x subjects was significantly lower than that of placebo subjects (p=0.023). There was no significant difference in T-cell proliferation responses between Vacc-4x and placebo groups, however, the percentage of subjects showing proliferative CD4 and CD8 T-cell responses to Vacc-4x peptides increased over time only for the Vacc-4x group. ICS analysis showed a predominance of CD8-mediated T-cell responses to p24 that were significantly increased from baseline for the Vacc-4x group ($p<0.043$) but not for the placebo group($p>0.05$). There was also a trend towards higher numbers of polyfunctional T-cells in the Vacc-4x group compared to the placebo group (p=0.188).

Conclusion: These findings suggest Vacc-4x immunization can influence the quality of immune responses to HIV-1 p24 irrespective of HLA status, and contribute to a reduction in viral load.

Oral Abstract Session 12: Vaccine Concepts – Vectors and Inserts

OA12.01

DNA Vaccines Expressing Conserved Elements Provide Potent and Broad Immune Responses

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Background: Immunodominance and sequence diversity are major hurdles in the development of effective HIV vaccines. We tested the hypothesis that a vaccine candidate composed of strictly Conserved Elements (CE) of the HIV proteome excluding the variable regions would help overcome problems of viral sequence diversity and potential negative effects of immunodominance. Seven CE were identified in $p24^{gag}$. Vaccination of macaques with $p55^{gag}$DNA failed to elicit cellular or humoral immune responses to the CE, while epitopes outside of the CE were immunogenic.

Methods: Two HIV $p24^{gag}$CE DNA plasmids were generated, providing potential epitopes found in >99% of all HIV-1 M group sequences. DNA vectors, optimized for gene expression were used to immunize mice and macaques by IM injection followed by in vivo electroporation.

Results: Vaccination with $p24^{gag}$CEvac DNAs elicited potent, cross-clade cellular and humoral immune responses. Highly cytotoxic CE-specific T cells, capable of Granzyme B production and degranulation, were generated. Importantly, boosting of the CEvac-primed macaques with $p55^{gag}$DNA greatly augmented the CE-specific cellular responses (up to 10-fold) as well as humoral responses, despite the failure of $p55^{gag}$DNA vaccine to induce de novo CE-specific responses. CEvac DNA prime-$p55^{gag}$DNA boost in mice led to similar conclusions. Interestingly, mapping analysis showed differential increase of the CE-specific responses by the $p55^{gag}$DNA boost, demonstrating changed hierarchy of CE responses in macaques.

Conclusion: Vaccination with the $p24^{gag}$CEvac DNA overcame the problem of diversity by generating strong cross-clade Gag-specific immune responses, and of immunodominance, eliciting responses to subdominant but highly conserved elements, and also by broadening the $p55^{gag}$DNA induced immunity. $p55^{gag}$DNA did not induce de novo responses to the CE, but was able to significantly boost pre-existing CE-induced responses and alter the hierarchy of these responses. Translation of this concept into clinical trials may elicit cross-clade cellular immune responses against components of the viral proteome with limited capacity for immunological escape.

OA12.02

Reinventing the Nucleic Acid Vaccine with Self-Amplifying RNA

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Background: Self-amplifying RNAs (replicons) of positive-strand viruses such as alphaviruses are potentially safe and useful vectors for delivering vaccine antigens. We previously showed that recombinant alphavirus replicon particles (VRP), used in prime-boost regimen with Env in MF59 protein protected rhesus macaques against mucosal challenge with SHIVSF162P4 (J. Virol. 84:5975, 2010).

Novartis recently developed a synthetic self-amplifying mRNA (SAM™) vaccine platform, using cell-free RNA production and non-viral vaccine delivery systems. The SAM™ platform avoids the limitations of cell culture production that complicate production of the alphavirus VRPs and other viral vector systems. Safety concerns associated with the potential generation of replication competent virus (RCV) are eliminated, and the absence of viral structural proteins reduces issues associated with anti-vector immunity, a major limitation of other vectored vaccine systems.

Methods: HIV- SAM™ vaccines were evaluated in small animals and nonhuman primates (NHP).

Results: Here we show that SAM™ vaccines encoding HIV antigens induced potent systemic and mucosal immune responses in small animals and nonhuman primates (NHP). Humoral and cellular responses elicited in mice with HIV-SAM™ vaccines were superior to naked DNA or RNA and comparable to those seen with alphavirus replicon particles (VRPs). Robust binding and neutralizing antibodies were seen following two immunizations with a HIV-SAM™ vaccine encoding subtype C TV1 gp140 in rabbits. The same vaccine elicited both IFNγ and IL2 T-cell responses, B-cell ELISpots, and Env-specific antibody responses in rhesus macaques, also after only two immunizations. Importantly, the vaccines were well-tolerated with no local or systemic adverse events observed.

Conclusion: These results provide the first evidence in a primate species that vaccination with formulated self-amplifying RNA is safe and immunogenic, eliciting robust immune responses.

The safety, immunogenicity, and ease of production provided by SAM™ vaccines provide a rationale for accelerated evaluations of this platform in the context of HIV vaccines.

OA12.03

Adenovirus Serotype 26 Utilizes CD46 as Primary Cellular Receptor and Only Transiently Activates T Lymphocytes Following Vaccination of Rhesus Monkeys

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Background: Adenovirus serotype 5 (Ad5) utilizes coxsackievirus and adenovirus receptor (CAR) as its primary cellular receptor. However, the cellular receptor utilized by Ad26 and the inflammatory responses elicited following Ad26 vaccination remain unclear.

Methods: Receptor usage was assessed using CD46 transgenic mouse cells, as well as by CAR- and CD46-specific mAb blocking studies using human PBMC. Twelve adult rhesus monkeys were inoculated with of 10^{11} viral particles (vp) of replication-competent Ad5 and Ad26 (N=6) or saline (N=6) at weeks -8 and -4, and were vaccinated intramuscularly with 3×10^{10} vp replication-incompetent Ad26-Gag/Pol/Env vectors. At week 2, monkeys were sacrificed to assess immunologic and inflammatory responses at mucosal surfaces.

Results: Transduction by Ad26 and Ad35 vectors was markedly enhanced in CD46 transgenic mouse cells compared with wild type mouse cells. Moreover, transduction of human PBMC by Ad26 and Ad35 vectors was efficiently blocked by the anti-CD46 mAbs 13/42, M177 and MEM-258, but not by the anti-CAR mAbs RmcB and E1-1. Monkeys with and without baseline Ad5/Ad26 immunity exhibited similar magnitude and only transient activation (1-2 weeks) of vector-specific $CD4^+$ T cell responses in both PBMC and colorectal biopsies. Inflammatory cell infiltrates in colorectal and foreskin mucosa were comparable in baseline and vaccinated animals regardless of baseline Ad5/Ad26 immunity.

Conclusion: Ad26 utilizes CD46 and not CAR as a primary cellular receptor for infection. We also observed no increased mucosal cellular activation or vector-specific $CD4^+$ T lymphocytes in baseline Ad5/Ad26-seropositive monkeys as compared with baseline seronegative monkeys following Ad26 vaccination. These data contribute to our understanding of the biology of Ad26 as a candidate vaccine vector.

OA12.04

Full-Length HIV-1 Immunogens Induce Greater T Lymphocyte Responses to Conserved Epitopes Than Conserved-Region-Only HIV-1 Immunogens in Monkeys

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Background: A global HIV-1 vaccine will need to induce broadly reactive immune responses against conserved HIV-1 regions. It is currently unclear how best to elicit these responses by vaccination. We therefore compared the immunogenicity of a bivalent full-length HIV-1 Gag/Pol/Env mosaic vaccine, a trivalent full-length HIV-1 Gag/Pol/Env mosaic vaccine, and a bivalent mosaic vaccine containing only conserved HIV-1 Gag/Pol/Env epitopes in rhesus monkeys.

Methods: We immunized 18 rhesus monkeys with rAd35 (prime) and rAd26 (boost) vectors expressing bivalent full-length (N=6), trivalent full-length (N=6), or bivalent conserved-region-only (N=6) HIV-1 Gag/Pol/Env mosaic immunogens. We assessed HIV-1-specific and conserved-region-specific cellular immune responses by ELISPOT using global PTE and vaccine-matched peptides. Responses were mapped to individual epitopes and were identified as CD4+ or CD8+ through cell-depletion assays. Comparisons were performed by Wilcoxon rank-sum tests.

Results: There was no difference in the breadth of HIV-1-specific T lymphocyte responses elicited by the bivalent and trivalent full-length mosaic vaccines (P=.686). However, the bivalent full-length vaccine generated a greater breadth of HIV-1-specific CD8+ T lymphocyte responses than the conserved-region-only vaccine (P=.007). The bivalent full-length vaccine also generated equivalent breadth of CD8+ T lymphocyte responses to conserved HIV-1 epitopes compared to the conserved-region-only vaccine (P=1.000), and surprisingly, the responses generated by the full-length vaccine to conserved HIV-1 epitopes were greater in magnitude than those generated by the conserved-region-only vaccine (P=.008).

Conclusion: These data demonstrate that an HIV-1 mosaic vaccine expressing full-length antigens elicited greater responses to conserved epitopes than a mosaic vaccine expressing only concatenated conserved HIV-1 regions. In addition, the bivalent and trivalent full-length mosaic vaccines generated comparable breadth of HIV-1-specific CD8+ T lymphocyte responses. These results support the clinical development of the bivalent full-length HIV-1 mosaic vaccine.

OA12.05 LB

Rational Immunogen Design to Target Specific Germline B Cell Receptors

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Background: VRC01 and a number of other broad and potently neutralizing CD4 binding site antibodies have been isolated from HIV positive individuals. These antibodies utilize VH1-2 and make the majority of their contacts via the framework portion of the heavy chain. Recently, it has been noted that the germline precursors to these VRC01-like antibodies do not bind to HIV Env nor does Env stimulate B cell lines expressing these germline precursors. This lack of interaction between germline antibodies and Env may represent a significant block for re-elicitation of these antibodies.

Methods: We engineered a modified Env to have affinity for the VH1-2 germline antibodies. We believe this antigen will selectively activate B cells that have the potential to elicit VRC01-like antibodies.

Homology modeling and computational protein interface design was used to predict mutations to modify GP120 to have affinity for the VH1-2 germline antibodies. Mutations identified during the computational design were used to generate directed libraries that were screened on the surface of yeast to optimize binding against the germline predicted precursors of several VRC01-like antibodies as well as their mature counterparts.

Results: Using the strategy outlined above, we have modified a GP120 outer domain to have sub-micromolar affinity for several VH1-2 germline antibodies while maintaining high affinity for the VRC01-like matured antibodies. We have shown in a cell-based assay that, when multimerized, the engineered immunogen stimulates B-cell lines expressing germline VRC01 and other VH1-2 germline antibodies.

Conclusion: Our immunogen offers a novel approach to re-elicit VRC01-like antibodies. We have demonstrated proof of principle that immunogens can be rationally directed to target specific germline B cell receptors. If this approach proves successful, it could become a generally applicable strategy to selectively activate desirable antibodies when creating new vaccines.

Topic 1: Adjuvants, Immunogens and Inserts

P01.01

Live, Attenuated Rubella Vectors Expressing HIV and SIV Vaccine Antigens

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Background: Despite progress in identifying targets of neutralizing antibodies and T cell immunity, many HIV antigens have been weak immunogens. We have developed rubella as a live viral vector to enhance immunogenicity by presenting HIV and SIV antigens in the context of an acute infection. These vectors are based on the rubella vaccine strain RA27/3: safety and immunogenicity have been established in millions of children around the world. If the vector loses its insert, it would revert to the vaccine strain. It is immunogenic: one dose protects for life (against rubella). It elicits mucosal and systemic immunity. Rubella readily infects rhesus macaques, and these animals can test vector growth, immunogenicity and protection against SIV or SHIV challenge.

Methods: Starting from the live, attenuated vaccine strain, we have identified two insertional sites, where foreign genes can be expressed without compromising rubella protein expression or titer.

Results: Rubella can accommodate foreign inserts at either of two sites. Inserts at the nonstructural site were expressed as a fusion protein with rubella nonstructural protein p150. At the structural site, inserts were expressed with the structural polyprotein, processed to free protein, and incorporated into virions. Foreign antigens, including GFP, SIV Gag, and HIV MPER, were stably expressed for >10 passages. Six recombinant rubella vectors were selected for testing in vivo. Two of these grew well, infecting 3 out of 3 macaques while expressing HIV or SIV antigens.

Conclusion: Live, attenuated rubella vectors provide a good vaccine platform for evaluating SIV and HIV antigens in vivo and comparing prime/boost strategies for immunogenicity and protection. The results may also apply to man, since the same vectors grow well in human cells and could be substituted for current rubella vaccines, or they could be given prior to the age of natural rubella infection (9 years).

P01.02

HIV-1 Capture and Antigen Presentation by Dendritic Cells: Enhanced Viral Capture Does Not Correlate with Better T-Cell Activation

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Background: During HIV-1 infection, dendritic cells (DC) facilitate dissemination of HIV-1 while trying to trigger adaptive antiviral immune responses. We examined whether increased HIV-1 capture in DC matured with lipopolysaccharide (LPS) results in more efficient antigen presentation to HIV-1–specific $CD4^+$ and $CD8^+$ T cells. In order to block the DC-mediated trans-infection of HIV-1 and maximize antigen loading, we also evaluated a non-infectious integrase-deficient HIV-1 isolate, the $HIV_{NL4\text{-}3\Delta IN}$.

Methods: Immature DC (iDC), mature DC (mDC) activated with IL-1β, TNF-α, IL-6, and PGE2 (ITIP) or LPS during viral uptake, and fully mDC matured with ITIP or with LPS for 48 h before viral loading were tested. Antigen presentation to HIV-1-specific $CD4^+$ and $CD8^+$ T cell clones was quantified by IFN-γ ELISPOT. DC-associated $p24^{Gag}$ HIV-1 and DC-mediated HIV-1 trans-infection were also evaluated in parallel.

Results: We showed that higher viral capture of DC did not guarantee better antigen presentation or T-cell activation. Greater $HIV_{NL4\text{-}3}$ uptake by fully LPS-matured DC resulted in higher viral transmission to target cells but poorer stimulation of HIV-1–specific $CD4^+$ and $CD8^+$ T cells. Conversely, maturation of DC with LPS during—but not before—viral loading enhanced both HLA-I and HLA-II HIV-1–derived antigen presentation. On the other hand, DC maturation with ITIP during viral uptake only stimulated HIV-1–specific $CD8^+$ T cells. Integrase-deficient $HIV_{NL4\text{-}3\Delta IN}$ was also efficiently captured and presented by DC through HLA-I and HLA-II pathways, but in absence of viral dissemination.

Conclusion: Hence, DC maturation state, activation stimulus, and time lag between DC maturation and antigen loading impact HIV-1 capture and virus antigen presentation. Our results demonstrate a dissociation between the capacity to capture HIV-1 and to present viral antigens. $HIV_{NL4\text{-}3\Delta IN}$ seems to be an attractive candidate to be explored. These results provide new insights into DC biology and have implications in the optimization of DC-based immunotherapy against HIV-1 infection.

P01.03

CYPA, a Novel and Potential Genetic Adjuvant Enhanced HIV-1 DNA Vaccine Immunoreactivity

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Background: Recent research highlight that newly synthesized HIV-1 capsid protein is required to induce dendritic cell activation through a pathway involving interaction with peptidyl-prolyl cis-trans isomerase cyclophilin A (CYPA),which subsequently induce an antiviral type I interferon response and activation of T cells.

Previous studies also revealed HIV Gag-CYPA interaction inhibited recognition by host restriction factors. In another perspective, the inhibition effect might be benefit for HIV Gag immunogenicity.

Consequently, we assume CYPA might be a potential genetic adjuvant co-inoculation with HIV-1 Gag DNA vaccine, which not only circumvent the restriction of host factors, but also activate dendritic cell and enhance adaptive immune response.

Methods: In this study, 6-8-week-old female BALB/C mice were administrated thrice intramuscularly with pDRVI4.0-Gag HIV-1 DNA vaccine co-formulated with pDRVI4.0-CYPA plasmid at two weeks interval. IFN-γ ELISPOT, Gag-specific ELISA, IgG isotype ELISA detected cellular and humoral immunological response.

Results: The regimen of HIV-1 Gag DNA premixed with CYPA DNA induced robust immune responses in mouse model. IFN-γ ELISPOT result demonstrated Gag DNA alone only generated 180 ± 63(mean ± SD) SFCs/million. However, Gag DNA combined with CYPA DNA strategy produced 353 ± 80(mean ± SD) SFCs/million. In humoral immune response, Gag DNA co-inoculation with CYPA DNA showed high level antibody titer (GMT=22800),whereas Gag DNA alone induced slight antibody response (GMT=8000). IgG isotype results confirmed that co-vaccination with CYPA DNA induced Th1-bias immune response, however, Gag DNA alone activated Th1/Th2 response in balance.

Conclusion: This is the first report demonstrating that mixture of CYPA DNA and HIV-1 gag DNA vaccine could induce robust cellular and humoral immune response in the mouse model. Ongoing studies are focusing on construction of dual expression cassette DNA vaccine, which including both Gag and CYPA gene. Further research will also construct others HIV DNA vaccine involving human or non-human primates CYPA.

P01.04

Cross-Clade Neutralization of HIV-1 by a Monoclonal Antibody Obtained by Immunization with Liposomes Containing Lipid A and a Synthetic MPER Peptide

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Background: Development of vaccine formulations that induce cross clade neutralizing antibodies to the membrane proximal external region (MPER) of gp41 similar to 4E10 and 2F5 have been extensively investigated. We previously demonstrated that immunization with liposomes containing MPER and lipid A as an adjuvant could induce antibodies to the MPER and IgM monoclonal antibodies obtained from the mice bound to both lipids and MPER and neutralized HIV-1 (Matyas et al., 2009 AIDS 23, 2069-77). We now report a new IgG monoclonal antibody WR324 obtained from mice immunized with a similar vaccine formulation that binds to MPER and can neutralize multiple clades of HIV-1.

Methods: Mice were immunized with liposomes containing phosphatidyl inositol-4-phosphate and MPER with lipid A as an adjuvant. The peptide, LELDKWASLWNWFDITNWLWYIK (aa 661-684) was derived from gp41 (HXB2 stain). Spleen cells were fused with SP2/O cells and cloned. WR324 was purified and the binding specificities were assessed by ELISA. HIV-1 neutralization was assessed in PBMC using replication competent, Renila reniformis luciferase (LucR)-expressing HIV-1 reporter viruses (LucR-IMC) and in a human monocyte derived macrophage (MDM) assay with purified viruses.

Results: WR324 is an IgG2b with a kappa light chain and it bound to the MPER peptide, but not to lipids or recombinant gp41. WR324 neutralized clade B, BaL and SF162, clade CRF01_AE, CM235, and clade C, GS 014 IMC viruses in the PBMC assay using two different donors. It also neutralized primary viruses, US-1, BaL, and clade CRF01_AE, M066 in the MDM assay using 3 different donors. WR324 did not bind to the surface of MDM cells, but binding to HIV-1 was observed by electron microscopy with immuno-gold labeling.

Conclusion: Immunization with liposomes containing MPER and lipid A as an adjuvant induced an IgG monoclonal antibody with specificity to the MPER that neutralized multiple clades of HIV-1 in two different assay systems.

Topic 1: Adjuvants, Immunogens and Inserts

P01.05

IL-12 Plus CTB in Intranasal DNA-MVA Schemes Improved Magnitude and Quality of Both Systemic and Mucosal HIV Cellular Immune Responses

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Background: Mucosal tissues are the major route of HIV transmission. Therefore, designing immunization regimes aimed to induce mucosal immune response is needed. The aim of this study was to analyze the activity of IL-12 alone or in combination with the cholera toxin B subunit (CTB), applied in DNA-prime/MVA-boost intranasal immunizations.

Methods: Balb/c mice were intranasally immunized with DNA expressing HIV-1 EnvB plus DNAIL-12 alone or in combination with CTB (10ug, applied at prime and booster doses). Groups receiving CTB, complete cholera toxin (CT) or non-adjuvants (control) were included. All groups received MVAEnvB as boost dose. Immune responses were evaluated 14, 30 or 53 days after immunization.

Results: IL-12 plus CTB generated the highest response, showing a synergistic effect for both adjuvants, measure by IFN-g and IL-2 ELISPOT, in spleen (7-fold increment), in regional (cervical) lymph nodes (LN), genital LN (iliac, GLN) and, more importantly, in genital tract mucosa (GT). At memory phase, we found that in the IL-12+CTB group IFN-g and IL-2 secreting cells were two to three-fold higher in both systemic and mucosal compartments (GLNs and GT) (p=0.001).

IL-12+CTB improved several quality features of the response: i) Higher levels of T-cell polyfunctionality in spleen and GT. ii) % of specific proliferating cells was increased at 10, 30 and 53 days. iii) Enhanced in vivo citotoxicity: median 53% vs 16.4% for control group. iv) Higher T-cell avidity in spleen cells (p=0.01). v) T-cell responses with a superior breadth: cross-reactivity against different Env subtypes was superior.

Conclusion: We demonstrated that IL-12 plus CTB generated a cooperative adjuvant effect on the cellular immune response against Env applied in DNA-MVA intranasal immunizations. The improvement observed was not only in magnitude, but also in the breadth and quality of the responses induced. These results are important due to the need to develop mucosal vaccine strategies against HIV.

P01.06

Engineered gp120 Immunogens That Elicit VRC01-like Antibodies by Vaccination

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Background: One of the great challenges for an HIV vaccine is to elicit broadly neutralizing antibodies specific for conserved epitopes from which the virus cannot easily escape. The CD4 binding site is one such epitope against which several antibodies (e.g. b12, VRC01) have been isolated. In macaques infected with SHIV, passive immunization with these CD4-directed neutralizing antibodies fails to control the virus, but prophylactic administration is highly protective. Similarly, patients who generate neutralizing antibodies over the course of an HIV infection derive no clinical benefit from them, but eliciting such antibodies prophylactically by vaccination may prevent the virus from establishing its lethal foothold.

Methods: Yeast surface display is a powerful method for rapidly engineering complex glycoproteins. We have developed a stripped core gp120 that presents a functional CD4 binding site when displayed on yeast, and an accompanying suite of tools with which to map conformational epitopes of neutralizing antibodies, design novel immunogens, and monitor the specificity of serum following immunization.

Results: We map the epitopes of the anti-gp120 antibodies VRC01, b12, and b13, and uncover subtle energetic differences in their nearly-overlapping epitopes that are not obvious from existing crystal structures. With this information, we design novel gp120 immunogens that share the VRC01 epitope but whose other surface amino acids are highly diverse. When mice are immunized with these immunogens in a heterologous prime-boost format they elicit VRC01-competitive antibodies and not the competing immunodominant specificities seen with a single immunogen. The antisera are tested for their binding to a panel of gp120, and their neutralization potential is assayed.

Conclusion: We have engineered a novel set of immunogens that elicit CD4 binding site-directed antibodies upon immunization. The yeast display tools may be used to design future immunogens.

P01.07

TLR-3 and TLR-7/8 Ligands Indirectly Activate Langerhans Cells When Intradermally Injected by Triggering the Recruitment of Inflammatory Cells

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Background: TLR-3 and TLR-7/8 agonists are promising vaccine adjuvants but their effects on immune cells remain to be define in vivo. We analysed the modifications in the Langerhans cell (LC) network after intradermal injection of these TLR ligands in non-human primates used as a model for assessing human vaccines. LCs, which are the only antigen-presenting cells in the epidermis, are endowed with great ability to induce immune responses.

Methods: Intradermal injection of poly(I:C), R848 or PBS in cynomolgus macaques was followed by biopsy of the injection site at different time points. Epidermal and dermal sheets were dissociated, and epidermal and dermal cells were extracted before flow cytometry and culture. In situ immunofluorescence was performed on snap-frozen skin biopsies. Macaque polymorphonuclear neutrophils (PMNs) and monocytes were isolated from blood.

Results: Cynomolgus macaque epidermis contains CD45+, DR+, CD1a, CD207+ LC, and dermis contains DR+, CD11c+ dermal dendritic cells (featuring CD1a + CD14- dermal DCs and CD1a-CD14+ DCs) and DR+, CD11c-, CD163+ macrophages. R848 intradermal injection results in LC maturation and activation (increased expression of CD80, CD83 and CD86) and migration (in situ immunofluorescence) out of the skin. PMNs and macrophages were massively recruited locally (up to 17% and 20% of total leucocytes, respectively, whereas steady state values are below 3%); PMN recruitment and LC activation were significantly correlated suggesting the role of R848 induced inflammation on LC changes and migration. Similar results, with lower intensity, were obtained after poly(I:C) injections. The involvement of inflammatory cells was confirmed by the lack of TLR-7/8 expression in LCs and the effect of secreted cytokines.

Conclusion: Better understanding of the dynamics of local inflammation induced by TLR ligands in relevant animal models is critical for improving human vaccine adjuvants.

P01.08

Fusion of CTA and CTB Gene to Immunogen Significantly Enhances the Immunogenicity of DNA Vaccine

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Background: Cholera toxin and its two subunits (CTA,CTB) have been intensively investigated as mucosal adjuvants for protein based vaccine. In this study we evaluated the adjuvanticity of CTA and CTB in modality of either mixing their encoding plasmids with DNA vaccine or fusing their encoding genes to immunogen encoding gene.

Methods: DNA and recombinant vaccinia vaccines expressing HIV-1 AE strain tat, rev, intergrase(C-half), vif, nef fusion gene(designated as TRIVN) have been constructed. For the construction of fusion gene of CTA/CTB to TRIVN, overlapping PCR was employed to link CTA/CTB and TRIVN gene, the fused genes(TRIVN-CTA and TRIVN-CTB)were cloned into eukaryotic expression plasmid vector(pSV1.0). Six groups of female BALB/c mice were immunized with mock control, pSV-TRIVN, pSV-TRIVN-CTA, pSV-TRIVN-CTB, pSV-TRIVN mixed with CTA or with CTB respectively in a DNA priming-recombinant vaccinia boosting regimen. Two weeks after the final injection, mice splenocytes were collected and IFN-γ ELISPOT assay were used as readout for specific T cell response. Statistical analysis was performed by using Prism5.0 software.

Results: Our data showed that all constructed plasmids are capable of efficiently expressing their inserted genes. All groups immunized with vaccines raised significant more T-cell response than mock control. T-cell responses elicited by pSV-TRIVN-CTB(1548±330SFCs/106splenocytes) and pSV-TRIVN-CTA (1642±514SFCs/106splenocytes) were significantly higher than that by pSV1.0-TRIVN (520±150SFCs/106splenocytes), pSV1.0-TRIVN mixed with CTA (692±220SFCs/106splenocytes) and pSV1.0-TRIVN mixed with CTB (734±240SFCs/106splenocytes). Though TRIVN-CTA and TRIVN-CTB fusion vaccines mounted comparable level of total IFN-γ+ T-cell responses, only TRIVN-CTB elicits significantly T-cell responses against Tat, which is a subdominant component in the fusion immunogen. No significant differences were observed among groups inoculated with TRIVN alone or adjuvanted by CTA/CTB subunit proteins.

Conclusion: CTA and CTB could serve as potent adjuvants for DNA vaccine in immunogen-CTA/CTB fusion modality. Compared with CTA, CTB may enhance T-cell responses against subdominant epitopes in the immunogen and broaden the T-cell immune responses.

Topic 1: Adjuvants, Immunogens and Inserts

P01.09

HIV-1 gp120 Impairs the Induction of B Cell Responses by TLR9-Activated Plasmacytoid Dendritic Cells

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Background: Plasmacytoid dendritic cells (pDCs) play a central role in innate and adaptive immunity to viral infections, including HIV-1. pDCs produce substantial quantities of type I IFN and proinflammatory cytokines upon stimulation by Toll-like receptors (TLR), specifically TLR7 or TLR9. We have studied how gp120 affects human pDC responses to TLR9 agonists, and the subsequent ability of the pDCs to stimulate B cells, with the goal of learning how better to induce B cell responses to Env protein vaccines.

Methods: pDCs were isolated from human peripheral blood using CD304 magnetic beads, and then treated with endotoxin-free recombinant gp120 during stimulation with TLR9 agonists. IFN-α, IL-6, TNF-α, IRF-7 and BAFF were quantified at the protein or mRNA level. Co-cultures were performed to study how gp120-treatment of the pDCs affected their abilities to stimulate B cell responses, specifically proliferation, differentiation to plasma cells and IgG/IgM production.

Results: We found that gp120 impaired IFN-α production by pDCs in response to TLR9 (CpG-ODN), but not TLR7, stimulation. Receptor-blocking studies showed the inhibitory effects were mediated via CD4 and the C-type lectin receptor BDCA-2, but not via CCR5 or CXCR4. Treatment with gp120 inhibited CpG-induced pDC maturation, TNF-α and IL-6 production and IRF-7 and BAFF mRNA expression. The gp120-treated, CpG-activated pDCs also had impaired abilities to induce B cell proliferation, plasma cell differentiation and Ig production, due at least in part to decreased expression of BAFF and other cytokines.

Conclusion: Taken together, our data show that HIV-1 gp120 impairs pDC functions and B cell activation, and imply that TLR9 ligands may not be good adjuvants to use in combination with Env-based vaccines.

P01.10

Soluble and Bacteriophage T4 Displayed gp41 Mutant Proteins as HIV-1 Vaccine Candidates

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Background: HIV-1 envelope protein gp41 is a very attractive vaccine target as its epitopes are recognized by three broadly neutralizing antibodies. However, the extreme hydrophobicity and very transient exposure of neutralizing epitopes during infection has hampered its usage in HIV-1 vaccine development. Our goal is to design a soluble trimeric gp41 vaccine stabilized as a pre-hairpin intermediate.

Methods: A gp41AVERY-minus recombinant was constructed, which contained both gp41 ecto-domain and cyto-domain, while the immunodominant AVERY region was deleted to reduce elicitation of non-neutralizing antibodies. Another protein, gp41-5M, was constructed by introducing 5 point mutations to gp41AVERY-minus, at the exposed hydrophobic residues of the trimeric coiled coil region, in order to destabilize the interaction between HR1 and HR2 helices and therefore mimic the gp41 pre-hairpin intermediate. A 'foldon' structural tag was fused at the C-terminus of gp41-5M to facilitate trimer formation, and phage T4 small outer capsid (Soc) protein was fused at the N-terminus for arraying gp41 on T4 capsids. The ability of purified Soc-gp41-5M proteins to inhibit 4E10 and 2F5 neutralizing activity was tested in the TZM-bl assay. Immunization experiments were performed in rabbits using soluble as well as T4 displayed gp41 antigens to determine their immunogenicity.

Results: gp41 mutant proteins were over-expressed in E. coli and purified in soluble form following denaturation, renaturation, and Histrap affinity chromatography. The Soc-gp41-5M protein formed trimers and other oligomers, and effectively competed with native epitopes present on HIV-1 virus for binding to 4E10 and 2F5 antibodies in the TZM-bl assay. The sera from all immunized rabbits showed binding antibodies to gp41 while some also showed neutralizing antibodies.

Conclusion: Soluble near full-length trimeric gp41 immunogens can be produced by interfering with the HR1-HR2 interactions while retaining the 4E10 and 2F5 binding epitope conformations. These proteins might be good vaccine candidates to elicit broadly neutralizing antibodies.

P01.11

Evaluation of Latent Membrane Protein 1 as a Novel Vaccine Adjuvant

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Background: The EBV protein Latent Membrane Protein-1 (LMP1) is known to constitutively activate B cells. The LMP1 signaling pathway mimics that of CD40, a molecule involved in dendritic cell activation and maturation. Therefore we decided to evaluate the use of LMP1 as a vaccine adjuvant for both dendritic cell therapeutic vaccines and DNA-based vaccines for HIV.

Methods: To determine activity, LMP1 was analyzed using a luciferase report assay for NF-kB and IFN-β. To establish if LMP1 could activate human monocyte-derived dendritic cells (DC), LMP1 transfected DC were analyzed for activation/maturation markers and cytokines. DC migration was determined using a transwell-migration assay. LMP1 was also evaluated in a DNA vaccination/flu challenge mouse model. To determine the benefits of incorporating LMP1 into a DC therapeutic vaccine, LMP1 was tested in a tumor DC therapy mouse model.

Results: LMP1 activated high levels of NF-kB and IFN-β when evaluated using a luciferase reported assay. On primary DC, LMP1 induced DC activation, maturation, and proinflammatory cytokines. LMP1 induced 2-fold higher migration rates compared to the mature-DC control. As a DNA vaccine for flu, the addition of LMP1 provided superior TNF-α and IFN-γ responses. LMP1 vaccinated animals cleared virus more quickly and in the high-dose lethal flu challenge, LMP1 afforded more protection. Finally, LMP1 enhanced a DC therapeutic vaccine in a tumor model. Tumor progression was slowed compared to antigen-loaded DC alone and positive control mimic-matured DC.

Conclusion: These data suggest that LMP1 is an effective vaccine adjuvant. LMP1 can enhance the activation, maturation, and functional activity of DC. LMP1 can inducing a strong CD8+ T cell response in several mouse models, most notably the flu viral challenge model. LMP1 increased antigen-specific CD8+ T cells, improved survival to lethal flu high-dose challenge, and slowed tumor progression. These results suggest that LMP1 is a promising adjuvant for prophylactic vaccines for HIV.

P01.12

Potent Induction of Antibody-Secreting B-Cells by Human Dermal-Derived CD14+ Dendritic Cells Triggered by Dual Toll-like Receptor Ligation

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Background: A goal of HIV-1 vaccine development is to induce broadly neutralizing antibodies. However, the Env complex is poorly immunogenic and requires potent adjuvants. Given the pivotal role of TLRs and DCs in initiating and tuning adaptive immune responses, TLR agonists are attractive adjuvants. CD14+ dermal DCs (CD14+ DDCs) have a natural capacity to stimulate naïve B-cells, so targeting these cells with TLR ligands is a rational approach to inducing humoral responses.

Methods: Migratory cells were collected after culturing skin for 24 h. CD14+ DDCs were purified using CD14 magnetic beads, stimulated with TLR ligand(s) and analyzed for cytokine expression (ELISA/qPCR) and phenotype after 48 h. Naïve B-cells were stimulated with TLR ligand(s) plus CD40L and IL-2, either alone or in the presence of CD14+ DDCs, and analyzed for proliferation, phenotype and IgG/IgA secretion. TLR-ligand stimulated DDCs were incubated with allogeneic naive CD4+ T-cells for 6 days before T-cell derived cytokines were quantified.

Results: CD14+ DDCs express mRNA for TLRs 1–9, but respond differentially to single or paired TLR ligands. Compared to single ligands, some combinations were particularly effective, increasing the expression of B-cell stimulatory cytokines and maturation of the DDCs. These combinations were R-848 plus Poly(I:C); R-848 plus LPS; Pam3CSK4 plus Poly(I:C); LPS plus Poly(I:C). Selected TLR agonist pairs (R-848 plus either LPS or Poly(I:C)) were superior to individual agents at boosting the capacity of CD14+ DDCs to induce naïve B-cells to proliferate and differentiate into CD27+CD38+ B-cells that secrete high levels of IgG and IgA. These selected TLR ligand combinations also induced CD14+ DDCs to promote differentiation of Th1, but not Th2, Th17 or TFH cells.

Conclusion: Two TLR ligand combinations potently activate CD14+ DDCs to have enhanced B-cell stimulatory capacity, and could be used to improve humoral immune responses to HIV-1 Env.

Topic 1: Adjuvants, Immunogens and Inserts

P01.13

Recombinant IL-21 Induces Perforin and Granzyme B in Total and Virus Specific CD8 Tcells in Acute and Early Stages of SIV Infection in Rhesus Macaques

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Background: We have recently demonstrated that the cytokine IL-21 enhances the cytotoxic potential of CD8 T cells in chronically SIV infected rhesus macaques (vaccines 2011).

Methods: In this study, 12 RM were infected with SIVmac239 (i.v., 300 TCID50). rMamu IL 21-Fc fusion protein (50mg/kg) was given s.c on a weekly basis post infection (pi) for 5 doses on days14, 21, 28, 35 and 42pi to 6 animals, designated as "treated", with 3 mamuA01+ animals each in treated and control groups. Samples of PBMC, bone marrow (BM), rectal biopsy (RB) and peripheral LN (LN) were collected before infection (d-11), and at various times post infection.

Results: Compared to controls, IL-21 treated animals demonstrated increases in frequency and MFI of Perforin (Perf) and granzyme B (GrB) at d45 in total CD8 T cells in PBMC, LN and RB, particularly in CM and Effector subsets; these were sustained up to d70pi. Perf and GrB levels increased in virus specific Tet+ CD8 T cells at d 45 in PBMC (p=0.029), LN (p=0.015), and RB (p=0.024). In the CD4 T cells, GrB induction was more prominent in the PBMC, LN, BM and RB. Frequencies of CD4 (p=0.011) and CD8 (p=0.031) CM T cells increased in PBMC at d70pi. T cell inhibitory molecule PD-1 and proliferation marker Ki67 were similar in treated and control animals. In treated animals, 2/6 showed a decline in post-peak viremia that was sustained up to d70pi follow up.

Conclusion: In summary, IL-21 given s/c to SIV infected RM during early stages of infection led to augmented T cell cytotoxic granules perf and GrB in total and virus specific CD8 T cells in various anatomical sites. IL-21 should be explored further in vaccine strategies as an immunomodulating adjuvant.

P01.14

Inverse Dose-Response to gp140 YU2 Foldon Trimer Formulated with Aluminum Phosphate and ISCOMATRIX® Adjuvants

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Background: Conventional vaccine approaches based on delivery of HIV-1 envelope (Env) proteins or peptides derived from Env sequences have failed to generate broadly neutralizing antibodies (bNAbs) to the virus. Even with large doses (200 ug) of adjuvanted gp120 proteins administered multiple times to human volunteers, the subsequent antibody response boosts only moderately with each succeeding vaccination, and titers drop precipitously thereafter. We hypothesized that the usual practice of administering a moderate to high antigen doses may be counter productive to the goal of eliciting durable, high-affinity antibody responses.

Methods: We conducted a rabbit immunogenicity study in which we compared the anti-gp120 antibody response of rabbits immunized with low (1 ug), medium (10 ug) and high (100 ug) quantities of gp140 YU2 foldon trimer (FT) formulated with aluminum phosphate (alum) alone or in combination with ISCOMATRIX® adjuvant. In addition, we used a more protracted vaccination regimen by administering the vaccine at 0, 8, and 24 week time points.

Results: Antibody responses elicited by the different YU2 FT vaccination regimens were quantified by ELISA against JRCSF gp120 protein after vaccination showing weak responses after the first two vaccine doses. However, after 3 doses, the responses to vaccines co-formulated with both ISCOMATRIX® and alum were markedly higher than the corresponding responses to the antigen formulated with alum only. Interestingly, at 4 weeks post-dose 3, there was a reverse dose response effect, with the 1 ug dose group having higher titers than the 10 and 100 ug groups. At 12 weeks post-dose 3, the antibody GMTs were 28,078 (1 ug), 9,681 (10 ug) and 7,253 (100 ug).

Conclusion: The results showing that the low dose group maintained a response ~4 fold higher than the high dose group suggests that durability of the antibody response to HIV Env may be a function of antigen dose.

Topic 1: Adjuvants, Immunogens and Inserts

P01.15

Robust Antibody and Cellular Responses with an Improved DNA Vaccine Alone

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Background: The recent results of the RV144 trial demonstrate that HIV-specific antibodies may provide protection from infection. DNA vaccines may represent an important HIV vaccine platform since they are safe, relatively inexpensive to manufacture, and stable at room temperature. However, the platform has been used largely as a prime modality limited to induce low level CD4 T cell responses in NHP and humans. In this study we sought to improve our current DNA vaccines to induce HIV-specific antibodies with plasmid vaccination alone.

Methods: Groups of 5 Indian Rhesus Macaques were vaccinated with pHIV consensus gag, pol, and clade C envelopes delivered IM with in-vivo electroporation at weeks 0, 4 and 12. Immunogenicity was measured two weeks after each dose.

Results: Three doses of an HIV DNA vaccine alone induced robust cellular and antibody responses. Despite using clade C based enveloped, high binding titers were detectable against gp120s from multiple clades. Neutralizing titers were in the 100's range to a panel of clade B and C tier 1 viruses. These data establish that designed DNA envelop antigens can drive functional immunity in NHP.

Conclusion: Multiple improvements to DNA vaccine technology have significantly enhanced the immunogenicity of the platform. Just three doses of a plasmid based HIV vaccine induced robust binding and neutralizing antibodies as well as effector T-cell responses in NHP. We are currently expanding the immunity induced by these constructs through novel DNA adjuvants as well as in prime-boost combinations.

P01.16

Adjuvant-Dependent Cytokine Profiles in the Context of a DNA Prime-Protein Boost HIV-1 Vaccine

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Background: Heterologous prime-boost vaccinations have emerged as a promising strategy to generate protective immunity against a variety of pathogens. Our previous clinical work has demonstrated that an HIV-1 gp120 DNA prime-protein boost vaccine, DP6-001, elicits enhanced neutralizing antibody responses as well as cell-mediated immune responses in humans. However, the roles of adjuvants remain largely unknown in the context of such combination vaccines.

Methods: In a mouse model, we studied the effects of adjuvants QS-21, Alum, and MPL, in the context of DP6-001. Both gp120-specific antibody and T cell responses were monitored by ELISA and intracellular cytokine staining (ICS), respectively. Innate cytokine profiles were determined in sera collected 6 hours post-immunization by Cytometric Bead Array (CBA) and Luminex assays.

Results: Serum anti-Env IgG titers were comparable between adjuvant groups. All immunized animals demonstrated comparable positive gp120-specific CD4+ and CD8+ T cell responses by ICS. Adjuvant profiles were largely determined by sera cytokines following protein boosting. QS-21 was distinguished by elevated IL-4, IFNγ, MIP-1β, and IL-1β. MPL was characterized by elevated G-CSF, KC, and RANTES. Both adjuvant groups demonstrated elevated IL-6. Production of these sera cytokine profiles required DNA priming.

Conclusion: Our data indicated that different adjuvants generate unique patterns of biomarkers, indicating that different mechanisms are involved in their action. Our results also provided useful guidance in the selection of an adjuvant for inclusion in future prime-boost strategies, with the goal of enhancing immunogenicity while minimizing reactogenicity.

Topic 1: Adjuvants, Immunogens and Inserts

P01.17 LB

Neutralizing Antibodies Elicited In Rabbits By Patient-Derived Env Trimer Immunization

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Background: Eliciting broad cross neutralizing antibodies (bNAb) remains the primary and most challenging goal in HIV-1 vaccine development. So far no vaccine candidate has induced such bNAb. Selecting Env vaccine candidates will require both antigenic and immunogenic optimization and testing in relevant animal models.

Methods: Based on in-vitro neutralizing activity in serum, patients (n=6, subtype A and B infected) were selected and Env sequences of early HIV-1 variants, still sensitive to autologous neutralization, were used to generate soluble Env as immunogens. Gp140 trimeric proteins were expressed (293T cells) and purified. Rabbits (4/group) were immunized s.c. at weeks 0, 2, 4, 8 with 100µg trimer adjuvanted with cationic CAF01. Control groups received 20µg and 100µg trimer plus/minus CAF01 respectively. Sera collected at weeks 0, 2, 4, 8, 12 and 14 were screened in gp120-IIIB ELISA and IgG was analyzed in the TZMbl neutralization assay.

Results: All rabbits generated a gp120-IIIB specific IgG response 2 weeks after the first immunization and titers were boosted after each subsequent immunization. IgG titers measured 4 weeks after the last immunization clearly differed between groups (n=5) receiving 100µg/immunization (Geometric mean titer (GMT) : 152.601) and the group receiving 20µg/immunization (GMT : 13.262) or the group omitting CAF01 (GMT : 27.262). Only IgG from rabbits receiving the highest dose and in the presence of CAF01 were able to neutralize Tier 1 pseudoviruses of different subtypes.

Neutralizing activity was detected after the 2nd immunization and was boosted after each immunization. No significant differences were observed between the different trimers.

Conclusion: Gp140 trimers based on HIV-1 variants of patients with bNAb in serum elicited gp120-IIIB specific IgG and NAb given that enough immunogen was administrated in the presence of CAF01. These results indicate that the development of HIV-1 Env specific NAb is dose dependent and strengthen the rabbit model for HIV vaccine studies.

P01.18 LB

Rationally Designed HIV Envelope Glycoproteins Delivered in a Novel Adjuvant Elicited More Broadly Reactive Antigen-Specific Antibody Responses

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Background: The identification of optimal antigen(s) and adjuvant combination(s) to elicit potent, protective, and long-lasting immunity has been a major challenge for the development of effective vaccines against HIV-1.

Methods: Here, we designed disulfide-stabilized recombinant HIV-1 subtype B (SF162) envelope glycoproteins (Env), gp120 and gp140, by insertion of site-specific cysteine pairs between two layers (layer 1 and 2) in inner domain of gp120. In addition, we identified a novel adjuvant approach using Carbopol 971P, a cross-linked polyanionic carbomer, in combination with the Novartis proprietary oil-in water adjuvant, MF59, to augment humoral immune responses to the Env glycoprotein. We performed thorough in vitro analysis of the disulfide-stabilized Env glycoprotein followed by in vivo evaluations of the adjuvanted-Env glycoprotein boost in rabbits.

Results: Intramuscular immunization of rabbits with disulfide-stabilized Env glycoproteins formulated in Carbopol 971P plus MF59 gave significantly higher titers of binding and virus neutralizing antibodies as compared to immunization using Env glycoprotein with either MF59 or Carbopol 971P alone. In addition, the antibodies generated were of higher avidity. Mapping of serum antibodies to determine epitope specificities showed that the disulfide-stabilized gp140 proteins elicited broader Env glycoprotein-specific antibody responses directed against epitopes that included the CD4-binding site, CD4-induced site and V1V2-loop. Importantly, the use of the novel adjuvant, Carbopol plus MF59, did not appear to present any obvious tolerability issues in animals upon intramuscular administration.

Conclusion: Hence, the use of rationally stabilized Env-antigens in potent Carbopol 971P plus MF59 adjuvant may provide a benefit for evaluations of future vaccine against HIV-1.

P01.19 LB

CCL28 Induces Mucosal Homing of HIV-1-Specific Iga-Secreting Plasma Cells in Mice Immunized With HIV-1 Virus-Like Particles

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Background: MEC/CCL28 (CCL28) binds to CCR3 and CCR10 and recruits IgA-secreting plasma cells (IgA-ASCs) in the mucosal lamina propria. Virus-like Particles (VLPs) are a novel vaccine approach based on non-pathogenic particles that mimic the structure of virus particles with effective induction of both arms of the immune response. The suitability of CCL28 as an adjuvant for the elicitation of optimal mucosal and systemic immunity was assessed in mice immunized with HIV-1 VLPs.

Methods: Balb/c mice were immunized intramuscularly with a prime-boost regime based on VLP containing gp160 from HIV-1 IIIB in the presence/absence of CCL28 and of the parental control CCL19. Flow citometry evaluation of CCR3 and CCR10 expression was performed on purified splenocytes. Th1 and Th2 cytokine production was performed on splenocytes and either colon, lungs or uterine cervix, whereas antigen-specific IgG and IgA antibodies were evaluated in sera and mucosal secretions by ELISA. Immune sera and mucosal secretions were tested for ex vivo neutralization activity against HIV-1 either subtype B or C strains. IgA-ASC recruitment at the mucosal level was verified with immune-histochemistry.

Results: The following parameters were significantly augmented in VLP-CCL28 mice compared to control groups: the percentage and the surface density of CCR3 and CCR10 on CD19+ splenocytes; IFN-γ, IL-4 and IL-5 production in splenocytes and mucosal specimens; total IgA titers in sera and in mucosal secretions; antigen-specific IgG and IgA titers in sera and in mucosal secretions. Sera and mucosal secretions from VLP-CCL28 mice showed a significantly augmented neutralizing activity against homologous and heterologous viruses. IgA-ASCs were significantly increased in mucosal tissues of VLP-CCL28 mice.

Conclusion: CCL28 used as an adjuvant has a robust immunomodulatory effect on potentially beneficial mucosal and systemic immune responses. These findings suggest that CCL28 could play a useful role in increasing the efficacy of preventive vaccines for mucosally transmitted viral infections.

P01.20 LB

NMR Spectroscopy of HIV-1 gp120 Outer Domain

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Background: The outer domain (OD) of HIV-1 gp120 has been proposed as a minimal immunogen to elicit broadly neutralizing antibodies. However, OD is heavily glycosylated, contains many flexible regions, and immunization with a number of different OD variants has thus far failed to elicit neutralizing antibodies. An understanding of the conformational space sampled by the OD in its unliganded state, however, may assist in the use of OD as an immunogen.

Methods: We developed a method to isotopically enrich glycoproteins using a mammalian expression system that exploits the high level of protein expression obtained from an adenoviral vector, and employed heteronuclear NMR spectroscopy to obtain structural and dynamic information of unliganded OD. Multidimensional NMR experiments were recorded on uniformly labeled $^{15}N/^{13}C$ OD as well as on samples selectively enriched in ^{15}N-labeled Gly, Ile, Leu and Val. Experiments for backbone assignments were also recorded on an OD sample enriched in $^{15N/13}C$ for Ile, Leu and Val.

Results: We successfully produced isotopically labeled OD samples, suitable for NMR analysis. We also identified Gly, Ser, Val, Leu and Ile residues using samples selectively enriched in ^{15}N for Gly, Val, Leu and Ile. Standard triple resonance NMR experiments on the isotopically labeled OD were combined with backbone experiments recorded on a second sample – that was selectively enriched in $^{15}N/^{13}C$ for Ile, Leu and Val – to assign HN, C', C_{alpha} and N backbone resonances in about 80 of the 220 residues of OD.

Conclusion: We succeeded in assigning ~1/3 of the backbone for unliganded OD with triple resonance experiments. Extension of these assignments with NOESY experiments is now proceeding. Our results indicate that a solution structure of the highly glycosylated HIV-1 gp120 OD is feasible.

Topic 1: Adjuvants, Immunogens and Inserts

P01.21 LB

Immunogenicity of Native And CD4 Liganded Monomeric And Trimeric Envelope Glycoproteins Based on HIV-1 Subtype C Consensus Founder Virus Sequences

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Background: The ability to induce a broadly neutralizing antibody (bNAb) response following vaccination is regarded as a crucial aspect in developing an effective HIV-1 vaccine. This study describes the design and construction of a subtype C founder virus consensus Env immunogen derived from newly transmitted/ founder virus sequences, and its immunogenicity testing in the presence or absence of liganded CD4, in small animals.

Methods: Monomeric (gp120), dimeric (gp120GCN4) and trimeric (gp140GCN4 +/-) founder virus conformations were expressed in mammalian cell culture. Unliganded or 2dCD4^{S60C} liganded Env glycoproteins were purified by lectin affinity chromatography, followed by conformation and complex purification using size exclusion chromatography. Immunogens/ immune complexes were evaluated by ELISA, SDS-PAGE, Native PAGE and Surface Plasmon Resonance. Immunogenicity of each conformation alone or complexed to 2dCD4^{S60C} was evaluated in rabbits. Breadth and potency of the rabbit sera was tested against 12 pseudoviruses (Tiers 1-3), derived from HIV-1 subtype B and C Env, using the PhenoSense Neutralizing antibody assay (Monogram Bioscience Inc.).

Results: Minimal neutralizing breadth was obtained from animals immunized exclusively with Env conformations. However, animals that received the Env/2dCD4^{S60C} complex showed extensive neutralizing capacity against all 12 viruses tested, including the tier 2 and 3 virus strains. End-point ELISA titre results revealed that the rabbits that were immunized with Env/2dCD4^{S60C} produced both Env and 2dCD4 specific titres, but those directed towards 2dCD4 were on average 10x lower than the 2dCD4 control group. This implies a proportion of the neutralizing antibody activity is directed towards conserved epitopes exposed on the Env/2dCD4^{S60C} immunogens.

Conclusion: The ability to induce bNAb activity in previous immunization studies utilizing Env/CD4 complexes was attributed to the induction of high anti-CD4 titres. By contrast, in our study the relatively low anti-CD4 titres compared to anti-Env titres and neutralization profiles suggest an alternative mechanism of neutralization other than a response directed to CD4 alone.

P01.22 LB

Multivalent Adenoviral Vectors which use an Antigen Capsid-Incorporation Strategy for HIV Vaccination

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Background: Adenoviral (Ad) vectors have been used for a variety of vaccine applications. Traditionally, Ad-based vaccines are designed to express antigens through transgene expression. However, in some cases these conventional Ad-based vaccines have had sub-optimal clinical results. These sub-optimal results are attributed in part to pre-existing Ad serotype 5(Ad5) immunity. To circumvent the need for transgene antigen expression, the "antigen capsid-incorporation" strategy has been developed and used for Ad-based vaccine development. In addition, to increase the magnitude and/or breadth of antigen-specific antibody response, this strategy can be utilized. The major capsid protein hexon has been utilized for antigen display due to hexon's natural role in the generation of anti-Ad immune response and its numerical representation within the Ad virion.

Methods: Based on our abilities to manipulate Ad5 HVR2 and HVR5, we sought to manipulate Ad5 HVR1 in the context of HIV antigen display. More importantly, peptide incorporation within HVR1 was utilized in combination with other HVRs. In order to create a multivalent vaccine vector, we created vectors that display antigens within HVR1 and HVR2 or HVR1 and HVR5. To date this is the first report where dual antigens are displayed within one Ad hexon particle. These vectors utilize HVR1 as an incorporation site for a seven amino acid region of the HIV glycoprotein 41; in combination with a six Histidine (His6) incorporation within HVR2 or HVR5.

Results: Our study, illustrates that these multivalent antigen vectors are viable, present HIV antigen as well as His6 within one Ad virion particle. Furthermore, mouse immunization with these vectors; demonstrate that these vectors can elicit a HIV and His6 epitope-specific humoral immune response.

Conclusion: Our study focuses on generation of proof of concept vectors that can ultimately result in the development of multivalent vaccine vectors displaying dual antigens within the hexon of one Ad virion particle.

P01.23 LB

Hyperglycosylated Resurfaced Stabilized gp120 Core as an Immunogen Elicits Antibodies Targeted at the CD4-Binding Site

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Background: HIV-1 utilizes multiple mechanisms to evade immune surveillance. This has hindered the development of an effective vaccine. The CD4bs represents a highly conserved vulnerable site on HIV envelope that serves as an important target in HIV vaccine development. Novel concepts, such as resurfacing and glycan masking are some of the current approaches used to refocus the immune responses.

Methods: In this study, we used structural information of HIV gp120 core, together with the computer-assisted protein design tool to strategically add various glycosylation sites on the protein surface and create a panel of resurfaced stabilized core (RSC3)-based derivatives. The modified proteins were expressed in 293F or GnTi(-/-) cells and purified by Nickel-affinity and gel-filtration chromatography. To verify the glycosylation sites and antigenic analysis, the purified proteins were subject to mass spectrometry and SDS PAGE analysis using a panel of well-defined monoclonal antibodies. The proteins with desirable antigenicity and well-tolerated glycan additions were tested for their immunogenicity in mice and NHPs.

Results: Two hyperglycosylated mutants containing five or six extra glycans (RS3.Y5 and RSC3. Y6.1) elicited CD4bs antibodies in significant quantities. Variant Y6.1 was further tested in the NHP model, in which Y6.1 elicited non-neutralizing CD4bs antibodies. In addition, a novel glycan mutant Y8.2_1 was developed based on Y6.1 by incorporating 3 extra glycans. Antigenic analysis indicated that the protein was able to eliminate the binding of non-neutralizing or less potent CD4bs antibodies such as b12 and b13, but still retain the binding capacities to potent CD4bs antibodies such as VRC01 and VRC-PG04.

Conclusion: It is now feasible to induce CD4bs antibodies by vaccination with immunogens that have exogenous N-linked glycans added to mask the “undesired” regions of the immunogen and have an exposed surface that targets the CD4bs epitope. Further optimization will be necessary to obtain CD4bs antibodies with neutralizing activity.

Topic 2: Animal Models and Preclinical Trials

P02.01

Macaques Primed with Self-Amplifying RNA Vaccines Expressing HIV-1 Envelope and Boosted with Recombinant Protein Show Potent T- and B-Cell Responses

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Background: Self-amplifying RNAs (replicons) of positive-strand viruses are useful vectors for delivering vaccine antigens. Novartis has developed a self-amplifying mRNA (SAM™) vaccine platform to take advantage of cell-free RNA production and synthetic non-viral delivery systems. In this study, the safety, immunogenicity, and efficacy of HIV-SAM™ vaccines encoding HIV-1 clade C TV1 gp140 envelope glycoprotein were evaluated in rhesus macaques using two non-viral delivery systems: lipid nanoparticle (LNP) and a Novartis proprietary 2nd generation delivery technology (CNE).

Methods: Five groups of six macaques were primed at weeks 0, 4 and 12 with HIV-SAM™ vaccine formulated with LNP or CNE, alphavirus replicon particles (VRP), recombinant TV1 gp140 glycoprotein in MF59 adjuvant, or with vector controls encoding an irrelevant Ag. All treatment groups were boosted intra-muscularly at weeks 24 and 36 with TV1 gp140 in MF59, and controls with irrelevant protein in the same adjuvant. Systemic and mucosal responses were measured throughout the study. All macaques will be given a repeated low dose intra-rectal challenge with the heterologous clade C SHIV-1157ipd3N4 challenge.

Results: After priming immunizations, both IFNγ and IL2 T-cell responses and B-cell ELISpots were higher in HIV-SAM™-CNE macaques than those in HIV-SAM™-LNP, VRP and glycoprotein alone groups. Systemic Env-specific antibody responses were also detected by ELISA at week 6 in the RNA-immunized groups and increased after subsequent immunizations. Neutralization, ADCC, epitope mapping, and antibody isotyping assays are underway to further evaluate the antibody responses in these animals. No adverse responses to RNA immunizations were observed.

Conclusion: These studies provide the first evidence in nonhuman primates that vaccination with formulated self-amplifying RNA is safe and immunogenic, eliciting both humoral and cellular immune responses.

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P02.02

GM-CSF Co-expressing DNA/MVA Vaccine, Prevention of Acquisition by Two Series of SIVE660 Challenges Followed by a Series of SIV251 Challenges

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Background: In 2010 we reported prevention of acquisition of a repeated SIVE660 challenge in rhesus macaques vaccinated with a SIV239 DNA/MVA vaccine that co-expressed GM-CSF and VLP in the DNA prime. The reduced risk of infection correlated with the avidity of Env-specific IgG. Here we report studies on the longevity and breadth of this protective response.

Methods: Following the initial 12 challenges, 5 uninfected rhesus were monitored for one year, boosted with $1x10^8$ pfu of MVA/SIV239, and re-challenged 6 months later with 12 weekly rectal doses of SIVE660. The resulting four uninfected macaques were held an additional 6 months and challenged with 12 weekly rectal doses of SIV251. Avidity of Env-specific IgG was determined using a NaSCN elution ELISA. Per exposure efficacy was estimated using a leaky effects model.

Results: Per exposure efficacies were 90% and 94% for the 1st and 2nd SIVE660 series, respectively, and 72% for the SIV251 series of challenges. For the SIVE660 series, 50% infection was reached by the 3rd challenge for controls, but never reached in vaccinated animals. For the SIV251 series, 50% infection was reached by the 2nd challenge for controls but not until 10 challenges for vaccinated animals. Both SIVE660 and SIV251 series showed transient low "blips" of virus. None of five E660 "blips" resulted in anamnestic systemic Ab, whereas two of three SIV251 blips resulted in such. Correlates also differed for the two infections with the avidity of Env-specific IgG correlating with prevention of acquisition for SIVE660 but not SIV251.

Conclusion: A DNA/MVA vaccine in which GM-CSF is co-expressed in the DNA prime can provide substantial prevention of acquisition against serial challenges over a three year period of time. Our results also reveal SIVE660 and SIV251 rectal challenges differing in their ability to initiate systemic Ab responses and in their correlate for prevention of acquisition.

P02.03

Efficacy of Vaccine-Induced Vif-Specific CTL Responses Against SIVmac239 Infection: Implications for Antigen Design in AIDS Vaccines

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Background: Optimization of antigens as well as delivery system is crucial for development of an effective T-cell based AIDS vaccine. Our recent results suggested higher anti-viral efficacy of Vif- and Nef-specific CTLs as well as Gag-specific ones (JEM 199:1709, 2004; AIDS 24:2777, 2010). Here, we examined efficacy of Gag-specific or Vif/Nef-specific CTL induction by vaccination against SIV infection.

Methods: All 17 animals used in this study were Burmese rhesus macaques sharing MHC-I haplotype 90-010-Ie, which mostly show typical AIDS progression after SIVmac239 challenge (geometric means of setpoint plasma viral loads: 10^5 copies/ml; mean survival periods: 2 years). These animals were divided into three groups consisting of unvaccinated (n = 6), Gag-vaccinated (n = 5), and Vif/Nef-vaccinated (n = 6); the latter two were subjected to DNA-prime/Sendai virus vector-boost vaccination. We compared these three groups after an intravenous SIVmac239 challenge.

Results: After challenge, 3 out of 5 Gag-vaccinated and 3 out of 6 Vif/Nef-vaccinated animals controlled SIV replication. The SIV control was associated with Gag-specific CTL responses in the former and Vif-specific CTL responses in the latter.

Conclusion: This is the first report indicating efficacy of vaccine-induced Vif-specific CTL responses against SIV replication. Our results imply that not only Gag but also Vif may be a promising antigen for T-cell based AIDS vaccines.

P02.04

Induction of HIV-1 Gag-Specific Memory T Cells in Chacma Baboons by MVA Prime and VLP Boost Vaccine Regimen

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Background: We previously reported induction of HIV-specific responses in Chacma baboons following immunization with SAAVI MVA-C (MVA) and HIV-1 Pr55 Gag virus-like particles (VLPs) in a prime-boost vaccination strategy. In the current study, we characterised the vaccine specific memory T cells by flow cytometry.

Methods: Peripheral blood mononuclear cells (PBMC) from baboons primed with MVA and boosted with VLPs (n=3) or vaccinated with VLPs only controls (n=2) were stimulated with HIV-1 Gag peptide pools. T cell cytokine production (multiplex TNF-a, IFN-y and IL-2) and memory phenotype was determined by flow cytometry. Human anti-CD28 and CD95 antibodies were used to delineate effector memory (Tem) and central memory (Tcm) T cells.

Results: Vaccine specific memory responses were detectable one week after MVA prime. At peak T cell response (four weeks after VLP boost), the frequency of cytokine producing cells in prime-boost animals (mean response: 0.21%±0.012 and 0.242% ±0.049 of CD4+ and CD8+ cells respectively) was higher than in control animals (mean response: 0.066%±0.005 and 0.034%± 0.016 of CD4+ and CD8+ cells respectively). Gag-specific CD4+ cells from the prime-boost animals were significantly skewed towards a Tcm phenotype (>95%) of total cytokine responses compared to the Tem phenotype (<2%). A similar memory distribution profile of Gag-specific CD4+ cells was maintained 20 weeks after the VLP boost. At this time, Gag-specific CD8+ cells were evenly distributed between Tcm (~40%) and Tem (~60%) phenotypes. Vaccine specific memory responses were preserved 20 weeks after the VLPs boost (mean: 0.128%±0.025 and 0.147%± 0.039 of CD4+ and CD8+ cells respectively) in the prime-boost animals.

Conclusion: In conclusion, the MVA prime and VLP boost induced Gag-specific cytokine producing Tcm and Tem defined by expression of CD28 and CD95. These cells were detected up to 20 weeks post vaccination suggesting these vaccines could be potential HIV-1 vaccine candidates.

Topic 2: Animal Models and Preclinical Trials

P02.05

Construction of SHIVs Expressing Quaternary Neutralization Epitopes in Env and Mimicking the Neutralization Phenotype of Typical HIV-1 Isolates

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Background: Infection of macaques with chimeric Simian/Human Immunodeficiency Viruses (SHIVs) provides a powerful model for HIV pathogenesis and vaccine development. A limitation of the SHIVs routinely used for vaccine studies, e.g. SHIV-1157ipd3N4, SHIV-SF162P3, and SHIV-BaL, is that they don't express quaternary neutralization epitopes (QNEs) that are targets of broadly neutralizing antibodies like PG9, PG16, and CAP256 sera. We sought to introduce the QNEs into functional SHIVs to study how such antibodies develop during infection and help guide the creation of antigens capable of eliciting such antibodies.

Methods: Pseudovirus neutralization assays were used to map the QNE-resistance determinants of SHIV-1157ipd3N4 Env and to test the sensitivities of mutant derivatives of SHIV-1157ipd3N4 and SHIVSF162P3 to anti-QNE antibodies. Full-length SHIVs expressing the mutant Envs were tested for growth in PBMCs, and high titre stocks from those that grew were used to infect pigtail macaques.

Results: The double mutant SHIV1157 Env, Q170K/I192R, gained sensitivity to PG9, PG16, and CAP256 while maintaining resistance to CD4bs and anti-V3 antibodies. The full-length SHIV expressing this Env was able to replicate in macaque PBMCs while maintaining sensitivity to these antibodies, indicating there was no selection against these targets in the monkey cells. An update on the course of infection will be provided. Six changes in V2 (S164E, G166R, N167D, M169K, Q170K and N192R) were required to introduce the QNEs into SHIV-SF162P3 Env, and these dramatically increased sensitivity to anti-QNE antibodies. However, the full-length SF162P3 SHIV carrying this mutant Env failed to grow in macaque PBMCs; the basis of this growth defect is being studied.

Conclusion: We created an infectious QNE-expressing SHIV with overall neutralization phenotypes similar to those of typical primary HIV-1 isolates. This SHIV will allow the analysis of the development of anti-QNE antibody responses during the course of infection, and should provide an improved model for HIV-1 vaccine evaluation.

P02.06

SIVconsv DNA Prime - TLR7/IFNα Adjuvanted Long Peptide Boost Induces Potent CD4+ Ab Responses and Protects Against High Dose Intrarectal SIV Challenge

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Background: Because of the extreme variability of the HIV-1 genome, a successful vaccine has to effectively recognize diverse infecting HIV-1 strains in the population and must deal with ongoing virus escape in infected individuals. We have selected conserved regions within SIV, corresponding to HIVconsv (Letourneau PLoS ONE 2007) that show little variation across the different isolates and incorporated these in a peptide based vaccine strategy.

Methods: In this protocol rhesus macaques were immunized subcutaneously with 46 synthetic peptides of about 30 amino acids in length, which contain epitopes that can trigger helper as well as cytotoxic T-cell responses. Peptides were formulated in Montanide ISA-720. Pegylated Type I interferon plus Imiquimod, which triggers innate responses via toll like receptor 7, were given locally at the vaccine sites as additional immune stimulatory signals. Peptides were either given alone or after two times priming with DNA expressing the same conserved regions from a RNA and codon optimized gene.

Results: After two immunizations with only peptides strong immune responses of 2000-3000 spot forming units (SFU) per 10*6 PBMC were observed. DNA priming followed by peptide boosting generated up to 8000 SFU/10*6 PBMC, comparable to what is achieved in reported adenoviral vector systems. Predominantly CD4 T-cell responses were generated (up to 10% of cells reactive to SIVmac251), which were polyfunctional (30% IFNgamma/IL-2/TNFalpha triple production), and mainly mediated by central memory T-cells. Furthermore, peptide specific antibody responses were induced, capable of recognition of Env protein. Eight weeks after the last immunization, animals received a high dose intrarectal SIVmac251 challenge. 2/6 animals in the DNA prime/peptide boost group were protected against infection, while all 6 animals in the control group and the peptide only group were infected.

Conclusion: Vaccine induced strong CD4/antibody focused immune responses directed against conserved regions of SIV afford protection against high dose intrarectal SIVmac251 challenge.

P02.07

Modelling the Neuropathological Consequences of HIV Vaccines That Confer Partial Protection

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Background: Effective management of peripheral viral loads with anti-retroviral drugs can delay or halt CD4 cell loss for decades. However, patients still face the potential of developing significant HIV-1 associated neurocognitive disorders (HAND). Challenges obtaining relevant clinical samples limit the detailed investigation of the aetiology and neuropathology of HAND and therefore we do not understand the impact of vaccines that confer partial protection on long term neuropathology.

Methods: We are using immunohistochemical analysis of brain sections from the experimental infection of macaques with SIV to model neuropathology in situations where peripheral viral loads are under effective control.

Results: We have established that chronic infection with attenuated SIV, where peripheral viral loads are below detection, still results in pathological changes (astrogliosis, microglial activation, viral persistence). We have now extended these studies using a unique conditional live attenuated, doxycycline dependent virus, SIVrtTA. Removal of doxycycline 3 weeks after infection, at the end of the primary viremia results in detectable neuropathological changes in astrocytes, oligodendrocytes, microglia and perivascular macrophage 40 weeks later.

We are also investigating the effects of vaccines that confer partial protection on the neuropathology of wild-type SIV infection. A vaccine study using SIV Gag based vaccines comprising three primes with rDNAgag followed by a rAdgag boost resulted in significant delay in acquisition of SIVmac251 administered by low-dose intra-rectal challenge. Furthermore, there was a significant blunting of the primary viremia, but no significant suppression of set-point viral loads compared with naive challenge controls. We are determining the impact of vaccination on the frequency of virus infected cells in the brain collected 20-30 weeks after infection and also comparing the frequency and intensity of neuropathological changes in infected vaccinated and control macaques.

Conclusion: These data will enable us to establish the likely neurological benefit of vaccines that do not provide protection against detectable infection.

P02.08

Optimizing Delivery of HIV-1 Conserved Region-Derived Immunogen for Induction of T and B Cell Responses in Rhesus Macaques

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Background: The complexity of candidate HIV-1 vaccine formulations is increasing due to extreme challenges faced when trying to prevent or control HIV-1 infection.

Methods: Immunogen HIVconsv based on the most conserved regions of the HIV-1 proteome was used to explore combinations of seven distinct vaccines modalities in heterologous prime-boost regimens delivered to rhesus macaques to optimize induction of T cell and antibody responses. These include plasmid DNA (P), Semliky Forest virus replicons delivered as DNA (DREP; D) or virus particles (VREP; V), modified vaccinia virus Ankara (MVA; M), adenoviruses of human (HAdV-5; A) and chimpanzee origin (ChAdV-63; C) and adjuvanted synthetic long peptides (SLP; S).

Results: A number of observations were made. Thus, a very potent combination for induction of HIV-1-specific T cells was an adenovirus vector (A or C) followed by poxvirus M. S boost broadened T cell responses, but did not prime T cells efficiently. D was a stronger prime than P. PPP was the best prime for T cells, while PSS was best for induction of antibodies. Even very complex regimen PPPAMSSCMV continued to recruit new T cell clones into the response to a single epitope, although a ceiling for immunodominant responses was reached; subdominant responses could be boosted up to the last V delivery. Finally, PPSS, but not SSSS could protect 2/6 animals from SIVmac251 acquisition.

Conclusion: These results will guide initial design of human trials. So far, human studies in Oxford testing CM, PPPCM and PPPMC regimen concur with observations made in rhesus macaques.

Topic 2: Animal Models and Preclinical Trials

P02.09

A Single Dose of SAAVI MVA-C Re-boosts Rhesus Macaques After More Than 3 Years Post DNA-MVA Prime-Boost Vaccination

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Background: We have previously reported induction of robust immune responses in rhesus macaques following a prime boost immunization with candidate HIV-1 vaccines, SAAVI DNA-C (DNA) and SAAVI MVA-C (MVA). These vaccines are already in clinical evaluation. In the current study, we investigated whether re-boosting these animals with a single MVA inoculation after more than 3 years was sufficient to restore previous magnitudes of HIV-specific immune responses.

Methods: Seven rhesus macaques which had been vaccinated with three doses of DNA vaccine (4mg DNA/dose) and two doses of MVA (10^9 pfu MVA/dose) in a past study, >3 years previously, were re-boosted with a single dose of MVA. HIV-1-specific responses were quantified in the peripheral blood using an IFN-gamma ELISPOT assay.

Results: A peak magnitude of response (1146±240 sfu/10^6 PBMC) was reached 1 week after vaccination with the first dose of MVA. The second MVA inoculation did not increase these responses which declined to undetectable levels by 1 year post vaccination. After re-boosting with MVA after 3.5 years post the second MVA, all animals responded, with a peak response (1824±672 sfu/10^6 PBMC) being reached 1 week after vaccination. Although the mean magnitude of the second peak was not significantly higher than the one seen in the first peak, boosting of responses in 3 of 7 animals with an apparent broadening of the breadth of responses was observed.

Conclusion: These preliminary data suggest a long-term preservation of vaccine memory following a prime-boost vaccination regimen with SAAVI DNA-C and SAAVI MVA-C vaccines.

P02.10

Maturation of Protective Immunity Induced by SIVΔnef Correlates with Differential Expression of Transcription Factors in SIV-specific CD8+ T Cells

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Background: Protective immunity against vaginal challenge in SIVΔnef-vaccinated macaques develops at 20 weeks after vaccination, whereas the magnitude of SIV-specific CD8+ T cell responses peaks at 5 weeks. SIV-specific CD8+ T cells phenotypically mature from week 5 to 20, as characterized by upregulation of CCR7 and CD127, suggesting that the quality of the CD8+ T cell response may correlate with protection.

Methods: Highly parallel qRT-PCR was used to characterize the expression of 21 transcription factors (TFs) in T cells sorted into naïve, central, transitional, and effector memory subsets, and in SIV Gag CM9 and Tat SL8-specific CD8+ T cells obtained at wk5 and wk20 after SIV239Δnef vaccination.

Results: Unsupervised clustering organized T cell samples into groups concordant with cell surface phenotype. SIV-specific CD8+ cells segregated into wk5 and wk20 clusters. 11 of 21 TFs were expressed at significantly different levels at wk20 than at wk5. Wk20 cells exhibited increased levels of TFs associated with both quiescence and maintenance of effector function. Furthermore, 7 TFs were significantly differentially expressed between SIV Gag and SIV Tat-specific wk20 populations. Principal component analysis suggests the Gag-specific cells may be more effector-like and the Tat-specific cells more transitional or central memory-like.

Conclusion: Our data indicate distinct transcriptional profiles of different memory T cell subsets and clear differences between wk5 and wk20 SIV-specific CD8+ T cell transcriptomes. The mature wk 20 CD8+ T cell response temporally correlated with protection is characterized by the expression of transcription factors associated with both central memory and effector memory T cells. Additionally, wk20 Gag-specific cells exhibit a more effector-like expression profile than Tat-specific cells, which is consistent with the Tat epitope exhibiting more rapid CTL escape kinetics than the Gag epitope. Analysis of transcription factor expression therefore provides a valuable complement to the analysis of memory cell differentiation based on classical phenotypic markers.

P02.11

Mapping of SIVmac T-Cell Epitopes in Cynomolgus Macaques Immunized with Auxo-GTU-MultiSIV DNA by the Intradermal Route Followed by Electroporation

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Background: Intradermal immunization with electroporation using auxo-GTU-MultiHIV DNA encoding Gag, Nef, Rev and Tat induces strong and sustained T cell responses in cynomolgus macaques. Here, we used an equivalent vaccine encoding SIVmac239 antigens (Gag, Nef, Rev, Tat, Vif, Vpx and Vpr) and we described the breath of this T cell response.

Methods: Naïve male mauritian cynomolgus macaques were immunized with auxo-GTU-MultiSIV DNA encoding Gag, Nef, Rev and Tat (n=14) or by a combination of auxo-GTU-MultiSIV DNA and auxo-GTU-SIV-vifvprvpx (n=12) by the intradermal route followed by electroporation. MHC class I and class II haplotypes were determined by microsatellite analysis. T-cell epitopes were mapped by IFN-γ ELISPOT in PBMC by using matrix of 15-mer (overlapping by 11) SIVmac239 Gag, Nef, Rev, Tat, Vif, Vpx and Vpr peptides. Sequence and length of candidate epitopes were further optimized.

Results: Immunization induced intense and sustained IFN-γ ELISPOT responses in peripheral blood. T-cell responses against MHC class I epitopes were evidenced: two in Gag p15 (KA10(28-37) presented by Mafa-B*011:01 and EL10(58-68) presented by a MHC-Ia molecule from haplotype M3), two in Gag p27 (HL9(146-154) presented by Mafa-B*075:01 and LA9(189-197) presented by a MHC-Ib from haplotype M1), three in Nef (AS10(4-13) presented by a MHC-Ib molecule from haplotype M3; RM9(103-111) presented by Mafa-A1*063:02; LD10(146-155) presented by a MHC-Ia from haplotype M1M2M3), one in Rev (SP10(59-68) presented by Mafa-B*075:01), one in Tat (CF9(59-67) presented by Mafa-A1*063:02), four in Vif and two in Vpx. One MHC class II epitope was identified in Gag p27 (haplotype M3). Among these, to our knowledge, AF11 is a newly described epitope in cynomolgus macaques.

Conclusion: Auxo-GTU-MultiSIV DNA vaccination followed by electroporation induced multi-epitopic T cell responses, essentially CD8+ but also CD4+. Determination of SIV T-cell epitopes in cynomolgus macaques facilitates the monitoring of specific immune responses and pre-clinical vaccine development in this model.

P02.12

Development of a HIV-1 Vaccine Using an Orally-Administered, Replication-Competent Adenovirus Serotype 4 Vector Expressing Env Clade C Glycoprotein

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Background: Our hypothesis is that the replicating Ad4 vector approach, may be the best strategy for an effective HIV-1 vaccine due to advantages of demonstrated clinical safety and immunogenicity of both the Ad4 backbone and an Ad4 H5N1 vector influenza vaccine evaluated in Phase 1. Unlike other vectors, it can be bioengineered to express full-length HIV-1 Env gp160. More than 50% of global HIV-1 infections are caused by clade C viruses and therefore we initiated development of Ad4-Env160 vaccine using an Env clade C sequence obtained from CHAVI.

Methods: The Ad4-Env viruses were evaluated for: 1) genetic stability; 2) Env protein expression by Western blot analysis; 3) cell-surface Env recognition by broadly neutralizing antibody (bnAb); and 4) immunogenicity in rabbits.

Results: Genetically stable Ad4 recombinant viruses were generated which expressed the Env gp120, gp140, and gp160 proteins. A549 cells infected with Ad4-Env160 virus expressed cell-surface Env that was recognized by bnAb specific for MPER, CD4bs, V2-V3 loop sequences. Following immunization of rabbits, Env-specific binding antibodies were induced as measured by ELISA; 160>140>120.

Conclusion: An Ad4 virus expressing full-length Env160 was generated and evaluated for genetic stability, protein expression, recognition by bnAb and immunogenicity. These results represent substantial progress towards defining a replicating Ad4 vector, recombinant protein vaccine prime/boost approach for HIV-1 that could eventually undergo clinical testing. Funding: NIH/NIAID SBIR 1R43AI091546-01; NIH/NIAID Contract No. HHSN266200400045C.

Topic 2: Animal Models and Preclinical Trials

P02.13

Differential Induction of Activation and Apoptosis by TCR Signaling in Sooty Mangabeys and Rhesus Macaques

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Background: We previously showed that an increase in CD4+ T lymphocyte apoptosis and elevation of plasma tumor necrosis-receptor associated apoptosis-inducing ligand (TRAIL) occurs in rhesus macaques (RM) but not sooty mangabeys (SM) during acute SIV infection.

Methods: To further examine the mechanisms underlying differential apoptosis in SIV-infected RM and SM, we compared the in vitro responses to TCR signaling in seven SIV-negative SM and seven SIV-negative RM. PBMC were cultured for 18 hours with cross-linked CD3 and CD28 and subsequently analyzed by flow cytometry for upregulation of CD69, TRAIL and active Caspase 3, and for production of cytokines.

Results: Following TCR stimulation, there was a significant increase in TRAIL on T cell subsets, NK cells and myeloid DC cells (mDCs) in both species. However, levels of membrane TRAIL were significantly higher in CD8+ T lymphocytes and NK cells of SM compared to RM suggesting that they may be more cytotoxic on activation. TCR stimulation also resulted in an upregulation of CD69 and production of IFNγ, IL-2 and TNFα by T cell subsets in both species with greater levels being observed in SM. In contrast to SM, RM showed significantly higher frequencies of apoptotic mDCs both ex vivo and following TCR stimulation. In vivo inoculation of RM with SIVmac239 resulted in increased frequency of ex vivo apoptotic mDCs at 2-3 weeks post-infection. Increased apoptosis was also observed after overnight culture in medium but was abrogated by addition of soluble death receptor 5 indicating that it was TRAIL-mediated.

Conclusion: Overall, these data show an increased susceptibility to apoptosis of mDCs in RM, and a disconnect between T cell activation and apoptosis in SM. Elucidating the mechanisms by which SM are protected from apoptosis will be important for understanding the basis of nonpathogenicity in natural hosts of SIV infection.

P02.14

Assessing the Protective Efficacy of Antibodies to the HIV gp41 Region by Active Vaccination

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Background: The gp41 cluster I is a conserved immunodominant loop connecting the heptad repeat 1 (HR 1) and heptad repeat 2 (HR2) of the HIV-1 the envelope glycoproteins (Env). Following HIV-1 infection or vaccination with gp41-containing Env immunogens, this region elicits relatively high titers of antibodies generally considered to be non-neutralizing in nature. However, in a recent passive immunization study using a cluster 1 antibody, partial protection against SHIV SF162 P4 challenge was observed. In the present study, we sought to determine if vaccine-elicited cluster 1 antibodies might afford some protective capacity by active vaccination, presumably by binding to non-functional spikes on the virus and slowing the viral entry process in vivo.

Methods: To generate cluster 1-specific antibodies, we added residues flanking the cluster I cysteine-loop region to allow it to assume its preferred structural conformation. The resultant 20 residues peptides were expressed on the genetically modified Q-beta bacteriophage particles and also chemically coupled to KLH. Sera from rabbits immunized with these antigens were analyzed by ELISA for the binding to cluster 1 peptides and by cross-competition with the known cluster I antibodies.

Results: The cluster I region was found to be immunogenic and, interestingly, a version of the epitope in which alanines were substituted in place of the small cysteine-linked loop was found to be more immunogenic than the wild-type cysteine-cysteine motif. The sera from rabbits inoculated with either carrier cross-competed with the known cluster I antibodies such as F240. Though the sera did not neutralize JR-FL viruses, they serum antibodies were able to capture many different viruses in vitro.

Conclusion: We conclude that we have specifically elicited antibodies directed to the cluster 1 region of gp41 possessing properties similar to the known monoclonal antibodies. Active immunization of non-human primates by both the intranasal and intramuscular routes, followed by SHIV

P02.15

Expression and Function of FcγRII on NK Cells in Rhesus Macaques

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Background: Natural Killer (NK) cells play a crucial role in ADCC response through FcγRⅢa (CD16), which is able to bind to Fc region of IgG displayed on target cells. The presence of another class of low-affinity receptor for IgG, called FcγRⅡ(CD32), has also been reported in human. It raises the possibility of CD32 expression on NK cells in non-human primate models. Efforts to get a clearer understanding of the CD32 marker which might be related to ADCC function of NK cells in rhesus macaques will be helpful in evaluation of vaccines in non-human primate models.

Methods: PBMCs were obtained from a total of 42 healthy macaques in the study and cellular immunology of NK cells was analyzed by flow cytometry. CD32 Blocking antibodies were added to PBMCs for FcγRⅡ blockade assay.

Results: The expression of CD32 was observed on macaque NK cells (Median, 1.45%; range, 0.96%-2.20%) and was negatively correlated with CD16 expression ($P<0.001$). The downregulation of CD16 after stimulation by antibody-coated target cells was viewed as an important indicator of NK-cell activation, for its strong positive relationship with CD107a, IFN-γ or TNF-α measured in ADCC assay ($P<0.001$ for all). Either the percentage or MFI of CD16 expression on NK cells decreased in the presence of CD32 blocking antibodies, especially in early stage of ADCC. Surprisingly, after CD32 blocking, the percentage of CD107a+ NK cells shifted from increase at the very beginning of ADCC activity ($P<0.05$) to decrease when NK cells approached the complete activation by antibody-coated target cells ($P<0.01$).

Conclusion: $CD32^+$ NK cells defined a novel NK-cell subset in macaques and we found that CD32 had dual function with a time-course manner in ADCC activity. CD32 may attenuate or slow down the activation of macaque NK cells at first, then up-modulate function of NK cells at the late stage of ADCC.

P02.16

Protection Against Highly Pathogenic SIV by BCG-SIV Recombinant Priming and Attenuated Replicating Vaccinia-SIV Recombinant Boosting

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Background: We constructed recombinants of the Japanese-licensed Tokyo 172 strain of BCG and the replication-competent vaccinia virus strain LC16m8Δ (m8Δ) (a genetically stable variant of Japanese licensed smallpox vaccine LC16m8) to express SIV genes. We then evaluated the protective efficacy of these recombinants against challenge with pathogenic, neutralization resistant SIVmac251.

Methods: Indian rhesus macaques were immunized with rBCG-SIV expressing SIV Gag, Env, or Rev-Tat-Nef (RTN) fusion proteins via subcutaneous injection, followed with two boosts with the Gag-, Env-, RT-, or RTN-expressing m8Δ by skin scarification. Eight weeks after the 2nd boost, macaques were challenged up to 5 times with a low dose of SIVmac251 by the rectal route. Cellular and humoral immune responses were analyzed by standard methods. Plasma SIV RNA and cell-associated SIV proviral DNA in peripheral blood and various tissues were monitored by quantitative PCR.

Results: Env binding antibodies were elicited at similar levels in all vaccinated macaques after m8Δ boost, but neutralizing Ab against SIVmac239 was not detected. Robust SIV-specific CD4+ and CD8+ effecter memory T cell responses were elicited and maintained at high level until SIV challenge. One vaccinated animal had potent CD8+ T cells that suppressed SIVmac239 replication in vitro. Plasma viraemia was not detected in this animal, even after CD8+ T cell depletion throughout the follow-up period. Protection was confirmed by lack of detectable cell-associated provirus in various organs. A second vaccinated monkey became infected, but viral load remained from one to two logs lower than control monkeys.

Conclusion: Vaccine-induced SIV specific T cell responses appear to be effective against SIV challenge. Importantly, our results suggest that a vaccine regimen based on an rBCG prime and vaccinia m8Δ boost (both liscensed vaccine platforms with a long track record of safety in humans) should be explored as a safe and valuable means for efficacious HIV/AIDS vaccine

Topic 2: Animal Models and Preclinical Trials

P02.17

A Prime-Boost Immunization with rBCG Expressing HIV-1 Gag, RT and gp120 and SAAVI MVA-C Elicits Immune Responses in Blood and MALT of Rhesus Macaques

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Background: BCG pantothenate auxtroph (ΔpanCD) is safer to use as a live vaccine vector than wild-type BCG. We constructed 3 recombinant BCGΔpanCD candidate vaccines expressing HIV-1 subtype C Gag, RT and Env (gp120). The current study investigated immune responses in rhesus macaques following a prime with a mixture of these rBCG vaccines and a boost with SAAVI MVA-C (MVA).

Methods: Chinese rhesus macaques (n=8) were primed twice with a mixture of rBCG, 12 weeks apart. A control group (n=4) was mock-primed with a control BCG. Both groups were boosted with MVA. Two weeks after the MVA vaccination, two macaques from the rBCG-primed group were euthanased and jejunum, spleen and inguinal, mesenteric, iliac and bronchial lymph nodes were harvested for isolation of mononuclear cells. HIV-1-specific IFN-gamma ELISPOT responses were measured in the blood and these tissues using pools of overlapping HIV-1 peptides.

Results: Vaccination with rBCG elicited modest HIV-specific responses in the blood in 5 of 8 animals, 4 of which responded after the first rBCG vaccination. These responses were to either Env or to both Env and Gag proteins and the cumulative responses ranged from 50 to 172 sfu/10^6 PBMC. After boosting with MVA, HIV-specific responses were detected in 6 of the 8 animals (mean: 932±1100 sfu/10^6 PBMC). These responses were directed to Gag, RT, and Env proteins but not Nef or Tat. No responses were detected in the control animals before or after MVA vaccination. At necropsy, HIV-specific responses were detected in the peripheral blood, spleen, inguinal, iliac and bronchial lymph nodes of 1 of 2 animals. The cumulative responses ranged from 112 to 714 sfu/10^6 cell input.

Conclusion: These data demonstrate that our rBCGΔpanCD candidate vaccines, when given in a prime-boost combination with SAAVI MVA-C, induce vaccine-specific immune responses in both the peripheral blood and MALT.

P02.18 LB

Passive Immunization With Polyclonal Anti-SHIV Igg: Partial Protection or Increased Acquisition of Heterologous Tier 2 SHIV – Depending on Igg Dose

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Background: While passively administered broadly neutralizing monoclonal antibodies (bnmAbs) prevented SHIV acquisition, polyclonal Abs with high neutralizing titers provided only moderate protection in primates.

Methods: We tested whether passive immunization with polyclonal IgG raised in rhesus monkeys (RMs) with chronic clade C SHIV infection, termed SHIVIG, could protect RMs against multiple low-dose intrarectal challenges with the R5 tier-2 SHIV-2873Nip carrying an HIV clade C envelope heterologous to the viruses/envelopes against which the IgG responses had been elicited. We compared in vitro SHIVIG characteristics with in vivo protection.

Results: In vitro, SHIVIG demonstrated binding to SIV Gag, HIV Tat and Env of different clades, contained b12 and 4E10-like Abs and neutralized tier-1 and 2 viruses, including SHIV-2873Nip. NK-cell depletion decreased neutralizing activity in PBMC assays 20-fold. SHIVIG completely inhibited viral replication by ADCVI assay, but showed only 35% target-cell killing by ADCC assay.

Four groups of RMs were given SHIVIG at different doses: Group 1 (400 mg/kg), Group 2 (675 mg/kg), Group 3 (25 mg/kg) and Group 4 (none; virus-only control) followed by weekly low-dose challenges with SHIV-2873Nip. All controls and all SHIVIG-treated animals became systemically infected. RMs given 400 mg/kg of SHIVIG showed significantly lower peak viral RNA loads compared to controls. Surprisingly, single-genome analysis revealed a significant increase in the number of transmitted variants in Group 3 compared to controls (P=0.032), suggesting increased acquisition. Complement-mediated Ab-dependent enhancement of infection (C'-ADE) at low SHIVIG concentrations was observed in vitro.

Conclusion: Lack of protection and possibly increased acquisition has been reported for a passive immunization study that tested the efficacy of HIV hyperimmune globulin in preventing infection in Ugandan infants born to HIV-positive women (Onyango-Makumbi, JAIDS 2011). Thus, our primate model data paralleled clinical phase III results and suggest that polyclonal anti-HIV-1 Abs play a dual role upon virus encounter.

P02.19 LB

Vectored ImmunoProphylaxis Protects Humanized Mice from Mucosal HIV Transmission

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Background: Recently, a number of antibodies capable of broadly neutralizing HIV have been isolated from HIV infected patients, stimulating efforts to develop vaccines capable of eliciting their production in naive individuals. As an alternative to vaccination, we recently described vectored immunoprophylaxis (VIP) as an approach capable of generating high serum concentrations of a desired monoclonal antibody in mice following a single intramuscular injection of a specialized adeno associated viral vector (AAV). Mice that received VIP encoding b12 and VRC01 antibodies demonstrated long-term circulating antibody expression in serum, and VIP-treated humanized mice exhibited remarkable protection against high dose, intravenous challenge with CXCR4-tropic HIV. However, most human infections are initiated by transmission of CCR5-tropic strains through mucosal tissues.

Methods: To measure the efficacy of VIP against clinically relevant strains, we humanized VIP-treated mice by adoptive transfer of peripheral blood mononuclear cells (PBMC) and challenged these animals with CCR5-tropic HIV strains including JR-CSF, as well as REJO.c, a transmitted molecular founder. To determine the ability of VIP to prevent mucosal transmission of HIV, we developed a repetitive intravaginal challenge model in VIP-treated BLT humanized mice that were challenged weekly with JR-CSF and monitored for infection.

Results: PBMC humanized mice expressing either b12 or VRC01 were protected from intravenous challenge with JR-CSF. In contrast, the b12-resistant REJO.c strain readily infected PBMC humanized mice expressing b12 antibody, while mice expressing VRC01 demonstrated nearly complete protection following challenge. Intravaginally challenged BLT animals expressing a luciferase negative control protein all became infected over the study period while a majority of animals expressing VRC01 had no detectable HIV infection despite fourteen intravaginal challenges with JR-CSF.

Conclusion: VIP is capable of protecting humanized mice from challenge by diverse HIV strains and can substantially inhibit mucosal transmission. These findings warrant continued development of VIP as a novel approach for HIV prevention in humans.

P02.20 LB

Characterization in Rabbits & Nonhuman Primates Of The Neutralizing Antibody Response Elicited by DNA & Protein Vaccination With SIVmac251 & SIVsmE660

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Background: Partial success of the human RV144 clinical trial underscored the importance of envelope antibody responses for an effective HIV-1 vaccine. Immunogenicity studies with SIV envelope proteins typically neutralize TCLA isolates. Properly folded trimeric envelope proteins delivered with appropriate adjuvants may successfully elicit antibodies with broad neutralization specificity

Methods: We evaluated immunogenicity of DNA and protein vaccines encoding SIVmac251 and SIVsmE660 gp145 in rabbits and rhesus macaques. DNA vaccines encoding wild type gp145 or mutated gp160 truncated at Q708 were used. Trimeric wildtype gp145 proteins, stably expressed and purified from 293T cells, were used with Advax delta inulin adjuvant to boost after DNA immunization. Macaques were electroporated with wild type DNA of both isolates followed by adjuvanted homologous protein boosts. Rabbits received DNA vaccine alone, delivered by electroporation. Neutralization assays were performed in TZM-bl cells with SIVmac251 and SIVsmE660 isolates that are partially resistant to neutralization.

Results: Anti-envelope responses to SIV mac251gp145 and SIVsm660 gp145 were detected in macaque sera following DNA immunizations and response were enhanced significantly after adjuvanted homologous gp145 protein boost. At peak response, low to moderate neutralizing activity was observed against SIVmac251/M766, SIVsmE660-BR/CG7V and SIVmac251.30 clones. Immunization of rabbits with DNA encoding, either wild type or mutated envelope elicited strong antibody responses and sera neutralized both SIVmac251/766 and SIVsmE660-BR/CG7V to a limited extent, with a comparable response noted for both wild type and mutant envelope.

Conclusion: Antibody response elicited by DNA prime/Advax adjuvanted protein boost vaccine with oligomeric envelopes from both SIVmac251 and SIVsmE660 neutralized SIVmac251 and SIVsmE660 isolates of partially resistant phenotype. These envelopes together with other antigens that elicit cellular responses will be tested against SIV challenge in future efficacy studies.

Topic 3: B Cell Immunology and Antibody Functions

P03.01

The Breadth of Maternal HIV-1 Specific Neutralizing Antibodies Is Not Associated with a Lower Risk of Mother-to-Infant Transmission

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Background: It has been hypothesized that neutralizing antibodies (nAbs) should have broad specificity to be effective in protection against diverse HIV-1 variants. The mother-to-child transmission of HIV-1 is a model that provides the opportunity to examine whether the breadth of maternal nAbs would be associated with protection of infants from infection.

Methods: Samples were obtained at delivery from 57 transmitting mothers (T) matched with 57 non-transmitting mothers (NT) enrolled in the multicenter French Perinatal Cohort (ANRS EPF CO1) between 1990 and 1996. The mothers did not receive antiretroviral therapy during pregnancy, and did not breastfeed their infants. Sixty-eight (59.6%) and 46 (40.4%) women were infected by B and non-B viruses, respectively. Neutralization assays were carried out in TZM-bl cells using a panel of 10 primary isolates of 6 clades (A, B, C, F, CRF01_AE, CRF02_AG) selected for their moderate (tier 2) or low (tier 3) sensitivity to neutralization. The presence and titers of Nab to each strain, and the breadth of maternal nAbs at delivery were compared between T and NT mothers.

Results: Although there was a trend for both higher frequency and higher titers of nAbs in NT mothers vs T mothers for almost all the primary isolates that were tested, the differences were not statistically different when considering the entire population. However a few statistically significant differences were observed with higher frequency or higher titers of nAbs toward several individual strains in NT mothers when analyzing separately the B-infected or non-B infected mothers.

Conclusion: Our study confirms that the breadth of maternal nAbs is not associated with protection of infants from infection. However it suggests that, depending of the population, some primary isolates could be indicators of nAbs associated with a lower risk of MTCT.

P03.02

Continuous Evolution of HIV-1 More Than Ten Years After Infection in an Elite Neutralizer

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Background: The viral evolution of HIV-1 and its escape to autologous neutralizing antibodies (Nabs) during the early years of infection have been analyzed in depth. In contrast, little is known about neither the long-term evolution of the virus in patients who developed broadly Nabs (bNabs) nor the mechanism of escape in presence of these bNabs.

Methods: We have studied the viral population infecting an HIV-1 infected long term non progressor (LTNP) who had developed Nabs toward all tier 2/3 viruses (6 clades) tested, 9 years after infection, and was then followed up over 7 years. Sixty-nine env clones issued from sequential blood samples collected from 9 years to 16 years post-infection were obtained. Thirteen infectious clones representative of the genetic diversity of variants present at the different time-points were selected. Pseudotyped viruses harboring these different envelopes were generated and their sensitivity to neutralization was analyzed.

Results: Evidence of ongoing viral evolution was found, supported by both the phylogenetic analyses that showed a continuous diversification and an increasing divergence overtime. The mean autologous neutralization titers of the sequential sera toward the 13 env variants significantly increased during the period of late follow-up. The env pseudoviruses displayed a broad range of sensitivity to the autologous sera, with the most resistant variant identified at the last visit suggesting that it represented a late emerging escape variant. We identified 5 amino acids substitutions that appeared associated with escape to bNabs. They were V319I/S, R/K355T, R/W429G, Q460E and G/T463E, in V3, C3 and V5 regions.

Conclusion: This study showed that HIV-1 may continue to evolve in presence of both broadly neutralizing antibodies and increasing autologous neutralizing activity more than 10 years post-infection. Such material may provide opportunities to reveal the molecular determinants of escape of HIV-1 to highly potent broadly neutralizing antibodies.

P03.03

V5 Region in the HIV-1 Envelope Glycoprotein Determines Viral Sensitivity to the Broadly Neutralizing Monoclonal Antibody VRC01

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Background: VRC01, a broadly neutralizing monoclonal antibody (bnmAbs), is capable of neutralizing a diverse array of HIV-1 isolates through recognition of the loop D and the V5 regions within the CD4 binding site on envelope glycoprotein gp120. Nonetheless, resistant strains have been identified. Here, we examined two closely related envelope clones derived at a single time point from a CRF08_BC infected patient which displayed an over 20-fold difference in VRC01 neutralization sensitivity.

Methods: A total of 15 chimeric envelope clones were generated by interchanging the loop D and/or V5 regions between the original envelopes or by single alanine substitutions within each region. The resultant effects on VRC01 neutralization sensitivity were subsequently studied in the context of pseudotyped viruses.

Results: Our results showed that interchanging the V5 region between the two clones completely swapped their neutralization sensitivity profiles, while exchanging the loop D region alone had minimal impact. Mutagenesis analysis revealed that the potential N-linked glycosylation site (PNGS) at position 460 in the V5 region contributed to over 90% of observed resistance, while other amino acid changes made no discernible differences. Furthermore, changes in resistance were found to positively correlate with VRC01 binding activity to the corresponding envelope glycoprotein. None of the substitutions, however, significantly altered binding and neutralization sensitivity to bnmAb b12 or soluble CD4. Of note, a mutation that removed the PNGS at position 463 in the V5 region increased resistance to ibalizumab, a non-immunosuppressive monoclonal antibody that binds CD4 and has been shown to inhibit entry of diverse HIV-1 isolates.

Conclusion: In summary, our data indicates that amino acid residues in the V5 region play a critical role in determining viral sensitivity to VRC01. Increased length, glycosylation and long side-chain of amino acids in the V5 region may collectively create steric hindrance that lowers binding affinity, thereby increasing resistance to VRC01 neutralization.

P03.04

Comprehensive Mapping of SIVmac239 Envelope Antigenic Determinants Recognized by Specific Polyclonal Antibodies in Vaccinated and/or Infected Macaques

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Background: The RV144 trial has demonstrated a modest level of protection against HIV infection, which was partially related to binding antibodies targeting V2 loop. Along with several broad HIV-neutralizing antibodies identified recently, it is of great importance to understand the major differences between vaccination and infection in eliciting humoral immune responses.

Methods: We adopted a robust mapping technique for a quantitative measurement of antigenic determinants of entire SIVmac239 envelope glycoprotein displayed on the surface of the yeast as a combinatorial antigen library. Positive yeast clones recognized by the immunized and/or infected serum were identified and obtained by FACS followed by sequencing and structural analysis.

Results: The finding of reactive determinants ranges from 30 to 240 amino acids, which allows some conformational determinants being evaluated as a major technical improvement. The antigen profile of the two types of vaccine-induced sera before viral challenge shared two dominant domains that concentrated at the V1V2 stem of gp120 and the ecto-domain of gp41. Interestingly, using a FACS-based antibody binding assay, there were no significant differences of titer and MFI of anti-V1V2 antibodies between the two vaccination groups, suggested a minimal role of these antibodies in controlling viral replication post SIVmac239 challenge. A major distinct domain, however, was identified near the V3 loop and the main CD4 binding region which was significantly recognized by the immune serum of the effective vaccination regimen but not of the non-effective vaccination regimen or natural SIV infection. Unexpectedly, the anti-V1/V2 antibody responses were shifted to the ecto-gp41 region when infected macaques developed AIDS.

Conclusion: We present a comprehensive analysis of antigenic determinants recognized by specific antibody responses generated by vaccination and/or SIVmac239 infection. Our findings have significant implications to help understanding the humoral immunity against neutralization-resistant SIVmac239 infection and simian AIDS, and to guide rational vaccine immunogen identification and design.

Topic 3: B Cell Immunology and Antibody Functions

P03.05

Recognition and Penetration of the HIV-1 Env Glycan Shield by Potent Broadly Neutralizing Antibodies

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Background: Human monoclonal antibodies have been characterized recently that potently neutralize HIV-1 isolates across all clades. These exciting new antibodies (PGT series) were derived from direct functional screening of B cells from IAVI protocol G donors (Theraclone/Monogram) and are unusually potent with binding predicted to be to novel glycan-dependent epitopes on Env.

Methods: Structures of these new PGT antibodies are being determined by x-ray crystallography and electron microscopy with further characterization using binding and mutagenesis assays.

Results: The crystal and EM structures so far have been elucidated for many of these antibodies. Work on the others are in progress, focusing on Fab complexes with glycans, gp120 core and fragment constructs, as well as Env trimers.

Conclusion: Structural characterization and biochemical analysis of these antibodies have uncovered novel specificities to new glycan-dependent epitopes and reveal further mechanisms for viral neutralization. These new epitopes provide additional insights for neutralization of HIV-1 and how antibodies can bind and penetrate the glycan shield, a novel framework for structure-assisted vaccine design.

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P03.06

Neutralization and Enhancement of Trans Infection by Erythrocyte-Bound HIV with Antibodies and Complement

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Background: Antibodies to HIV envelope glycoproteins, with or without neutralization when assayed in standard neutralization assays, are reported to have potential to exert either inhibitory or enhancing effects through interactions with complement and/or Fc receptors. Although most of the standard neutralization assays use free virus rather than carrier-bound HIV as the infectious agent; a cell line as the target for infection; and no complement (C), these conditions may not reflect in vivo events that would include antibody-dependent innate effector mechanisms.

Methods: We investigated the Fc-receptor-mediated and the complement-mediated antibody-dependent enhancement in trans infection neutralization assays using two broadly neutralizing monoclonal antibodies, 4E10 and b12; primary HIV; PBMC target cells naturally expressing Fc receptors and complement receptors; and with or without complement.

Results: In the absence of complement, the 4E10 mAb did not neutralize erythrocyte-bound HIV, and the b12 mAb neutralized erythrocyte-bound HIV less effective than the free virus. At low concentrations, 4E10 caused enhancement of infection. In contrast, in the presence of complement, 4E10 neutralized erythrocyte-bound HIV, or caused enhancement of trans infection of erythrocyte-bound HIV, depending on the mechanism of binding of HIV to erythrocytes.

Conclusion: Our results are consistent with the concept that the binding of HIV to erythrocytes represents a mechanism of establishing a “safe harbor” for HIV from the adverse effects of antibodies and complement that might otherwise be detrimental to free circulating HIV. Our data shows that broadly neutralizing antibody 4E10 can cause both C-mediated neutralization and enhancement of trans infection. This suggests that erythrocyte-bound HIV-1 serves as a sort of battleground between neutralization and enhancement by antibodies in the presence of C. Because of this, we propose that induction of neutralizing vs. enhancing antibodies can only be differentiated by utilization of a trans infection neutralization assay that might yield more relevant results for in vivo conditions.

P03.07

Design of Epitope-Specific Probes for Sera Analysis and Antibody Isolation

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Background: The design of gp120 monomeric probes with modified antigenic profiles that are specific for a target epitope has been successfully used for the isolation of broadly neutralizing HIV-1 antibodies. Existing probes, however, do not possess sufficient specificity and can bind antibodies with undesired properties (e.g., weakly neutralizing antibodies targeting an overlapping epitope). To achieve improved epitope specificity, positive and negative design stages can be incorporated into the probe design process.

Methods: Here, we apply a combination of structure- and sequence-based methods for improving the epitope specificity of gp120 monomeric probes. Specifically, structure-based redesign using the OSPREY protein design software suite was used to predict gp120 knock-out mutations for selected antibodies. Additionally, using a sequence-based mutual information approach, knock-out mutations were designed by identifying gp120 residues that are predicted to associate with neutralization resistance for a given antibody.

Results: Using stabilized (Ds12F123) and resurfaced stabilized (RSC3) HXB2 gp120 cores as templates, we designed a set of mutants with improved epitope specificity. In particular, RSC3 (a prototypic probe previously used for the isolation of VRC01 and other CD4-binding-site antibodies) was redesigned to specifically bind CD4-binding-site antibodies that are broadly neutralizing (VRC01, VRC-PG04: positive design) but not moderately/weakly neutralizing (b12, b13, HJ16: negative design). Additionally, CD4i-specific probes were designed by introducing mutations that destabilize binding to the entire class of CD4-binding-site antibodies (negative design), while retaining binding to CD4i antibodies (positive design). The desired epitope specificity of the redesigned probes was confirmed by ELISA binding. The probe design approach was further validated with knock-out mutations for a diverse set of antibodies, including PG9 and 2F5.

Conclusion: Probes with enhanced epitope specificity can select more precisely for antibodies with desired properties. A set of our redesigned probes are currently being utilized for the isolation of antibodies targeting different epitopes of interest on the HIV-1 Envelope.

P03.08

Critical Role for Monocytes in Mediating HIV-Specific Antibody-Dependent Cellular Cytotoxicity

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Background: Antibodies (Abs) that mediate antibody-dependent cellular cytotoxicity (ADCC) activity against HIV-1 are of major interest. Considerable evidence supports a role for ADCC activity in the control of HIV-1 infection and in the context of vaccination. One method widely used to assess the role of ADCC is the rapid and fluorometric antibody-dependent cellular cytotoxicity (RFADCC) assay. In the RFADCC assay specific killing of target cells by PBMC is assessed by loss of intracellular CFSE but retention of membrane dye PKH26 (CFSE-PKH26+), which is assumed to be derived from CFSE+PKH26+ target cells killed by NK cells. We have revisited this assay to assess the role of effector cells in mediating ADCC.

Methods: Multi-color flow cytometry was used to analyse gp140-pulsed, CFSE and PKH26 double labeled CEM.NKr-CCR5 cells incubated with HIV+ plasma or purified IgG samples (n=57) and co-cultured with PBMC, purified NK cells, or monocytes prepared from healthy donor blood. Effector/target cell interaction was visualized using image stream flow cytometry and live cell imaging.

Results: Backgating analysis and phenotyping of CFSE-PKH26+ cells identified CD3-CD14+ monocytes as the major effector cell type. This was confirmed for all 57 HIV+ plasma samples tested. Emergence of the CFSE-PKH26+ cell population was observed following co-culture with purified monocytes but not purified NK cells. No significant IFNγ production or CD107a degranulation was detected in NK cells in this assay. Image flow cytometry and microscopy confirmed a monocyte-specific interaction with target cells. Monocytes acquire PKH26+ cell membrane presumably derived from killed target cells without typical morphological changes associated with phagocytosis, suggesting monocyte-mediated ADCC.

Conclusion: Our studies advance the understanding of the cellular events underlying HIV-specific ADCC. The RFADCC assay primarily reflects Ab-mediated monocyte function and has to be treated with caution in regard to NK cell-mediated ADCC. Further studies on the biological importance of HIV-specific monocyte-mediated ADCC are warranted.

Topic 3: B Cell Immunology and Antibody Functions

P03.09

HIV-1 Envelope Glycoprotein Characteristics That Correlate with the Development of Cross-Reactive Neutralizing Activity in HIV-1 Infected Individuals

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Background: Cross-reactive neutralizing activity (CrNA) is elicited in around 30% of HIV-1 infected individuals and is most likely directed against conserved regions of the envelope glycoprotein complex (Env). We hypothesized that the induction of CrNA may at least to a certain extent depend on the phenotypic characteristics of Env from viruses early in infection.

Methods: We selected 34 patients from the Amsterdam Cohort Studies (ACS) who had varying levels of CrNA at 2-4 years after seroconversion (SC). We retrospectively generated Env sequences from clonal HIV-1 variants that were isolated between 2 and 14 months after SC and analyzed the length of the variable regions and the number of potential N-linked glycosylation sites (PNGS).

Results: For 31 out of 34 patients we observed a correlation between a higher level of CrNA and on one hand a shorter variable region 1 (P=0.04) and on the other hand a increased number of NXS sequons relative to the number of PNGS (P=0.04), which decreases the probability of glycosylation at that site. In contrast, in the 3 patients with the most potent CrNA, defined as elite neutralizers, the viral V1 region was longer with a higher number of NXT sequons relative to the number of PNGS. These viral characteristics are similar to those in patients with low potency of CrNA, rather than to those in patients with higher potency of CrNA in their serum.

Conclusion: Our results suggest that in general the development of CrNA in HIV-1 infected patients is associated with a more open structure of the viral Env, mediated by a short V1 loop and a low probability of glycosylation. However, our three elite neutralizers were exceptions to this rule. The identification and understanding of Env characteristics that are involved in the development of CrNA should help the rational design of an effective antibody-based vaccine immunogen.

P03.10

Cross-Group Neutralization of HIV-1 and Evidence for Conservation of the PG9/PG16 Epitopes Within Divergent Groups of HIV-1

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Background: HIV-1 has been classified into 4 groups: M, N, O and P. The aim was to revisit the cross-group neutralization using a highly diverse panel of primary isolates (PI) and human monoclonal neutralizing antibodies (mAb).

Methods: The panel of viruses included 9 HIV-1 group O PIs, 1 recombinant M/O PI, 1 group N PI, 1 group P PI, 2 group M (subtype B) PIs and the HIV-1 group M adapted strain MN. All the viruses were tested for neutralization in TZM-bl cells, using a panel of sera issued from patients infected by HIV-1 group M viruses (n=11), HIV-1 groups O (n=12) and P (n=1). The mAbs were b12, 2G12, 2F5, 4E10, PG9, PG16, VRC01, VRC03 and HJ16.

Results: The 12 group O sera neutralized from 1 to 6 group O viruses, and 6 of them cross-neutralized one group M PI. Five of the 10 group M sera cross-neutralized from 4 to 9 group O PIs. The group N and P viruses were neutralized by 1-4 of 12 and 4-5 of 11 sera from groups O and M patients, respectively. The human mAbs did not show any cross-group neutralization, except PG9 and PG16. Two group O PIs were neutralized by both PG9 and PG16, and one group O PI was neutralized by PG9 only. The group N PI was highly sensitive to neutralization by PG9 and PG16. The N-linked glycans at positions 156 and 160 and the cationic residues of strand C of the V1/V2 domain that have been identified as part of the PG9 epitope are conserved among the group N.

Conclusion: The cross-group neutralization of HIV-1 has been demonstrated. The conservation of the PG9 and PG16 epitopes between groups provides an argument for their relevance as components of a potentially efficient HIV vaccine.

P03.11

Residue 315 Regulates V3 Exposure and V3 Antibody Recognition on HIV Subtype B and C Viruses

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Background: V3 mAbs often neutralize HIV subtype B viruses but exhibit poor neutralizing activity against subtype C viruses. This limited activity is typically attributed to masking of the V3 region on subtype C viruses. However, while relatively much effort has been devoted to exploring accessibility of the V3 region on subtype B viruses, V3 exposure and the mechanism(s) that might restrict V3 exposure on subtype C viruses has yet to be understood. Here we have focused on exploring the significance of the conserved V3 tip motifs GPGR and GPGQ of subtype B and C viruses for antibody recognition.

Methods: Position 315 in representative subtype B (SS1196) and subtype C viruses (ZM249M, CAP45) was switched to Gln and Arg, respectively, to assess the effect of the conserved Arg/Gln at position 315 on V3-specific neutralization. Neutralization sensitivities of the parental and mutant viruses were assessed in a single-round pseudovirus neutralization assay using a panel of neutralizing V3-specific mAbs with varying fine specificities for the V3 tip.

Results: Subtype B virus SS1196 was neutralized by all V3-specific mAbs tested here (B4e8, 2219, 268-D, 2557, 3074 and HGN194). In contrast, though as expected, mutant SS1196_R315Q was resistant to neutralization by mAbs B4e8 and 268-D, both of which require the Arg at position 315 for binding. Unexpectedly, the remaining V3 mAbs were also unable to neutralize SS1196_R315Q, despite not requiring an Arg residue at position 315 for binding. For the subtype C viruses the exact opposite was observed; both ZM249M and CAP45 were generally insensitive to antibody neutralization yet the Q315R mutants were strikingly sensitive.

Conclusion: The results suggest that V3 may be more accessible to antibody than previously appreciated in at least some subtype C viruses. However, the data also suggest that a Gln at position 315 modulates exposure of V3. Further elucidation of this mechanism is underway.

P03.12

Plasma Antibodies That Cross React to Subtype-B and C Third Variable (V3) Region Develop in Indian HIV-1 Infected Individuals with Time

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Background: Subtype-C alone accounts for approximately 50% of global and more than 95% human immunodeficiency virus-1(HIV-1) infection in India. Identification of antigenic epitopes that induce antibodies with cross-clade activity will be crucial to address the HIV-1viral diversity.

Methods: 80 HIV-1 infected drug naive patients were recruited for this study. The study was approved by the institute ethics committee and informed consent was obtained from all the participants. The relative binding of anti-V3 polyclonal plasma antibodies to 35 mer consensus¬-B and C V3 peptides was done by ELISA binding assay. Statistical analysis was performed by Graphpad Prism 5.

Results: Assessment of the relative binding revealed that 86% (69/80) of the plasma were able to reach an IC50 binding titer with consensus-B V3 peptide with substantially low antibody titers compared to binding with consensus-C V3 (mean IC50 V3-C=12611 versus V3-B=2736) ($p<0.0001$), implying that although majority of the antibodies were subtype specific, a good proportion of cross reactive anti-V3 antibodies also exist in these plasma (range=1-97%, mean=23%). We observed a strong correlation between percent cross reactive anti-V3 antibodies and days from first diagnosis (n=80: r=0.29 p=0.008) while no such association was found with other clinical and immunological parameters like plasma viral load (n=53: r=0.16 p=0.24), CD4 count (n=80: r=0.10 p=0.34), total plasma IgG levels (n=65: r=-0.09 p=0.45) and eventually with the V3 sequence of donor viruses.

Conclusion: This is the first study to demonstrate the presence of cross-clade reactive anti-V3 antibodies and their association with time in the plasma of HIV-1 infected Asian Indians from north India.

P03.13

Cross-Reactive Neutralizing Activity in HIV-1 Infected Injecting Drug Users

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Background: Vaccine elicited HIV-1 specific cross-reactive neutralizing humoral immunity may provide protection against acquisition of HIV-1. Several studies have focussed on a better understanding of cross-reactive neutralizing activity (CrNA) in natural infection, but only in homosexual and heterosexual HIV-1 transmission cases.

Methods: To analyze the prevalence and characteristics of CrNA in HIV-1 infected individuals who reported injecting drug use as the only risk factor, we screened serum samples from 50 male and 35 female participants of the Amsterdam Cohort Studies (ACS) on injecting drug users (IDU) for CrNA across a heterologous 6-viral panel. For comparison, similar data from the ACS on men who have sex with men (MSM) were available.

Results: HIV-1 infected IDU showed significantly lower geometric mean IC50 values and a lower proportion of individuals that was capable of neutralizing the majority of viruses in the panel as compared to MSM. This difference was however no longer observed when women were excluded from the IDU group. Interestingly, three out of 50 in the male IDU population qualified as elite neutralizer as compared to 1 out of 299 among MSM. Multivariate analysis with viral load at setpoint, CD4+ count at setpoint, gender and transmission route as co-variates revealed only CD4+ count at setpoint as independently associated with CrNA in serum.

Conclusion: CrNA prevalence and potency in HIV-1 infected IDU was lower than in MSM. This could be attributed to the presence of women in the cohort, rather than the route of exposure, which could be explained by the fact that women had a higher CD4+ T cell count at setpoint, which was the only independent predictor for the presence of CrNA. Our data may implicate that the ability of an HIV-1 vaccine to elicit CrNA may be lower in women and testing for gender dependent future vaccine efficacy may be recommended.

P03.14

Structural Definition for a New Modality of Broad and Potent Antibody Neutralization at the CD4-Binding Site on HIV-1 gp120

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Background: The initial site of CD4-attachment on HIV-1 gp120 is vulnerable to neutralizing antibodies, and a number of such antibodies have been found that target this site. One set of antibodies, represented by VRC01, mimic CD4 in their recognition and utilize a common V-gene origin (VH1-2*02). Another set of antibodies, represented by the recently identified VRC13, derives from VH1-69*01 and is able to neutralize over 90% of circulating HIV-1 isolates, including isolates resistant to VRC01. Do the VRC13-like antibodies also mimic CD4, or do they represent a new modality of effective CD4-binding-site neutralization?

Methods: To define the mode of recognition used by VRC13, we crystallized its antigen-binding fragment in complex with HIV-1 gp120, from both VRC01-sensitive and VRC01-resistant strains, and determined these X-ray structures.

Results: The structure of VRC13 indicates a mode of recognition rotated by 45 degrees and translated ~10 Å from that of VRC01, although both VRC01 and VRC13 utilize similar angles of approach. Unlike VRC01-like antibodies, which feature gp120 contacts primarily in the heavy chain 2nd complementarity determining region (CDR H2), VRC13 utilizes a long heavy chain CDR H3 to contact the CD4-binding site. Overall, the structural details of VRC13 do not mimic those of CD4.

Conclusion: Broad and potent neutralization at the CD4-binding site is not limited to the VRC01-mode of CD4 mimicry. A new mode of effective HIV-1 neutralization, which is defined by the VRC13-gp120 structure and utilizes CDR H3 recognition, may serve as an additional template for the design of an effective HIV-1 vaccine. The natural diversity of the CDR H3 – a product of V-D-J recombination – may provide advantages in the elicitation of VRC13-like antibodies.

P03.15

The Impact of Differences in Viral Entry and Trafficking on the Mechanism of HIV-1 Neutralization

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Background: Cellular entry of HIV-1 occurs through fusion at the plasma membrane (PM) or via endocytosis. PM fusion, resulting in deposit of nucleocapsid into the cytoplasm, typically establishes productive infection. In contrast, entry through endocytosis results in either lysosomal degredation, or productive infection through endosomal membrane fusion. While viral entry and permissivity are known to differ between host cells, the influence of these differences on antibody-mediate neutralization is unknown. Here we explore the relationships between HIV-1 entry, neutralization sensitivity, and the mechanisms by which antibodies act.

Methods: Differences in viral entry pathways between peripheral blood mononuclear cells (PBMC) and TZM-bl cells were visualized by electron microscopy (EM). Cells were incubated with HIV, with or without antibody, for 1 hour at 4oC, then viral entry was allowed for 5 min at 37oC. Cells were treated with pronase and either separated into cytosolic and membrane fractions, or cultured to assess viral growth and neutralization. The fraction where nucleocapsid localized was determined by antigen capture and microscopy techniques were used to visualize HIV-1 within cells.

Results: TZMbl cells are 30-fold more efficient at internalizing HIV-1 than PBMC with differences in entry observed between viruses. In TZMbl cells, a greater fraction of p24 localized in the membrane indicating endocytic entry, whereas in PBMC, SF162 localized predominantly in cytosol, indicating PM fusion, while Bal localized equivalently in membrane and cytosolic fractions. Both viruses are neutralized by 2G12 and b12, however, neutralization by 2G12 was not associated with entry inhibition; in fact, after the 5 minutes infection, entry appeared to be enhanced. In contrast, b12 decreased viral entry.

Conclusion: Our data suggest that, for certain antibodies, inhibition of HIV-1 may occur post-entry, and differences in entry mechanisms may impact neutralization. Experiments to elucidate the role of host restriction and post-entry neutralization on inhibition of HIV-1 are ongoing.

P03.16

Rate and Affinity Binding Constants Determined by SPR Spectroscopy Reveal Differential Antigenicity of HIV gp120 and gp140

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Background: The development of a practical, effective AIDS vaccine that can produce enduring broadly protective immunity to natural exposure by diverse strains of HIV-1 remains the ultimate goal for controlling the ongoing AIDS epidemic. The success of an effective AIDS vaccine will likely depend on designing immunogens that elicit broadly neutralizing antibodies to circulating HIV-1 strains, and it is important to thoroughly understand the mechanism of binding of HIV antigens and neutralizing antibodies. The current study was designed to determine differences in the specificity and quantitative properties of antibody binding to gp120 or gp140 envelope proteins derived from natural HIV isolates.

Methods: We previously described novel assays of Env-specific serum antibodies elicited by animal lentiviral and HIV infections and immunizations that measure qualitative properties of avidity and conformational dependence in an ELISA format. However, recent data have emphasized limitations of this format. To provide a more sensitive, specific, and reproducible format for our antibody assays, we have recently developed novel procedures using SPR spectroscopy as measured in the Biacore system to characterize real time reaction kinetics (association/ dissociation rates).

Results: The assays conducted under physiological conditions (37°C, nondenaturing conditions, etc.) clearly indicate the potential of these kinetic measurements of antigen-antibody binding to provide novel data not achieved with standard assays. We have used these binding assays and a panel of well-characterized monoclonal antibodies both to linear and conformational binding domains to characterize differences in reactivity between variant gp120 and gp140 antigens as determined by kinetic rates and affinity of antibody binding. We have identified significant distinguishing differences in several binding characteristics of monoclonal antibody binding to gp120 vs gp140 antigens.

Conclusion: These kinetic parameters offer novel insights into the fundamental interactions of reference monoclonal antibodies and variant Env proteins and have the potential to provide novel correlates of neutralization in vitro and protection in vivo.

Topic 3: B Cell Immunology and Antibody Functions

P03.17

Neutralizing Anti-HIV Antibodies Develop In a Humanized Mouse Model of HIV-1 Infection

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Background: In BLT (bone marrow-liver-thymus) humanized mice, human thymocytes are educated by autologous human thymic tissue, resulting in functional human T cells capable of rapidly selecting for CTL escape mutations in HIV. In contrast, limitations to B cell maturation have been noted. But despite this, we show for the first time that HIV infected BLT mice can produce class-switched anti-HIV antibodies with neutralizing activities.

Methods: Humanized BLT mice were generated by transplanting irradiated NOD-scid/IL2rgnull (NSG) mice with fetal thymus and liver fragments and then injecting them with autologous human CD34+ stem cells. BLT mice were then infected with HIV_{JRCSF} and bled at various time-points. HIV neutralizing activity was measured using Tat-induced luciferase reporter TZM-bl cells.

Results: Human transitional B cells were present in greater frequencies in BLT mice than adult humans. Most of these cells had a T1 phenotype in the blood and spleen. But despite this B cell maturation defect, class-switched IgG Abs against various HIV proteins were detected by Western Blot in HIV-infected BLT mice. Using ELISA to determine anti-p24 IgG Ab titers, Abs were present as early as 8 weeks post infection (p.i.), with peak Ab titers seen after 15 weeks. One infected mouse demonstrated a peak titer similar to that seen in a chronically infected human. Finally, plasma samples from infected BLT mice after 22 weeks p.i. demonstrated neutralizing activities against the challenge virus. Average IC50 neutralizing titers in these mice were similar to those from infected human samples.

Conclusion: The ability of humanized BLT mice to generate functional humoral immune responses may be further improved by strategies to improve their B cell maturation, which will further improve the potential of these mice to become a model system to study candidate HIV vaccines and therapies.

P03.18

A Comprehensive Binding and Neutralization Analysis of Plasma of HIV-1 Subtype-C Infected Donors from India Suggest MPER Directed Neutralization

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Background: Dissecting the specificities of the anti-HIV-1 neutralizing antibodies will assist in identifying targets for an HIV-1 subunit vaccine which undoubtedly remains ultimate goal for vaccine development.

Methods: We tested 30 HIV-1 drug naive plasma samples for neutralization against a broad panel of subtype-A, B and C tier 1 and tier 2 viruses in a TZM-bl assay. Three broadly neutralizing plasma (bNP) samples (AIIMS206, AIIMS239 and AIIMS249) were tested for ELISA binding with a set of 211 consensus-C gp160 overlapping peptides (NIH AIDS Research and Reference Reagent Program). The competition and depletion experiments (for neutralization) were carried out with peptides corresponding to consensus-C V3 (35mer), IDR (19mer) and MPER (24mer).

Results: Approximately 25% of the plasma/virus combinations showed neutralizing activity with a predominance of subtype C specific neutralization compared to subtype B (p=0.001). Immunoglobulin-G fractions from bNP were shown to mediate neutralization exclusively and were shown to retain the binding to subtype-A, B and C recombinant gp120 proteins. Based on the Max50 ELISA binding titers, the immunoreactivity of the three bNP mapped to second variable (V2), second constant (C2), third variable (V3), fourth constant-fifth variable (C4-V5), fifth constant (C5) regions of gp120 and fusion protein (FP), immunodominant region (IDR), C-heptad region (CHR), membrane proximal external region (MPER) and C-terminal (CT) of gp41 protein. In the depletion and competition assays, the bNP AIIMS206 and AIIMS239 showed dependence on MPER directed antibodies with four and six (out of eight) viruses respectively while the V3 and IDR peptides showed minimal effect on neutralization as compared to untreated and mock depleted plasma controls. Further, the mapping of IgG fractions from bNP with overlapping MPER peptides showed 2F5 like binding specificity for AIIMS206 plasma.

Conclusion: Our study demonstrates that MPER directed antibodies in HIV-1 subtype-C infected patients play a crucial role in viral neutralization.

P03.19

Highly Efficient Neutralization of Human Immunodeficiency Viruses by Plasma from Antiretroviral Drug Treated Patients Is Mediated by IgG Fractions

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Background: Little is known about the neutralizing activity in patients on antiretroviral therapy (ART), as most recent studies have focused on drug naïve individuals. ART may lead to a significant increase in B cell numbers and normalization of B cell subpopulations, providing a possible explanation for improved B cell responses after ART.

Methods: Thirty-four HIV-1 seropositive patients on ART (25 males and 9 females) within the age range of 20-55 years were recruited in this study. The patients had a median CD4 count and viral load of 283 cells and 178 RNA copies respectively, and were on treatment for a few days up to two years. Heat inactivated plasma samples were tested for neutralization against a panel of 14 subtype-A, B and C tier 1 and tier 2 viruses in TZM-bl assay.

Results: Of the 34 plasma samples, remarkably all the plasma samples were able to neutralize at least one virus while 32 (94%) samples were found to neutralize ≥50% viruses tested. Clustering analysis revealed that AIIMS253 (a clade-C virus) was the most sensitive while RHPA4259.7 (a clade-B isolate) was most resistant to antibody neutralization. The Immunoglobulin-G fractions from two representative samples AIIMS221 and AIIMS265 were shown to mediate neutralization exclusively. The IgG fractions retained binding to subtype-A, B and C recombinant gp120 proteins. We did not find any association of mean reciprocal ID50 neutralization titers with the plasma levels of ART drugs and clinical and immunological variables like CD4 count (p=0.35), viral load (p=0.37) and plasma total IgG (p=0.46). However we observed a positive association of neutralization with duration of ART (p=0.02) with a similar trend in two follow up patient samples.

Conclusion: Plasma antibodies from patients on ART display high neutralizing activity most likely due to an improved B cell function induced by ART despite low antigenic stimulation.

P03.20

Modulation of Antibody Secreting Cells and Neutralizing Ab Activity in HIV Infected Individuals Undergoing Structured Treatment Interruptions

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Background: HIV-1 infection generates numerous abnormalities in the B cell population. The majority of these defects are reverted by antiretroviral therapy. Our aim was to evaluate the effects of re-exposure to HIV antigens on the frequency and functionality of antibody secreting cells (ASC) in patients undergoing structured treatment interruptions (STI). As re-exposure to viral antigens may also boost the production of (neutralizing) antibodies, we also assessed the neutralizing activities during STI cycles.

Methods: Retrospective study of 10 patients undergoing 3 cycles of STI with 2 weeks on and 4 weeks off HAART. ASC frequencies were determined by flow cytometry in samples obtained at the beginning and the end of STI. Neutralization capacity, total IgG concentration and anti-gp120-IgG titres were evaluated.

Results: Median viral loads were higher at the end of STI compared to time of treatment stop: 20[20-1200] vs 615[20-452000] respectively for the first STI, 20[20-654] vs 3655[20-45900] ($p<0.05$) for the second and 35[20-82] vs 290[20-17400] ($p<0.05$) for the third STI. The frequency of ASC followed the same trend: 0.35%[0.17-1.15] at the beginning of first STI vs 0.27[0.20-4.95] at treatment restart, 0.35[0.10-1.40] vs 0.82[0.30-3.25] for the second and 0.30[0.10-0.70] vs 0.40[0.15-1.85] for the third STI. Eight out of 10 patients maintained stable total IgG levels during the study. HIV-neutralizing activity was observed in two patients concomitantly with high anti-gp120 titters. In one patient the neutralizing activity remained constant while the second patient showed elevated neutralizing Ab after first STI and once treatment was reinitiated after the 2nd STI.

Conclusion: Our data suggest that STI and its associated transient increases in viral load drive the frequencies of ASC in an antigen-specific manner. In some subjects, this re-exposure to autologous virus boosts the presence of neutralizing antibodies, albeit in a somewhat delayed manner.

Topic 3: B Cell Immunology and Antibody Functions

P03.21

Construction and Characterization of Neutralizing Antibody Fragments for Efficient Access to V3 Epitope

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Background: The neutralizing antibody against HIV-1 is important for the vaccine development. However, primary isolates of HIV-1 have resistance to antibody neutralization. While a neutralization epitope in V3 is exposed by conformational change of gp120 following CD4-binding, anti-V3 IgG molecules are too large to access V3-epitope. The purpose of our study is to develop Fab and scFv fragments from the anti-V3 monoclonal neutralizing antibody 5G2 for efficient access to V3-epitope.

Methods: RNA was extracted from clonal cells producing 5G2, and cDNA was prepared by reverse transcription. Using primers for variable region of heavy and light chains, the antibody genes were amplified by PCR, and inserted into pComb3X vector. Fab and scFv were produced in the transformed E. coli by induction with isopropyl-β-D-thiogalactoside, and purified by nickel chelate chromatography.

Results: Binding of recombinant 5G2 Fab and scFv to V3 epitope was shown by enzyme-linked immune-sorbent assay against V3 peptide. The flow cytometry analysis using cells expressing HIV-1 Env revealed the higher intensity by smaller antibody fragment, suggesting the increased accessibility of small fragment to V3 epitope. Neutralizing activity of these 5G2 fragments were observed against both primary HIV-1JR-FLwt and neutralization-sensitive variant, JR-FLL175P. In the case of JR-FLwt, scFv showed a significant improvement of neutralization.

Conclusion: The use of smaller antibody fragments, such as scFv and Fab, may be one strategy to overcome steric constraints that confer neutralization resistance of primary isolates. We are now evaluating an effect of small antibody fragments on neutralization against various primary isolates.

P03.22

Affinity Maturation Pathway of an Anti-MPER Neutralizing mAb, CAP206-CH12

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Background: The membrane proximal external region (MPER) of HIV-1 is an important target of broadly cross-reactive mAbs. CAPRISA participant, CAP206, developed anti-MPER antibodies early that became cross-neutralizing at 18 months post-infection. This coincided with neutralization of the C4 HIV-2/HIV-1 chimera containing the W670 residue, suggesting changes in an antibody paratope may have resulted in the acquisition of breadth. A neutralizing mAb (CAP206-CH12) was isolated, providing an opportunity to determine which somatic hypermutations contribute to breadth.

Methods: The putative CAP206-CH12 reverted unmutated ancestor (RUA) was inferred using SoDA (www.dulci.org). Batch transient transfections were used to generate recombinant antibodies. Neutralization breadth and potency was tested against autologous, Tier 2, and HIV-2/HIV-1 MPER chimeric viruses using the TZM-bl assay. Binding to MPER peptides was assessed by ELISA and surface plasmon resonance (SPR).

Results: CAP206-CH12_RUA bound the MPER.03 peptide with a Kd of 120nM, 15-fold weaker than CAP206-CH12 binding, but had no neutralizing activity. Since CAP206-CH12 and its RUA differed by 19 residues in the heavy chain and 9 in the light chain, we designed an intermediate precursor (IP) where changes near the CDRs in CAP206-CH12 were reverted back to the germline sequence (11 in the heavy and 5 in the light chain). This CAP206-CH12_IP did not neutralize the C4 chimera suggesting that changes responsible for the affinity-matured CAP206-CH12 neutralizing capacity were among these 16 residues. Chimeric pairs of the light chain IP with the heavy chain of CAP206-CH12 or CAP206-CH12_RUA showed binding to MPER with differences in binding kinetics.

Conclusion: The reduced binding and neutralizing activity of CAP206-CH12_RUA and CAP206-CH12_IP compared to CAP206-CH12 suggests a correlation between affinity maturation, neutralization breadth and potency. Ongoing work will assess the affinity maturation of CAP206-CH12 by determining moieties in the antibody paratope associated with effective epitope recognition and the effects of somatic mutations on the evolution of neutralization.

P03.23

Comparison of the Neutralization Sensitivity of South African and Indian HIV-1 Subtype C Viruses to South African Plasma Antibodies

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Background: HIV-1 subtype C is the predominant subtype in India and South Africa (SA) responsible for explosive epidemics. An understanding of the neutralization sensitivity of viruses from these regions will help to define shared epitopes in the envelope protein, which may be important for inclusion in an effective subtype C-specific vaccine.

Methods: Plasma from 44 chronically HIV-1 subtype C infected SA individuals in the CAPRISA 002 Acute Infection cohort were used to assess the neutralization sensitivity of 10 SA and 9 Indian Tier 2 viruses in a TZM-bl assay. Intra-subtype neutralization activity between SA and Indian viruses was determined. The envelope sequence of the Indian panel was analyzed in order to identify epitopes known to be targeted by the neutralizing antibodies in a subset of the CAPRISA plasmas.

Results: South African and Indian viruses were generally sensitive to the most potent plasmas. However, SA viruses showed overall greater sensitivity compared to Indian viruses; neutralization of 50% of the SA virus panel was achieved by 15/44 (34%) of the plasmas compared to only 6/44 (14%) against the Indian panel. Furthermore, neutralization titers against SA viruses were higher, with 9/44 (21%) of plasmas neutralizing at >1:200 compared to 5/44 (11%) against the Indian panel. An analysis of the gp160 sequences suggested that genetic differences in key neutralization epitopes between SA and Indian viruses were in part responsible for differences in sensitivity.

Conclusion: These data suggest the presence of common neutralization epitopes between SA and Indian viruses although there is also evidence for regional neutralization determinants within subtype C. The finding that SA viruses were generally neutralized at higher titers, suggests that the exposure of common epitopes on envelope may vary between SA and Indian viruses. Overall, these data support the development of a subtype C-specific vaccine that can be used in both SA and India.

P03.24

Biophysical Dissection of the Antigen-Antibody Interaction of the Broadly Reactive Anti-V3 Human mAb 447-52D

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Background: The immunogenic third variable region (V3) of HIV-1 gp120 is a target for AIDS vaccines. V3 is recognized by mAb 447-52D, known for its ability to neutralize viruses with a GPGR beta turn motif at the apex of V3, which is characteristic of clade B viruses. Interestingly, 447-52D can also bind non-clade B V3 peptides containing a GPGQ motif. A detailed biochemical and biophysical dissection of the antigen-antibody interaction of 447-52D was undertaken to understand this disparity.

Methods: We cloned and produced large amounts of the Fv fragment of 447-52D and a panel of mutations. We then measured their epitope binding characteristics by Isothermal Titration Calorimetry (ITC).

Results: We assessed the Fv-V3 binding by ITC for the following mutations in residues of the mAb that are thought to mediate three key interactions: (i) Y^{H100j} of the heavy chain (H) to T ($Y^{H100j}T$) or $Y^{H33}A$. These two aromatic residues form a pi-cation interaction, sandwiching the side chain of R^{315} of the GPGR motif in the V3-peptide. These mutations reduce binding affinity by 56 and 171-fold, respectively. (ii) W^{L91} of the light chain (L) to A ($W^{L91}A$). This residue packs against P^{313} of the V3 GPGR turn. This mutation reduces binding 230-fold. (iii) $D^{H95}R$ of the heavy chain or $R^{315}Q$ of the epitope. These two residues form a salt bridge between the antigen and the antibody. These mutations reduce binding by 224 and 171-fold, respectively. These data suggest a hierarchy of interactions and the salt bridge plays an important role in the affinity.

Conclusion: mAb 447-52D binds non-clade B peptides with the R315Q variation with much less affinity, explaining why it cannot neutralize non-clade B viruses. Through probing specific contributions of individual residues by mutagenesis and ITC, we were able to fully characterize the interactions between V3 and 447-52D.

Topic 3: B Cell Immunology and Antibody Functions

P03.25

Characteristics of HIV-1 gp120 Molecules That Bind Ancestor, Intermediate and Mature Forms of VRC01-like Antibodies

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Background: A group of highly effective neutralizing antibodies, which target the site of CD4 binding on HIV-1 gp120, have recently been identified. These antibodies – called VRC01-like antibodies – all originate from the same VH1-2*02 germline gene and, while the mature antibodies have undergone extensive maturation via non-homologous pathways, their recognition of the CD4-binding site of gp120 is similar. An efficacious vaccine that elicits VRC01-like antibodies will likely be required to bind to VH1-2*02-derived B cells to initiate their expansion and subsequent maturation, however, binding studies with reverted-ancestor VRC01-like antibodies and HIV-1 gp120 molecules typically show binding that is too weak to initiate B cell maturation.

Methods: To identify HIV-1 gp120 molecules capable of interacting with reverted-ancestor molecules with sufficient affinity to initiate B cell maturation, we screened large panels of HIV-1 pseudoviruses for sensitivity to reverted-ancestor forms of VRC01-like antibodies. Identified HIV-1 strains (and related gp120s) were then analyzed for recognition to a panel of diverse VRC01-like antibodies.

Results: No HIV-1 strains were identified which could be neutralized by reverted heavy chain- and light chain-ancestors of VRC01-like antibodies. Chimeric forms of the VRC01-like antibodies with reverted and mature heavy/light chain mixtures did, however, neutralize a small subset of HIV-1 isolates. Characterization of gp120s from the sensitive subset found measurable affinity to the ancestral forms of VRC01-like antibodies. In comparison, typical gp120 molecules, e.g. YU2 gp120, fail to bind low-divergent forms of the VRC01-like antibodies, i.e. those with less than 10% divergence from germline.

Conclusion: Select strains of HIV-1 can interact with ancestral forms of VRC01-like antibodies. Defining the specific characteristics of these select strains should enable identification of gp120-derived immunogens capable of productive interactions with VH1-2*02-derived B cells.

P03.26

Optimization and Validation of the HIV-1 Neutralizing Antibody Assay in A3R5 Cells

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Background: A3R5 is a highly sensitive cell line for the detection of neutralizing antibodies (Nabs) against tier 2 strains of HIV-1. This cell line is particularly useful for the detection of weak Nab responses in preclinical and clinical trials of candidate HIV-1 vaccines. All methods used for endpoint analyses in clinical trials should be validated and demonstrably fit for purpose, in compliance with ICH Q2 (R1) guidelines. Here we describe the optimization/qualification and validation of the HIV-1 Nab Assay in A3R5 cells.

Methods: A3R5 is a human lymphoblastoid cell line naturally expressing CD4 and CXCR4 and engineered to express CCR5. Nab assays in A3R5 cells are performed with Env.IMC.LucR viruses containing a reporter gene in the viral genome, whose expression is induced by viral Tat protein soon after infection. Luciferase activity is quantified by luminescence and is directly proportional to the number of infectious virus particles present in the viral inoculum. The assay is performed in 96-well culture plates for high throughput capacity.

Results: We determined the stability of the cell line over time in culture for receptor and coreceptor expression, susceptibility to infection, and sensitivity to neutralization. The assay was optimized for cell density, input virus dose, length of incubation time and use of DEAE-dextran. We also determined the stability of a set of reference reagents, for validation experiments and for future competency and proficiency testing that express a broad spectrum of neutralization phenotypes. A prospective validation plan with pre-set pass/fail criteria was composed and implemented that addressed key assay parameters, including accuracy, precision, limit of detection and quantitation, specificity, linearity and range, robustness and specificity. Results of validation experiments were statistically analyzed and used to generate a final validation document.

Conclusion: This validated assay will be used to identify correlates of protection in HIV vaccine trials conducted globally.

P03.27

Crystal Structure Analysis of Anti-V2 Mab 2158 Suggests a Conformational Epitope Involving an N-Linked Glycan

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Background: Many structural elements of HIV-1 gp120 have been revealed by X-ray crystallography, NMR and electron microscopy; however, several key immunogenic regions are still not well understood. Data from the recent RV144 trial indicated that antibodies targeting the V1V2 region correlate with a lower risk of infection. A detailed characterization of anti-V2 antibodies, in concert with recent V1V2 structural information is critical to the design of a V2 immunogen.

Methods: We have determined a crystal structure of the uncomplexed Fab fragment of anti-V2 mAb 2158. To understand the antigen binding mode of mAb 2158, we computationally created twenty V1V2 models from a panel of gp120s that were tested previously in ELISA for binding reactivity with mAb 2158. Subsequently, we docked these models in silico to the antigen binding site of mAb 2158.

Results: The structure of Fab 2158 revealed that its antigen binding site consists of a hydrophobic surface comprised with residues from CDRs H2 and H3 with a deep pocket between CDRs H1 and H3. Surface energy analysis suggests that the heavy chain and the binding pocket are likely to dictate the antigen binding mode of Fab 2158. Our model-building of the 20 V1V2 structures revealed that the residues previously suggested as the mAb 2158 epitope all mapped to a single face on the V2 domain next to glycosylation site N186. Correlating our computational analysis with ELISA data suggests that the glycosylation of N186 plays a key role in binding and our docking results indicates that the mAb 2158 binding pocket can accommodate the mannose glycan harbored by N186.

Conclusion: Structural analyses of Fab 2158 suggest that binding to a conformational epitope containing an N-linked glycan. Our results allow us to hypothesize a binding motif signature for anti-V2 mAb 2158 and form a framework for designing V2 immunogens.

P03.28

Detection of Antibodies to the α4β7 Integrin Binding Site on HIV-1 gp120 V2 Loop Using A Novel Cell Adhesion Assay

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Background: The gut mucosal homing integrin receptor α4β7 present on activated CD4+ T-cells interacts with the HIV-1 gp120 second variable loop (V2). Case control analysis of the RV144 phase III vaccine trial showed that antibodies induced by the vaccine bound to a MuLV-gp70 scaffolded V1V2 loop of gp120 (V1V2-gp70) and correlated inversely with infection. These, and other data, generate the hypothesis that the vaccine-elicited antibodies may have been involved in limiting HIV-1 acquisition. We have developed a high-throughput assay to evaluate antibodies that block α4β7 binding. We have named this the RAP assay.

Methods: Plates were coated with either MAdCAM-1, the natural ligand of α4β7, or streptavidin followed by the addition of biotinylated cyclic-V2 peptides (strain 92TH023 or MN). Plasma and purified IgG antibodies from RV144 volunteers, conformational mAbs specific for V2 (697), for V2 and V3 (PG9, PG16), or for V2 linear epitopes (CH58 and CH59, cloned from RV144 vaccinee B cells) were then added to the peptide-coated plates followed by RPMI8866 cells, which constitutively express α4β7. Cell binding/inhibition was assessed by AlamarBlue. Anti-α4β7-specific mAb (ACT-1) served as a control. In separate experiments, plasma, IgG, or mAbs were tested in a competition assay using V1V2-gp70.

Results: ACT-1 inhibited the binding of both MAdCAM-1 and cyclic-V2 peptides to α4β7 by 65-85%, while CH58 and CH59 inhibited the α4β7-cyclic-V2 peptide binding by 37-45% in a dose-dependent manner. PG9, PG16, and 697 did not inhibit the binding of V2-peptides to the cells. However, in the competition assay, 697 and PG9 mAbs inhibited V1V2-gp70 binding to α4β7. Some of the RV144 plasma and IgG inhibited binding to 92TH023 or MN-V2-peptides.

Conclusion: We have developed a novel high-throughput reproducible assay for assessing α4β7-specific blocking antibodies. The above results raise the hypothesis that anti-V2 loop antibodies may play a role in regulation of gp120-α4β7 interaction.

Topic 3: B Cell Immunology and Antibody Functions

P03.29

Superinfection by Discordant Subtypes of HIV-1 Does Not Enhance the Neutralizing Antibody Response Against Autologous Virus

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Background: Recent studies have demonstrated that both the potency and breadth of the humoral anti-HIV-1 immune response in generating neutralizing antibodies (nAbs) against heterologous viruses are significantly enhanced after superinfection by discordant HIV-1 subtypes, suggesting that repeated exposure of the immune system to highly diverse HIV-1 antigens can significantly improve anti-HIV-1 immunity. We investigated whether sequential plasma from subjects superinfected with discordant HIV-1 subtypes, who exhibit broad nAbs against heterologous viruses, also neutralize either or both of their discordant early autologous viruses with increasing potency.

Methods: Sequential blood samples were collected from superinfected and singly infected subjects in Cameroon. RNA was extracted; env was amplified by nested RT-PCR and cloned in env-expression vectors. Purified vector was used to co-transfect 293T cells with the Q23-delta-env HIV-1 backbone vector. The pseudovirus supernatant was tested in neutralization assays with TZM-bl cells using patient plasma samples before and after superinfection.

Results: Comparing the neutralization capacities of sequential plasma obtained before and after superinfection of 4 subjects to those of matched plasma obtained from 4 singly infected control subjects, no difference in the increase in neutralization capacity was observed (p=0.328). Overall, neutralization increased over time in 3 of the 4 singly infected patients (mean change in IC_{50} titer from first to last plasma sample: 183.4) and in 3 of the 4 superinfected subjects (mean change in IC_{50} titer from first to last plasma sample: 66.5). Analysis of the Breadth-Potency Scores confirmed that there was no significant difference in the increase in superinfected and singly infected study subjects (p=0.234).

Conclusion: These studies suggest that while superinfection by discordant subtypes induces antibodies with enhanced neutralizing breadth and potency against heterologous viruses, the potency to neutralize their autologous viruses is not better than those seen in singly infected patients.

P03.30

Hyperglycosylated gp120 Mutants Elicit Improved CD4-Binding Site Directed Antibodies in a Heterologous Prime:Boost Regimen

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Background: The CD4-binding site (CD4bs) on gp120 is targeted by broadly neutralizing antibodies (nAbs) and is therefore of interest for vaccine design. Insight derived from molecular interactions of CD4bs-specific antibodies with gp120 has guided structure-based protein design and the development of a number of immunogens. Of equal interest are strategies to improve potency and durability of desired nAb responses to such immunogens.

Methods: We generated truncated, hyperglycosylated gp120 mutants designed to selectively present nAb epitopes overlapping the CD4bs. To help focus antibody responses to these epitopes, we conducted a heterologous prime:boost immunization using two mutants (termed ΔN2mCHO and ΔN2mCHO(Q105N)) in combination with a resurfaced gp120 core protein (RSC3) that preferentially presents the CD4bs neutralizing face. Groups of animals were primed with ΔN2mCHO, unappended or N-terminally appended with one of three immunostimulatory sequences known to amplify humoral responses – PADRE, N10 or C3d. The animals were boosted with RSC3 and then ΔN2mCHO(Q105N). Serum specificities were dissected using CD4bs and non-CD4bs mAbs and responses were followed at the cellular level by phenotyping the memory B cell compartment after each injection.

Results: Relative to other groups, PADRE-ΔN2mCHO elicited significantly more rapid and higher titres against gp120 and the immunogens, suggesting that PADRE has superior immunoactivating properties. The PADRE- antibodies also bound greatest to epitopes overlapping the CD4bs. Unexpectedly, only sera from N10- animals exhibited significant neutralizing activity against select tier 1B and 2 viruses. Unanticipatedly also, no significant differences were observed at the memory B cell level between the groups for gp120 specificity.

Conclusion: Our results show that selective exposure of conserved epitopes through the use of varied immunogens fused with immunostimulatory sequences, in particular PADRE, can boost desired antibody responses. Together, our data highlight the importance of not only immunogen design but also formulation on directing antibody responses to conserved epitopes.

P03.31

Structural Comparison of Somatically Related PG9 and PG16 in Complex with Their Epitope Reveals Differences in Glycan Recognition

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Background: The somatically related antibodies, PG9 and PG16, neutralize 70-80% of HIV-1 isolates and bind a glycosylated epitope in the V1/V2 domain of HIV-1 gp120. Mutations in V1/V2, and sometimes V3 depending on the HIV-1 strain, affect neutralization and a glycan on Asn160 is required for neutralization. Both antibodies also preferentially bind the native trimer over monomeric gp120, especially PG16. The structure of PG9 in complex with its epitope, a scaffolded V1/V2 from HIV-1 strain ZM109, was recently solved and showed that PG9 targets a site of vulnerability comprising 2 glycans and a β-strand.

Methods: To understand the differences in binding properties from these two somatically related antibodies, we first assessed their binding to monomeric gp120 and scaffolded V1/V2 proteins with different glycan types (oligomannose, hybrid, and complex). In order for PG16 to bind the scaffolded V1/V2, the protein had to be expressed in mammalian cells in the presence of swainsonine, which inhibits glycan maturation past the hybrid state. A stable complex could be obtained between PG16 and a scaffolded V1/V2 domain from ZM109, and this complex was crystallized.

Results: Although the structure of PG16 bound to scaffolded V1/V2 resembled that of PG9, some differences were seen: 1) PG16 binding to the β-strand is weaker than PG9 with fewer charged interactions, 2) PG16 interacts with a hybrid glycan at position N173. The difference in binding recognition of PG9 and PG16 to monomeric gp120 depends on the type of glycans present. PG16 binds the protein portion of V1/V2 weaker than PG9 and this might explain the higher affinity of PG9 for the monomer. PG16 has evolved a second glycan site to compensate for weaker peptide interaction.

Conclusion: The results show the importance of polyclonal response in infected individual to combat HIV-1, and in this case, to differential glycosylation.

P03.32

Neutralizing and Non-Neutralizing Antibody Responses in HIV-1 Subtype C Chronically Infected Patients with Divergent Rates of Disease Progression

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Background: Development of an efficacious HIV-1 vaccine able to elicit the production of broadly neutralizing antibodies (nAbs), capable of retaining potent activity against a diverse panel of viral isolates remains a significant challenge. The evolutionary forces that shape envelope and ensuing nAb and non-neutralizing antibodies in HIV-1 subtype C are incompletely understood and these two parameters have been rarely studied concurrently.

Methods: We characterized patterns of virus-specific nAbs and non-neutralizing antibodies in four slow progressors and four progressors with chronic HIV-1 subtype C infection, over a median of 21 months. Single cycle neutralization assays was performed. In addition, the binding affinities of HIV-specific immunoglobulins (IgGs) and the affinities of the IgGs to various Fcγ receptors (FcγRs) were assessed.

Results: NAbs evolved significantly in progressors ($p=0.003$) from study entry to study exit. NAb IC50 titers significantly correlated with amino acid lengths for V1-V2 ($p=0.04$), C3-V5 ($p=0.03$) and V1-V5 ($p=0.04$). Both groups displayed preferential heterologous activity against the subtype C panel. Both groups displayed preferential heterologous activity against the subtype C panel. There were no significant differences in breadth of responses between the groups for either subtype A or C. Neutralization breadth and titers to subtype B reference strains was significantly higher in progressors compared to slow progressors (both $p<0.03$) with increasing nAb breadth from study entry to study exit in progressors. Progressors had cross-reactive neutralizing antibodies that targeted V2 and V3. Binding affinities of non-neutralizing antibodies to HIV-specific gp120, gp41 and p24 and to activating and inhibitory Fcγ receptors (FcγRs) were similar in both groups. However, in slow progressors, CD4 T-cell counts correlated inversely with antibody binding affinity for the activating FcγRIIa ($p=0.005$).

Conclusion: Overall, the data suggest that neither nAbs nor non-neutralizing antibodies could be directly associated with disease attenuation. However, continuous evolution of nAbs was a potential marker of disease progression.

P03.33

Structural Analyses of Antigen Binding Similarities and Differences Between Rabbit and Human Anti-gp120 V3 mAbs

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Background: The rabbit is a commonly used animal model to study antibody responses in AIDS vaccine development. However, little is known about the relationship between epitopes recognized by the rabbit and human immune systems. Structural knowledge of antigen-antibody interactions of rabbit and human mAbs will help us understand the similarity of these two immune systems in recognizing HIV antigens, thus provides a guidance in using the rabbit for AIDS vaccine development.

Methods: Complex structures of anti-V3 mAbs R56 and R20, generated by immunizing a rabbit with JR-FL gp120 using a DNA prime-protein boost regimen, were determined and analyzed in comparison with human mAbs from HIV-1 infected patients against the same V3 immunogenic regions.

Results: The epitope of R56 is structurally mapped to the N-terminal region of the V3 crown, overlapping with the epitopes recognized by human IGHV5-51 germline anti-V3 mAbs. Both R56 and the human mAbs bind the highly conserved V3 residues, consistent with their broad neutralization activities. However, while the human antibodies can bind the whole beta-hairpin of the crown, R56 only binds the N-terminal half of the hairpin. The epitope of R20 is located in the V3 C-terminal region near the two highly conserved glycosylation sites at the V3 base. This epitope overlaps with that of human mAb PGT128. A long beta-hairpin CDR H3 of R20 stands at the center of its antigen-binding site in a manner similar to several potent human mAbs such as 2909, and interacts with the epitope by a beta-sheet-type interaction.

Conclusion: Structural analyses of immunization-generated rabbit antibodies show that they can recognize immunogenic regions of gp120 and mimic the binding modes of human antibodies. However, optimized immunization schemes need be tested in rabbits to produce antibodies with sufficient affinity maturation to recognize Env epitopes as complex as that of human antibodies generated in chronic infected patients.

P03.34

Refined Identification of Neutralization-Resistant CRF02_AG Viruses and Their Sensitivity to Anti-MPER Neutralizing Antibodies

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Background: The first antibody-inducing HIV-1 vaccines are unlikely to protect against all HIV-1 isolates. There is thus a danger that a vaccine will select for HIV-1 viruses that are highly resistant to antibody-mediated neutralization. We sought to identify and characterize such viruses.

Methods: A diverse panel of 24 HIV-1 pseudoviruses was tested for neutralization resistance using two sets of samples from ARV-naive HIV-1-infected individuals selected for good neutralizers: sera from South Africa donors (n=68, infected >1 year, subtype C predominant area) and CRF02_AG-infected plasma samples from Cameroon donors (n=12, good neutralizers selected from 22 samples).

Results: Sensitivity to South Africa sera by subtype was C>B≈CRF02_AG>A. Importantly, and in contrast to previous reports, CRF02_AG plasma neutralized CRF02_AG viruses better than other panel viruses ("within-subtype neutralization"). This included three (257-31, 251-18 and 33-7) of five CRF02_AG viruses previously designated as tier 3 (most resistant). This within-subtype neutralization testing showed that the other two tier 3 CRF02_AG panel viruses, 253-11 and 278-50 were highly resistant. Most CRF02_AG viruses, including 253-11 and 278-50 were sensitive to two membrane proximal external region (MPER)-specific monoclonal antibodies and soluble CD4 (sCD4), suggesting targets for neutralization of even these highly resistant viruses. This information may help design a global HIV-1 vaccine. We also propose testing viruses with within-subtype samples selected for good neutralizers in order to evaluate their neutralization resistance.

Conclusion: Some but not all CRF02_AG viruses are sensitive to neutralisation by CRF02_AG-derived plasma, even though most are previously reported as highly resistant (Tier 3). Further work is necessary to properly characterize such Tier 3 viruses. If research focus is not placed on such resistant viruses, a future partially effective HIV-1 vaccine may select for them.

P03.35

Antibody-Dependent Cellular Cytotoxicity-Mediating Antibodies from an HIV-1 Vaccine Efficacy Trial Preferentially Use the VH1 Gene Family

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Background: The ALVAC-HIV/AIDSVAX-B/E RV144 vaccine efficacy trial showed an estimated efficacy of 31%. The immune correlates analysis raised the hypothesis that the observed protection in RV144 may be partially due to Antibody-Dependent Cellular Cytotoxicity (ADCC)-mediating antibodies in the presence of low levels of Env IgA antibodies. In this study we analyzed the Ig VH family usage of vaccine-induced ADCC mAbs isolated from memory B cells of vaccinees.

Methods: From a total of 321,945 memory B-cells of 6 vaccinees we obtained 23 mAbs that mediated ADCC using IgG+ memory B-cell cultures (n=9) and Env-specific flow cytometric single memory B-cell sorting (n=14). ADCC activity was measured using both E.CM243 gp120-coated and E.CM235-infected target cells in a flow-based assay.

Results: ADCC-mediating mAbs displayed a disproportionate usage of VH1 family genes (17/23; 74%), in particular the VH1-2 gene segment (10/17; 59%), as recently observed for CD4bs broadly neutralizing antibodies (HAAD bNAbs). In contrast, only 17.1% of 111 heavy chains isolated from cultures that did not mediate ADCC used the VH1 gene. VH1 ADCC-mediating mAbs showed a high degree of V(D)J amino acid similarity to both the VH (68-84%) and VL (70-87%) HAAD motifs. V(D)J rearrangements displayed modest levels of affinity maturation (0.5-5.1% for heavy chains and 0.4-4.3% for light chains). While none of the VH1 ADCC-mediating mAbs was capable of mediating HIV-1 neutralization, the strength of their ADCC activity correlated with the levels of heavy chain somatic mutations (p=0.02). We produced the reverted unmutated ancestor antibodies of two VH1 ADCC-mediating mAbs: one bound to B.MN Env and both reacted against autoantigens.

Conclusion: ADCC-mediating antibodies induced by the ALVAC-HIV/AIDSVAX-B/E vaccine underwent limited affinity maturation, and preferentially used VH1 gene segments which share the HAAD motif with CD4bs bNAbs. These observations raise the hypothesis that HIV-1 Env preferentially selects VH1 family usage for distinct subsets of antibodies with different functions.

P03.36

High-Resolution Crystal Structure of the Fv of Quaternary Neutralizing Epitope mAb 2909 Reveals Atomic Details of Its Antigen-Binding Site

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Background: Human mAb 2909 is in a class of potently neutralizing mAbs against the HIV-1 quaternary neutralizing epitope (QNE) preferentially presented by the native Env trimer complex. Its distinctive feature is a long CDR H3 loop with 2 sulfated tyrosines that are suggested to play a key role in antigen binding. Two structures of the Fab fragment of 2909 have been published, but at only 3.3Å and 3.2Å resolution, respectively, some atomic-level details of the antigen binding sites of these structures are contradictory.

Methods: After crystallizing a recombinant Fv (rFv) of mAb 2909, expressed as a single chain in E. coli and refolded from inclusion bodies, we solved and refined its structure to 1.9Å resolution. We also characterized the neutralizing activity of the rFv against pseudotyped virus SF162.

Results: Despite lacking the native sulfation of 2 tyrosine residues at the apex of CDR H3, rFv 2909 retains neutralization activity against SF162 pseudoviruses. Our high-resolution structure features a series of 5 tyrosine residues decorating one face of H3 like rungs of a spiral staircase, as seen in the Spurrier structure. The presence of this feature, despite different crystal packing around the H3 loop, suggests that the stacking pattern is not an artifact of crystallization, and that these tyrosine side chains play an important role in epitope recognition.

Conclusion: Our structure of rFv 2909 at 1.9Å resolution reveals additional atomic-level details of its antigen-binding site, allowing further analysis of its binding mode. Our data demonstrate that rFv can be used as a tool to obtain high-resolution structures of antigen-binding regions, and may be useful for experiments requiring molecular weights smaller than that of a full Fab fragment, such as ITC and NMR spectroscopy.

Topic 3: B Cell Immunology and Antibody Functions

P03.37

Gaining Access to the Sterically Occluded CD4-Induced Epitopes

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Background: A preventive vaccine is potentially the most effective way to control the HIV pandemic. Such a vaccine needs to successfully harness humoral immunity and produce cross-reactive anti-envelope antibodies that mediate direct virus neutralization and/or Fc receptor-dependent killing. The targeted HIV envelope spikes are covered by a glycan shield, which masks most of the surface from the humoral immune system, leaving very few sites that are antigenic. Among the vulnerable sites of HIV Env are intermediate structures formed after gp120 interaction with target CD4+ cell. The capacity of these CD4i antibodies to carry out their functions in clearing HIV infection is dependent on the timing, duration and extent of cognate epitope exposure during the attachment and entry processes.

Methods: We employed confocal microscopy to visualize the temporal appearance and disappearance of CD4i epitopes during HIV-1 JRFL – TZM-bl cell interaction. We also examined the location of these exposed epitopes with ~20nm precision using super resolution microscopy.

Results: We find that CD4i epitopes recognized by A32, 17b, and C11 were exposed on HIV-1JRFL within 5 minutes of interaction with TZM-bl cells, and persisted up to 60 minutes. 3D examination of confocal images revealed that these epitopes were exposed at sites distal to the virus – cell interface. CD4i epitope exposure was greatly reduced on mutant HIV-1 JRFL with a defective virus matrix (MA) as it interacts with TZM-bl cells.

Conclusion: CD4i antibodies are thought to be sterically occluded from the virus – cell interface. Our results show that these epitopes appear distal to this site, where they can be accessed by antibodies involved in humoral and/or cell-mediated immunity. HIV Env gp120 engagement of target cell CD4 led to perturbations of virus MA that resulted in CD4i epitope exposure on other spikes of the virus away from gp120 – CD4 contact points.

P03.38

Low Levels of Anti-MPER Antibodies Are Detectable in Viremic HIV Infected

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Background: Antibodies against the CD4 binding site (CD4bs) in gp120 and the membrane proximal extracellular region (MPER) of gp41 are associated with broadly neutralization capacity. While the former have been identified in a large number of HIV infected individuals, the latter show a much lower prevalence.

Methods: 31 HIV-infected individuals with detectable viremia were selected for the study. Two samples separated by at least one year were analyzed for each individual. The presence of anti-CD4bs was screened using a competitive flow cytometric assay with a CD4 IgG fusion protein. The presence of anti-MPER antibodies was screened using a sensitive flow cytometric assay that measures antibody binding to different cell lines stably expressing two different truncated forms of gp41. These molecules properly expose the MPER epitope, as assessed by staining with control antibodies 4E10 and 2F5.

Results: Detectable levels of both anti-CD4bs antibodies and anti-MPER antibodies were observed in plasma samples from all groups. Of note, most samples showed recognition of MPER with a strong correlation between the recognition of the two different forms of truncated gp41 used ($r=0.65$, $p<0.0001$), suggesting that the assay was robust enough for the detection of these antibodies. However, no correlations were found between the level of anti-MPER antibodies, the neutralizing capacity of plasma samples, the viral load and the CD4 T-cell counts.

Conclusion: Anti-MPER antibodies can be detected in viremic chronic HIV infected individuals. The level of these antibodies does not appear to correlate with control of viremia or clinical progression. These data may suggest that anti-MPER antibodies are elicited in the course of HIV infection, but they do not reach the necessary threshold to be easily detectable or to impact infection.

P03.39

Antibody Subclass Skewing Predicts Enhanced ADCC Activity in Both Natural Infection and Vaccination

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Background: The innate immune recruiting property of antibodies are elicited following an Fc/Fc-receptor interaction. We previously demonstrated that HIV-specific antibodies from elite controllers (ECs) robustly recruit NK cells to mediate antibody dependent cellular viral inhibition (ADCVI) compared to antibodies from chronic progressors. To gain further insights into the biophysical properties of EC antibodies that enable them to recruit Fc-effector functions so robustly, we performed at 24 dimensional analysis (including specificity, isotype, Fc-receptor affinity, and function) of the antibody profiles mounted in ECs and HIV-progressors to define the humoral signature(s) associated with most robust innate immune recruiting activity

Methods: A total of thirty donors were included in this study (10 untreated chronics, 10 treated chronics, 10 ECs). Anti-gp120, p24, gp41 and gp140 antibody binding titers were quantified by ELISA and a customized-multiplex HIV binding assay against HIV recombinant gp120, p24, gp41 and gp140. Fc-receptor affinity was analyzed by Biacore. ADCVI, ADCC, and ADCP were quantified as previously described

Results: While no differences were observed in the antibody binding titer between ECs and chronic progressors against gp120 or gp41, we showed that ECs exhibited a higher level of p24-specific antibodies, associated with robust ADCVI activity. Moreover, humoral responses in ECs were skewed toward IgG1 and IgG3 responses, compared to chronic progressors, that strongly predicted enhanced ADCVI activity. Similar skewing of antibody responses were observed in RV144 vaccinees, strongly suggesting that specific cues elicited within this vaccine trial may have resulting in the induction of highly potent innate immune recruiting antibodies similar to those found in ECs

Conclusion: Overall, ECs elicit a skewed humoral immune response marked by the preferential selection of HIV specific IgG1 and IgG3 antibody subclasses, that parallels the humoral immune responses observed in RV144 vaccinees. Therefore, characterizing these unique antiviral capacities may provide critical information on humoral responses that could potentiate vaccine-induced responses

P03.40

A Novel Rabbit Monoclonal Antibody Platform to Dissect the Diverse Repertoire of Antibody Epitopes for HIV-1 Env Immunogen Design

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Background: The majority of available monoclonal antibodies (mAbs) in the current HIV vaccine field are generated from HIV-1 infected people. In contrast, preclinical immunogenicity studies have mainly focused on polyclonal antibody responses in experimental animals. Although rabbits have been widely used for antibody studies, there has been no report of using rabbit mAbs to dissect the specificity of antibody responses for AIDS vaccine development.

Methods: Here we report the production of a panel of 12 mAbs from one NZW rabbit that was immunized with a HIV-1 JR-FL gp120 DNA prime and protein boost vaccination regimen.

Results: These rabbit mAbs recognized a diverse repertoire of epitopes. Besides the traditional highly immunogenic V3 region, these mAbs recognized several previously underappreciated epitopes in the C1, C4, and C5 regions. Nine mAbs showed cross-reactivity against gp120s of clades other than clade B. At least three mAbs showed neutralizing activities with various breadth and potency. Increased somatic mutation percentage and long CDR3 were observed with some of the rabbit mAbs. More interestingly, phylogenic tree analysis showed that the heavy chain of mAbs recognizing the same region on gp120 were segregated into an independent subtree, implicating that these mAbs may derive from the same B cell precursor. Crystal structures of several rabbit mAbs suggested that these rabbit mAbs generated from vaccines mimic the binding modes of well-characterized human mAbs isolated from infected individuals.

Conclusion: Therefore, isolation of mAbs from vaccinated rabbits provides us an opportunity to study the evolution and affinity maturation of HIV-1 Env-specific mAbs elicited by candidate AIDS vaccines.

P03.41

Recombinant Env Proteins That Bind the Quaternary-Specific, V1/V2-Directed PGT Antibodies

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Background: Antibodies PGT141-145 are broadly neutralizing and recognize a glycan-dependent epitope in the V1/V2 loop, similar to antibodies PG9 and PG16. Collectively, this class of antibodies binds preferentially to the functional viral spike. Although PG9, and to a lesser extent, PG16, bind monomeric gp120s and V1/V2 scaffolds, to date no recombinant env-derived proteins have been identified that bind to antibodies PGT141-145.

Methods: As a first step toward obtaining structural information of the epitope recognized by PGT141-145, we have created and characterized novel gp140s and epitope scaffolds designed to present the V1/V2 conformation recognized by PGT141-145. To date, over 70 recombinant proteins have been expressed and tested for antibody binding.

Results: We have identified one V1/V2-scaffold protein that binds to PGT142. The binding is dependent on the HIV-1 strain used in the scaffold. We have also produced trimeric, cleaved gp140 constructs and evaluated them for binding to PGT141-145.

Conclusion: Proteins that accurately mimic V1/V2 conformations of the functional viral spike are crucial to obtaining structures of the PGT antibodies in complex with their epitopes, and may be ideal immunogens for eliciting broadly neutralizing, V1/V2-directed antibodies in a vaccine setting.

P03.42

Antibody Lineages with Evidence of Somatic Hypermutation Persisting for >4 Years in a South African Subject with Broad Neutralizing Activity

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Background: The origins and maturation pathways of broadly neutralizing antibodies (bnAbs) are unknown. Only ~20% of HIV-1-infected subjects develop bnAbs, suggesting their development may require unusual or protracted maturation pathways.

Methods: Two subjects were followed from the time of HIV-1 infection to >3 years; one developed broad neutralization (CAP206) while the other did not (CH040). Memory B cells were sorted as single antigen-specific cells, Ig heavy and light chain genes were amplified by PCR, and recombinant mAbs produced. Variable heavy chain gene (V_H) 454 pyrosequencing was performed on 5-10 samples spanning the 3-5 years of infection.

Results: From CAP206 we isolated 13 Env-reactive mAbs of which 6 (46%) used V_H1-69; from these we identified three V_H1-69 clonal lineages. One clonal lineage contained a neutralizing antibody (CAP206-CH12) while the other two lineages had non-neutralizing antibodies (CH64, CH82). All three clonal lineages were mutated (range 5.2-11.8% V_H mutation), and members of these lineages could be detected by 454 sequencing as early as one month after infection, with persistence as late as 57 months after infection. In contrast, an autologous neutralizing antibody clonal lineage from CH040 was found only over a one month period, and was not detected in three additional samples over 48 months. Of 18 additional CH040 Env clonal lineages, lineage members from 9 were found only at a single time point, 8 lineages had members found over two time points, and only 1/18 lineages were detected spanning 48 months.

Conclusion: Multiple Env-reactive antibody clonal lineages persisted for up to 5 years in broad neutralizer CAP206 while the autologous neutralizing antibody clonal lineage and other Env-reactive lineages did not persist in non-neutralizer CH040. These data raise the hypothesis that a high degree of clonal persistence was required for the development of broad neutralization, and imply a predisposition for this trait in broad neutralizers.

P03.43

Systematic Profiling of Polyclonal HIV Antibodies and Prediction of Effector Functions

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Background: Antibody-dependent effector functions (ADEF) with the ability to recruit the innate immune response may play an important role for the spread of HIV infections. ADEF are mediated by the antibody Fc and depend on antibody class and glycosylation status. We describe the systematic profiling of the Fc regions of HIV-specific antibodies isolated from different patient sera.

Methods: A LuminexTM-based suspension array was used to capture HIV antibody fractions binding to various HIV antigens on beads and probe them for binding to (1) antibody class-specific binding reagents, (2) Fc receptors, (3) complement proteins and (4) different lectins. The obtained binding profiles are correlated with other measures of effector function and may help to understand which ADEF are crucial to provide protection.

Results: Subclassing data has been used to correlate patients with enhanced measures of effector function such as phagocytosis or ADCVI. In addition, results of the Fc-gamma-receptor binding assay have been correlated to traditional measures of affinity such as SPR, showing that our method is giving useful results. We have also used epitope-specific reagents like the resurfaced stabilized core form of gp120 to isolate and probe eppitope-specific antibodies.

Conclusion: We hope to use our system as a means to quickly evaluate antibodies induced in an HIV vaccine setting. Being able to get a rapid profile of the polyclonal antibody response should be a useful predictor of vaccine efficacy. In addition, the assay we have devised is easily customizable, as antigens and receptors can be tailored to fit our interests.

P03.44

Characterizing the Fitness Cost of Viral Escape from the HIV-1 Broadly Neutralizing Monoclonal Antibody VRC01

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Background: The receptor-binding site on the HIV glycoprotein gp120 is a highly conserved epitope, and certain antibodies directed against this CD4 binding site (CD4bs) can potently neutralize the majority of circulating HIV-1 isolates. One such antibody, VRC01, was isolated from a slow progressor HIV-1 infected donor who maintained low to moderate viral load without treatment. We recently described that almost all viruses in this donor plasma had escaped VRC01 neutralization. This raised the question of whether viral escape from a broadly reactive CD4bs antibody results in reduced affinity for CD4 and thus, a fitness cost to viral replication.

Methods: Env-pseudoviruses and infectious molecular clones (IMC) were constructed using near-full length gp160 env genes from three circulating VRC01-resistant viruses and their complementary revertants (where VRC01-sensitivity was restored through mutations in the CD4 binding loop, Loop D and V5) as well as from autologous env genes from the VRC01 donor (both sensitive and resistant to VRC01 neutralization). Cell entry was quantified by infectivity into cell-lines expressing varying levels of the CD4 receptor, and replication kinetics of IMC were assessed by in vitro infection of primary CD4 T cells.

Results: Two of the three reverted VRC01-sensitive viruses demonstrated more efficient CD4 receptor mediated entry and greater replication in CD4 T cells, than the parental VRC01-resistant Envs. However, analysis of five VRC01-resistant and four VRC01-sensitive autologous Envs from the VRC01 donor revealed no significant difference in replication kinetics or efficiency of CD4 usage in infectivity assays.

Conclusion: Some VRC01-resistant viruses appear to have impaired replicative fitness, possibly caused by reduced CD4-mediated cell entry. However, VRC01-resistant Envs derived from the VRC01 donor did not display this deficiency, suggesting that compensatory changes over time may partially or fully restore CD4 usage and replication.

Topic 3: B Cell Immunology and Antibody Functions

P03.45

Conformational Study of Quaternary Epitope Region of V1/V2 Loop: Influence of Disulfide Bonds and Glycosylations

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Background: The HIV-1 envelope spike, which consists of a compact, heterodimeric trimer of the glycoprotein gp120 and gp41, is the sole viral target of neutralizing antibodies. The gp120 component of the viral spike is known to be heavily glycosylated, and glycosylation can affect the conformation of envelope spikes. V1/V2 variable loops of gp120 are key target regions for a number of broadly neutralizing human antibodies, such as PG9 and PG16, CH01-CH04, and PGT141-145. Two glycosylation sites (N156 and N160) have been shown by mutagenesis studies to be important in forming the PG9 and PG16 epitopes. Recently, Peter Kwong and coworkers have resolved crystal structure of V1/V2 domain of HIV-1 gp120 from strains CAP45 and ZM109 complexes with antigen-binding fragment of PG9.

Methods: We employ enhanced molecular dynamics sampling methods (eg. replica exchange molecular dynamics) to dissect the influence of disulfide bond and glycosylation on the conformational landscape of an indel free epitope region of V1/V2 loop. These methods are expected to capture the influence of glycosylation, solvent, rest of the gp120 protein and scaffold constructs on the conformation of V1/V2.

Results: We evaluate the backbone conformational preferences and solvent accessibility of each residue in the selected V1/V2 region and compare them to the antibody-bound conformation of this region. Both the disulfide bond that links V1 and V2 loops and the nearby glycosylations affect the beta sheet formation propensity of that region. Further characterization indicates that glycans predominantly influence the entropy of the V1/V2 loops.

Conclusion: Our studies reveal how glycans can impact the electrostatic and hydrophobic surfaces of the V1/V2 regions that have been proposed to form the epitope for broadly neutralizing antibodies. Along with proximal disulfide bonds, glycans tend to affect the local beta-sheet propensities that can further contribute to the quaternary nature of the epitope.

P03.46

Short Constrained Peptides Derived from Phage Display Libraries as Epitope Models: The Case of mAb 2F5

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Background: Since the monoclonal antibody 2F5 (mAb 2F5) was isolated in the early 90's, its epitope have continued to be the focus of extensive investigations attempting to elucidate the mechanism by which impedes viral entry into host cells. Because the DKW-flanking amino acids are strongly conserved in viruses, it is not clear whether the DKW only satisfies the 2F5 epitope recognition demand.

Methods: We used phage display technology involving biopanning of a pIII-type 7-mer constrained peptide library (not screened in previous experiments with 2F5) for its epitope mimics. After peptides selection and widely characterization of several phage-peptide clones, some of them were used as immunogens. Polyclonal antibodies were evaluated as cell-cell fusion inhibitors of the CD4-Env complex interactions.

Results: We found that the specificity of recognition of the epitope depends on the structural context in which the cognate epitope sequence is presented. The antibody does not tolerate any replacements of the DKW-flanking epitope amino acids and binds exclusively to the (L)DKWA sequence provided by a 7-mer constrained peptide exposed by the M13 phage pIII protein. Additionally, immunization data supports the notion that the binding and neutralizing immunogenic structural features of the described epitope model do not coincide.

Conclusion: In this study, we show that when mAb 2F5 screens a pIII-type phage display 7-mer constrained peptide library for its epitope mimics, it demands an epitope sequence longer than DKW and does not tolerate substitutions in the epitope amino acid sequence as has been suggested in previous reports.

P03.47

Mapping Epitopes on CRF01_AE Viruses Recognized by Broadly Neutralizing Antibodies in Sera from Elite Neutralizers from North America and Thailand

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Background: The RV144 trial has rekindled interest in defining the epitopes responsible for the neutralization of CRF01_AE (clade E) viruses. In this study we used a novel method, swarm analysis, to map epitopes on Thai viruses recognized by broadly neutralizing (bN) antibodies in HIV+ sera. Swarm analysis relies on the swarm of closely related quasi-species that evolve in each HIV+ individual. In these studies we analyzed the bN activity in sera from elite neutralizers (ENs) against Thai viruses.

Methods: Libraries of envelope genes were amplified from 30 Thai subjects who became infected with HIV in the VAX003 clinical trial. 12-24 clones from each individual were tested for sensitivity and resistance to neutralization by HIV+ EN sera obtained in Thailand and the USA. Pairs of neutralization sensitive and resistant viruses were identified from each individual, and the amino acids responsible for neutralization sensitivity localized by mutagenesis.

Results: Novel mutations were identified that conferred neutralization sensitivity or resistance to antibodies from ENs. Unlike previous studies with clade B viruses, the mutations in clade E viruses that altered neutralization sensitivity and resistance did not appear to affect the CD4 binding site or gp41. The mutations identified were located in V1, V2, and V3 domains of gp120 and had no effect on neutralization by bN monoclonal antibodies such as VRC01, b12, PG9, PG16, and 2G12.

Conclusion: Clade E viruses from Thailand can be neutralized by HIV+ sera from ENs infected with clade B or clade E viruses. The epitopes on clade E viruses recognized by bNAbs from ENs appear to be distinct from those defined with most of the bN monoclonal antibodies described to date. The results suggest that antibodies to the V1 domain as well as the V2 domain should be considered in the RV144 correlates analysis.

P03.48

Isolation of Broadly Neutralizing HIV-1 Antibodies from High-Throughput Single B Cell Culture

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Background: The isolation of broadly neutralizing HIV-1 antibodies that arise during infection has provided insights into the design of vaccine immunogens capable of eliciting similar antibody response. The use of HIV-specific sorting probes resulted in the isolation of antibodies to vulnerable viral epitopes, such as the CD4-binding site, but the use of such probes does not explore the subject's immunoglobulin repertoire breadth.

Methods: To identify new HIV-1 monoclonal antibodies (mAbs), we developed a B-cell culture system to isolate and screen thousands of B cells. Memory B cells were isolated by negative selection and individually cultured in 384 well plates with irradiated feeder cells expressing CD40 ligand. The addition of cytokines IL-2 and IL-21 stimulated proliferation and immunoglobulin secretion.

Results: After 14 days in culture, approximately 35% of the B cell clones secreted >100 ng/ul of IgG which met the sensitivity threshold to screen each clone in an automated micro-neutralization assay. B cell clonal expansion allowed recovery of the immunoglobulin heavy and light chains by RT-PCR, with subsequent cloning into expression vectors and mAb testing against a large panel of viruses. In one experiment screening approximately 8600 B cells, 9 clones were identified as potential contributors to the neutralization detected in the patient's serum. One of the isolated clones produced mAb VRC22 with 30% neutralization breadth and moderate potency. Further studies revealed that VRC22 was sensitive to JRCSF glycan mutants N332A and N301A but not N160K. VRC22 utilizes VH4-34 with a single amino acid CDR1 deletion and a mutation frequency of 7% which is a lower level of affinity maturation than observed for most known HIV-1 neutralizing antibodies.

Conclusion: This B-cell culture system allows efficient screening of thousands of individual B cells and the recovery of antigen specific mAbs. This approach can be used to isolate human mAbs to diverse pathogens.

Topic 3: B Cell Immunology and Antibody Functions

P03.49

Characterization of V1/V2-Specific Antibodies Present in Broadly Neutralizing Plasma Isolated from HIV-1 Infected Individuals

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Background: Recent studies of antibodies in human plasma from HIV-1-infected and immunized individuals have revealed an important role for the V1/V2 region of gp120 in an antiviral response, and recent evidence that protection in the RV144 vaccine trial correlated with the presence of V1/V2-specific antibodies suggests that the V1V2 region is an important target for candidate HIV-1 vaccines. However, the function of V1V2-specific antibodies is not well understood. In this study we present data about the development, subtype specificity, and neutralization activity of such antibodies present in plasma from several hundred infected North American and African subjects.

Methods: Human plasma from infected individuals were screened for neutralization activity versus a panel of subtype B, subtype C, and other Tier 2 pseudoviruses, and were titrated for binding activity against the consensus subtype B, C and A/E V1/V2 fusion glycoproteins, expressed via fusion to a fragment of the MuLV gp70 sequence. This system expresses V1/V2 domains in their native glycosylated and conformational forms. V1V2-specific antibodies were isolated from selected plasma by immunoaffinity chromatography on gp70-V1/V2 antigen columns, and characterized for neutralizing activity against various HIV-1 pseudoviruses.

Results: Most (>80%) of the HIV-1 infected subjects possessed robust levels of V1/V2 binding activity versus the three antigens. Interestingly, the development of V1/V2-reactive antibodies tracked with the development of autologous neutralizing antibodies in several subjects infected with subtype C viruses. Immunoaffinity-purified V1/V2-specific antibodies from selected broadly neutralizing plasma samples also possessed broad neutralization activities, with IC_{50}s generally in the 1-20 µg/ml range.

Conclusion: Highly cross-reactive V1V2-specific antibodies were present in almost all broadly neutralizing human plasmas at large concentrations, and frequently possessed modest neutralizing activities against a range of isolates, including tier 2 viruses. Additional information about the nature of these antibodies and their target epitopes would help elucidate their potential roles in protection against infection.

P03.50

Impact of IgA Constant Domain on HIV-1 Neutralizing Function of Monoclonal Antibody F425-A1g8

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Background: With the majority of HIV infections resulting from mucosal transmission of HIV, induction of an effective mucosal immune response would be pivotal in preventing transmission. HIV-specific IgA but not IgG has been detected in genital tract, seminal fluid, urethral swabs, urine and vaginal wash samples of HIV-negative sex workers and HIV-status discordant couples. Although present at low levels, purified mucosal and plasma IgA from HEPS individuals demonstrated cross-clade neutralizing activity and were able to inhibit HIV mucosal transcytosis.

Methods: The monoclonal antibody F425-A1g8 was generated in our laboratory by hybridoma technique and showed binding activity to the CD4i site of gp120. We isolated the variable genes of heavy chain (VH) and light chain (VL) from the hybridoma cell line and cloned the VH fragment into the vectors pHC-HuCγ1 and pHC-huCα1 individually, as well as cloned VL into the vector pLC-huCk. Both of VH and VL plasmids were co-transfected into CHO-K1 cells in equimolar amounts and established F425-A1g8 IgG1 and IgA1 expressing cell lines. We characterized the impact of different isotype variants of F425-A1g8 to HIV neutralizing activity by direct HIV viral neutralizing assay and antibody dependent cell-mediated viral inhibition.

Results: The switching constant domain of F425-A1g8 to construct IgG1 and IgA1 isotypes do not impact their binding activity with the CD4 site binding site of HIV. The result of neutralization showed that in contrast to little neutralization by F425-A1g8 IgG1 in the absence of sCD4, the IgA1 variant of the antibody displayed significant neutralization activity against a range of HIV clade B isolates.

Conclusion: This research clearly suggests that IgA isotype utilizing its unique molecular structure plays an important role in HIV neutralization. The studies of the neutralizing function of IgA isotypes may also serve to inform the design of vaccine strategies that may be more effective at preventing mucosal transmission.

P03.51 LB

Broad and Potent Neutralization of HIV-1 by Human-Llama Fusion Antibodies Derived from Immunized Llamas

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Background: Llamas naturally produce heavy chain only antibodies in addition to conventional antibodies. The variable regions of the heavy chain (VHH) demonstrate comparable affinity and specificity for antigens with conventional immunoglobulins. To date, immunizations in human and animal models have yielded only antibodies with limited ability to neutralize human immunodeficiency virus (HIV)-1.

Methods: A VHH (J3) isolated from a llama multiply-immunized with recombinant trimeric HIV-1 envelope proteins (Env) was found to neutralize 96 of 100 HIV-1 strains, encompassing subtypes A, B, C, D, BC, AE, AG, AC, ACD, CD and G. Isolation involved expression of VHH in E. coli and analysis of neutralization ability in TZM-bl reporter cells.

Results: Newly isolated VHH from multiple immunized llamas also have broad and potent HIV-1 neutralization activity. J3 targets HIV-1 via the CD4-binding site and neutralization is seen when J3 is used in combination with VHH targeting other Env epitopes. VHH-human FC fusion heavy-chain only antibodies (VHH-FC) have been constructed and J3 activity is not only preserved in this context but enhanced.

Conclusion: This study shows that experimental immunization with recombinant HIV-1 Env can elicit broad neutralizing heavy-chain only antibodies and supports the development of VHH and VHH-FC as anti-HIV-1 microbicides and therapeutics.

P03.52 LB

Humoral Immune Response Profiling with Peptide Microarrays

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Background: In a case-controlled analysis designed to identify immune correlates of infection risk in the RV144 HIV vaccine trial, 2/6 primary variables showed significant correlation. One, the binding of IgG antibodies to the V1/V2 loop of HIV-1 env protein appears to protect against HIV-1 infection. Consequently, data from a detailed mapping of antibody reactivities in response to vaccination on a sub-protein level might be a predictor for vaccine efficacy. In contrast to assays relying on whole antigens, peptide microarrays are efficient tools to deliver such information. Besides, complex peptide libraries can cover the HIV sequence diversity. We present peptide microarray data from a human clinical trial.

Methods: A library representing Env-gp160 consensus sequences from clades A,B,C,D,M,CRF1, and CRF2 was produced. Serum samples of vaccinees from groups receiving different doses of a prototype Ad26 vector-based vaccine expressing clade A-HIV-1 Env (Ad26.EnvA.01) were evaluated. For the calculation of signals the signal intensity per peptide at baseline was substracted from the signal intensity at week 28 after vaccination.

Results: All groups of vaccinees show a clear pattern of antibody reactivity after vaccination. This pattern depends on the dose and the number of doses given. From the lowest doses of $1x10^9$ viral particles (vp) a cross-clade reactivity towards the V3 region of gp120 is observed. At doses above $1x10^{10}$ vp the magnitude of signals is enhanced and new regions of gp120 are targeted by patient antibodies, e.g. towards the V2 loop region. The representation of different clades on the peptide microarray allows for a detailed investigation of the clade specificity of the antibody response after vaccination.

Conclusion: Costly vaccination studies require consideration of all possible factors for success. The results of peptide microarray experiments may facilitate the design and dosing regimen of vaccines in clinical trials and shed light on the underlying protective mechanisms.

Topic 3: B Cell Immunology and Antibody Functions

P03.53 LB

Study on the Functional Role of Immunoglobulin E as Surrogate Marker for HIV/AIDS Infection

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Background: IgE class of antibodies has been found in mammals and plays an important role in allergic and hypersensitivity reactions. Certain viral infections are known to produce specific IgE antibodies, to the extent that significant changes in the level of total serum IgE may occur. Study attempts to associate the Level of Ig E in HIV progression.

Methods: The study involves fifty HIV seropositive patients attending Anti-Retroviral Therapy Centre, Department of Sexually Transmitted Disease, Rajaji Government Hospital, and Madurai,India subjected for the present study. The individual involves 27 HIV/ AIDS Male patients, 23 HIV/ AIDS Female patients. The control sample comprises 15 HIV sero negatives. The samples were collected at the informed consent of the patients. Serum sample were collected and IgE was quantified using MAGIWELL IgE quantitative solid phase Enzyme- linked Immunosorbent assay (ELISA).

Results: The study documents highest percentage of deviation from the control observed in Male HIV seropositives (43.7%) and age-wise influence documents highest percentage of deviation in the age group 15- 29 years (56%).

Conclusion: Serum IgE level in the present study found to be elevated from the normal range documents the existence of imbalance between Th 1 and Th 2 and associated with T-cell dysfunction and a hypergammaglobulinemia. The present results suggest that elevation of circulating IgE levels may be due, at least in part, to specific IgE directed to the HIV virus rather than as a result of a nonspecific phenomenon. HIV infected adults indicate that total IgE is also increased during the early stages of disease, and this elevation appears to be independent of CD4 counts and is not correlated with the levels of other immunoglobulins, suggesting an important role for IgE as a surrogate marker of disease progression Further research need to be exploited to bring out the exact role of IgE in HIV pathogenesis.

P03.54 LB

Pre-existing Humoral Immunity In Military Smallpox Vaccinees Temporally Effects MVA-Vectored Transgene Expression in Dendritic Cells

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Background: Modified Vaccinia Ankara (MVA), a vector-based vaccine that targets dendritic cells (DC) and induces cell-mediated immunity, is a promising HIV vaccine candidate. As MVA vaccination strategies continue to be explored, concerns arise regarding transferability to individuals with pre-existing immunity to vaccina, particularly military personnel. Prior reports suggest long-lasting vaccinia immunity after childhood vaccination. This study, conducted in a unique cohort of adult primary vaccinees, explores how pre-exisiting humoral immunity to vaccinia affects entry and transgene expression of MVA-vectored vaccines in a primary human DC infection model.

Methods: Serum from military personnel vaccinated against smallpox with either Dryvax or ACAM2000 vaccines were obtained at 4 time points post-vaccination (n=50 per time point, 400 total). As a comparator, n=25 individuals with longitudinal sera available at corresponding time points were studied to compensate for inter-individual variability in response over time. Sera were tested for inhibition of infection of DCs in vitro using either MVA-GFP or MVA-CMDR, an HIV vaccine candidate with env/gag/pol inserts, currently in clinical trials. Vaccinia naïve sera served as a negative control. Vaccinia binding titers were measured by ELISA.

Results: Vaccinia binding antibody titers waned after 5 years and were undetectable 10 years after vaccination. Neutralizing activity, as measured by transgene expression in DCs, confirmed this finding. Expression and neutralization of HIV p24 (gag) expression data in DC were equivalent to that of GFP. No differences in neutralization activity were detected between Dryvax and ACAM2000 vaccinee sera at corresponding time points.

Conclusion: In an adult, military, primary vaccinee population, humoral responses to smallpox vaccination do not persist as long as reported in the civilian population vaccinated during childhood. This data suggests that pox-vector based vaccines may be used in the military population, and that the age of primary vaccination influences durability of humoral immunity.

P03.55 LB

Light Chain Plays A Role In Neutralizing Antibody In b12 H Chain Knock-In Mouse

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Background: HIV vaccine design has focused on epitopes seen by broadly neutralizing antibodies (bNAbs). The key to developing a robust vaccine capable of eliciting bNAbs is to identify the epitopes appropriate for incorporation into vaccines and to exclude irrelevant or harmful epitopes.

Methods: To assess bNAb B cell responses, we have developed series of bNAb B cell lines and b12 'knock-in' mouse.

Results: Antibodies targeting the CD4 binding site, including PGV04 and b12, expressed higher levels of surface Ig compared to MPER targeting antibody 4E10. Correlating with the cell line data, in b12 heavy chain knock-in mice, B cells could develop robustly (this was not the case in 4E10 mice). 10-20% of b12 transgenic B cells bound to YU2 gp120 monomer and lacked expression of CD93, indicating that they matured normally and were non-anergic. Upon immunization with YU2 trimer, b12 mice exhibited strong gp120-specific IgG responses. Gp120-binding cells used Vk10-96 and Vk19-93, but were skewed to usage of Jk2 and Jk4, which are normally used less frequently than Jk1 and Jk5. Cells in the gp120- non binding fraction frequently expressed Vk1-135. Interestingly, the CDR1 length of Vk1-135 is 11 amino acids in length, whereas in Vk10-96 and Vk19-93 genes it is 6 amino acids, suggesting that a short CDR1 is important for gp120 binding. Several anti-gp120 hybridomas were established from LPS-stimulated B cells. However, compared to the original b12 antibody, these hybridoma cells did not show strong neutralization activity.

Conclusion: The antibodies targeting CD4 binding site are apparently not autoreactive, supporting the notion that this is a desirable epitope for vaccine target. B12 H chain knock in mouse data showed light chain is important for bNAb function. B12 knock in mice and recently generated b12 germline strains are suitable for vaccine study.

P03.56 LB

HIV-1 Neutralizing Antibodies Display Dual Specificity for the Primary and Coreceptor Binding Sites and Preferential Recognition of Fully-Cleaved Env

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Background: The gp120 CD4 binding site (CD4bs) and coreceptor binding site (CoRbs) are two functionally conserved elements of the HIV-1 envelope glycoproteins (Env). We previously defined the presence of CD4bs neutralizing antibodies (nAbs) in the serum of an HIV-1 infected individual and subsequently isolated the CD4bs-specific monoclonal antibodies (mAbs) VRC01 and VRC03 from the memory B cell population. From the same patient sera, we detected that there was also a fraction of potent nAbs specific for elements of the Env CoRbs by differential protein adsorption followed by neutralization analysis.

Methods: In this study, we employed a differential FACS-based sorting strategy using a stabilized gp120 core and a mutant gp140 possessing a CoRbs "knockout" mutation (I420R) to isolate CoRbs specific B cells.

Results: The mAb VRC06 was recovered from these cells and its genetic sequence allowed us to identify a clonal relative, VRC06b, which had been isolated from a prior cell sort using a resurfaced core gp120 probe. VRC06 and VRC06b neutralized 22% and 44% of circulating viruses tested, respectively. Virus neutralization assays revealed that VRC06/VRC06b better neutralized autologous viruses compared to VRC01 and VRC03. More potent autologous neutralization was associated with a 7 amino acid residues insertion in the framework of the VH gene-coding region. Epitope mapping studies demonstrated that the two mAbs were sensitive to mutations in both the gp120 CD4bs and the CoRbs, including the gp120 bridging sheet and the base of the third major variable region (V3). Interestingly, cell-surface binding assays demonstrated their preferential recognition of fully-cleaved Env trimers compared to un-cleaved trimers.

Conclusion: VRC06 and VRC06b are novel neutralizing mAbs that bind to a region of gp120 that overlaps with both the primary and secondary HIV-1 receptor binding sites, preferentially recognize fully-cleaved Env, and complement the neutralizing capacity of other CD4bs bNAbs isolated from the same individual.

P03.57 LB

Antibody responses to V2 loop Are Induced by CRF01_A E and not Clade B Envelopes

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Background: The RV144 vaccine trial of canarypox vCP1521 (ALVAC-HIV) prime and bivalent HIV-1 envelop gp120 protein subtype B/CRF01_AE boost (AIDSVAX B/E) demonstrated a significant effect in preventing HIV-1 infection. A case-control analysis suggested that variable loops 1 and 2 (V2) of gp120 may have contributed to protection against HIV-1 acquisition. Two other vaccine trials using gp120 only– VAX003 (AIDSVAX B/E) and VAX004 (AIDSVAX B/B) failed to show protection

Methods: Binding antibody responses induced by the RV144, VAX003 and VAX004 vaccine regimens were compared using ELISA. Recombinant gp120 envelope proteins MN (subtype B), 92TH023 (CRF01_AE), A244 (CRF01_AE) and cyclic V2 peptides were used as capture antigens

Results: After two protein injections, VAX004 had the highest geometric mean titers (GMT) against MN (25,600), VAX003 against A244 (21,378) and RV144 against 92TH023 (6,263). Antibody responses against V2 (CRF01_AE) were detected in plasma samples from RV144 and VAX003 with GMTs of 972 and 1100, respectively. However, VAX004 failed to generate antibodies against CRF01_AE V2. None of the three vaccines generated antibodies against MN V2 after two protein immunizations.

Compared to VAX004, VAX003 had higher antibody responses against all three recombinant proteins: 2-fold (MN), 4-fold (A244) and 4-fold (92TH023) when two additional protein injections were administered. Two additional protein inoculations in the VAX trials failed to increase antibody titers against, CRF01_AE V2, but generated a small response against MN V2 (GMT, 76) in VAX003

Conclusion: Antibody responses against V2 were induced by CRF01_A E recombinant proteins as there were no responses induced by the AIDSVAX B/B vaccine regimen. Repeated protein immunization increased the magnitude of responses against recombinant proteins in VAX003 but failed to increase titers against CRF01_AE V2. If antibodies against V2 are protective against HIV-1 acquisition, designing antigens with greater V2 antigenicity would be critical

P03.58 LB

Partial Germline Reversions Can Increase VRC07 Potency and Breadth

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Background: VRC01 and related antibodies target the CD4 binding site (CD4bs), are broadly neutralizing and highly potent, and have undergone high levels of somatic hypermutation. To optimize such antibodies for passive immunization and to further understand antibody development, we reverted three CD4bs antibodies towards their putative germlines and analyzed the effects on breadth and potency. Interestingly, we also identified key germline reversion mutations that increased neutralization potential.

Methods: Structure/function-based analyses were used to design partially reverted heavy and light chains based on the clonally-related antibodies VRC01, NIH45-46, and VRC07. Mature CDRs were maintained and framework regions were back-mutated. The antibodies were expressed, purified, and tested for binding to gp120 by ELISA. Neutralization against a panel of tier 2 HIV-1 pseudotyped viruses was determined for select antibodies.

Results: The heavy chains of VRC01, NIH45-46, and VRC07 are 42%, 41%, and 44% somatically mutated from their germline precursor, while the light chains are 29% (VRC01/07) and 27% (NIH45-46) somatically mutated. We began by reverting over half of the heavy chain somatic mutations and over one-third of the light chain somatic mutations to their germline residue identities. An iterative design approach was used, and we systematically re-introduced mature residues to the partial germline reversions. Most mutants retained the ability to bind gp120 and neutralize diverse HIV-1 pseudoviruses, albeit with lower breadth and/or potency than their mature counterparts. Additionally, we found 3 partial-germline reversion mutations that increased VRC07 potency.

Conclusion: Here, we showed that in most cases mature framework regions in addition to mature CDRs were required for highest neutralization potency and breadth. However, three framework germline reversion mutations increased potency 2-3 fold. These partial reversions are being combined with other mutations, including those that modulate Fc effector function, to optimize the antibody function for passive transfer in NHPs and humans.

P03.59 LB

Regulatory B Cells Are Induced In Untreated HIV-1 Infection and Suppress HIV-1 Specific T Cell Responses

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Background: Regulatory B cells (Breg), the B cells producing interleukin 10 (IL-10), have been identified in mice and humans. Mouse Breg can suppress innate and T cell responses and are implicated in pathogenesis of some autoimmune diseases and immune evasion of some pathogens. However, the role of Breg in humans is less clear.

Methods: PBMC and gut biopsy samples were obtained from healthy donors and HIV infected individuals. Flow cytometry and Luminex were used to quantify cytokine production. Flow cytometry were used to analyze Breg's phenotype.

Results: Breg were elevated in both peripheral blood and gut tissue of untreated HIV-1 infected individuals and the elevation correlated with viral load in early HIV-1 infection. Breg from HIV-1 infected individuals were $CD19^{+}TIM\text{-}1^{+}$.Anti-retroviral therapy could reduce elevated Breg frequency. Treatment of B cells from healthy donors with microbial translocation products could differentiate them toward a Breg phenotype. Ex vivo Bregs from HIV-1 infected individuals suppressed cytokine production /degranulation of HIV-1 specific T cells that was in part IL-10 dependent.

Conclusion: Our findings show that Bregs are induced early in HIV-1 infection, which may play a role in inhibiting effective HIV-1-specific T cell responses.

P03.60 LB

Heterologous Neutralization Breadth Persists Despite B-Lymphocyte Dysfunction in Chronic HIV-1 Infection

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Background: Of the millions globally infected with HIV-1, only 20-30% will develop broadly neutralizing antibodies. To date, no one has measured this phenomenon in a cohort of subjects for which multiple aspects of B-lymphocyte dysfunction have been evaluated in parallel.

Methods: In 16 viremic seroconverters, the cross-clade neutralizing activity of plasma was investigated using a panel of thirteen clade A, B, and C HIV-1 envelope (Env) pseudotyped virions, which represented three tiers of sensitivity. The neutralization IC50 was calculated for each plasma-Env combination, and these data were used to determine a breadth (how many Envs were neutralized) and potency (the strength of neutralization) score for each seroconverter. Additionally, the level of plasma antibodies that bound to the monomeric form of a subtype B Env gp120 (HIV-1 BaL) was quantitated.

Results: A range of neutralization breadth emerged: three plasma samples (19%) demonstrated widespread neutralizing activity against this panel of Envs, while five subjects (31%) exhibited a complete lack of detectable neutralization at the lowest dilution of plasma tested (1:100). No correlation was observed between neutralization breadth or potency and parameters of B-lymphocyte dysfunction (PD-1, BTLA), immune activation (Ki-67, CD95), or disease progression (CD4 T cell count, plasma viral load). The level of total IgG in each plasma sample, however, did significantly correlate with both neutralization breadth and potency. Like total IgG, anti-gp120 binding antibodies also positively correlated, but, in this case, the correlations only trended toward significance. Anti-gp120 binding antibodies did not correlate with parameters of B-lymphocyte dysfunction, immune activation, disease progression, or total IgG level.

Conclusion: These findings demonstrate that even in chronically HIV-1-infected subjects in whom B-lymphocytes display multiple indications of dysfunction, antibodies that mediate cross-clade neutralization breadth (particularly anti-gp120 binding and other IgG antibody specificities) continue to circulate in plasma.

Topic 3: B Cell Immunology and Antibody Functions

P03.61 LB

Sequential Exposure to Specific Antibody Escape Mutations May Program Neutralization Breadth During Subtype A HIV-1 Infection

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Background: Mechanisms that expand the otherwise narrow neutralization capacity observed during early HIV-1 infection are currently undefined, but multiple lines of evidence suggest that the ability to elicit broad and potent neutralizing antibodies (nAbs) via vaccination could increase the protective efficacy of immunization.

Methods: Here we characterized the initial nAb response in a subtype A HIV-1-infected Rwandan seroconverter and investigated how consequent immune events influenced the downstream development of cross-clade breadth. Autologous envelope (Env) glycoproteins from the transmitted/founder virus and twenty longitudinal nAb escape variants were utilized to define the neutralization targets of autologous plasma and monoclonal antibodies (mAbs), the latter of which were also examined genetically and structurally through crystallization. Heterologous Env glycoproteins from nine cross-clade variants were used to determine neutralization breadth.

Results: Initially, nAbs targeted a single region of gp120 at the base of V3 involving the alpha2 helix. A single amino acid change at one of three positions conferred early escape from plasma nAbs. Then, two autologous mAbs, revealed to have flat epitope contact surfaces, typified the second wave of nAb pressure and neutralized escape Envs carrying the defined V3/alpha2 helix substitutions in a manner dependent on immunoglobulin light chain variable domain modifications. Subsequent mAb resistance arose in later Envs through alteration of two glycan motifs previously implicated in the development of nAb breadth. Finally, three-year autologous plasma displayed moderate neutralization breadth and most potently neutralized heterologous Envs containing the altered glycan motifs.

Conclusion: Our data demonstrate that the V3-proximal nAb epitope originally recognized in this individual elicited strain-specific mAbs and that glycan-mediated escape from these mAbs likely initiated the development of heterologous neutralization breadth. These findings suggest that epitope localization and the resultant routes of viral immune evasion, which include exposure to a specific sequence of nAb escape variants, drive humoral immune responses toward cross-clade viral recognition.

P03.62 LB

Analysis of gp41 Epitopes in Model Viral Membranes to Study HIV-1 Neutralization

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Background: The membrane proximal external region (MPER) of the HIV surface glycoprotein gp41 is the binding site for several potent broadly neutralizing antibodies. These antibodies recognize highly conserved linear peptide epitopes and antigens derived from this region have the potential to be important components of a vaccine directed against HIV-1. Highly conserved peptide epitopes for these antibodies are sterically obscured from the immune system by an intimate association with the viral membrane and by the presence of a large trimeric glycoprotein spike. As a result, previous attempts to immunize with linear peptides have failed to elicit broadly neutralizing antibodies.

Methods: In order to better mimic the structural features of the MPER domain with an eye towards better understanding of epitope-antibody interactions near membranes, peptide mimics derived from HIV gp41 have been synthesized with a variety of structural changes including positioning of the epitope with respect to the membrane as well as the presence and nature of an anchoring transmembrane domain. These peptide mimics have been incorporated into membrane bilayer mimics called "Nanodiscs," which are ~10 nm-diameter structures composed of a phospholipid bilayer ringed by an apolipoprotein-derived scaffold protein.

Results: We have examined the affinity of HIV-1 neutralizing antibodies to these membrane-bound peptides to better understand the binding of these neutralizing antibodies to epitopes on or near a membrane. Significant differences in binding have been observed between peptides with different transmembrane domains.

Conclusion: We have chemically synthesized peptide mimics of gp41 and incorporated them into artificial membrane bilayers. The peptides are anchored with both native and artificial transmembrane domains, and both the presence and nature of the transmembrane domain modulates antibody binding. Membrane presentation of peptide antigens with an appropriate transmembrane domain may be an important feature of vaccine design.

P03.63 LB

Mutations in the V1 Domain of Thai CRF01-AE Viruses That Confer Sensitivity/Resistance to Broadly Neutralizing Antibodies

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Background: Antibodies to the V1/V2 domain of gp120 have recently been identified as a correlate of protection in the RV144 clinical trial. To better understand the specificity of broadly neutralizing antibodies) to the V1/V2 domain of Thai CRF01_AE viruses, we analyzed the specificity of antibodies in HIV+ elite neutralizer (EN) sera by swarm analysis.

Methods: Swarm analysis makes use of the swarm of closely envelope variants that evolve in each HIV-1 infected individual, as a source of naturally occurring and biologically relevant mutations that confer neutralization sensitivity/resistance. Envelopes from clade B and CRF01_AE viruses were tested for neutralization sensitivity/resistance with sera from ENs infected with clade B and CRF01_AE viruses.

Results: We found five mutations in the V1 domain that affected neutralization sensitivity/ resistance of CRF01_AE viruses. This differed from clade B viruses in which mutations altering neutralization sensitivity/resistance clustered in the V2 domain. Structural studies have shown that the V1/V2 domain of gp120 consists of a four-stranded _-sheet structure. We found that mutations affecting neutralization sensitivity/resistance in Thai CRF01_AE viruses clustered around the exposed turn at the junction of the A-B strands. In contrast, the mutations that altered neutralization sensitivity/resistance in clade B viruses clustered around exposed turns at the junction of the B-C and the C-D strands.

Conclusion: The present studies suggest that there is a major difference in the antigenic structure of the V1V2 domain between clade B and CRF01_AE envelope proteins. These results suggest that antibodies to the V1 domain of CRF01_AE envelope proteins should be evaluated as a correlate of protection in the RV144 trial. For this purpose, studies using novel proteins and scaffolds, that replicate the structure of conformation- and glycoform- dependent epitopes in the V1/V2 domain, are under investigation.

P03.64 LB

V1/V2-Directed Antibodies Elicited in RV144 Vaccinees Bind to a Structurally Polymorphic Site

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Background: An immune correlates study of the RV144 vaccine trial demonstrated that elicitation of V1/V2-directed antibodies was inversely associated with infection risk. In addition, a sieve analysis of breakthrough infections identified residue 169 in the V2 loop as a site of immune pressure. Antibodies CH58 and CH59 were isolated from RV144 vaccinees and were shown to neutralize some Tier 1 isolates.

Methods: The epitopes for CH58 and CH59 were identified by peptide-mapping and alanine-scanning. Antigen-binding fragments (Fabs) were generated by proteolysis, and crystal structures of the CH58 and CH59 Fabs in complex with a linear V2 peptide were determined to 1.7 and 1.5 Å, respectively. Surface plasmon resonance was used to determine the kinetics of antibody binding to recombinant env-derived proteins.

Results: The crystal structures reveal that CH58 recognizes V2 residues 167-176 as an α-helix and residues 177-181 as an extended coil. In contrast, CH59 recognizes residues 168-173 as coil, with residues 174-176 as a short 3_{10} helix. Both antibodies form hydrogen bond or salt-bridge interactions with the side chain of Lys169, the imputed site of immune pressure. The conformations of V2 recognized by CH58 and CH59 differ markedly from the β-strand conformation recognized by the broadly neutralizing antibody PG9. All three antibodies bound with high affinity to the same gp120 protein, suggesting that V2 residues 167-176 can adopt multiple conformations on a shed gp120.

Conclusion: Since PG9 is broadly neutralizing and recognizes V2 as a β-strand, and because CH58 and CH59 neutralize only some Tier 1 isolates and recognize alternative conformations of V2, these data suggest that the β-strand conformation of V2 may be favored in the viral spike, whereas alternative V2 conformations may be favored on shed gp120s. If true, then vaccine immunogens may need to have the V1/V2 region stabilized in the β-strand conformation in order to elicit broadly neutralizing antibodies.

Topic 4: Clinical Vaccine Trials and Trial Site Challenges

P04.01

Priming with a "Simplified Regimen" of HIV-1 DNA Vaccine Is as Good as a "Standard Regimen" When Boosted with Heterologous HIV-1 MVA Vaccine

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Background: Intradermal priming with DNA prior to MVA boosts gives strong and broad immunogenicity, however that required 5 injections at each immunization. A higher concentration of DNA might allow a simpler administration.

Methods: This double blind, placebo-controlled trial, enrolled 120 (12 placebo) HIV-uninfected volunteers, in Dar es Salaam and Mbeya. Two pools of DNA plasmids were used (pool1 EnvABC + RevB, pool2 GagAB + RTB) boosted with MVA CMDR EnvE, GagA, PolA. Volunteers were randomized in three groups of 40, primed with either two injections of 300ug, one in each arm, (total 600ug) of DNA with combined plasmids (group IA) or two injections of 300ug with one pool in each arm (total 600ug) of DNA (group IIA) "simplified regimens" or five injections, 2 (pool1) and 3 (pool2) injections in the right and left arm respectively, (total 1000ug) of DNA (IIIA) "standard regimen". DNA/Placebo priming was administered by a needlefree (Biojector) device intradermally at weeks 0, 4 and 12. All volunteers were boosted intramuscularly with 10 pfu of recombinant MVA/placebo at weeks 30 and 46. The primary end point was the number of ELISpot responders to Gag and Env, 2 weeks post the last vaccination.

Results: There were no safety concerns. The response rate to Gag and/or Env was 27/32 (84%) in group IA vs 31/33 (94%) in group IIA (p=0.26). The response rate to Gag and/or Env when comparing the 'simplified regimens' vs 'standard' regimen was 58/65 (89%) vs 32/32 (100%) p=0.09. In responders the median magnitude (IQR) response to Gag was 165 (100,365) SFC/million PBMC vs 210 (120,320), p=0.46 while the magnitude for Env was 150 (92,225) vs 110 (80,160) p=0.17 for the 'simplified' vs 'standard' regimens.

Conclusion: The simplified HIV-1 DNA regimens primed as well as the standard regimen for cellular immune responses following boosting with with MVA.

P04.02

Barriers to Participation in HIV Vaccine Trials and Cancer Trials: A Cost-Gain Analysis

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Background: Previous studies examining barriers and willingness to participate in HIV vaccine trials have demonstrated the role of factors identified by the Health Belief Model. Barriers to cancer trials can also be understood in terms of a theoretical framework consisting of the locus of the barrier (personal vs. social) and the nature of the barrier (risk vs. cost). In this systematic review of review articles, we extend this framework to another life-threatening disease, cancer. The purpose is to improve conceptual clarity about volunteering in clinical trials by comparing and contrasting barriers in these two areas.

Methods: In 2012, two people independently searched the Cochrane Database for Systematic Reviews, Pubmed, Embase, and Google Scholar to identify review articles examining cancer trial barriers to participation. Search terms used were: "cancer", "oncology", "cancer trials", "oncology trials", "clinical trials", "medical research", "willingness to participate", "barriers". Review articles were also retrieved from our search examining motivators to participation in cancer research and from bibliographic references.

Results: We retrieved 19 review articles from 2000-2012 examining barriers to participation in cancer trials. "Reduced quality of life" / "distrust of institutions" / "loss of control" were personal risks (PR). "Perceptions of the provider" / "subjective norms" were social risks (SR). "Side effects" / "experimental nature of the trial" were personal costs (PC). Misconceptions included "confidentiality concerns". Consistent with HIV vaccine trials, most barriers with regards to cancer trials were related to PR and PC. More misconceptions were identified in HIV vaccine preparedness studies (VPS).

Conclusion: Personal risk, PC, and SR barriers were similar to those identified in HIV VPS, but more misconceptions were identified in the latter. Understanding barriers can result in better recommendations on how to overcome these barriers. A limitation is that cancer populations are different than those affected by HIV.

P04.03

Motivators to Participation in HIV Vaccine Trials and Cancer Trials: The Application of Personal and Social Categorization

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Background: The Health Belief Model provides a framework to understand motivators for volunteering for medical research. Motivators can take the form of social benefits, including macrosocial (pertaining to one's greater society), mesosocial (pertaining to one's larger social world), and microsocial (pertaining to one's immediate social network). Personal benefits can be sub-divided into psychological, physical, and financial well-being. In this systematic review of review articles, we extend our conceptual framework from motivators regarding participation in HIV vaccine trials to another life-threatening disease, cancer.

Methods: In 2012, two people independently searched the Cochrane Database for Systematic Reviews, Pubmed, Embase, and Google Scholar to identify review articles examining cancer trial motivators to participation. Search terms were: "cancer", "oncology", "cancer trials", "oncology trials", "clinical trials", "medical research", "willingness to participate", "motivators", "incentives". Review articles were also retrieved from our search examining barriers to participation in cancer research and from bibliographic references.

Results: We retrieved 12 review articles from 2000-2012 examining motivators to participation in cancer trials. Personal benefits were most often psychological such as "coping with symptoms". Social benefits included "advancing research", "helping other cancer patients", and "for their family". These categories also apply well in HIV vaccine trials. Personal/psychological benefits are most commonly cited as motivators for both types of research. In cancer research, "coping with symptoms" and "extending life expectancy" were motivators; in HIV vaccine trials, it was to "reduce the risk of becoming HIV positive".

Conclusion: While specific motivators vary between considerations of cancer research and HIV vaccine trials, these motivators fall into similar categories at similar frequencies. Personal / psychological benefits are most common in each. Altruistic factors found at a macrosocial level outnumber factors relevant to more proximal levels, such as family and other patients. Participant recruitment must be mindful of these categories of motivators for both HIV vaccine and cancer research.

P04.04

A Social Ecological Model of Willingness to Participate in HIV Vaccine Trials Among Men Who Have Sex with Men in Chennai and Mumbai, India

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Background: Recruitment of low- and middle-income country volunteers from most-at-risk-populations in HIV vaccine trials is essential to vaccine development. In India, men who have sex with men (MSM), at 20-fold higher risk for HIV infection than the general population, are a crucial population for recruitment. Research on willingness to participate (WTP) in HIV vaccine trials has focused predominantly on individual-level determinants (e.g., safety concerns, mistrust, altruism) in high-income countries. We used a social ecological framework to explore multi-level determinants of WTP among MSM in India.

Methods: We conducted 9 focus groups (n=49) with low socioeconomic MSM (aged 20-46 years, mean=28), including peer outreach workers, in Chennai (in Tamil) and Mumbai (in Marathi/Hindi), and 7 key informant interviews with MSM community leaders and healthcare professionals. Focus groups/interviews were recorded, transcribed and translated into English. Two independent bilingual investigators conducted narrative thematic analysis using line-by-line coding and a constant comparative method, with member-checking by community representatives.

Results: Structural-level determinants of WTP included poverty, compensation for trial-related harms and having financially-dependent family members. Community-level factors were negative societal attitudes about same-sex sexuality and community engagement in HIV vaccine research. Interpersonal factors included anticipated peer and familial reactions and perceived HIV stigma. Individual-level determinants of WTP included knowledge about vaccines, openness about one's sexuality and altruistic beliefs.

Conclusion: WTP in HIV vaccine trials among MSM in India is associated with multi-level factors. Ubiquitous interpersonal and community-level concerns characteristic of the sociocultural context may complicate individual-focused conceptualizations of WTP. Interventions to reduce stigma and discrimination against MSM and PLHIV, capacity-building of MSM service organizations, and sustained and transparent communications tailored to the knowledge, concerns and educational level of local communities may support meaningful engagement of MSM in HIV vaccine trial preparedness. Attention to providing fair yet non-coercive compensation and healthcare benefits are warranted to support ethical conduct of trials.

Topic 4: Clinical Vaccine Trials and Trial Site Challenges

P04.05

A Decade of Partnering to Stop HIV in West Africa: GAIA VF Prevention, Education, Access to Care and Vaccine Trial Site Development in Bamako, Mali

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Background: GAIA Vaccine Foundation (GAIA VF) has been working alongside the Department of Health (DRS), HIV expert clinicians, and basic scientists in Mali to prepare a site for Phase I-III HIV vaccine trials. The proposed location for the Phase I-III trials to be conducted in this collaboration is Sikoro, a multi-ethnic village with a population of over 40,000 located near Bamako, Mali.

Methods: To date, GAIA VF, the DRS, and Malian clinicians and scientists have collaborated on: (1) HIV education in Sikoro and Bamako, (2) HIV prevention in Sikoro, (3) access to HIV treatment in Sikoro, and (4) vaccine readiness studies in Bamako and Sikoro. Regular meetings and extensive collaborations have been established among GAIA VF, regional scientists, and government officials.

Results: An HIV transmission prevention (MTCTP) program was established in the Sikoro prenatal care clinic (Chez Rosalie) in 2005; the Hope HIV Care Center (Bloc Espoir) was completed in 2008, and GAIA VF established the first 'primary-care-setting' HIV treatment program in Mali in 2009. >10,000 pregnant women have been screened for HIV infection, >200 have been diagnosed with HIV; continuing access to HIV treatment and nutritional support was made available for these women and their spouses and children in Bloc Espoir. Peer educators distributed >47,000 condoms and provided peer-to-peer education to >22,000 individuals over 5 years. >300 'walk-in' patients have been screened for treatment at the on-site care center, and >195 individuals are currently receiving antiretroviral therapy in this low resource setting.

Conclusion: GAIA VF has established successful local and regional partnerships in Mali, performed vaccine readiness research, and implemented a comprehensive HIV care program in anticipation of performing HIV vaccine trials. A Phase IV HPV vaccine trial in Mali is anticipated in 2012, which will build local expertise and capacity for future HIV vaccine trials.

P04.06

Electroporation (EP)-Related Technical Errors Experienced During an HIV Vaccine Clinical Trial Conducted in Rwanda and Uganda: Lessons Learned

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Background: Intracellular DNA vaccine delivery is essential for antigen expression and induction of immune response. Unfortunately, conventional intramuscular injection provides low efficiency DNA uptake and suboptimal immunogenicity. Electroporation-based DNA vaccination can enhance potency by 100-1000 fold. In this trial we used the TriGrid Delivery System by Ichor Medical Systems. We describe the electroporation technical errors that occurred during administration of an HIV pDNA vaccine in a clinical trial conducted at sites in Rwanda and Uganda and how those errors were resolved.

Methods: The site physicians were trained and qualified to use the EP procedure during site initiation visits and further practiced mock EPs later. We examined the technical errors that occurred by reviewing volunteer files between 19th December 2011 and 27th March 2012.

Each administration of the HIV pDNA intramuscularly by electroporation required two injections (one in each deltoid muscle) at each vaccination time point. Should an error occur during EP procedure, the respective error codes are shown on the monitor. Once the procedure is complete, 'procedure complete' is indicated on the monitor.

Results: Six errors out of 90 eletroporations occurred. Four occurred because of improper insertion of the electrodes or injection needle. These errors were resolved by repeating the procedure. Two occurred because of improper insertion of the cartridge into the applicator and on both occasions the errors were captured before switching on the enable button on the pulse stimulator. These were resolved by properly re-inserting the cartridge into the applicator. Most of the other potential errors were caught before the procedure started by the second person observing the set up

Conclusion: We conclude that errors related to EP procedure can be minimized or avoided when there is appropriate quality control immediately prior to vaccination. We advise to have two trained and qualified staff present during the procedure for quality control purposes

P04.07

High Prevalence of ECG Variations and Abnormalities in Young and Healthy TaMoVac 01 HIV Vaccine Trial Volunteers from Tanzania

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Background: Vaccinia immunizations have caused peri-/myocarditis. As a result, volunteers receiving Modified Vaccinia Ankara(MVA) are monitored with ECGs. Early Repolarization Syndrome(ERS) has been reported in 1-2% and Left Ventricular Hypertrophy(LVH) in <10% of young, healthy populations across different ethnicities. These changes have been described as normal ECG variations, and according to early reviews, are more frequent in African and Asian populations. A prevalence of 90% has been reported for ST-Elevation in young males in several studies from industrialized and developing countries.

Methods: ECG was performed in healthy HIV negative volunteers during screening for the Phase II "TaMoVac01" HIV Vaccine Trial in DarEsSalaam and Mbeya, Tanzania. ECG variations that could potentially interfere with the later interpretation of myocarditis/pericarditis were confirmed by a panel of international cardiologists and led to exclusion from the study. These were ST-segment elevation, T-wave abnormalities, signs of LVH and ERS.

Results: 263 Volunteers (mean age 24.4 years, 63.5% males) had a baseline ECG evaluation performed. 19% of ECGs showed ERS, 20.3% showed LVH and 77% showed ST-elevation. 22.1% of volunteers were screened out due to ECG findings, none of whom had a history of cardiac disease, although one participant had a systolic murmur which led to echocardiogram. Dilation of the left ventricle was diagnosed.

Conclusion: Although the prevalence of ST elevation was no higher than expected in young males, ERS and LVH was far more common than the literature suggested in this cohort of clinically healthy, young Tanzanians.Exclusions were due to the presence of more than one abnormality. None had symptoms that were clinically relevant, although a significant cardiac finding was revealed through echocardiogram in one participant.

The clinical benefit of ECG screening in the context of vaccine studies in healthy volunteers remains to be determined, but it is clear from this study that it added considerably to the number of screenouts.

P04.08

Immune Response After Vaccination of HIV Infected Individuals Receiving HAART with Overlapping Gag Peptides Pulsed on Autologous Cells

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Background: HIV Gag specific CD4+ and CD8+ T cell responses are important for HIV immune control. Pulsing overlapping Gag peptides on autologous cells (Opal) has proven immunogenic and effective in reducing viral loads in multiple macaque studies, warranting clinical evaluation.

Methods: We performed a phase I, single centre, placebo-controlled, double-blinded and dose-escalating study to evaluate the safety and preliminary immunogenicity of a novel vaccine approach Opal-HIV-Gag(c). This vaccine is constituted by 120 15mer peptides, overlapping by 11 amino acids spanning the entire HIV Gag C Durban consensus sequence proteome, pulsed on white blood cells enriched from whole blood using a closed system, followed by intravenously reinfusion. Patients with well controlled HIV on HAART received four vaccinations administered at week 0, 4, 8 and 12, and were followed up for 12 weeks post-treatment. Eighteen people were enrolled in three groups: 12mg (n=6), 24mg (n=6) or matching placebo (n=6). An additional group (48mg, n=2) was not evaluable. Immunogenicity was assessed by IFNγ ELIspot/ICS.

Results: The constituent peptides in Opal-HIV-Gag(c) were antigenic in vitro using peptide stimulated PBMCs (median 30 fold increase). However, after vaccination with Opal-HIV-Gag(c), 1/6 at 12mg and 1/6 at 24mg had a 2 to 3 fold increase from Baseline of Gag specific CD8+ T cells at Week 14, compared to 0/6 placebo recipients. No Gag specific CD4+ T cell responses or overall change in Rev, Nef, Tat and CMV specific responses were detected. Marked, transient and self-limiting lymphopenia was observed immediately post-vaccination (4 hours) in Opal-HIV-Gag(c) but not placebo recipients, with median 1.72 to 0.67 million lymphocytes/mL for active groups (P<0.001), compared to 1.70 to 1.56 for placebo group (P=0.16).

Conclusion: Despite the promising effect found in several Macaca nemestrina studies using this approach, Opal-HIV-Gag(c) was not significantly immunogenic in this population and improved methods of generating Gag-specific T-cell responses are required.

Topic 4: Clinical Vaccine Trials and Trial Site Challenges

P04.09

Sexual Behavior Among Volunteers Enrolled in a Phase I HIV Vaccine Trial: Experience of Projet San Francisco in Kigali, Rwanda

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Background: Phase 1 HIV vaccine trials are conducted among HIV-uninfected, healthy volunteers at low risk for HIV. Study volunteers are counseled to maintain low risk behavior for HIV acquisition. The objective of this study was to assess sexual behavior of volunteers in a Phase 1 vaccine trial, conducted in Kigali, Rwanda.

Methods: Concordant HIV-negative couples who were counseled and tested together were considered as low risk group for HIV acquisition and were invited to participate in Phase 1 HIV vaccine trial to evaluate safety and immunogenicity of a multiclade HIV-1 DNA plasmid vaccine followed by a recombinant, multiclade HIV-1 adenoviral vector vaccine (IAVI V001). HIV risk reduction counseling, assessment of sexual behavior and screening for sexually transmitted infections (STI) were conducted quarterly. After completion of the vaccine trial, participants and their partners were invited to participate in a long-term 5-year follow-up study from their last vaccination for further assessment of sexual behavior and continuous risk reduction counseling.

Results: Between November 2005 and May 2006, 57 volunteers (36 men and 21 women) were enrolled in the clinical trial. All volunteers completed their trial visits. During the first twelve months following the first vaccination, none of the study participants reported sex with other partners nor was treated for STI. After unblinding, 55 volunteers agreed to continue in long-term follow up study. Through January 2012 6 men and 2 women reported having sex at least once with other partners; two were treated for STI, and one subsequently acquired HIV. (HIV incidence rate: 0.3/100 person-years).

Conclusion: This trial and subsequent follow-up study confirm our previous findings that concordant HIV-negative couples are at low risk of HIV infection and suitable for enrolling in Phase 1 HIV prevention trials. Ongoing HIV risk reduction counseling should be provided during the trial as well as after the trail, if possible.

P04.10

Developing Standards of Care for HIV Prevention Research in Developing Countries – A Case Study of Ten Research Centers in Eastern and Southern Africa

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Background: Standards of care in general vary across countries and communities and thus may affect decisions about standards of care provided by research centers in HIV prevention research. To serve as a basis for clarifying and improving standards, a systematic survey of practices at 10 experienced research centers affiliated with the International AIDS Vaccine Initiative in Eastern and Southern Africa was conducted between 2008 and 2010.

Methods: A survey tool was developed to collect qualitative and quantitative data on types of services provided, recipients of services, referral systems and barriers to referral uptake. Focus group discussion and semi-structured interviews were conducted with key research centre staff. Qualitative data were coded and enumerated where appropriate. Quantitative data were categorized and tabulated using STATA.

Results: All research centres consistently provided HIV prevention and care services but had varied practices on family planning options, and general medical care to research volunteers, screen out volunteers, partners of volunteers, and former volunteers. Services that were less consistently provided included provision of female condoms and anti-retroviral post-exposure-prophylaxis to volunteers in case of rape. All research centres either had referral points for treatment and care for volunteers who become infected with HIV or provided the services on-site. Limited referral points were available for psychosocial services and adult male circumcision. The greatest challenges for referral uptake included transportation and health care costs, poor quality and inconsistency of services at some referral points. While all research centres covered the cost of health services for study-related adverse events, policies varied on covering the cost of other health services.

Conclusion: These findings informed the development of standards of care across the 10 research centers and for IAVI-sponsored research. In developing such standards, balance should be made between scientific priorities, considerations of fairness, contextual realities, community expectations, and cost-effectiveness of conducting clinical trials

P04.11

Immunogenicity of a Universal HIV-1 Vaccine Vectored by DNA, MVA and CHADV-63 in a Phase I/IIA Clinical Trial

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Background: The major challenge facing both antibody and T cell-eliciting vaccines against HIV-1 is the extreme variability of the HIV-1 genome: a successful vaccine has to effectively target diverse HIV-1 strains circulating in the population and then must deal with ongoing virus escape in infected individuals. To address these issues, we assembled a vaccine immunogen HIVconsv from the functionally most conserved regions (not epitopes) of the HIV-1 proteome.

Methods: A gene coding for the HIVconsv immunogen was inserted into plasmid DNA (D), modified vaccinia virus Ankara (MVA; M) and non-replicating adenovirus of a chimpanzee origin ChAdV-63 (C). Currently, combined heterologous prime-boost regimens of these vaccines, namely CM, DDDCM and DDDMC, are being evaluated in a phase I/IIa trial HIV-CORE002 in healthy HIV-1/2-negative volunteers in Oxford

Results: Preliminary data indicate that the vaccines are well tolerated and show high immunogenicity. Following the CM regimen, vaccine-induced T cell frequencies reached a median of 5150 (range 1475 to 16495) SFU/10^6 PMBC ex vivo one week post MVA vaccination. DNA priming increased subsequent T cell responses to ChAdV-63 vaccination (median: C 577 and DDDC 1328 SFU/10^6 PBMC) and ELISpot responses again peaked 1 week following MVA (median 4500; range 2260-7960 SFU/10^6 PBMC). Matrix analyses of the participants following CM vaccination showed that T cells responded to a range of peptides across the length of HIVconsv. The CM regimen elicited IFN-γ in both CD4+ and CD8+ T cell subsets and polyfunctional (IFN-γ & TNF-α) responses to HIVconsv peptides.

Conclusion: Presented data will be very much work in progress. Nevetheless, the HIVconsv vaccines have so far induced T cell responses superior to other HIV-1 vaccine candidates tested to date. ChAdV-63 is the first adenovirus of chimp origin delivering an HIV-1-derived immunogen that has reached the clinic.

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P04.12

Using an Internet Consumer Marketing Strategy to Reach Men Who Have Sex with Men for Participation in a Preventive HIV Vaccine Clinical Trial

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Background: Sustaining research subject recruitment in biomedical HIV prevention trials requires continual innovation. Since June 2009, the University of Pennsylvania HIV Vaccine Trials Unit (UPenn HVTU) has employed multiple strategies to recruit men who have sex with men (MSM) for a phase IIb HIV vaccine trial, HVTN 505.

Methods: Between 12/1/2011 and 1/3/2012, the HVTU contracted with a consumer marketing company to recruit potential trial participants. For $3000, the company emailed MSM a scripted message inviting them to participate in a clinical HIV vaccine trial, and the company provided the trial site with contact information for those who responded. Site staff emailed and phoned each respondent to provide study information and conduct a phone-screen interview for the trial. Those eligible were scheduled for in-office screening appointments.

Results: 266 MSM were emailed; 118 viewed the message, and 109 responded that they were interested in participating. 83% of responders were White, and 71% earned >$50,000/year. Staff successfully contacted 64 individuals, and 58 completed phone-screens. Of these, 17 were eligible for, and 9 completed, screening visits. Five enrolled in HVTN 505 during January-February, 2012. Of 41 phone-screened ineligible, primary reasons for ineligibility were: being HIV-positive (n=20; 49%), not meeting protocol-specified sexual risk criteria (n=11; 27%), out of age range (n=6; 15%), and/or being uncircumcised (n=5; 12%). Ineligible participants were referred to phase I or future prevention trials as appropriate.

Conclusion: This strategy reached MSM not engaged by previous efforts, and doubled site HVTN 505 enrollment over two months. Respondents included a higher proportion of White MSM than the population screened for HVTN 505 at the HVTU. This approach has wider potential use for recruitment in biomedical HIV prevention trials. To understand the true utility of this approach, respondent HIV risk data and financial costs associated with this strategy must be carefully examined.

Topic 4: Clinical Vaccine Trials and Trial Site Challenges

P04.13

New HIV Peptide-Based Immunoassay Resolves Vaccine-Induced Seropositivity in HIV Vaccine (Phase III) Trial Participants

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Background: HIV Vaccine trials bring the significant risk of vaccine-induced HIV seropositivity(VISP) resulting in negative personal consequences for vaccinees. The overall rate of VISP in licensed EIA tests is reported as 41.7%(JAMA 2010;304:275-283). We have developed and modified the peptide-based HIV Selectest immunoassay(J.Virol 2006;80:2092-2099), which discriminates VISP from true HIV infection, in a format suitable for routine laboratory use, and have evaluated its performance on samples from three Phase III HIV vaccine trials.

Methods: The HIV Selectest incorporated five synthetic peptides in a single well microplate ELISA. Serum panels evaluated comprised well-characterized HIV-positive sera from clades A,B and C, worldwide panels comprising all major clades, blood donor controls, and sera from vaccine and placebo recipients in RV144, Vax003 and Vax004 trials.

Results: 360 serum samples from the RV144 vaccine trial, including 170 samples from vaccinated subjects at the peak immune response, 120 pre-immune samples, and 70 subjects from the placebo group were tested on the HIV Selectest. One (1) subject(0.6%) among the vaccine recipient group yielded false-positive results, while 3 placebo recipients (4.3%) and 1 pre-immune serum sample (0.8%) were also false positive in the HIV Selectest. All false-positive samples demonstrated broad non-specific cross-reactivity that was not restricted to a particular HIV-specific peptide.

Similar results were obtained with samples from the VAX003 and VAX004 vaccine trials. One subject out of 87(1.2%) tested after the final vaccination(7thvisit) at the peak of the immune response was detected as false positive. Two additional samples out of 96(2.1%) taken after the 4thvisit were likewise detected as false-positive, bringing the average false-positive rate for both groups to 1.6%.

Blood donors yielded a statistically equivalent false-positive rate of 1.2%. Detection sensitivity for HIV positive samples was 96% among 648 serum samples representing different clades.

Conclusion: The HIV Selectest ELISA has demonstrated significantly better discrimination of VISP than currently licensed HIV serologic assays.

P04.14

A New Method for Integrated Analysis Applied to Gene Expression and Cytokines Secretion in Response to LIPO-5 Vaccine in HIV-Negative Volunteers

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Background: We present an integrated analysis of gene expression and cytokine secretion using a novel statistical method applied to the ANRS VAC18 trial.

Methods: The statistical approach combines multilevel and multivariate analyses. The statistical approach is based on a variance decomposition followed by a sparse Partial Least Square Discriminant Analysis (sPLS-DA) to select the discriminative features (genes, cytokines) separating the classes in a supervised framework, or sPLS to select subsets of correlated features (genes and cytokines) in an integrative framework.

HIV-LIPO-5 vaccine is a mix of five synthetic HIV-1 peptides (from Gag, Pol, Nef), each coupled to a palmytoil tail. PBMCs from 12 HIV-negative volunteers were collected before (W0) and after vaccination (W14). PBMC were stimulated with either i) the HIV-LIPO-5 vaccine; or ii) a pool of 15-mer Gag peptides included in the HIV-LIPO-5 vaccine (Gag+); or iii) a pool of 15-mer peptides not included in LIPO-5 vaccine (Gag-). Production of 10 cytokines was assessed at day 11 (MILLIPLEX MAP kit, Millipore). Gene transcription in PBMC was assessed after 6- and 24-hour stimulations (Illumina Human HT12-v4 chips).

Results: After vaccination, the multilevel discriminant analysis led to the selection of 290 genes over three components that gave a good separation of the four groups of stimulation. The first component that distinguished LIPO5 stimulation from the others included a cluster of genes belonging to the metallothionein family (MT1M, MT2A,…) possibly linked to the effect of the palmytoil tail. The second component separated Gag+ from other stimulations. The multilevel sPLS showed similar profiles of correlations between gene expression and cytokine secretion for TH2 (IL5 and IL13), IL21 and IL1b, TNF and IL6.

Conclusion: This new statistical approach helps in analyzing complex designs. In VAC18, it revealed the differential responses to vaccine peptides and the lipid adjuvant. Gene expression signatures associated with cytokine responses were identified.

P04.15

Uptake and Tolerability of Repeated Mucosal Specimen Collection In Two Phase 1 AIDS Preventive Vaccine Trials In Kenya

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Background: Mucosal specimens are useful to evaluate local immune responses in AIDS preventive vaccine trials, but the acceptability and tolerability of mucosal sampling in Africa remains unknown.

Methods: The Kenya AIDS Vaccine Initiative (KAVI) initiated two AIDS preventive vaccine trials in Nairobi in 2011. After informed consent for a mucosal substudy, participants were asked to provide any of several types of mucosal secretions: saliva, oral fluids, semen, cervico-vaginal and rectal. Specimens were collected at baseline, one month after final vaccination, and at the next scheduled trial visit. A tolerability questionnaire was administered at the final visit.

Results: Of 80 trial participants, 65(81.3%) consented to the mucosal sub-study and provided at least one specimen, 7/65(10.8%) gave all specimens at least once and 2/65(3.1%) gave all possible specimens at all visits. Saliva and oral fluids were given at all time-points by 62/65(95.4%) participants. Of 48 men, 21(43.8%) provided semen at baseline, 18/21 completed all 3 time-points. Of 17 women, 15(88.2%) gave vaginal sponge and SoftCup specimens at least once; 8/15(53.3%) gave both at all eligible time-points. Rectal sampling was the least acceptable method: 13/65(20%) participants agreed at baseline [4/17 women (23.5%), 9/48 men (18.8%)]. Of these, 4 men and 2 women gave samples at all time-points. The most common reason for accepting mucosal sampling was a desire to contribute to HIV research and for refusal, embarrassment/emotional discomfort.

Conclusion: Repeated saliva, oral fluid, semen and cervico-vaginal mucosal sampling in AIDS vaccine preventive trials in Kenya is feasible; this study however re-affirms the challenge of repeated rectal mucosal sampling in low-risk participants, noted in an earlier observational study at KAVI (AIDS Vaccine 2010 P10.07). Possible explanations include cultural and religious reasons contributing to embarrassment and emotional discomfort in low-risk participants. Including more qualitative research in vaccine trials with mucosal sampling could help elucidate these findings.

P04.16

Feasibility of Recruiting High-Risk Women in the US for HIV Vaccine Efficacy Trials (HVTN 906)

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Background: Identifying women in the US with sufficient risk of HIV infection for inclusion in HIV vaccine efficacy trials has been challenging. Using geography and sexual network characteristics to inform new recruitment strategies, HVTN 906 determined the feasibility of recruiting and retaining women at high risk and assessed HIV incidence.

Methods: HIV uninfected women were enrolled in Chicago, New York City and Philadelphia if they were 18-45 years, not pregnant or intending to become pregnant for 18 months and reported unprotected vaginal/anal sex in the prior six months and either i) resided or engaged in risk behavior in local geographical HIV risk pockets; and/or ii) had a male partner who had either been incarcerated or injected drugs in the last year or had concurrent sex with another partner in the last six months. Behavioral risk assessment, risk reduction counseling, HIV and pregnancy testing were done at baseline, 6, 12 and 18 months.

Results: Among 799 women, 71% were from local high-risk pockets and had high-risk male partners, 18% were from local high-risk pockets only and 10% had high-risk male partners only. Median age was 37 years; 79% were Black and 15% Latina. At baseline, the median number of male partners was 3 (25%,75%: 2,7), 76% had unprotected sex while intoxicated (alcohol or drugs), and 52% exchanged sex for money or drugs. Retention at 18-months was 80%. Pregnancy incidence was 11% with 48% of pregnancies occurring during the first 6 months of follow-up. HIV incidence was 0.31% (95% CI: 0.06,0.91). Risk behaviors decreased between screening and 6 months with little change thereafter.

Conclusion: Women recruited using new strategies based on geography and sexual network characteristics did not have a substantial HIV incidence, despite baseline levels of risk behaviors. New strategies to identify women at high risk in the US are needed.

Topic 4: Clinical Vaccine Trials and Trial Site Challenges

P04.17

Pattern of HIV Risk Behavior in a Cohort of High Risk Women in East Africa

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Background: Development of high risk cohorts is critical for advanced HIV vaccine testing. Prevention interventions during follow up of such cohorts could influence the pattern of risk behavior leading to unmet study outcomes. We examined risk behaviors among high risk women enrolled in a prospective cohort to determine changes over time.

Methods: Adult women self identified as sex workers or bar workers were enrolled in an open cohort at three sites in East Africa. HIV risk factors were assessed at baseline and every six months for 1½ years, using Audio Computer Assisted Self Interview (ACASI). Participants were also evaluated twice weekly to identify HIV infection. HIV counseling was done every 3 months and when required during twice weekly visits. Male condoms were made available at all visits. Data was analyzed using Fisher's exact test.

Results: Data is available for 1158 HIV negative participants at baseline and 771(66.6%), 537 (46.4%), 403 (34.8 %) participants at 6, 12 and18 months respectively and 37 acute HIV infections. Overall, the risk status of Tanzania women was lower compared to Kenya and Uganda. There was a significant drop in proportion of participants reporting sex with ≥ 3 Non-spouse/Non-cohabitating male partners and sex with high risk partners at 6 months (25.3%, 39.0%) compared to baseline (55.4 %, 62.0%)(p< 0.0001, <0.0001) but no decline subsequently. Most participants (76.8%) used alcohol during sex with male partners at baseline and throughout the study (73.3%, 72.8%, and 72.9% at 6, 12 and 18 months). There was a significant increase in proportion of participants using condoms at 6 months in Tanzania (p<0.006). Incidence rates were 3.3, 2.7 and 1.5 per 100PYs during 0-6, >6-12 and >12-18 months.

Conclusion: HIV prevention interventions among high risk individuals may result in significant decreases in risky behavior that could have implications for future trials

P04.18

Experience in Recruiting Youths in HIV Vaccine Trials in Tanzania: The TaMoVac 01 Study

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Background: Muhimbili University of Health Allied Science has been conducting HIV Vaccine trials since 2007. The first trial the HIVIS 03 study began in February 2007 that recruited Police. We believe that the population to be prevented from HIV would be youths. We describe our experice in recruiting youths

Methods: Sensitization meetings were conducted at the youth clinic.Pre screening workshops were conducted at Muhimbili National Hospital. Youths who showed interest in volunteering were asked to come for screening at the clinic located at Muhimbili National Hospital.

Results: Enrolment of youth volunteers in the study was not a problem. Among 60 volunteers recruited 25 were youths. We recruited 5 males and 20 females who were recruited over 1 year. There were challenges encountered in recruiting youths. These included inability for independent decision to join the trail though we noted that the parents were supportive after being well informed. 4/25 youths relocated in search for jobs this resulted in additional costs to call in the volunteer for safety assessments. 2/25 female youths became pregnant during the study period.

Conclusion: We noted that it was easy to recruit youths in the trial after the parents were well informed. However, there is need for continuous education so as to address pregnancy prevention.

P04.19

Tolerability and Acceptability of Electroporation During a Phase 1 Vaccine Trial at Two Sites in Uganda and Rwanda

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Background: DNA vaccines are weakly immunogenic in humans and one way of increasing their immunogenicity is by administering the vaccines using electroporation (EP). This technique increases the uptake of the DNA into the cell by producing an electrical field which makes the cellular membrane more permeable. In the ongoing trial, we are using the ICHOR TriGrid Delivery system (TDS-IM) to administer bilateral injections of an HIV pDNA vaccine by EP into the medial deltoid muscles.

Methods: We assessed the acceptability and tolerability of Electroporation among 37 volunteers who received two injections by EP/IM at their first vaccination time point by administering a questionnaire after the procedure. Tolerability was assessed at 4 time points: i) before the vaccine was injected, ii) at the time of electrical stimulation, iii) 10 minutes and iv) 30 minutes after the procedure. The pain was graded as none, light, uncomfortable, intense, severe or very severe.

We also sought their opinion on acceptability of the procedure as a method of vaccine administration.

Results: Of the 37 volunteers, 45.9% felt no pain at time before vaccine was injected. At time of electrical stimulation, 59.5% felt light pain, 29.7 felt uncomfortable and 10.8% felt intense pain. Ten minutes after the procedure, 56.8% felt light pain, 29.7 felt uncomfortable and 13.5% felt no pain. Thirty minutes after procedure, 70.3% felt light pain, 13.5% felt uncomfortable and 2.7% felt intense pain but this was associated with multiple device applications to the site prior to achieving administration. Majority of the volunteers (94.6%) thought that vaccination by EP would be acceptable if the vaccine protected people from acquiring HIV and contributed to scientific knowledge on vaccine administration.

Conclusion: Electroporation among African volunteers in this trial is tolerable and acceptable. EP may be considered in future for the administration of HIV vaccines.

P04.20

A Dose-Escalation Clinical Trial to Evaluate the Safety and Immunogenicity of a Replication-Defective HIV-1 Vaccine-HIVAX

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Background: Replication-defective SIV elicited protective immunity in animals. In this first-in-human therapeutic vaccination study, a replication-defective HIV-1 vaccine was tested in HIV-1 infected subjects under antiretroviral therapy.

Methods: A010 is an ongoing randomized, placebo-controlled dose-escalation clinical trial to evaluate the safety and the immunogenicity of two doses of a replication defective HIV-1 vaccine (HIVAX™) in subjects receiving stable highly active antiretroviral therapy (HAART) who have an HIV-1 RNA <50 copies/ml and CD4 cell count >500 cells/mm3. Following the randomized placebo-controlled vaccination phase subjects who received active vaccine and who meet eligibility will undergo a 12-week analytical antiretroviral treatment interruption.

Results: HIVAX™ is well tolerated in HIV infected subjects. Only mild injection site reaction occurred with transient duration. No medical treatment is necessary. High level of cell-mediated immune responses measured by ELISPOT assay was noticed after vaccination.

Conclusion: The replication defective HIV vaccine appears no severe adverse effect in HIV-1 infected subjects. High level of cell-mediated immune response was elicited in the vaccinees. HIVAX™ is worth for further evaluation of protective efficacy.

Topic 4: Clinical Vaccine Trials and Trial Site Challenges

P04.21

Evaluating Short-Term Patient Outcomes After HIV Care Interventions in a Low Resource Setting: Preparing for an HIV Vaccine Trial Site in Bamako, Mali

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Background: HIV treatment in Mali is constrained by limited access to HIV experts. In 2009, GAIA Vaccine Foundation's "Hope Center Clinic" (HCC), a community health center in Mékin-Sikoro, became one of the first "front-line" clinics to offer HIV treatment in Mali. To assess our HIV prevention strategies and prepare for an eventual HIV vaccine trial, we performed a retrospective study of ten clinical parameters in patient charts.

Methods: The charts of 54 patients receiving antiretroviral therapy (ART) at HCC between 04/2009-09/2011 (30 months) were reviewed. Information on the presence of opportunistic infections (OIs), weight changes, CD4 counts, BMI, viral load (VL), CD4 T-cell counts, hemoglobin (Hb), alanine aminotransferase, leukocyte counts, and platelet counts were tabulated and analyzed in Excel.

Results: The mean age of the subjects in the study was 33; 85% were women, of whom 57% received mother-to-child transmission prevention at HCC. 93% had HIV-1; 33% were WHO Stage I, 11% Stage 2, 24% Stage 3, and 4% Stage 4. 48 (89%) patients improved in at least one parameter. 35 (65%) patients gained weight. 28 (70%) patients had increased CD4 counts (74% of patients had two counts recorded). 13 (59%) patients had decreased VL (41% of patients had two VL recorded). OIs were common among subjects (61%) prior to clinical intervention, but decreased significantly by months 7-15 to affect only 17% of subjects.

Conclusion: These data affirm that village-level HIV care is both feasible and associated with positive patient outcomes. Providing care allowed GAIA VF to reinforce the rapport between clinic staff and community members and assess clinical interventions for HIV-positive patients. Importantly, this develops strategies for treatment distribution and adherence monitoring, patient follow-up and retention, and study implementation and analysis that lay the groundwork for the development of a Phase I-III HIV vaccine trial site in this region of Mali.

P04.22

Developing Community Advisory Board Guidelines for AIDS Vaccine Trials in China

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Background: Community Advisory Boards (CABS) have played an important role in ensuring community oversight of AIDS vaccine trials and are an accepted part of the ethical conduct of AIDS vaccine trials. China's political system limits community engagement in trial communities and wholesale application of western CAB approaches does not accomplish the intent of a CAB. We adapted the CAB concept to China's political system through a multi year process of research and pilot testing in collaboration with six provincial CDCs conducting AIDS prevention research studies.

Methods: Using questionnaires developed by IAVI for a global CABS study, we carried out an assessment of existing CABS in six provinces in China. The questionnaire examined membership, process, and governance along with other aspects of CABS operations. The results were used to revise standard CABS guidelines developed by IAVI for use in Africa and India. The revised guidelines were pilot tested in three sites, in Yunnan, Guangxi, and Shanxi. In depth interviews were also conducted with CAB members and local CDCS.

Results: A revised version of Chinese CABS guidelines was published in October 2011 and publicized at China's AIDS Prevention Conference by China's AIDS Ethical Review Board and IAVI. The CABS guidelines are now being promoted as part of the ethical requirements for AIDS prevention clinical trial research by China's National Center for AIDS Prevention and Control.

Conclusion: Developing and adapting ethical tools for ensuring community input and oversight of clinical trial research in China is necessary given China's political system. These newly adapted CABS guidelines provide a mechanism to accomplish the intention of a CAB even while different in form and governance from standard CABS elsewhere.

P04.23 LB

Screen Failure in Phase I HIV Clinical Trials In Soweto, South Africa: An Opportunity For Care

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Background: Reasons for screen failures are evaluated for three phase 1 HIV vaccine clinical trials recruiting healthy low-risk participants at the Perinatal HIV Research Unit: SAAVI102/HVTN073 and SAAVI103/HVTN086 (the first trials evaluating a Clade C vaccine, Novartis Subtype C gp140 with MF59 adjuvant boosting SAAVI DNA-C2 and SAAVI MVA-C vaccine) and IAVIB003/HVTN091 (Ad26 and Ad35 ENV vaccines). Recruitment strategies involved a pre-screening programme, clinics and community outreach.

Methods: Protocol-specific eligibility was determined using assessments of understanding, risk behaviour, medical history, physical examination, blood and urine testing, and for HVTN073 and 086, electrocardiograms. Descriptive analysis and multivariate logistic regression of age, trial group and gender were performed.

Results: Between June 2009-2012, 225 participants (females=24%), median age 22 years (IQR:20-25) were screened. Overall 53% were ineligible, 60% of females vs. 51% of males (p=0.2). Site screening-to-enrolment ratios for 073, 086 and 091 were 2.1:1, 2.3:1 and 1.7:1 respectively. Medical abnormalities contributed 59% (n=70) of ineligibility reasons, chiefly urine abnormalities (n=12/70 where eleven displayed microscopic blood/haemoglobin, seven with leucocyte esterase and one had proteinuria), abnormal ECG (n=12), raised liver enzymes (n=10), raised blood pressure (n=9), low white cells (n=8) and hepatitis B (HBsAg+ve) or C (anti HCV+) (n=7). Other criteria excluded 41%(n=49) e.g. incomplete screening before enrolment closure (n=16), high-risk sexual behaviors (n=15), inability to comply with protocol (n=11), enrolment in another study (n=3), substance abuse (n=2, both cannabis-users), and poor understanding (n=2). In multivariate analysis, increasing age (OR 1.081, CI:1.007-1.16, p=0.032) predicted ineligibility but gender did not (OR: 0.67, CI: 0.35-1.3, p=0.24). HVTN073&086 participants were more likely to be ineligible than HVTN091 (OR 2.2, CI:1.1-4.5, p=0.023).

Conclusion: Screen failures in phase 1 vaccine trials in Soweto provide young people opportunities for care, especially through blood pressure, urine, risk behaviour and hepatitis B/C screening. Older participants and those in protocols stipulating ECG criteria were more likely to fail screening.

P04.24 LB

HIV Vaccine Trial Safety And Retention Among 18-20 Year Olds In The HVTN 503/Phambili Study Support The Inclusion Of Adolescents In Future Trials

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Background: Worldwide, many adolescents, especially women, acquire HIV before age 18. Yet to date, no HIV vaccine trials have enrolled adolescents. Reasons for excluding adolescents from these trials include regulations protecting vulnerable subjects and concerns regarding informed consent, social harms, adverse events, and loss to follow-up.

Methods: Using data from the HVTN 503/Phambili study, a multisite phase 2b double-blind RCT in South Africa, motivations for joining the trial, adverse events, social harms, and loss to follow-up were compared between young adults (18-20 years-old) and adults ≥21 years-old using bivariate and multivariate analyses.

Results: Young adults (n=238) were less likely than older participants (n=563) to report joining the vaccine trial for monetary incentives (10.5% vs. 16.3%, p<0.05), and no differences were seen for altruistic motivations such as the desire to help the community (95.4% vs. 93.1%, p=.22) or to find an effective vaccine (98.3% vs. 97.0%, p=.34). There was no significant difference in social harms by age (3.8% vs. 6.9%, p=0.09). No differences were seen between 18-20 year-olds and older participants for adverse events that were definitely, probably, or possibly related to study product (5.0% vs. 7.8%, p=0.16) or for adverse events that required expedited reporting to the study sponsor (2.1% vs. 2.5%, p=0.74). Furthermore, loss to follow-up did not differ between 18-20 year-olds and older participants in either bivariate analysis (21.4% vs. 24.2%, p=.40) or in a multivariate model that controlled for both gender and study site (OR .95, CI .65-1.38).

Conclusion: Participants who were 18-20 years old were less likely to report financial motivations for joining the HVTN 503/Phambili study and were no more likely to experience adverse events, social harms, or loss to follow-up than participants ≥21 years-old. These data are reassuring and support the safe and feasible inclusion of younger adolescents in future HIV vaccine efficacy trials.

P04.25 LB

rAd5/NYVAC-B is Superior to NYVAC-B/rAd5 and is Dependent on rAd5 Dose for Neutralizing Antibody Responses Against HIV-1

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Background: HVTN 078 is a phase 1b clinical trial of heterologous vector prime/boost vaccine regimens (NYVAC-B/rAd5 vs. rAd5/ NYVAC-B) in healthy, HIV-1 uninfected, Ad5 seronegative adults. The rAd5 expressed a clade B Gag-Pol fusion protein and secreted gp140s of HIV-1 strains 92RW020 (clade A), HxB2/Bal-V3/_V1V2 (clade B) and 97ZA012 (clade C). The NYVAC-B expressed a clade B Gag-Pol-Nef polyprotein and the secreted gp120 of Bx08 (clade B). A total of 80 participants were randomized into a placebo group (P) and four treatment groups: T1, 2x NYVAC-B/1x rAd5 (10^{10}); T2, 1x rAd5 (10^{8})/2x NYVAC-B; T3, 1x rAd5 (10^{9})/2x NYVAC-B; T4, 1x rAd5 (10^{10})/2x NYVAC-B.

Methods: Binding and neutralizing antibodies were assessed at 2 weeks post-final boosting. Neutralization was assessed with tier 1 and tier 2 Env-pseudotyped viruses in TZM-bl cells, and with tier 2 Env.IMC.LucR viruses in A3R5 cells.

Results: A dose effect for increasing anti-Env binding antibodies was seen, with higher doses of rAd5 being optimal. For neutralizing antibodies, positive response rates/median titers across the treatment groups were highest against MN.3 (69.3%/116) followed by SF162.LS (42.1%/54), BaL.26 (18.4%/15.5), MW965.26 (14.5%/31) and Bx08.16 (11.8%/19.5). Five subjects neutralized all 5 tier 1 viruses, 5 subjects neutralized 4 viruses, 7 subjects neutralized 3 viruses, 14 subjects neutralized 2 viruses (MN.3 and SF162.LS) and 18 subjects neutralized 1 virus (MN.3). Aggregate magnitude-breadth scores across the tier 1 panel were strongest for T4 followed by T3, T1 and T2. Differences were significant for T1 vs. T3 (p=0.048) and T1 vs. T4 (p=0.004). Responses against tier 2 viruses were weak and sporadic in the A3R5 assay and were nearly absent in the TZM-bl assay.

Conclusion: A 10^{10} dose of rAd5 was superior to the two lower doses of 10^{9} and 10^{8} for both binding and neutralizing antibodies. At the highest rAd5 dose tested, rAd5/NYVAC-B was superior to NYVAC-B/rAd5 for neutralizing antibodies.

P04.26 LB

DNA Plasmid HIV Vaccine Design, Number Of Doses, Participant Gender, And Body Mass Index Affect T-Cell Responses Across HIV Vaccine Clinical Trials

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Background: Numerous studies have evaluated individual DNA plasmid vaccines. We evaluated data from 10 HIV vaccine clinical trials that utilized common, validated immunogenicity assays to objectively investigate factors that influence DNA plasmid-induced T-cell responses.

Methods: This retrospective analysis included data from 1218 healthy, HIV-1 uninfected adults enrolled in 10 DNA HIV vaccine clinical trials conducted within the HIV Vaccine Trials Network. HIV-specific T-cell responses from peripheral blood mononuclear cells were measured using validated IFN-γ ELISpot and intracellular cytokine staining assays. The effects of DNA vaccine HIV antigens, number of doses, gender, body mass index (BMI), and age were evaluated.

Results: When plasmids expressing Gag, Env and Pol were co-administered, the highest T-cell response rate was against Env (38.1%) and much less to Gag (4.6%) or Pol (4.5%). Comparing 2, 3, and 4 DNA injections, 3 vaccinations compared to 2 improved the magnitude of Env-specific CD8+ T-cell response (p=0.048), but not CD4+ T-cell responses, and a 4th vaccination had no additional effect. HIV-specific CD4+ T-cell response rates were higher in females (50.9%) than in males (27.7%, p=0.0002). Having lower BMI (p=0.025) was independently associated with higher HIV-specific CD4+ T-cell response rates. There were no significant differences in HIV-specific CD8+ T-cell response frequencies by gender or BMI. Lower HIV-specific CD4+ T-cell responses were seen with the 18-20 (36.4%) and 41-50 (23.3%) age groups compared to the 21-30 (44.0%) and 31-40 (41.%) groups, but these differences were not significant (p=0.0565) and no differences were seen in HIV-specific CD8+ T-cell responses by age.

Conclusion: Pooled data across DNA HIV vaccine clinical trials indicate that plasmid inserts, number of doses, gender, and BMI can affect T-cell responses. These factors should be considered in vaccine research.

P04.27 LB

First-In-Human Phase I Clinical Trial Of A Recombinant Vesicular Stomatitis Virus (rVSV)-Based Preventive HIV-1 Vaccine

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Background: Replicating viral vectors are promising HIV vaccine candidates that may enhance immunogenicity through prolonged antigen expression. VSV is the first replicating viral vector after vaccinia to be tested clinically as an HIV vaccine; here we present preliminary safety and immunogenicity data from a phase 1a trial.

Methods: HVTN 090 enrolled sixty healthy, HIV-1-uninfected adults in a randomized, double-blinded, placebo-controlled dose escalation study. Groups of 12 participants received rVSV Indiana HIV Gag vaccine at 5 dose levels (4.6×10^3 to 3.4×10^7 PFU) (N=10/group) or placebo (N=2/group), delivered intramuscularly at 0 and 2 months. Reactogenicity over 7 days, adverse events (AEs), and viral cultures from whole blood, urine, saliva and swabs of oral lesions were collected. HIV-1-specific CD4+ and CD8+ T-cell responses to Gag peptides were measured 1 and 2 weeks post-boost by intracellular cytokine staining.

Results: The study is ongoing and data are blinded. The median age was 24; 47% were female and 37% were non-white. Local and systemic reactogenicity was self-limited, mild to moderate in intensity and increased with dose, with headache reported most commonly (52%). At the highest dose, 92% reported systemic symptoms, including flu-like syndrome (41%), fever (41%), and moderate chills (33%). Lymphadenopathy, decreased neutrophil count, oral ulceration, and presyncope were each seen in > 1 participant. No severe reactogenicity, encephalitis, or product-related SAEs were reported, and all VSV cultures were negative at all doses tested. Low frequency HIV-specific CD4+ (9%) and CD8+ (3%) T-cell responses were detected post-boost at the first 3 dose levels.

Conclusion: Immunization with an attenuated, replicating rVSV Indiana HIV-1 vaccine has an acceptable reactogencity and safety profile to date. Preliminary data reveal few T-cell responses at lower doses. Immunogenicity of the vaccine at the highest doses and in heterologous prime-boost regimens will guide future vector development.

P04.28 LB

A Mixed-Methods Assessment Of Understanding (AoU) Tool For AIDS Vaccine Trials In Sub-Saharan Africa: Results From A Pilot Study

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Background: Assessments of understanding (AoUs) in clinical trials are often composed of true/false multiple choice questions, however, these tools can be difficult for volunteers with limited education or without prior testing experience.

Methods: 35 adults were recruited at two research centers in Southern Africa. A within-subjects, repeated measures design was used, whereby each volunteer served as his /her own control. An AoU tool with closed- and open-ended questions was administered within a hypothetical AIDS vaccine trial setting. Performance on closed- and open-ended questions was compared using correlations and repeated-measure t-tests, limited to 4 complex concepts: false sense of security, risk of false positive test, need for contraception, and potentially enhanced susceptibility.

Results: Mean scores of understanding for each concept assessed by closed-ended questions ranged from 0.73 (need for contraception) to 0.84 (risk of false positive test); and by open-ended questions from 0.4 (risk of false positive test) – 0.6 (need for contraception). Scores for the open-ended measure were all lower than the equivalent closed-ended measure. Correlations between the closed- and open-ended measures were generally low, achieving significance for false sense of security (r=0.377), potentially enhanced susceptibility (r=0.393), and total score across concepts (r=0.617). Volunteers' understanding as assessed by the closed- and open-ended methods differed significantly: false sense of security= -3.862; risk of false positive test= -7.210; need for contraception= -2.303; and potentially enhanced susceptibility= -8.007. The correlation with years of education was consistently and significantly higher for the open-ended measure than the true/false questionnaire with the exception of need for contraception.

Conclusion: The results suggest the qualitative measure better assesses understanding than the quantitative measure. The scores from the two assessment methods have limited interchangeability. The standard closed-ended questions appear to provide an inflated measure of volunteers' understanding. An assessment tool with closed- and open-ended questions is better suited to determine genuine understanding.

Topic 4: Clinical Vaccine Trials and Trial Site Challenges

P04.29 LB

DNA And Recombinant Adenovirus Serotype 35 And 5 Preventive HIV-1 Vaccines With Env A Inserts Elicit Cross-Clade Binding And V1V2 Antibodies

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Background: We previously reported that combinations of DNA, recombinant adenovirus serotype 5 (rAd5) and 35 (rAd35) HIV-1 vaccines with clade A Env inserts elicit strong T-cell responses. Here we investigate their ability to induce cross-clade IgG binding antibody (bAb) responses. Based on recent evidence that Ab to the V1V2 loop in Env were correlated with reduced risk of HIV infection in the RV144 efficacy trial, we also evaluated those responses.

Methods: HVTN 077 was a placebo-controlled, double-blinded trial that randomized 192 healthy, HIV-uninfected, Ad35 neutralizing antibody (nAb) seronegative participants into placebo (n=28) and 4 vaccine groups: rAd35/rAd5 (T1, n=34), DNA/rAd5 (T2, n=48), and DNA/rAd35 (T3, n=48) -- in persons seronegative for Ad5 nAb; and DNA/rAd35 (T4, n=34) seropositive for Ad5 nAb. Vaccines were given at 0,6 months (T1) and 0,1,2, and 6 months (T2-4). IgG bAb responses by multiplex assay against Group M Consensus (Con S), clade A (00MSA 4076), clade B (B.con.env03), and clade C (C.con.env03) gp140 and novel gp70 V1V2 scaffolds, V1V2 VRC A and V1V2 (Case A2), were measured 4 weeks post boost.

Results: High frequency responses were elicited against clade A (T1 95.8%, T2 100%, T3 97.4%, T4 100%), clade B (T1 95.8%, T2 95.0%, T3 92.1%, T4 96.3%), clade C (T1 92%, T2 76%, T3 76%, T4 78%), and Con S (T1-4 100%). There were no significant between-group differences in response frequency or magnitude. The majority also had responses to V1V2 clade A (T1 100%, T2 87.8%, T3 83.8%, T4 85.2%) and clade B (T1 58.3%, T2 65.9%, T3 54.1%, T4 51.9%). The mean fluorescent intensity was higher in T2 vs T1 (p=0.005) for clade A V1V2. No significant differences were observed between other groups.

Conclusion: All vaccine regimens tested elicited cross-clade bAb responses, including those that target V1V2. rAd-based HIV-1 vaccines, particularly using rare serotypes, warrant further development.

P04.30 LB

Rapid Development of Cross-Clade Neutralizing Antibody Responses After Clade B gp120/gp140 Protein Priming And Clade C gp140 Protein Boosting

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Background: Immunization with heterologous Env protein immunogens following an immunologic rest period has the potential to generate cross-clade neutralizing antibody responses. We identified individuals who had received a clade B Env protein with MF59 4-17 years earlier, most in combination with a DNA or ALVAC prime, and administered a clade C protein boost in an open label phase 1 trial.

Methods: Sixteen previously primed volunteers and 20 naïve volunteers each received 2 doses of a clade C TV1 trimeric Env protein with MF59 given 6 months apart. HIV-1 specific CD4+ and CD8+ T cell responses were measured by an intracellular cytokine staining (ICS) assay. Antibody responses were measured with a Luminex binding antibody assay and a neutralizing antibody assay in TZM-bl Cells.

Results: Despite the long interval, 31% of primed participants demonstrated CD4+ T cell responses to Env at baseline, which increased to 75% after a single protein boost. IgG and IgA responses to TV1 trimeric Env were present in 64% (IgG) and 7% (IgA) of primed participants at baseline, and rose to 93% and 85%, respectively, after one dose of protein. 71% of primed participants demonstrated neutralizing antibodies against Tier 1 clade B isolate MN at baseline. After a single booster dose of protein, 100% of the primed participants neutralized MN and 93% showed neutralizing activity against a clade C isolate, MW965.26. Unprimed participants did not demonstrate CD4+ responses or antibody responses to Env until after the second dose, which elicited IgG and IgA responses to TV1 trimeric Env in 88% and 50%, respectively. Neutralizing antibody developed to MN in 38% and to MW965.26 in 88% of the unprimed participants.

Conclusion: These results demonstrate the durability of vaccine-elicited HIV-1 specific antibody responses and support current efforts to enhance the breadth and magnitude of neutralizing antibodies through heterologous protein prime-boost regimens.

Topic 5: HIV Transmission and Viral Diversity

P05.01

Rising Epidemic of HIV-1 Infections Among General Populations in Fujian, China

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Background: Monitoring HIV transmission and viral diversity has significant impacts on guiding effective vaccine development. Until now, few large-scale studies have investigated HIV infections among the general populations of China.

Methods: 915,830 and 2,152,658 blood samples from various groups were collected in 2006-2007 and 2008-2009, respectively, in Fujian, a low prevalent region in China. Comprehensive HIV-1 epidemiology and molecular epidemiology studies were conducted.

Results: Our data revealed a significant rise of the overall prevalence of infections within a short time period, from 0.064% in 2006-2007 to 0.074% in 2008-2009 (p=0.003), which resulted in the double numbers of infections from 528 in 2006-2007 to 1129 in 2008-2009. Critically, the prevalence rate among general populations such as voluntary blood donors, recipients of blood transfusion and people during pre-surgery screening had significantly increased in recent years (p<0.001). Besides CRF01_AE as the dominant circulating subtype (61/86, 70.9%), 25 non-CRF01_AE strains were found contributing to increased HIV-1 genetic diversity including C/CRF07_BC/CRF08_BC (5.8%), B/B' (15.1%) and unique recombinant forms (8.1%). More than 30% (26/81) of subjects were found to contain various drug-resistant mutations.

Conclusion: The rising epidemic in recent years in Fujian is likely due to the increased prevalence of HIV-1 infections among general populations and multiple viral subtypes circulating. Our findings will be useful for helping to enhance the current surveillance system and to generate strategic prevention programs targeting general populations in China. Moreover, these results also have implications for AIDS vaccine research.

P05.02

A New Transmission Map of HIV-1 CRF07_BC in China: Analysis of Sequences from 12 Provinces over a Decade

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Background: HIV-1 CRF07_BC descended from subtypes B' and C represents one of the most prevalent HIV-1 strains in Asia and has been devastating in IDUs for more than a decade in China. Recently, several groups made their effort to address its origin and migration pattern, and had generated several enlightening conclusions, however, the limited available sequences may have restrained the precise depiction of global migration pattern of CRF07_BC. In this study, epidemic evidences and sequences analysis was combined to investigate the transmission linkages among 12 CRF07_BC epidemic regions in China and their tMRCA(time of most recently common ancestor).

Methods: 138 of 769 sequences covered 12 provinces (including newly generated 45 sequences) was identified as independent sequences and used for subsequent phylogeographic tree analysis, Bayes Factor test. Almost all Chinese literatures on early HIV-1 epidemic history are reviewed to confirm the results of sequences analysis.

Results: The initial transmission occurred in Guangxi (eastern neighbor to Yunnan) in 1994 and Xinjiang (northwest) in 1995 and Sichuan (northern neighbor to Yunnan) in 1996 after CRF07_BC generated in Yunnan around 1993. The subsequent transmissions occurred from Yunnan to Liaoning (northeast) in 1997 and Jiangsu in 1998. Interestingly, after the initial epidemic regions, including Guangxi, Xinjiang and Sichuan, served as secondary epicenters for further spreading, triggered further transmission into Gansu, Ningxia, Qinghai, Beijing and Hunan during 1999-2001. These analyzed results are in accordance with early epidemic investigations of HIV-1.

Conclusion: Our data indicated that both the origin site and secondary epicenters played important roles in the dynamic migration of CRF07_BC in China, and both drug traffic and population migration (which largely occurred from west to east and from other regions to capital city during last two decades) may have significantly contributed to the complicated transmission pattern of CRF07_BC.

Topic 5: HIV Transmission and Viral Diversity

P05.03

Low Selection Rate of HLA-Anchor Escape Mutations in HIV After Transmission of Subtype B and Recombinants BF Strains Patients from Argentina

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Background: The immune response of HIV-infected individuals shapes the evolution of the virus by selecting escape mutation. After transmission to a new host, the HLA-mediated immune pressure changes. The objective of our study was to characterize the dynamics of HLA-anchor escape mutations after transmission.

Methods: We studied 6 transmission events between members of serodiscordant couples collecting blood samples from the donor and the previously seronegative-partner at the moment of seroconversion, and a second sample at least 6 months post-infection. HLA-I typing was performed by the SSOP-PCR method. The viral gene gag was amplified by RT-PCR and cloned into the pGEM-T vector for viral quasiespecies analysis. Transmitted strain was identified by phylogenetic analysis. Escape mutation was defined as viral polimorphisms located in HLA-anchor position that eliminate an epitope predicted by the NetMHC (CBS Prediction Server). Significant variations in the number of escape mutations were assessed by Poisson´s probability distribution.

Results: Of the total epitopes available in transmitted gag for recognition by the recipient HLA alleles, a mean 7.25% of them selected escape mutations by the moment of the second sample collection in an anchor position of the epítope. Of the total of HLA-anchor escape mutations present in the second sample and absent in the subtype consensus, a mean 79,8% were already present in the first sample of the recipient and in the sample of the donors. Also, the majority (84%) of the escape mutations to the donor's HLA alleles persisted in time after transmission without reversion, even in the absence of the selecting HLA allele.

Conclusion: The low rate of newly selected escape mutations could be due in part to lack of immune pressure, but considering the high rate of transmission and persistence, our results suggest a high level of viral adaptation to the HLA system in subtype B and recombinants BF circulating in Argentina.

P05.04

The Efficiency of Bridging Sheet Recruitment Determines HIV-1 R5 Envelope Sensitivity to Soluble CD4 and Macrophage Tropism

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Background: HIV-1 R5 viruses vary extensively in their capacity to infect macrophages. R5 viruses that confer efficient infection of macrophages are able to exploit low levels of CD4 for infection and they predominate in brain tissue where macrophages are a major target for infection. HIV-1 R5 variants that are transmitted are generally non-macrophage-tropic. Here, we investigated the sensitivity of macrophage-tropic and non-macrophage-tropic R5 envelopes to neutralizing antibodies.

Methods: Env+ pseudovirion neutralization assays were carried out using HeLa TZM-bl cells. Envelope capture ELISAs assessed monoclonal antibody binding to monomeric gp120.

Results: We observed striking differences in the sensitivity of Env+ pseudovirions to soluble CD4 compared to neutralizing monoclonal antibodies that target the CD4 binding site. Macrophage-tropic R5 envelopes were sensitive to sCD4, while non-macrophage-tropic envelopes were significantly more resistant. In contrast, all envelopes were sensitive to VRC01 regardless of tropism, while mab b12 conferred an intermediate neutralization pattern with all the macrophage-tropic and about half of the non-macrophage-tropic envelopes sensitive.

Conclusion: CD4, b12 and VRC01 share binding specificities on the outer domain of gp120. However, these reagents differ in their ability to induce conformational changes on the trimeric envelope and in specificity for residues on the V1V2 loop stem and β20-21 junction that are targets for CD4 in recruiting the bridging sheet. These different binding specificities of CD4, b12 and VRC01 likely explain the striking differences in envelope sensitivity to inhibition by these reagents. However, they also provide an insight into the envelope mechanisms that control macrophage-tropism. We present a model where the efficiency of bridging sheet recruitment by CD4 is a major determinant of HIV-1 R5 envelope sensitivity to soluble CD4 and macrophage tropism.

P05.05

Evolutionary Dynamics of HIV-1 Subtype C Accessory and Regulatory Genes in Primary Infection

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Background: Studies addressing the dynamics of accessory and regulatory viral gene diversity and selection during early stage of HIV-1 infection are limited but crucial for progress towards vaccine research.

Methods: Intra-patient diversity and evolution was assessed during primary HIV-1C infection, viral quasispecies were obtained by single genome amplification (SGA) at multiple sampling time points up to one year post-seroconversion (p/s).

Results: The mean intra-patient diversity was found to be 0.11% (95%CI; 0.02 to 0.20) for vif, 0.23% (95%CI; 0.08 to 0.38) for vpr, 0.35% (95%CI; -0.05 to 0.75) for vpu, 0.18%(95%CI; 0.01 to 0.35) for tat exon 1 and 0.30% (95%CI; 0.02 to 0.58) for rev exon 1 during the time period 0 to 90 days p/s. The intra-patient diversity increased gradually in all non-structural genes over the first year of HIV-1 infection, which was evident from the vif mean intra-patient diversity of 0.46% (95%CI; 0.28 to 0.64), vpr 0.44% (95%CI; 0.24 to 0.64), vpu 0.84% (95%CI; 0.55 to 1.13), tat exon 1 0.35% (95%CI; 0.14 to 0.56) and 0.42% (95%CI; 0.18 to 0.66) for rev exon 1 during the time period of 181 to 500 days p/s. Statistically significant increases in viral diversity were observed for vif (p=0.013) and vpu (p=0.002). Weak and sporadic associations between levels of viral diversity within the non-structural genes and HIV-1 RNA load during primary infection were found. Positive and negative selection patterns over the first year post-seroconversion were assessed in each of these genes, providing insight into the selection pressures on these genes which are crucial for viral replication in-vivo.

Conclusion: Our study highlights differential diversity and slower diversification across these HIV-1 genes. The most likely cause is different selection pressure imposed by host immune response to the encoded viral gene products that may result in different evolutionary rates.

P05.06

Incident Cases Characterization and Deep Sequencing Provide New Insight into Multiplicity of Infection and HIV Evolution in Very Early Acute Infection

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Background: RV217/ECHO aims at capturing HIV-1 incident cases during the very earliest stages of acute HIV-1 infection(AHI). Limitations in single genome amplification(SGA) sampling depth have the potential to confound the study of multiplicity of infection and viral evolution during AHI. Here we combined SGA and deep, next-generation sequencing(NGS) to investigate the prevalence of minor viral variants during pre-peak viremia and characterize subsequent viral evolution.

Methods: A male CSW from Thailand with documented nucleic acid testing(NAT)-conversion and seroconversion, and with measured viremia peak, was studied. Plasma viruses from pre-peak viremia(9 days after last negative NAT and 2 days after first positive NAT; pVL=891,251), immediate post-peak viremia(38 days after last negative NAT; pVL=512,861), and 6 months post-infection(pVL=181,970) were characterized by SGA and targeted NGS. A library prepared from 1,500 PCR-equivalent copies was analyzed using IonTorrent(LifeTechnologies).Reading coverage was>20K and the experimentally-measured error rate was<0.0028/base.

Results: Based on 47 env SGA sequences from pre-peak viremia, the infection was initiated by a single T/F virus (mean env pair-wise nt diversity:0.047%).A second variant, highly similar but distinguishable from the T/F (env nt distance:2.4%;env AA distance:3.4%), was detected during immediate post-peak viremia(18/56 SGA sequences). By 6 months post-infection, the viral population was dominated by the second variant along with numerous recombinants.

NGS analysis of multiple, independent subgenomic areas with concentrated sequence variation, revealed that the second variant was already established at 1% at pre-peak viremia. Outgrowth of the second variant, along with its derivatives, was confirmed by longitudinal NGS.

Conclusion: Supplementing SGA analysis with NGS allowed us to demonstrate the presence of two T/F viruses at pre-peak viremia with substantial frequency differences, in the study participant. Due to its implications, the prevalence of this phenomenon needs to be further explored. The application of NGS to the study of HIV-1 evolution during AHI promises to foster the advancement of vaccine development.

Topic 5: HIV Transmission and Viral Diversity

P05.07

Characterization of Envelope Function of Transmitted Viruses Circulating in Mbeya, Tanzania, and Its Impact on Disease Progression

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Background: An understanding of the biological characteristics of transmitted viruses provides important insights into HIV pathogenesis and informs vaccine development. The aim of the study was to characterize env function of transmitted viruses and its role in disease progression.

Methods: Ten sequences were generated from single genome amplicons from 10 individuals at acute infection (range = 3 - 6 months post-infection) and the sequence representative of the consensus was cloned and functional env clones from subtypes C (n= 6), D (n =1) and recombinants CD (n = 2), AC (n = 1) were generated. Pseudovirions were generated, and entry efficiency in TZM-bl cells, tropism, dependency on CD4 and CCR5 using HEK 293 dual-inducible Affinofile cells, and sensitivity to entry inhibitors, was measured.

Results: Half the envelope clones showed high levels of entry (52 -164% infection relative to Du151a, a reference env clone), and the remaining five had low entry efficiency (1- 18 %). We found an association between entry efficiency and viral load at 3 months (p = 0.0022) and 12 months postinfection (p = 0.0347). There was no significant correlation between entry efficiency and the IC50 of sCD4 (p = 0.5074), TAK779 (p = 0.4366) and enfurvitide (p= 0.5821), suggesting that the difference in entry efficiency was not due to CD4 and CCR5 binding, or membrane fusion. However, only 3/10 transmitted viruses from the group with high entry efficiency were able to infect cells with low of levels of CD4 and high levels of CCR5 receptors.

Conclusion: Transmitted viruses have a range of entry efficiency in TZM-bl cells (with high CD4 and CCR5 levels) with high entry efficiency associated with higher viral loads. When the expression of CD4 was lowered, only three viruses were able to enter target cells, suggesting that transmitted viruses most likely target cells with high CD4 levels.

P05.08

Subtype-Specific Differences in Human Immunodeficiency Virus Type 1 Co-receptor Usage in Northern Kenya

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Background: HIV-1 phenotype variability plays an important role in HIV-1 transmission and pathogenesis of AIDS. The basis of heterogeneity between the phenotypes is known to be the differential use of chemokine receptors as co-receptors for viral entry. Beta Chemokine receptor-5 (CCR5) using variants are associated with acute infections, macrophage tropism, non syncytium inducing (NSI) phenotype and slow progression to AIDS. Alpha chemokine receptor-4 (CXCR4) using variants evolve later in infection in about 50 % of the patients and are associated with T cell lines tropism, syncytium induction, accelerated T cell depletion and Rapid progression to AIDS. HIV-1 co-receptor usage and phenotypes are mapped in the third variable region (V3) of the gp120 env gene.

Methods: One hundred and thirty five (135) whole blood samples were collected from HIV-1 infected patients in Northern Kenya. Proviral DNA was extracted using DNazol and ethanol precipitation. HIV-1 V3 region was amplified by nested PCR using C2V3 primers. Amplicons were sequenced directly using Big Dye technology in the ABI Prism Genetic Analyzer to generate gp120 V3 loop sequences. Bioinformatics tools were used to determine co-receptor usage from the sequences obtained.

Results: CXCR4 usage was most dominant with 94 out of 135 samples(69.6 %) using this co-receptor compared to CCR5 which was used by 29 of the 135 (21.8 %) samples and dual co-receptor usage found in 12 of the 135(8.6%)samples tested.

Conclusion: Most patients were inferred to be in late stages of infection and likely to be rapidly progressing to AIDS. Based on these results from co-receptor usage data, HIV-1 incidence rate was likely to be low. Co-receptor usage was observed to be associated with HIV-1 subtypes. Poor alignment of the Northern Kenya sequences with the training data for the classifier software necessitates the need for development of Bioinformatic software relevant for locally circulating HIV-1 subtypes.

P05.09

Analysis of HIV-1 gp120 Quasispecies Suggests High Prevalence of Intra-subtype Recombination

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Background: Recombination between viruses of the same HIV-1 subtype has been understudied primarily due to the lack of reference sequences, as well as appropriate bio-informatics tools. The introduction of recombination detection program (RDP3) made the detection of HIV-1 intra-subtype recombination feasible

Methods: This study determined the prevalence and patterns of HIV-1 intra-subtype recombinants among female bar and hotel workers in Moshi, Tanzania. The HIV-1 env gp120 V1-C5 quasispecies from 45 subjects classified as pure HIV-1 subtypes A1, C, or D were analyzed for recombination events by RDP3.

Results: HIV-1 quasispecies with evidence for recombination were found in 89% of subjects infected with pure HIV-1 subtypes A, C, and D. Recombinant viruses were observed at both the baseline and the 12 month visits in 88% of the subjects; in 12% of subjects recombination was identified only at the later time point. Two major patterns were observed: 70% of subjects had unique recombination breakpoints without dominance of any particular variant, while in 30% of subjects a specific recombinant variant dominated in the pool of viral quasispecies.

Conclusion: A large proportion of female bar and hotel workers were infected with intra-subtype recombinant viruses. These results suggest that HIV-1 co- and super-infections are common in this population, and occur more frequently than previously thought. Intra-subtype recombination contributes to the increased viral diversity which poses a challenge to HIV-1 vaccine design.

P05.10

HIV-1 Diversity in Cameroon: New Insights on the Evolution of the Virus

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Background: Given the central role of HIV-1 diversity in the HIV pandemic and its impact on vaccine development, it is imperative that global molecular epidemiology surveillance continues. Constantly improving phylogenetics-based analytical techniques and rapidly expanding HIV sequence datasets promise to yield important insights into the origin, evolution and spread of HIV-1.

Methods: In an effort to study the phylogeography and phylodynamics of the HIV-1 M epidemic in the early 20th century, we analysed 50 plasma samples from HIV-infected blood donors from Cameroon. Full length gag sequences were generated and aligned using MUSCLE along with a representative selection of HIV sequences from the rest of the world and all published gag sequences from Cameroon and other West African countries. A maximum likelihood phylogenetic tree was constructed from these sequences following removal of recombinant sequence fragments by a blinded fully exploratory screen for recombination using RDP3.

Results: All the Cameroonian sequences were derived from HIV-1 M viruses. The phylogenetic tree indicated that at least one of the newly-sequenced CRF02_AG viruses is an outlier of the CRF02_AG clade and may help resolve the controversy surrounding the origins of this clade. Furthermore, isolates from Cameroon were spread throughout the phylogenetic tree clustering with different subtypes and circulating recombinant forms, a finding consistent with West Africa being the geographic origin of the global HIV epidemic. Importunately, our blind recombination screen suggested that many divergent Cameroonian viruses previously identified as being unique recombinant forms, may be divergent, relatively non-recombinant, but under-sampled subtype-level lineages.

Conclusion: Lineages diverging early after the origin of HIV-1 M are likely still circulating in Cameroon and could be suitable for retracing the movement and evolutionary dynamics of HIV-1 during the earliest stages of the pandemic. These lineages will be useful for reconstructing ancestral HIV-1M sequences for use as vaccine immunogens.

Topic 5: HIV Transmission and Viral Diversity

P05.11

Postnatally-Transmitted HIV-1 Variants Are Efficient at Dendritic Cell Trans-infection and Sensitive to Autologous and Heterologous Neutralization

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Background: Postnatal transmission via breastfeeding is a leading cause of infant HIV infection in the developing world. However, only a small minority of breastfed infants born to HIV-infected women become infected. As a genetic bottleneck severely restricts the number of postnatally-transmitted variants, genetic or phenotypic differences in the virus Envelope (Env) may play a role in its ability to breech the mucosal barrier in the infant gastrointestinal tract.

Methods: We examined the biologic properties of HIV Env pseudoviruses cloned from breast milk of postnatally-transmitting mothers (n=14 viruses), clinically-matched nontansmitting mothers (n=16 viruses), and early viruses from postnatally-infected infants (n = 6).

Results: There was no difference in epithelial cell attachment, internalization, or gp120 interaction with the putative HIV epithelial cell receptor, galactosylceramide, between milk HIV Env variants from transmitting and nontransmitting mothers. Similarly, there was no difference in the efficiency of milk Env variants to bind to monocyte-derived dendritic cells (DC). However, there was trend towards more efficient DC-mediated trans-infection of CD4-expressing target cells by milk Env variants of transmitting women compared to those of non-transmitting women ($p = 0.06$). Moreover, early infant Env variants were more efficiently transferred from DC than milk variants of nontransmitting women ($p = 0.0009$). The high-efficiency DC trans-infection was not attributable to higher infectivity or fusion efficiency of the postnatally-transmitted viruses. Infant Env variants were more sensitive to neutralization by broadly- neutralizing antibodies (HIVIG-C: p=0.02, PG-9: p=0.04, and VRC01: p=0.02) than Env variants from milk of nontransmitting women. In addition, Env variants from transmitting and nontransmitting mothers were equally-sensitive to neutralization by autologous plasma.

Conclusion: While resistance to broadly-neutralizing antibodies does not appear to be a defining feature of postnatally-transmitted Env variants, efficient HIV Env co-interaction with DCs and CD4-expressing target cells may be required for postnatal HIV transmission via breastfeeding.

P05.12

Envelopes Found Early After Acquisition Compared to Those in the Chronically Infected Partner Do Not Have Enhanced Alpha4 Beta7 Binding or Utilization

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Background: It has been hypothesized that the RV144 immune correlate, V1V2 antibodies, block binding to gut homing receptor, alpha4 beta7, which may limit virus access to gut associated lymphoid tissue. We hypothesized that if alpha4 beta7 binding is important during HIV-1 acquisition, viruses with envelopes isolated from newly infected subjects should have enhanced ability to bind the alpha4 beta7 integrin and/or infect cells with high levels of alpha4 beta7 receptor compared to variants present in the chronically infected transmitting partner.

Methods: Envelopes isolated from newly infected subjects and the transmitting partner were incorporated into NL4-3. Virus stocks were generated from peripheral blood mononuclear cell cultures. CD8+ and CD4+ T cells were activated with PHA, IL-2, and retinoic acid (RA). Binding was examined with quantitative PCR, and replication was assessed by estimating produced infectious virus on TZM-bl cells. Comparisons were done using Wilcoxon matched-pairs signed rank test.

Results: Samples from 9 newly infected subjects were collected a median of 93 days after estimated HIV-1 seroconversion (range 17 – 324 days), and all the transmitting partners had chronic persistent infection of more than two years duration at the time of estimated transmission. The alpha4 beta7 receptor expression increased in both CD8+ and CD4+ T cells by day 6. Viruses with recipient (median 9 copies, range 0 – 632) versus donor (median 9 copies, range 0 – 313) demonstrated no significant difference ($p = 0.9$) in binding to alpha4 beta7 expressing CD8+ T cells. Recipient viruses also demonstrated similar replication in CD4+ T cells compared to the donor envelope viruses ($p = 0.4$).

Conclusion: Enhanced alpha4 beta7 binding does not distinguish envelopes found early after acquisition compared to those circulating in chronically infected partners. Thus, the V1V2 antibodies found as a RV144 immune correlate likely does not provide protection by blocking access to the alpha4 beta7 integrin.

P05.13

HIV-1 Subtype B- and F1-Infected Subjects Display Higher Cross-Clade T-Cell Response Than Subtype C-Infected Subjects

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Background: The impact of the extensive genetic diversity of the HIV-1 group M isolates and its implications for vaccine design have long been discussed. Studies indicate that Gag and Nef conserved epitopes are commonly recognized and give rise to high cross-clade responses. The aim of this study was to compare T-cell responses to peptide pools derivate from subtype B, C and F1 consensus, among Brazilian subjects infected with those three HIV-1 subtypes.

Methods: The study included 32 subjects infected with HIV-1 subtypes B (n=13), C (n=11) and F1 (n=8). Gag and Nef-specific T cell responses were evaluated by IFN-γ ELISpot assay, using peptide pools based on subtype B, C and F1 Brazilians consensus.

Results: A high cross-clade response between subtypes B and F1 for both Gag and Nef regions was observed among HIV-1 subtype B- and F1-infected subjects. We also found no significant difference in magnitude of responses between subtype B and C consensus peptides in subtype B-infected subjects.In contrast, the magnitude of T cell responses to consensus C peptides in Gag region was significantly higher than to consensus B peptides among HIV-1 subtype C-infected subjects. In Nef, subtype C-infected subjects showed higher T cell responses to C than to F1 consensus peptides. Moreover, subtype F1-infected subjects presented lower responses to subtype C peptides than to subtype F1 and B ones.

Conclusion: Overall, the level of cross-clade response between subtypes B and F1 was higher than between subtype C and B or between subtype C and F1. Our data suggest that significance of the HIV-1 group M genetic diversity for vaccine design may be dependent of the subtypes involved.

P05.14

Emergence of Unique Recombinant Forms (URFs) in Indian HIV-1 Epidemic: Data from Nationwide Clinical Cohort Between 2007 and 2011

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Background: Current epidemiological studies in India have been limited to single or localized geographic settings within the country. In this study we aim to cherecterised the nationwide distribution pattern of the HIV-1 subtypes based on the data collected from clinical cohorts from 7 provinces from four regions in India (northern, north-eastern, central and southern)

Methods: Blood samples were collected from 212 HIV-1 seropositive subjects between 2007 and 2011. HIV-1 subtypes were determined using at least two or three viral genes, gag, pol, and env using maximum likelihood tree. Recombination events were identified using RIP ver 3 tools followed by breakpoints analysis in Simplot version 3.5.1 and fragment-specific phylogenetic analysis.

Results: When a single gene was used for subtype determination, the mean proportion of HIV-1C, B and A1 were 95.9%, 1.9% and 0.2% respectively while recombinants constituted 2.1%. The overall prevalence of URFs (BC/A1C) increased significantly to 10% when two ($p<0.01$) or three genes ($p=0.02$) were used. All the A1 and B strains, identified in the single-gene approach, were re-identified as URFs. Detailed analysis indicated that the B segment of the URF_BC probably originated from China/Thailand, while the A1 segments originated from eastern Africa. Among the four geographical regions examined, a high proportion of the recombinant strains based on the two-gene approach were identified in north-eastern (46.7%) and northern (18.5%) followed by southern India (5%), while central India appeared to have a concentrated subtype C epidemic (100%).

Conclusion: The rapid and continuous evolution of the HIV-1 epidemic in India was evidenced by the increase of recombinant strains. The use of multiple genes, rather than a single gene to identify HIV-1 subtypes can reduce the chances of false identification. These results warrant an urgent need for continued molecular surveillance to guide efficient disease intervention strategies and develop an effective subunit-based vaccine.

Topic 5: HIV Transmission and Viral Diversity

P05.15

Genetic Characterization of HIV-1 Subtype G Envelope Sequences by Single Genome Analysis

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Background: Subtype G is the sixth most prevalent subtype of HIV-1 and is responsible for an estimated 1,500,000 infections worldwide. Although systematic analyses of a wide range of HIV-1 envelope sequences and neutralization have been performed, subtype G viruses are severely underrepresented in these studies. There is thus an important need to study subtype G envelope sequences and their neutralization capacities.

Methods: 64 Plasma samples from Cameroon were used and 6 were found to be Subtype G by sequencing of gag and nef including one typed only for gag. Single genome analysis (SGA)-PCR was then performed and full length envelope genes were generated, which were then sequenced in the V1-V5 region

Results: Phylogenetic analysis of V1-V5 envelope sequences confirmed that 5 samples grouped within the expected subtype G and 1 with subtype A which we had been unable to type for nef. Sequences from within one sample were genetically related to each other, while samples were genetically distinct from each other. This suggests that the sequenced HIV-1 from any donor were generally from a single infection. Sequences from 4 of 5 samples grouped most closely to other West African subtype G sequences, while the fifth grouped in a cluster populated by sequences from Spain and few other African sequences. The V1-V5 region of the sixth sample clustered with subtype A and is thus presumed to be a recombinant.

Conclusion: These results suggests that our samples capture a substantial amount of the diversity within the subtype G envelope sequences and are largely different from each other. Therefore, forthcoming data concerning the neutralization patterns of these viruses will be able to give some of the sense of the diversity of neutralization pattern of subtype G viruses that will be needed to design a truly global vaccine.

P05.16

Enrolment and Logistical Challenges in TaMoVac 01 Phase I/II HIV Trial Despite the Completion of an Earlier (HIVIS-03 trial trial) in Dar es Salaam

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Background: Participation of sub-Saharan countries in HIV vaccine trials is important in the fight against HIV/AIDS, and has to be sustained by continued trials. Experiences from earlier trials are expected to influence the design and the performance of subsequent trials. Following completion of HIVIS-03 trial, TaMoVac- 01 trial was initiated. We compare enrolment experiences between the two trials.

Methods: The HIVIS-03 trial, conducted between 2007 - 2010 recruited 60 volunteers from the Police force in Dar es Salaam.

The subsequent TaMoVac- 01 trial has recruited 62 volunteers from the Police and Prisons force, and youths at IDC.

Results: Enrollment of volunteers into the HIVIS-03 took 12 months while the TaMoVac-01 trial took 13 months.

Screened: enrolled ratio was 3:1for HIVIS-03 trail, while for TaMoVac-01 8:1. Reasons for screen-out in the TaMoVac-01 trial were influence of family, misconception, clinical and laboratory abnormalities.

Recruitment of females was a challenge in the HIVIS-03 trial, but was unnoticed in the TMV-01 trial, this could be due to inclusion of youths. Misconceptions in the Police force remain an obstacle to recruitment despite regular education sessions.

Other challenges were: Poor adherence to schedules, due to competing prioritization of employment requirements; difficulties in communication with volunteers without phones.

Most challenges were addressed through collaboration with the concerned authorities in the respective cohorts

Conclusion: Recruitment challenges continued in the TMV-01 trial despite our experiences with HIVIS-03 trial. Enhanced Community engagement and timely action by the researchers is necessary to ensure a smooth conduct of the trials.

P05.17

Deep Sequencing Reveals an Association Between HIV-1 Subtype C Mutations in gp41 MPER Epitopes and Mother-to-Child Transmission

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Background: Enhanced HIV-1 mother-to-child transmission (MTCT) by high maternal anti-gp41 antibody titer led to the hypothesis that transmitting mothers would have greater diversity in membrane-proximal external region (MPER) and mutated amino acid residues associated with resistance to gp41 antibodies.

Methods: Pyrosequences of HIV-1 subtype-C gp41 heptad repeat-region 2 (HR2), MPER, and membrane-spanning domain (MSD) were generated from 2,000 plasma viral copies/subject from four mothers who transmitted via breast feeding (TM) and four non-transmitting mothers (NTM) in a matched case control study. A bioinformatic pipeline with rigorous quality controls generated ~50,000 quality pyrosequences/subject and provided 25-fold coverage of input virus populations. Population genetic algorithms clustered pyrosequences at 3% genetic distance to study biodiversity using rarefaction/Chao1. Frequency distribution of cluster sizes defined population structure. Consensus sequences constructed from bioclusters for each subject were aligned to an HIV-1 subtype-C consensus sequence to determine number and frequency of nonsynonymous substitutions at each position and to identify mutations by HIV Molecular Immunology Database. Groups were compared using paired t-test.

Results: Sequences in MPER were more polymorphic than in HR2 or in MSD. TM had more diverse MPERs than NTM ($p = 0.02$). The number of clusters calculated from rarefaction curves was 62(±35) for TM vs 35(±28) for NTM. The Chao1-estimated maximum number of variants within populations was 106(±51) for TM vs 59(±65) for NT. Low fit viruses (≤5 sequences/biocluster) contributed to differences in biodiversity between TM [71.1(±9.7)%] and NTM [63.5(±14.2)%]. Polymorphisms at residues within 4E10 (W672V, F673L, D674S, T676I, and W680G) and 2F5 (D664S) were confined exclusively to viruses from TM mothers. Viral variants with positively charged hydrophilic MPER occurred more frequently in TM than in NTM.

Conclusion: HIV-1 subtype-C variants with high biodiversity correlated with polymorphisms in MPER and were associated with MTCT, which may reflect increased immune selection and have implications for vaccine design.

P05.18

First Report on HIV Molecular Epidemiology in a Native Community from Argentina Reveals Transmission Clades in the Context of a Unique HLA Background

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Background: The Department of Orán is located on the North of Argentina and is populated by native settlements and urbanized areas. During the last twelve years, an increasing number of HIV infections have been detected. We previously reported a highly limited HLA diversity with a reduced number of HLA-A and B alleles present at high prevalence. The objective of the present study was to characterize the HIV epidemic through full-length genome sequencing of the virus in this population.

Methods: Samples from 147 HIV positive individuals collected in three campaigns to Orán were analyzed. Viral load, CD4-count and drug resistance were assessed and reported to the clinicians. Limiting-dilution full genome sequencing was performed from plasma on 65 of the samples. Viral diversity was analyzed by recombination (SimPlot) and Neighbor-Joining trees with bootstrap. HLA-A, B and C were characterized by SBT.

Results: HLA typing showed a limited genetic diversity even at the four-digits high-resolution typing. This allele distribution resembles the one reported previously for this community with two-digits and confirms that the individuals included were in fact native. SimPlot analysis of the 65 complete and nearly-complete genomes showed a high prevalence of BF recombinants(70.77%) and the presence of subtype B(23.08%), C(3.08%) and F(3.08%) virus. 7 main recombination structures were found repeatedly in not-epidemiologically-linked individuals within highly supported monophyletic clades suggesting transmission of these particular viral strains within the community.

Conclusion: This HIV epidemic seems to be characterized by a few introductions of viral variants that had spread on the community. Most of them have been BF recombinants with particular recombination structures. This work will allow us to further investigate the impact of the genetic features of this native community on viral evolution but also has facilitated the access of these individuals to clinical studies and counseling.

P05.19

Dynamics and Frequency of Gag Transmitted Polymorphisms in Zambia

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Background: HIV immune escape is not random and follows a predictable mutational path in response to the HLA alleles carried by an individual.

Methods: Using 143 epidemiologically linked transmission pairs from a Zambian cohort we assessed: (1) the frequency of Gag polymorphisms circulating in the population, (2) if the polymorphisms could be associated with the infected individual's HLA alleles, (3) the frequency at which polymorphisms are transmitted, and (4) the relevance of the transmitted polymorphisms (TP) to the newly infected individual's HLA-I alleles.

Results: We observed a median of 35 (range 23-66) polymorphisms per chronically infected individual in Gag and 42% of these polymorphisms could be associated with the individual's HLA (16% statistically linked, 26% epitope analysis). When transmission of these polymorphisms was assessed, we observed that the majority of these polymorphisms (84%) are transmitted to the epidemiologically linked partner and, of these TP, an equivalent fraction (43%; 11% statistically linked, 32% epitope analysis) were relevant to the newly infected individual's HLA-I alleles. In 81 transmission pairs observed during the first two years of infection, we observed a very low overall reversion rate (4%/year) of the TP. Reversion was not uniform and when p17, p24, p2, p7, p1, and p6 are examined individually, TP in p17 and p2 exhibited the highest frequency (2.5x) of reversion events based on amino acid length. Four CTL-targeted positions were identified where a majority of TP reverted to consensus over the two years of follow up, consistent with a reduction in fitness following transmission.

Conclusion: These data indicate: (1) HLA-I associated polymorphisms are stable and circulating frequently in the Zambian population, (2) individuals are acquiring HIV-I variants with a high frequency of polymorphisms relevant to their HLA-I alleles, and (3) reversion is slow and not evenly distributed across Gag.

P05.20

Limited Evidence for Alterations in Gag-Mediated HIV Replication Capacity Over the Course of the North American Epidemic (1979-Present)

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Background: The extent to which HIV replication capacity (RC) has changed over the epidemic's course, and the influence of HLA-associated immune pressure as its driving force remains unknown. We performed a comparative study of immune escape and RC in historic (1979-1989) and modern Gag subtype B sequences from North America.

Methods: Using phylogenetically-informed methods, we identified HLA-associated Gag polymorphisms in a historic cohort (N=239; 1979-1989). We also generated recombinant NL4-3 viruses encoding clonal plasma RNA Gag from 80 historic and 58 modern (2002-2008) sequences. Viral RC was measured using a GFP reporter T-cell assay and results were normalized to NL4-3 controls.

Results: 95% of HLA-associated polymorphisms identified in the historic cohort were consistent with published modern escape pathways. Overall, the prevalence of HLA-associated polymorphisms in the general population increased a median 1.3-fold between historic and modern sequences; however in many cases this was influenced by differences in HLA allele frequencies between HIV-infected populations examined. Of note, the prevalence of the B*27-associated R264K escape mutation increased from 0.4 to 1.3% in the general population over time despite B*27 allele frequency remaining constant at 2.5%. Modestly lower viral RC was observed for Gag recombinant viruses constructed from pre-1985 sequences (median 0.86 [IQR 0.78-0.97], N=24) compared to those from 1985-1989 (median 0.98 [IQR 0.87-1.05], N=56) and 2002-2008 (median 0.96 [IQR 0.83-0.1.10], N=58) (p=0.049). In both historic and modern cohorts, host expression of HLA-B*27 was associated with lower RC (p=0.007). Gag codons associated with lower RC, including S67A, were identified in an exploratory analysis.

Conclusion: Gag-mediated viral RC may have increased modestly since the beginning of the North American epidemic, despite limited evidence for HLA-driven viral sequence evolution during this time. Although mechanisms driving RC differences remain unclear, results do not support rapid and substantial accumulation of HLA-driven escape mutations in circulating North American HIV-1 sequences.

P05.21

Genetic Variability and Drug Resistance Mutations in HIV-1 Infected Individuals on HAART or Drug Naïve in Limbe, Cameroon

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Background: Cameroon is a country in West Central Africa with a population of about 20 million inhabitants, with an estimated HIV prevalence of 5.1% in the general population. The HIV epidemic in this region is marked by a broad genetic diversity dominated by Circulating Recombinant Forms (CRFs).

Methods: To characterize HIV-1 genotypes circulating in HIV positive individuals in Limbe, Cameroon, we phylogenetically analyzed blood samples from 116 HIV positive patients. Of 116 samples tested, 110 were amplified by nested PCR at the Gag, Pol and Env genes. Sequences obtained were phylogenetically analyzed with reference sequences from the Los Alamos database. The RT region of samples was also amplified to identify mutations that conferred resistance to Reverse Transcriptase inhibitors (RTIs) using the Stanford University Drug resistance database.

Results: Our results revealed a broad genetic diversity, dominated by circulating recombinant forms as follows: CRF02_AG 50.4% (n=54), CRF22_01A1 7.4% (n=8) F2 3.7% (n=4), D 2% (n=2), CRF43_02G 2.8% (n=3), CRF18_CPX 2.8% (n=3) and recombinant forms (RFs) 31.7 %(n=34). Most RFs contained CRF02_AG in one or two HIV loci analyzed. RT sequences of 3 patients on HAART and 15 drug naïve individuals harbored mutations which conferred resistance to RTIs.

Conclusion: CRF02_AG continues to be the most predominant strain in Cameroon, CRF22_01A1 on the increase. Identification of drug resistance strains in drug naïve patients suggest these viruses are being transmitted in the study population and highlights the need of drug resistance testing before start of ART for HIV patients.

P05.22

Intrasubtype C Superinfected Individuals Mount Delayed and Low-Titer Autologous Neutralizing Antibody Responses Prior to Superinfection

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Background: The potential role of neutralizing antibody in protecting against intra-subtype HIV-1 superinfection remains to be understood. We compared the early neutralizing antibody responses in three individuals, who were superinfected within one year of primary infection, to ten case-matched non-superinfected controls from a Zambian cohort of subtype C transmission cases. Sequence analysis of single genome amplified full-length env showed minimal diversification in the individuals who became superinfected with the same subtype of HIV-1 within year one post-seroconversion. We hypothesized that these superinfected individuals had a muted neutralizing antibody response that elicited little pressure on the founder virus to escape.

Methods: We molecularly cloned envs from virus at the time of seroconversion and from virus at the time point that superinfection was detected. Using a TZM-BL pseudovirus reporter assay, we tested plasma neutralization of these autologous variants over the first year of infection.

Results: Neutralization assays showed that autologous plasma NAb titers to founder virus were low to undetectable in all three superinfected individuals prior to superinfection. In contrast, neutralizing antibodies with a median IC50 of 1:1896 were detected as early as three months post-seroconversion in non-superinfected matched controls. There was no evidence, prior to superinfection, of cross-neutralization of superinfecting variants in any of the three cases, although cross-neutralization breadth and potency to the subtype C pseudovirus reference panel was also limited in the plasma from non-superinfected individuals. Although there was a trend towards superinfected individuals having reduced levels of gp120 binding antibodies prior to superinfection compared to non-superinfected controls, this difference was not statistically significant between the groups.

Conclusion: These data suggest that development of antibodies, as reflected in autologous neutralizing antibodies to the primary infection variants, may provide protection and decrease susceptibility to superinfection.

Topic 5: HIV Transmission and Viral Diversity

P05.23

Immunodominance and Viral Fitness in Gag May Contribute to Differential Viral Control in HLA-B*7 Supertype Individuals Acutely Infected with HIV-1C

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Background: HLA-B*7 supertype alleles are common among people of African descent and are associated with viral control. In particular, HLA-B*81 has been previously associated with reduced viral fitness. We analyzed the immunodominance of CD8+ T cell responses targeted by the B*7 supertype alleles, viral evolution and fitness dynamics over 1yr in acutely infected patients.

Methods: Six HLA-B*7 supertype participants [HLA-B*81 (n=2), HLA-B*4201 (n=3) and HLA-B*4202 (n=1)] identified with acute HIV-1 infection (antibody negative, vRNA positive) in KwaZulu-Natal, South Africa were studied. CD8+ T cell responses were measured by the IFN-γ ELIspot assay. Replication capacities of viruses encoding Gag-protease were measured. Full-length HIV-1 Gag clonal sequencing of plasma was performed at ~14 days post infection and 1yr later.

Results: The average viral set point of the 4 HLA-B*42 individuals was higher than the 2 HLA-B*81, 4.89 vs 4.16 respectively. Approximately 28 days after viral infection, CD8+ T cell responses were directed to an average of 2/5 (range 2-4) HLA-B*42 Gag-specific epitopes, median magnitude of 490 (range 170–2,480 SFC/million PBMCs). None of these 4 individuals had selected for escape mutations in the immunodominant TL9 epitope at 1yr post-infection. Interestingly, CD8+ T cell responses were only against the TL9 epitope for the 2 HLA-B*81 patients with a median magnitude of 950 (range 300–1780 SFC/million PBMCs). One patient had a single wild type epitope in the transmitted virus, compared to 4/5 wild type epitopes in the second patient. However, CD8+ T cell responses were only elicited at the TL9 epitope with a low magnitude against T186S in the 1 patient with a much lower viral fitness.

Conclusion: Strong, rather than broad immunodominant responses in HLA-B*7 individuals is desirable in viral control. Furthermore, this study emphasizes the advantage of early dominant CD8+ T cell immune responses and an attenuated virus in conferring clinical benefit among HLA-B*7 supertype individuals.

P05.24

HIV Disease Progression Compared by Linkage Status in Rwanda and Zambia

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Background: Recently HIV infected individuals are followed up at Rwanda-Zambia HIV Research Group (RZHRG) sites to establish markers of disease progression. Here we compare disease progression between seroconverters infected by their spouses (linked transmission) to those infected by non-spousal partners (unlinked transmission) where a greater fraction of individuals are infected by multiple virus variants.

Methods: Seroconverters (SC) were identified from HIV discordant couples enrolled from the Couples' VCT program into a prospective cohort study. Linkage of transmission was established by comparing viral DNA sequences in the SC to that of the suspected index partner. SCs were followed up to six years to collect clinical and laboratory data. Data analysis using STATA was done on CD4 and viral load trajectories and a comparison of disease progression with respect to time to endpoints of CD4 <350 cells/uL and initiation of ARVs. Log rank test of equality for survival function was performed for each endpoint and p-values calculated.

Results: From February 2006 to December 2011, 88 unlinked and 225 linked transmissions were identified. Across all RZHRG sites, SC males represented 56% of the unlinked and 55% of the linked group. Overall mean age at seroconversion was 33 years and 69% of HIV transmissions were acute infections (identified within 90 days of infections). Mean CD4 count and viral load at specified time points during a six year follow up period of the two groups were similar. Disease progression in the two groups (CD4 <350cell/uL or initiation of ARVs) showed no significant difference (p=0.21 and p=0.26) respectively.

Conclusion: Despite the fact that previous studies have shown that there is faster disease progression in individuals who were infected by multiple genetic variants from their partner, we show here that linkage status does not predict HIV disease progression in HIV seroconverting couples.

P05.25 LB

Transmitted HIV-1 variants in HIV infected Mother-Child Pairs, Carrying Different Subtypes

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Background: Mother-to-child transmission is the predominant route of HIV-1 infection in children. Prevention strategies are available in industrialized countries, but are limited in developing countries. Defining pertinent characteristics of transmitted HIV-1 from mother-child pairs, where the actually transmitted virus has been traced, is important in creating better preventive measures against transmission of HIV-1.

Methods: Two mother-child pairs of subtype A, three of subtype C, and three of CRF01_AE, were included. The V3-loop and flanking regions of the HIV-1 env gene were amplified from PBMC and plasma lysates. Virus isolates from several time points of pregnancy and in the child were sequenced, as well as 50 to 100 single HIV genomes per case.

Results: A total of 543 single genome sequences were obtained. The sequences were analysed phylogenetically, demonstrating at least one maternal sequence, identical to the child's first detected viruses or clones. In all cases an infant isolate sequence co-localized with maternal sequences, most probably related to transmission. The localization of other sequences from clones and/or virus isolates in the phylogenetic tree and their detailed amino acid composition provided a clear indication of the V3 loop amino acids of the respective transmitted virus. The fact that these sequences included an isolate will allow a greater genetic analysis of the complete envelope of transmitted HIV-1.

Conclusion: Since the sequence of the gp120 V3 loop is of paramount importance for virus entry, we can compare the amino acid properties across subtypes and test these viruses in neutralization assays. This knowledge can be exploited in prevention strategies and vaccine design.

Topic 6: Immunogenetic Factors

P06.01

The Viral Set Point in Primary HIV Infection Is Associated with Specific Amino Acids in Position 97 of MHC Class I

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Background: Sequence variations which affect the peptide binding groove of the major histocompatibility complex (MHC) class I allele HLA-B are strongly associated with viral control in chronic HIV infection. We sought to determine if these differences affect primary HIV infection and establishment of the early viral set point.

Methods: We longitudinally followed 428 individuals during primary HIV infection. In 110 individuals, we were able to determine the viral set point at six months following diagnosis. Associations between known genetic polymorphisms in HLA class I residues and HIV viral load and viral set point were identified.

Results: The identity of the amino acid at position 97 in the peptide binding groove of HLA-B was significantly associated with the level of initial viremia in acute infection (p=0.04) and the viral set point (p=0.025). Individuals with valine at position 97 had a nearly 10-fold lower mean viral set point than those with serine. This association was dependent on presence of the B*57 allele, which was also associated with a lower viral set point. The initial viral load at the time of primary HIV diagnosis was closely correlated with the level of the subsequent viral set point (R=0.4, p=<0.0001).

Conclusion: Control of HIV during acute infection and the viral set point are strongly associated with the amino acid in position 97 of HLA-B. These results provide evidence for genetic predictors of HIV control in primary infection and highlight the importance of sequence polymorphisms in the binding pocket of MHC class I which may be relevant to the development of future HIV vaccines.

P06.02

Sequence Based Typing of HLA-A and B Exons-2 and -3 in a HIV-Positive Native Community with Limited HLA Diversity from the North of Argentina

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Background: We previously reported a limited diversity of HLA-class I alleles in two-digits typing studies of HIV-positive native populations from Oran, North of Argentina. In the present study we determine whether the restricted diversity observed at low-resolution reflects also a restricted genetic diversity in HLA peptide groove.

Methods: We studied 65 HIV-positive patients whose HLA-A and -B genes were previously typed by SSOP technique. We set-up a sequence-based typing of the most prevalent alleles in Oran. HLA-A and B were initially PCR-amplified and exons-2 and -3 were sequenced. NCBI-SBT-Interpretation tool was used to confirm the two-digits typing with previous SSOP data. We designed a set of primers specifics for HLAs highly prevalent in Oran to achieve differential PCR-amplification of each allele in heterozygote patients. Phylogenetic analysis was used to assign exons-2 and -3 sequences to a high-resolution HLA group.

Results: Our results show that for HLA-A alleles, 85.1% of A*02 are A*02:01:01:01, 96.7% of A*31 are A*31:01:02 and 92.8% of A*24 are A*24:02:01:01. In the case of A*68, 50% are A*68:01:02 and 31.2% are A*68:17. For HLA-B alleles, B*35 was diverse: B*35:01:01:01 (15.8%), B*35:04:01 (15.8%), B*35:05:01 (21.1%) and B*35:19 (21.1%). 43.8% of B*39-alleles were B*39:05:01 and 25% were B*39:03. 52.9% of B*48-alleles were B*48:01:01 and 35.3% were B*48:03:01. 62.5% of B*51 alleles were B*51:01:01. The mentioned alleles represent the 73.1% of HLA-A genetic diversity and the 45.4% of HLA-B. All the polimorphisms observed lead to non-synonymous changes.

Conclusion: Our results show that two-digits typing of HLA-A usually corresponds with a specific allele in our population. For HLA-B alleles, observed within-subtype diversity was higher. The different protein sequence encoded by exons-2 and -3 may lead to different peptide specificities among alleles from the same HLA-B subtype that would be miss-classified as homogeneous in a low-resolution typing study.

P06.03

Interactions Between HLA-B and Leukocyte Immunoglobulin Like Receptors B2 (LILRB2) Correlate with HIV-1 Disease Outcomes

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Background: Recently, a genome-wide association study identified 3 amino acids (residues 67, 70, 97) in the HLA-B peptide binding groove that explain the classical HLA class I associations with HIV-1 immune control. However, how amino acid substitutions at these positions affect HIV-1 immune defense remains unclear. Leukocyte Immunoglobulin Like Receptors (LILR) are a class of immunoregulatory molecules that are expressed on myeloid dendritic cells and interact with HLA class I molecules as their physiologic ligands. Here, we assessed how different amino acids in the HLA-B binding groove may influence their binding to LILRs.

Methods: Using a cell-free assay, the binding strength between 50 HLA-B alleles and recombinant LILRB2-Fc fusion protein was experimentally determined. Average bindings scores to LILRB2 were calculated for HLA-B allotypes sharing identical amino acid residues at position 67, 70 and 97. For comparison purposes, binding scores to LILRB1 were similarly determined. The average binding scores were then correlated to odds ratios reflecting the impact of the respective amino acids residues at position 67, 70 and 97 on HIV-1 disease progression.

Results: Six amino acid variants at position 97, four amino acid variants at position 70, and five amino acid variants at position 67 were included into the analysis. Overall, we observed an almost perfect inverse correlation between the odds ratios of each amino acid variant at position 97 on HIV-1 control and LILRB2 binding scores of class I alleles carrying the respective amino acid variant (R=0.88, p=0.02). Similar findings were made for amino acid variants at position 70 (R=0.993, p=0.007) and position 67 (R=0.95, p=0.005). No significant correlations were observed when HLA-LILRB1 binding scores were used instead of LILRB2.

Conclusion: These data suggest that amino acid polymorphisms at position 67, 70 and 97 of the HLA-B binding groove influence HIV-1 immune control through altered interactions with the immunomodulatory LILRB2 receptor.

P06.04

The Prognostic and Diagnostic Use of MicroRNA Expression in Chronic HIV Infection

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Background: MicroRNA (miRNA) is engaged in the regulation of host immunity during HIV infection and may play an important role in the disease progression. We sought to identify a diagnostic/prognostic miRNA signature to diagnose/predict the outcome of chronic HIV infection.

Methods: The expression levels of 754 known human microRNA were quantified in whole peripheral blood by TaqMan low density array for 23 chronically HIV-infected subjects. Significance analyses of miRNA expression profiles were performed on groups with varied levels of CD4+ T cell counts or viral loads. The identified prognostic miRNA signature was further validated in a 51 HIV+ patient cohort naïve to ART with following up for 2 years. In addition, we assessed their association with progression-free rate. For the latter, various prediction algorithms were computed on the basis of weighted levels of the miRNAs forming the outcome signature.

Results: Of the 10 differentially expressed miRNAs ($p<0.001$), 3 were validated to contribute to the predictive value and thereby used as prognostic signature. Based on different prediction models (binary discrete model and linear models), the prediction accuracy ranged from 84.31% to 88.24% compared with subsequent 2-year follow-up. When applied all the computed classifiers to serial time-point samples of seven progressors and five non-progressors collected during the 2-year follow up, binary discrete model show a better prediction stability. Low miR-31, miR-29b, miR-590-5p were associated with poor progression-free rate, independent of age, gender and known prognostic factors including viral load and immune activation.

Conclusion: A unique microRNA signature has been associated with advance disease and identified as potential prognostic marker, and provides a new strategy to select patients who would benefit from earlier ART.

P06.05

Human Leukocyte Antigen Class I Supertypes and Viral Control in HIV-1 Infected Former Plasma Donors from China

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Background: The role of human leukocyte antigen (HLA) class I supertypes in controlling human immunodeficiency virus type 1 (HIV-1) infection in Chinese has not been established. The aim of this study is to examine the frequency of HLA-A and HLA-B alleles and supertypes of 222 HIV-1 infected former plasma donors in central China and to investigate their impact on HIV-1 viral control.

Methods: HLA-A and HLA-B alleles were genotyped with PCR-SSP and sequence-based typing assay to four-digit resolution. The HLA alleles were classified functionally to 4 HLA-A supertypes and 6 HLA-B supertypes according to their shared peptide binding properties. Plasma viral load was determined using the Roche Amplicor ultrasensitive assay which has a lower detection limit of 50 copies HIV-1 RNA per ml.

Results: HLA-A03 supertypes(A03s) and HLA-B62 supertypes(B62s) were associated with lower viral load (P=0.0206, P=0.0483), whereas HLA- A24 supertypes(A24s) appeared to have an association with higher viral load (P=0.0483). There was a highly significant correlation between the genotypic supertypes(GS) and viral load (Kendall's tau b = 0.180, P=0.000). The median viral load was lower among A*3001(P=0.0139)、A*1101(P=0.0096)、B*5101(P=0.0025)、B*3501(P=0.0091) or B*4601(P=0.001) carriers and higher in A*2301(P=0.0106) carriers.

Conclusion: HLA-A03s and -B62s may be associated with favorable HIV-1 viral control, A24s associated with unfavorable viral control; HLA-B*4601 within B62s and HLA-A*2301 within A24s might contribute to the outcomes of HIV-1 viral control.

P06.06

Association of Interleukin-10 Promoter Genetic Variants with T-Cell and B-Cell Activation in HIV-1 Infection

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Background: Interleukin-10 (IL-10) is a potent immunoregulatory cytokine, with promoter polymorphisms that have previously been associated with HIV-1 susceptibility and pathogenesis. Association of IL-10 SNPs with markers of CD4, CD8 and B cell activation has not previously been investigated.

Methods: Two IL-10 polymorphisms were genotyped by TaqMan allelic discrimination and markers of activation of CD4, CD8 and B cells were measured in 63 individuals using flow cytometry. The following monoclonal antibody combinations were used: anti-CD3 Pac-blue, anti-CD38 PE-Cy7, anti-HLA-DR ACP-Cy7, anti-CD95 PE, anti-CD19 Alexa-700, anti-IgG PE-Cy5, anti-PD-1 APC, anti-Ki67 FITC, anti-CD4 Qdot605 and anti-CD8 Qdot655.

Results: Previous studies on this cohort showed a significant association between -1082GG and higher median IL-10 expression, compared to the -1082AA/AG (p= 0.0006). The -592AA and -1082AA (both previously shown to be associated with low-IL-10 production) had significantly higher median expression of HLA-DR on CD4 and CD8 cells respectively, compared to the other genotypes (-592AA vs. -592CA p= 0.005, -592AA vs. -592CC p= 0.03 and -1082AA vs. -1082AG p= 0.02). The -592AA genotype also had a higher median expression of CD95 and PD-1 on CD4 cells (-592AA vs. -592CA p= 0.0227, -592AA vs. -592CC p= 0.0270 and -592AA vs. -592CA p= 0.01 respectively). The -592CC and -1082GG genotypes associated with higher median expression of IgG on the surface of B cells (-592CC vs. -592AA p= 0.0207 and -1082GG vs. -1082AG p= 0.0392, -1082GG vs. -1082AA p= 0.0051).

Conclusion: These data suggest that IL-10 polymorphisms that affect cytokine production and HIV pathogenesis may affect markers of immune activation and exhaustion in response to antigen, and suggest a beneficial role for IL-10 in chronic HIV infection. Further studies on association between IL-10 and the quality and magnitude of immune responses in HIV infection are needed.

Topic 7: Innate Immunity

P07.01

Two Independent Functions of Vγ2Vδ2 T Cells Discriminated by CD16 During HIV-1 Infection

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Background: Vγ2Vδ2 (Vδ2) T cells play a vital role in the control of HIV infection. Vδ2 T cells recognize phosphoantigens such as IPP, and they mediate ADCC through FcγRIIIa (CD16). Our goal is to understand how the heterogeneous repertoires of Vδ2 T cells are involved in both phosphoantigen -induced response and ADCC in HIV infection, especially in the early stage of HIV infection.

Methods: PBMCs were obtained from a total of 81 subjects, including 18 early, 42 chronic HIV-1 infected subjects (all treatment-naive) and 21 healthy subjects. Cellular immune functions of Vδ2 T cells were analyzed by flow cytometry.

Results: Circulating Vδ2 T cells comprised two functionally diverse subsets which were discriminated by the CD16 expression. Most cytotoxic molecules and IFN-γ were released by $CD16^-$ subset (98% in average) after IPP stimulation, while the $CD16^+$ subset was in charge of triggering ADCC via CD16 that was closely related to HIV-associated changes in Vδ2 T cell-mediated ADCC ($p< 0.001$). In early HIV infection, the $CD16^-$ Vδ2 T cells dramatically decreased in comparison with healthy controls ($p=0.02$), accompanied by the decline of IPP-responsive Vδ2 T cells ($p=0.01$). Interestingly, a dramatic functional switch of Vδ2 T cell-mediated ADCC with almost reverse profile of the CD107a and IFN-γ expression compared to uninfected group was observed since early HIV infection. Frequency of CD107a+ Vδ2 T cells from early-infected group was significantly higher than that from healthy controls ($p<0.05$). Although the IPP-activated Vδ2 T cells declined notably in chronic-infected individuals with CD4>500 (cells/μl), the percentage of antibody-dependent cytotoxic Vδ2 T cells was over threefold as high in CD4>500 individuals as in healthy controls ($p<0.05$ for both).

Conclusion: These data revealed the involvement of two Vδ2 T subsets with different functions during HIV infection and highlighted the plasticity of Vδ2 T cell-mediated ADCC in controlling HIV infection.

P07.02

KIR-HLA Footprints and NK Cell-Mediated Recognition of HIV-1

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Background: Increasing data suggest an important role of KIR+ NK cells in the control of HIV-1, however the precise mechanisms on how NK cells recognize HIV-1-infected cells remain poorly understood. KIRs can bind to HLA class I molecules, but the binding affinity of these interactions is dependent on the HLA class I presented epitope. Recent reports have suggested that sequence variations within HIV-1 epitopes presented by HLA class I can affect the binding of inhibitory KIRs expressed on NK cells, potentially modulating NK cell responses to infected cells. Here we investigated whether HIV-1 might adapt to the combined KIR/HLA genotypes on a population level to identify areas within HIV-1 that might be targeted by KIR+ NK cells.

Methods: HIV-1 Gag was sequenced in 390 untreated chronically clade C infected individuals from KwaZulu-Natal, South Africa. All study subjects were HLA class I and KIR typed. Phylogenetically-corrected logistic regression analysis of KIR/HLA associated Gag sequence polymorphisms was performed and q-values were used for multiple test correction.

Results: A total of 93 sequence polymorphisms significantly associated with the combined HLA/KIR genotypes were identified ($p<0.05$), 6 of them with a false-positive rate of less than 20% ($q<0.2$). These significant associations were independent of previously identified KIR or HLA-linked polymorphisms.

Conclusion: This study identified several sequence polymorphisms within HIV-1 Gag that were significantly associated with the expression of combined KIR/HLA genotypes at the population level, indicating adaptation of HIV-1 to NK cell mediated immune pressure. KIR/HLA class I binding studies in the context of the sequence polymorphisms and studies for NK cell function are ongoing to determine the consequence of these sequence changes for NK cell-mediated recognition of HIV-1-infected cells.

P07.03

Frequent and Strong Antibody-Mediated NK Cell Activation to HIV-1 Env in Individuals with Chronic HIV-1 Infection

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Background: Natural killer (NK) cells are critical in viral control and NK cells that respond to HIV-1 peptides have been described. However, it is unclear how NK cells recognize HIV-1 antigen. We investigated NK cell responses to HIV-1 peptides during early and chronic HIV-1 clade B infection.

Methods: NK cells responding to HIV-1 peptides were assessed by multiparameter flow cytometry using whole blood from 74 individuals with treated or untreated early or chronic HIV-1 infection. In addition, 15 HIV uninfected individuals were also studied.

Results: No NK cell responses to HIV-1 peptides were detected in HIV-1 uninfected individuals. The HIV-1 NK cell specific responses to peptide were less frequent during the first year of infection but were of high magnitude and frequent in individuals with controlled or progressive infection (22% vs 79%; P<0.00001). The activation of NK cells to peptide pools required the presence of plasma IgG and the responding NK cells had a low CD16 expression and high CD57 expression. Furthermore, plasma derived from HIV-1 infected individuals was sufficient to trigger a response to HIV-1 Env peptide pool by NK cells from healthy donors suggesting the role of antibodies in mediating this activity.

Conclusion: NK cell responses to specific antigens can be induced in HIV-1 infection. Large cohorts are needed to assess the consequences of these NK cells against HIV-1 control or protection from infection.

P07.04

TLR7/9 Antagonist Reduces HIV-1-Induced Immune Activation

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Background: T cell immune activation is a strong predictor of HIV-1 disease progression and HIV-1 transmission, and IFN-alpha production following TLR7 stimulation has been associated with elevated $CD8^+$ T cell activation (Meier et al., Nat Med 2009). We therefore hypothesized that modulation of TLR7 stimulation could be used to manipulate IFN-alpha production and subsequently reduce HIV-1-associated immune activation. TLR7/9-specific antagonists developed for treatment of auto-immune diseases were used in vitro and in vivo in a humanized mouse model.

Methods: Humanized BLT mice were generated by transplanting irradiated NOD/SCID/$\gamma c^{-/-}$ mice with human fetal thymus and injected with human hematopoietic stem cells isolated from matching liver tissue. Following reconstitution, cells were harvested from the mice to examine the effects of the antagonist in vitro. Humanized mice were also infected with HIV-1 and then either treated or untreated with TLR7/9 antagonist from Idera. T cell activation markers were examined pre-infection, following infection and during treatment with the antagonist. Additionally, responsiveness of DCs to TLR7/8 stimulation ex vivo following in vivo TLR7/9 antagonist treatment was assessed by intracellular cytokine staining.

Results: 16-20 weeks after transplant, human DC, monocyte and T cell populations were detectable in the mice. Ex vivo stimulation with TLR7/8 induced cytokine production by humanized mice DCs and monocytes similar to those observed from human PBMCs and this was significantly blocked by in vitro treatment with the TLR7/9 antagonist (P<0.05). HIV-1 infection of humanized mice led to increased T cell immune activation marker CD38 on human T cells in vivo, and treatment of infected mice with the TLR7/9 antagonist led to a significant reduction in CD38 expression.

Conclusion: Treatment of HIV-1-infected humanized BLT mice with a TLR7/9 antagonist resulted in a significant reduction of HIV-1-associated immune activation. This may have important implications in reducing viral transmission associated with higher immune activation in HIV-1.

P07.05

Tim-3-Mediated Signaling in NK Cells May Be Modulated by Increased Galectin-9 Expression in HIV-1 Infection

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Background: Natural Killer (NK) cells constitutively express high levels of Tim-3, an immunoregulatory molecule recently proposed to be a marker for mature and fully functional NK cells (Ndhlovu et al., 2012). Cytokines can induce Tim-3 expression on NK cells, and IFN-gamma production by Tim-3+ NK cells can be enhanced by exposure to Galectin-9 (Gleason et al. 2012), yet their ability to kill target cells is lost upon Tim-3 cross-linking. Moreover, up-regulation of Tim-3 on NK cells has been associated with reduced anti-viral properties in chronic hepatitis B infection (Ju et al., 2010). However, the impact of HIV-1 infection on Tim-3 expression on NK cells and on Tim-3-mediated NK cell function has not been studied yet.

Methods: Flow cytometry was used to analyze Tim-3 and intracellular galectin-9 expression in subjects with acute and chronic HIV-1 infection, in HIV-1 elite (VL <50 copies/ml) and viremic controllers (VL <2000 copies/ml), and in HIV-1 negative subjects. Plasma levels of Galectin-9 were quantified by ELISA.

Results: HIV-1 infection was associated with reduced expression of Tim-3 on NK cells, as early as in acute infection, and could be normalized by HAART. Importantly, percentages of Tim-3+ $CD56_{dim}$ NK cells correlated with CD4+ cell counts in untreated patients. Plasma concentrations of Galectin-9 were higher in HIV-1-infected individuals than in controls. Interestingly, Galectin-9 expression in immune cells was significantly elevated in acute infection, with monocytes and dendritic cells displaying the highest levels, which correlated with viral loads. In vitro, Galectin-9 triggered NK cell activation and Tim-3 down-regulation on NK cells.

Conclusion: Further investigations are warranted to determine whether increased Galectin-9 production alters Tim-3 function and contributes to NK cell impaired activity in chronic HIV-1 infection. Defining the role of NK cell receptors in the control of HIV-1 will offer novel therapeutic targets to manipulate and improve future HIV-1 vaccine strategies.

P07.06

Toll Like Receptor 7 Regulates Viral Loads and Cytokine Secretion During Acute Retroviral Infection

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Background: Acute HIV infection is characterized by a high viremia accompanied by a powerful wave of pro-inflammatory cytokines that affects the subsequent course of infection and pathogenesis. Thus, understanding the mechanisms that regulate cytokine secretion and viremia is a key priority. The innate immune receptor TLR7 has been identified a retrovirus-sensing protein, and is expressed in several key immune lineages. In vitro data suggests that HIV can trigger TLR7-dependent innate immune responses, but TLR7s role in vivo is unclear.

Methods: To determine whether TLR7 affects viremia or cytokine secretion during acute retroviral infection, we analyzed the plasma of wild type and TLR7 deficient mice infected with the model retrovirus, Friend virus (FV).

Results: We identified 16 cytokines that are significantly upregulated in the plasma of wild-type mice infected with FV, the majority of which are also upregulated during HIV infection. Individual cytokines have distinct kinetic profiles and intensities, with peak levels ranging from 5dpi to 14dpi. To examine the contribution of TLR7 to this response, we compared viral loads and cytokine levels of wild-type or TLR7 deficient mice. Surprisingly, we found that majority of the pro-inflammatory cytokines exhibited exacerbated secretion in the absence of TLR7, while only an early wave of the anti-inflammatory cytokine IL-10 was attenuated. This exacerbated cytokine storm was accompanied by an elevated viremia. Significantly, IL-10 deficient mice also exhibited elevated viremia and cytokine secretion during acute infection. By contrast, TLR7deficient mice exhibit an attenuated antibody response, while anti-viral antibody levels in IL-10 deficient mice were normal.

Conclusion: Our results demonstrate that TLR7 negatively regulates viral loads and cytokine secretion during acute retroviral infection by promoting an early wave of IL-10, and that TLR7 regulates the development of anti-viral antibodies independently of IL-10. These results reveal that TLR7 plays multiple roles in regulating the immune response to retroviral infection.

Topic 7: Innate Immunity

P07.07

Developing a Quantitative Model of Antibody Effector Function

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Background: While the induction of neutralizing antibodies remains a cornerstone of HIV vaccine development, the success of the Thai trail and evidence from non-human primate (NHP) studies has indicated that non-neutralizing antibodies may provide protection from infection. These non-neutralizing antibodies can act as molecular beacons to recruit innate immune cells to recognize infected CD4T cells or free virus as pathogenic. The ability of an antibody to efficiently recruit innate immune cells depends on complex interactions between the pathogenic target, the antibody, and an effector cell.

Methods: Here we present a quantitative model of natural killer cell mediated antibody dependent cellular cytotoxicity (ADCC), and examine the impact of core fucosylation, FcgR3a expression/ occupancy, serum IgG competition and mutations in the Fc binding domain.

Results: The results indicate that FcgR3a site occupancy can predict the percentage of cells killed on an ADCC dose response curve. In addition, it appears ADCC is initiated past a threshold of FgR3a site occupancy on the natural killer cell.

Conclusion: This provides key insight into the mechanism of the innate immune response and provides powerful evidence that the effector function of antibodies should be a key consideration in the development of HIV vaccines.

P07.08

Implications of Post-translational Modifications of IRF7 on pDC IFN-Alpha Response

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Background: We previously showed that plasmacytoid dendritic cells (pDCs) derived from females can produce significantly more IFN-alpha in response to HIV-1 and HIV-1-encoded TLR7/8 ligands than pDCs derived from males, resulting in stronger secondary activation of CD8+ T cells (Meier et al., Nat Med 2009). Given the crucial role of interferon regulatory factor 7 (IRF7) in the regulation of type I IFN production by pDCs, the goal of the current study was to investigate its impact on the observed differences.

Methods: Fresh PBMC were isolated from HIV-1-negative subjects enrolled at Massachusetts General Hospital and stimulated by either CL097 (synthetic TLR7 ligand) or AT-2 inactivated HIV-1. Phosphorylation levels of proteins involved in the TLR7 pathway including IRF7 were measured in pDCs by phospho-flow cytometry at baseline and at different time-points after TLR7 stimulation. The kinetics of IRF7 modifications in the TLR7 pathway were confirmed using mRNA expression of IFN-alpha

Results: Baseline levels of phosphorylated IRF7 were found to be similar between males and females. However we observed faster phosphorylation kinetics of IRF7 in females than in males using flow cytometry with phospho-IRF7 peaking in females at 20min post-stimulation and males at 30min.

Conclusion: These data indicate that sex differences in the kinetics of IRF7 phosphorylation might account for described higher IFN-alpha production upon TLR7 stimulation in females, providing new insights into the mechanisms underlying faster HIV-1 disease progression in females compared to males after controlling for viral load (Farzadegan et al., Lancet 1998).

P07.09

HLA-Cw*0102-Restricted HIV-1 p24 Epitope Variants Can Modulate the Binding of the Inhibitory KIR2DL2 Receptor and Primary NK Cell Function

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Background: Recently, it was shown that Natural Killer (NK) cell-mediated immune pressure can result in the selection of HIV-1 escape mutations contributing to accumulating evidence suggesting that NK cells play an important role in the control of HIV-1 infection. Selection of HLA class I-presented HIV-1 epitopes that allow for engagement of inhibitory killer cell Ig-like receptors (KIRs) might serve as a potential mechanism for NK cell escape. We therefore investigated the consequences of sequence variations within HLA-Cw*0102-restricted epitopes on the interaction with KIR2DL2 using a large panel of HIV-1 p24 Gag peptides.

Methods: A total of 217 decameric peptides spanning HIV-1 p24 Gag and overlapping by 9aa were screened for HLA-Cw*0102 stabilization by co-incubation with Cw*0102(+) TAP-deficient T2 cells using a flow cytometry-based assay. KIR2DL2 binding was assessed using KIR2DL2-Fc. Function of KIR2DL2(+) NK cells was flow cytometrically analyzed by measuring degranulation of primary NK cells after co-incubation with peptide-pulsed T2 cells.

Results: We identified 11 peptides stabilizing HLA-Cw*0102 on the surface of T2 cells. However, only one peptide (p24 Gag209-218) also allowed for binding of KIR2DL2. Notably, functional analysis showed significant inhibition of KIR2DL2(+) NK cell function in the presence of p24 Gag209-218-pulsed T2 cells, while degranulation of KIR2DL2(-) NK cells was not affected. Moreover, we demonstrated that sequence variations in position 7 of this epitope observed frequently in naturally occurring HIV-1 sequences can modulate binding to KIR2DL2.

Conclusion: Our results show that variations in HIV-1 peptides presented by HLA can modulate target cell recognition by NK cells. Understanding the mechanisms that determine NK cell-mediated recognition of HIV-1-infected cells will be a critical step for harnessing NK cell immunity for vaccine design, in particular given the recent discovery of virus-specific memory NK cells in mice (Paust et al., Nat Imm 2009). This study was supported by the NIH, Ragon Institute and HU CFAR.

P07.10

Dendritic Cell Mediated Inhibition of Lentiviral Infection

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Background: Lentiviral entry to quiescent lymphocytes represents a 'time bomb' waiting for cellular activation to spread infection. In order to undergo immune activation T cells interact with dendritic cells presenting peptide:MHC complexes 'sampling' them to look for agonist peptides and receiving survival signals from self peptides. This makes the dendritic cell:T cell interaction an ideal checkpoint to contain lentiviral infection of quiescent lypmhocytes.

Methods: We have used replication defective lentiviral vectors expressing reporter genes and/or HIV proteins to study the innate immune response to lentiviral infection in vitro using primary mouse and human cells and in vivo in B6 mice. We have used flow cytometry and PCR to quantify the infection rates in various culture and adoptive transfer conditions to identify conditions where successful viral infection is inhibited.

Results: Activating T cells exposed to dendritic cells display inhibited lentiviral vector infection compared to activated control cells. This infection reduction is mediated by dendritic cell:T cell contact. Dendritic cell mediated inhibition of infection reduces over time as cells become fully activated and proliferate. When using lentiviral vectors similar results are seen in primary human T cells in culture. However when replication defective vectors expressing HIV proteins are used to infect T cells de novo expression of HIV proteins in the host cell prevents DC mediated inhibition. We have gone on to use knockout mice to assess the role of established innate immune sensing pathways in DC mediated inhibition but have not yet identified the molecular sensor mediating this phenomenon.

Conclusion: The inhibition of productive T cell infection by lentiviral vectors caused by contact with dendritic cells represents a previously undescribed innate immune response which we have called DC mediated inhibition. The successful lentiviral pathogen HIV overcomes DC mediated inhibition enhancing its replication and obscuring this immune response.

Topic 7: Innate Immunity

P07.11

Chronic SIV Infection Induces Differentiation and Accumulation of Cytotoxic CD16+ NK Cells in Lymph Nodes Followed by Transmigration to the Mucosae

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Background: Natural killer (NK) cells inhibit lentiviral replication both directly and indirectly, but substantial evidence also indicates HIV/SIV can induce NK cell dysfunction. NK cells can be subdivided based on expression of CD56 and CD16. In blood, cytotoxic CD16+ NK cells are the dominant subpopulation, while cytokine-secreting CD56+ and double-negative (DN) NK cells are the primary NK cells found in lymph nodes (LN). Furthermore, CD56+ and DN NK are thought to be precursor populations, whereas CD16+ NK cells are terminally differentiated. The effects of HIV/SIV infection on NK cell distribution, trafficking, and development are unclear.

Methods: Macaque NK cells were isolated from blood, LN, and mucosal tissues of naive and chronically SIV-infected animals and then analyzed phenotypically by surface and intracellular flow cytometry, evaluated functionally by ICS and in a direct killing assay against MHC-devoid 721.221 cells. In situ analyses were performed by immunohistochemistry.

Results: In peripheral blood of chronically infected animals, we found a specific expansion of CD16+ NK cells, coupled with high frequencies of cytotoxic perforin+ CD16+ NK cells in LN, where they are normally absent. Interestingly, classic LN-trafficking molecules, CD62L and CCR7, were downregulated to undetectable levels on blood and LN CD16+ NK cells, suggesting they did not migrate from extralymphoid tissues. Furthermore, the putative NK cell precursors, CD56+ and DN NK cells, exhibited increased proliferation and activation, providing a potential source of differentiated CD16+ NK cells. CD16+ NK cells in LN also upregulated the mucosa-trafficking marker, α4β7, correlating with increased frequencies of cytotoxic CD16+ NK cells in colorectal and jejunum tissues of infected animals.

Conclusion: Our data suggest a novel mechanism whereby lentivirus infection induces differentiation of cytotoxic CD16+ NK cells, which are normally absent in LN, to differentiate in situ and then transmigrate to the gut mucosa, the primary site of virus replication.

P07.12

HIV-1 p24 Derived Epitopes Modulate KIR2DL2-Binding to HLA-Cw03

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Background: Recent studies have suggested that HIV-1 can evade Natural Killer (NK)-cell-mediated immunity by mutating viral epitopes to enhance engagement of inhibitory Killer Ig-like receptors (KIRs) expressed on NK cells. However, the precise mechanisms modulating the interaction of inhibitory KIRs and their HLA class I ligands, and the role that HIV-1 epitopes might play in this interaction are not well understood. In this study we investigated whether HLA-Cw3-presented epitopes within HIV-1 p24 Gag can modulate binding of KIR2DL2, an inhibitory KIR, to HLA-Cw03.

Methods: Using tapasin-deficient 721.220 cell line expressing HLA-Cw*0304 we initially screened for HIV-1 peptides that stabilized HLA-Cw*0304 expression using 222 10-mer peptides overlapping by 9 amino acids spanning the entire HIV-1 p24 Gag sequence. Peptides stabalizing HLA-Cw*0304 expression were thereafter investigated for their ability to facilitate binding of a KIR2DL2-IgG fusion construct.

Results: We identified several HIV-1 p24 epitopes that were able to stabilize HLA–Cw*0304 expression. A subset of these epitopes also allowed for binding of KIR2DL2. Currently we are investigating the consequences of KIR2DL2-binding to HLA class I presented HIV-1 epitopes for the antiviral function of primary NK cells from KIR2DL2+ individuals.

Conclusion: Taken together, these studies have identified epitopes within HIV-1 that enhance the binding of the inhibitory NK cell receptor KIR2DL2 to its ligand HLA-Cw3, and are in line with recent data suggesting that the sequence of the HLA class I presented epitope has an important impact on the interaction between KIR and HLA class I (Boyington et al. Nature 2000, Vivian et al. Nature 2011).

P07.13

Early Immune Events During Acute HIV Infection

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Background: We characterized plasma cytokine and chemokine profiles at baseline and during acute infection in a prospective high-risk cohort (RV217). PBMC collected in parallel allow for the longitudinal analysis of changes in immune cell populations during acute infection. These data can provide insight into the earliest events in host-HIV interaction and inform HIV vaccine development.

Methods: Semiweekly viral load (VL) testing in individuals at high-risk for HIV acquisition was performed to prospectively identify very early acute HIV infections (Fiebig stage I/II, NAT+/Ab-). Cytokines were assayed in plasma from pre-infection through early plasma viral load set point using the Q-Plex Multiplex Array or by traditional ELISA. Peripheral blood cells were longitudinally analyzed with multiparameter flow cytometry.

Results: African participants were all female and acquired subtype A, C and D recombinant HIV while participants in Thailand were male and acquired HIV subtype E predominantly. Longitudinal plasma samples from acutely infected individuals (n=24) showed similar trends in cytokine profiles with statistically significant increases in expression over time. VL setpoint, IL-10 and MCP-1 expression differed by region. Preliminary PBMC analysis revealed frequency and phenotypic changes in all antigen presenting cell populations. Significant decreases in plasmacytoid dendritic cells were observed at peak VL, which preceded a sharp decline in plasma IFN-α to near baseline values. MCP-1 and IP-10 also rose sharply in conjunction with fluctuations in T cell subsets and APCs. These early changes could impact adaptive cellular and humoral immune responses.

Conclusion: Understanding cytokine kinetics and VL dynamics during acute HIV infection may help identify key controls of early viral set point. Regional differences may reflect HIV subtype, gender, background cytokine expression levels and co-morbidities as these covaried. Analyses of correlations with immune cell phenotype/function and viral set point are underway. Efforts focus on examination of the evolution of the innate response after infection, prior to peak viremia.

P07.14 LB

Modulation of HIV-1 Replication In Primary Dendritic Cells In Contact With Autologous CD4 T-Lymphocytes

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Background: Immature dendritic cells (DCs), which reside in genital mucosal surfaces, are among the first cells that encounter HIV-1. These cells poorly replicate R5-tropic HIV-1, but have been shown to efficiently transfer the virus to autologous CD4 T-lymphocytes. We have shown that HIV-1 replication was enhanced in primary HIV-1-loaded DCs when they are in close contact with autologous CD4 T-lymphocytes. Recently, SAMHD1 has been proposed to restrict HIV-1 replication in myeloid cells by decreasing deoxynucleoside triphosphate (dNTP) pools. We aimed to decipher whether SAMHD1 was involved in the increased HIV-1 replication observed in DCs coculture with CD4 T-lymphocytes.

Methods: DCs were generated from human blood $CD14^+$ monocytes. After 2 hours of incubation with HIV-1, DCs were added to autologous primary CD4 T-lymphocytes. The percentage of infection was quantified by flow cytometry (intracellular p24 staining). The expression of intracellular SAMHD1 was measured by flow cytometry and by western blot. Virus-like particles containing Vpx (VLP-Vpx) was used as control to decrease SAMHD1 expression.

Results: HIV-1 replication was enhanced in DCs cocultured with autologous CD4 T-lymphocytes compared to DCs alone. Slightly lower levels of SAMHD1 expression were detected in infected cocultured DCs by flow cytometry. Moreover, addition of exogenous dNTPs to the culture significantly increased HIV-1 replication in DCs. As control, VLP-Vpx enhanced viral replication and decreased SAMHD1 expression in DCs.

Conclusion: Preliminary results suggest that the stimulation of HIV-1 replication in DCs observed in coculture conditions was partially associated to the concomitant decrease of SAMHD1 expression in DCs. As HIV-1 replication was inhibited by Abs in DCs, further investigations are required to determine if SAMHD1 modulates HIV-1 replication in the presence of these Abs. Cross-links between the restriction factors and humoral immune response should be considered in the development of an effective anti-HIV-1 vaccine.

Topic 7: Innate Immunity

P07.15 LB

Apoptotic Microparticles Generated During Acute HIV-1 Infection Inhibit Human Dendritic Cells Via CD44

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Background: Acute human immunodeficiency virus type 1 (HIV-1) infection results in dysregulated immunity which contributes to poor control of viral infection. Dendritic cells (DCs) are key regulators of both adaptive and innate immune responses needed for controlling HIV-1 and we surmised that plasma factors elicited during acute HIV-1 infection (AHI) may impede DC function. Such inhibitory factors present in AHI plasma include apoptotic microparticles (MPs), small membranous blebs derived from dying cells.

Methods: Plasma samples over sequential time points were obtained from AHI patients or healthy controls. Apoptotic MPs were isolated from supernatant of UV-irradiated PBMCs, AHI patient plasma or control plasma. Human DCs were treated with MPs or 10% plasma (control or AHI) and subsequently stimulated with various TLR agonists. DC activation was then assessed. MP-specific receptors were isolated from the DC surface and sequenced by mass spectrometry.

Results: AHI plasma inhibited TLR-stimulated DC cytokine production. The inhibitory capacity of AHI plasma occurs at time of viral ramp-up, whereas plasma at times before plasma viremia is not inhibitory. We determined this inhibition was not mediated by virus. Because apoptotic MPs are elevated in AHI plasma, we treated DCs with experimental and AHI plasma-derived MPs, both of which reduced DC activation. The inhibition of DCs by AHI plasma or MPs blocked DC capacity to prime IFNg-producing T helper 1 CD4+ T cells as well as NK cell activation. Mass spectrometric analysis revealed CD44 a MP receptor, and blocking CD44 on DCs relieves MP-mediated suppression. Direct ligation of CD44 also inhibits DC activation. MP-CD44 interaction activates Rac1, c-Abl, and AKt signaling.

Conclusion: Determining the factors in AHI which block DC function will provide potential targets to stimulate HIV-specific immunity. We are currently investigating molecular mechanisms of CD44-mediated DC inhibition and downstream signaling events that can be targeted to alleviate DC inhibition.

P07.16 LB

HIV Triggers Immunoregulatory Dendritic Cells And Regulatory T Cells Through The Non-Canonical NF-kB Pathway

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Background: HIV stimulates plasmacytoid dendritic cell (pDC) through TLR7 and induce the secretion of high levels of IFNα. pDC stimulated by HIV also upregulate the expression of the enzyme indoleamine 2,3 dioxygenase (IDO). IDO is critical for the induction of regulatory T cells (Treg) by HIV-activated pDC. We investigated the molecular mechanisms of IDO induction and its consequences for Treg function.

Methods: The cells used were purified primary pDC and the GEN pDC cell line. A combination of siRNA knock-down, immunoprecipitation of TLR signaling pathway molecules, IDO promoter engineering and chromatin immunoprecipitation was used to determine the molecular mechanisms of IDO induction in pDC. To analyze Treg function and interaction with conventional DC (cDC), blocking antibodies to CTLA- 4 and CTLA-4-Ig were used.

Results: We demonstrate that HIV induces activation of the non-canonical NF-kB pathway in pDC, and is essential for IDO induction. TLR7 triggering induces recruitment of TRAF3 to the TLR-MyD88 complex, followed by release of NIK and phosphorylation of IKKα. Activation of the non-canonical NF-κB pathway culminates in p52/RelB nuclear translocation and binding to the IDO promoter.

Furthermore, IDO-expressing pDC trigger the generation of Treg, which dampen cDC activation through CTLA-4. CTLA-4 also induces IDO expression in cDC in a NIK-dependent fashion, allowing cDC to induce Treg from naïve CD4+ T cells.

Conclusion: The non-canonical NF-kB pathway plays a central role in regulating IDO expression in pDC and cDC upon HIV infection, and may be a potential target for regulating Treg activity in chronic or acute HIV infection.

P07.17 LB

S100A9 Protein is a Novel Ligand for the Receptor CD85j and Their Interaction is Implicated in the NK cell-mediated control of HIV-1 Replication

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Background: CD85j is a receptor expressed by different cells of the human immune system including Natural Killer (NK) cells. Previous reports have shown that CD85j interacts with several MHC-I molecules, as well as with some viral proteins (Cosman D et al., 1997). We have also demonstrated that CD85j$^+$ NK cells efficiently control HIV-1 replication in monocyte-derived dendritic cells (MDDC) in vitro (Scott-Algara D et al., 2008). We hypothesize that the CD85j$^+$ NK cell-mediated anti-HIV activity in MDDC is specifically dependent on the interaction between CD85j receptor and unknown non-HLA class I ligand(s). Therefore we focused on the identification CD85j ligand(s) and its(their) implication in the control of HIV-1 infection.

Methods: To identify the CD85j ligand(s), lysates from MDDC infected or not by HIV-1 were co-immunoprecipitated using CD85j recombinant receptor and analyzed by SELDI-TOF-MS protein chip arrays. The interaction between CD85j and its ligand(s) where then confirmed by ELISA test. Surface expression of the putative ligand(s) was analyzed by flow cytometry. To confirm the implication of the interaction receptor-ligand in the control of HIV replication, NK cells were pre-stimulated with CD85j ligands and co-cultured with HIV-infected MDDC or CD4$^+$ T cells, then, intracellular and supernatant p24 were measured.

Results: We found that the CD85j receptor interacts with the calcium-binding protein S100A9. We further demonstrated that HIV-1 infection of MDDC modulates the expression of S100 proteins at the surface of MDDC. Pre-stimulation of NK cells with S100A9 monomers resulted in an increased control of HIV infection in MDDC and CD4$^+$ T cells. Moreover, pre-stimulation of NK cells with S100A9 tetramers resulted in a better and increased control of HIV-1 infection in CD4$^+$ T cells.

Conclusion: Triggering the inhibitory receptor CD85j on NK cells by S100A9 may be implicated in the establishment and/or the regulation of the specific anti-HIV-1 NK cell response.

P07.18 LB

Are the KIR3DS1homozygous and KIR3DL1*h/*y+HLA-B*57 Genotypes Associated Protection From HIV by Different Routes of Exposure?

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Background: We previously reported that HIV Exposed Seronegative (HESN) individuals have a higher frequency than HIV infected subjects of 2 genotypes: homozygosity for Killer Immunoglobulin-like Receptor (KIR) 3DS1 (KIR3DS1hmz) and homozygosity for high expression KIR3DL1 genotypes co-expressed with HLA-B*57 (*h/*y+B*57). KIR3DL1/S1 are Natural Killer (NK) cell receptors that influence NK functionality. Here, we assessed whether these genotypes were associated with protection by parenteral and mucosal routes of HIV exposure.

Methods: 473 Caucasian individuals were typed for HLA, KIR3DL1/S1 generic genotypes and KIR3DL1 allotypes. Of 88 HESN, n=69 were injection drug users (IDU) and 19 were sexually exposed (sHESN). Of 385 HIV seropositive subjects n=108 were IDU and n=277 were infected through sexual exposure. The frequency of KIR3DS1hmz and *h/*y+B*57 carriers was compared in HESN versus HIV susceptible subjects exposed through parenteral versus mucosal routes.

Results: KIR3DS1hmz were more frequent among HESN than HIV positive IDU (10% versus 2.7%, respectively, p=0.05). This genotype was also more frequent among HESN than HIV infected individuals exposed sexually (25% versus 5.7%, respectively, p<0.01). The *h/*y+B*57 genotype was more frequent among HESN than HIV positive IDU (7.2 vs. 0%, respectively, p<0.01). This genotype was not observed among any sHESN and was detected in 1.8% of mucosally HIV infected individuals (p=not significant).

Conclusion: The protective HESN KIR3DS1hmz genotype is associated with protection from HIV infection by both mucosal and parenteral routes. *h/*y+B*57 carriers are more frequent among IDU HESN than HIV susceptible subjects suggesting a protective effect via exposure by this route. Although there is no evidence that the *h/*y+B*57 genotype is protective at the level of sexual exposure the small number of sHESN precludes making firm conclusions on this point. Carriage of both these genotypes is linked to potency of NK cell function, which may influence early innate responses to HIV.

P07.19 LB

KIR/HLA Genotype Combinations Are Determinants Of Natural Killer (NK) Cell Mediated Antibody-Dependent Cellular Cytotoxicity (ADCC) Potency

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Background: Several allelic variants of the killer immunoglobulin-like receptor 3DL1 (KIR3DL1) in combination with their HLA-Bw4 ligands, are associated with protection from HIV infection and/or time to AIDS. The HLA-B*57+KIR3DL1*h/*y genotype combination (KIR3DL1*h/*y encodes high expression receptors) has the most potent effect on slowing time to AIDS in HIV infected individuals. Although this HLA/KIR combination confers natural killer (NK) cells with high functional potential, the mechanism of the AIDS protective effect remains unknown. Indeed, NK cells expressing 3DL1 fail to degranulate upon exposure to autologous HIV-infected CD4+ T-lymphocytes. As HLA-Bw4/KIR3DL1 combinations also endow NK cells with antibody-dependent cellular cytotoxicity (ADCC) functional potential, we hypothesized that NK cells from individuals carrying HLA-B*57+3DL1*h/*y mediate more potent ADCC than NK cells from individuals with less protective HLA/KIR combinations.

Methods: We compared the ability of NK cells from 39 HIV-uninfected individuals carrying HLA-B*57+3DL1*h/*y (n=11), other HLA-Bw4+3DL1 combinations (n=18), or 3DL1 in the absence of HLA-Bw4 (n=10) to mediate anti-HIV ADCC. Anti-HIV ADCC was measured as the ability of NK cells to deliver granzyme B to HIV gp120-coated CEM.NKr.CCR5 cells (i.e. percent of granzyme B positive cells) in the presence of a common source of anti-HIV antibodies. This GranToxiLux assay efficiently measures ADCC, as target cells do not receive granzyme B in the presence of antibodies from seronegative individuals or F(ab')2 fragments of anti-HIV antibodies.

Results: NK cells from HLA-B*57+3DL1*h/*y carriers mediate more potent ADCC (13.4+/-2.61%) than those from individuals carrying other HLA-Bw4+3DL1 combinations (5.15+/-1.54%) or 3DL1 in the absence of HLA-Bw4 (2.78+/-1.48%) ($p=0.002$, Kruskal-Wallis test, $p<0.05$ and $p<0.01$ respectively, Dunn's Multiple Comparisons test).

Conclusion: Therefore, HLA/KIR dependent NK cell "education" is a determinant of ADCC functional potential, and should be taken into consideration when evaluating ADCC as a correlate of disease progression or vaccine efficacy.

Topic 8: Mucosal Immunity

P08.01

Natural Killer Cells Present in Gut Mucosa as Potential ADCC Effector Cells

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Background: Data from the RV144 HIV vaccine trial showed a moderate protection from HIV infection in the absence of neutralizing antibodies or CTL activity. In contrast, all vaccinees mounted robust HIV-specific binding antibodies, that may have provided some level of protection through their capacity to recruit antibody dependent cellular cytotoxicity (ADCC). The capacity of an antibody to recruit ADCC relies on its ability to interact with the Fc-receptor, FCγRIII (CD16), expressed on Natural Killer (NK) cells. However, little is known about innate immune effector cells present within mucosa, and whether they have the capacity to be harnessed by ADCC inducing antibodies should they be elicited by a vaccine. Here we hypothesized that abundant numbers of CD16+ NK cells line the gut mucosa, providing a robust effector arm that could be harnessed by ADCC inducing antibodies.

Methods: The frequency and function of CD16+ cells in the colon was assessed by flow cytometry following enzymatic digestion of intestinal resections from HIV-uninfected subjects.

Results: Significantly fewer NK cells were found in colon resections compared to peripheral blood of healthy controls (median: 10.40% vs. 18.80%, p=0.0062, respectively). Furthermore, while both $CD56^{bright}$ immunoregulatory and $CD56^{dim}$ cytolytic NK cells able to mediate ADCC were present in the gut, the frequency of these 2 subsets was altered compared to the blood. Moreover, fewer NK cells expressed NKp46, NKG2A, KIR, CD8, perforin, and importantly CD16 in the gut compared to the blood. However, gut NK cells demonstrated similar to blood cytolytic activity upon stimulation.

Conclusion: Taken together, these data suggest that ADCC inducing antibodies present within the gut mucosa may likely recruit the antiviral activities of NK cells. Greater emphasis should be placed on developing innate immune recruiting antibody assays that measure the capacity of antibodies to recruit the antiviral activity of innate immune cells present within mucosal membranes where transmission occurs.

P08.02

Mucosal Prime with a Replicating Vaccinia-Based Vaccine Promotes Mucosal Immunity Against SIV

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Background: We previously demonstrated that vaccine prime with a recombinant replication-competent modified vaccinia Tiantan (rMVTTSIVgpe) was able to enhance the boost effects of a rAd5SIVgpe for eliciting protective immunity against SIV mucosal challenge in rhesus macaques. Whether this heterologous prime and boost regimen is able to elicit potent mucosal immunity specific to SIV remains less understood.

Methods: Different groups of mice were immunized with the following regiments: rMVTTSIVgpe-rAd5SIVgpe, rAd5SIVgpe-rMVTTSIVgpe and rAd5SIVgpe-rAd5SIVgpe. rMVTTSIVgpe was administrated through intraoral and intranasal routes (ioin) routes whereas rAd5SIVgpe was given through the intramuscular injection (im).

Results: Consistent with previous findings in macaques, mice immunized with the rMVTTSIVgpe-rAd5SIVgpe regimen generated significantly stronger systemic cellular immune responses as well as serum antibody responses than any other vaccine regimens. Furthermore, as compared with other groups, this rMVTTSIVgpe-rAd5SIVgpe regimen induced significantly higher frequencies of gut-homing CCR9+ Gag-specific CD8+ T cells as well as CCR6+ Gag-specific CD4+ and CD8+ T cells. This regimen also elicited the highest level of CD8+ T cell ELIspot responses against Gag, Pol and Env antigens in mesenteric lymph nodes (mLN). Besides, SIV-specific IgGs could be detected in the rectal wash of mice received rMVTTSIVgpe-rAd5SIVgpe immunization with detectable neutralizing activity.

Conclusion: These findings demonstrated that mucosal priming with rMVTTSIVgpe significantly promoted mucosal immunity against SIV, which may have implications to the effectiveness of the mucosal rMVTTSIVgpe prime-systemic rAd5SIVgpe boost vacciniation strategy in preventing mucosal infection of SIVmac239 in macaques.

Topic 8: Mucosal Immunity

P08.03

Increased Epithelial Thickness and Reduced HIV Receptor Expression in the Ectocervical Mucosa Is Associated with Relative HIV Resistance

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Background: The female genital tract is an important site of HIV acquisition, but the epithelial and submucosal tissue factors associated with HIV susceptibility have not been defined.

Methods: Ectocervical biopsies were obtained from HIV-exposed seronegative (HESN) women (n=20) and HIV-seronegative lower risk controls (n=20). Epithelial thickness and tissue distribution of immunological markers were assessed in situ by immunohistochemistry and measurement of mRNA expression was performed by quantitative PCR.

Results: The thickness of the ectocervical epithelium was significantly higher in HESN vs. lower risk subjects. CD4 and DC-SIGN mRNA expression was significantly lower in HESN than lower risk women, and in situ immunohistochemical analysis confirmed the reduced CD4 expression in HESN participants. In addition, immunohistochemistry demonstrated lower CCR5 and higher Langerin expression in the HESN subjects.

Conclusion: A thicker epithelial barrier and altered expression of HIV binding receptors in the ectocervix of HESN women may contribute to protection against HIV transmission.

P08.04

Intravaginal Immunization Using a Novel Antigen Delivery Device Elicits Robust Baccine Antigen-Specific Systemic and Mucosal Humoral Immune Responses

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Background: While it is relatively easy to elicit antigen-specific serum antibody it is much more difficult to establish meaningful levels of specific antibody at mucosal surfaces, the major route of viral invasion. We sought to determine if mucosal vaccination using topical vaginal application could initiate local antigen-specific immunity, enhance previously existing systemic immunity or re-target responses to the mucosae.

Methods: We used a silicone elastomer ring device to deliver a protein vaccine formulation to the vaginal mucosal surface. Cylindrical rod-shaped inserts (2 x 7mm) were prepared by freeze-drying an aqueous hydroxypropylmethylcellulose (HPMC) gel containing recombinant CN54gp140 (500µg) with and without the TLR7/8 agonist R848 (resiquimod – 500µg). Inserts were loaded into cavities within each ring such that only the ends of the inserts were exposed. Sheep received an intramuscular injection of 100µg HIVgp140 + 200µg R848 followed by three successive ring applications of one week duration, separated by one month intervals. Other sheep received only the ring devices without priming. Serum and vaginal mucosal fluids were sampled every two weeks and analysed by CN54gp140 ELISA. Antigen-specific cellular responses were determined at necropsy.

Results: Vaccine antigen-specific serum antibody responses were detected in both the intramuscularly primed and vaginal mucosally-primed groups. Those animals that received only vaginal vaccinations had identical IgG but superior IgA responses. Analysis revealed that all animals exhibited mucosal antigen-specific IgG and IgA with the IgA responses 30-fold greater than systemic levels. Surprisingly, very high numbers of antigen-specific B cells were detected in local genital draining lymph nodes.

Conclusion: We have elicited local genital cellular and humoral immune responses after topical application of an adjuvanted antigen formulation within a novel vaginal ring vaccine delivery device. This regimen and delivery method elicited high levels of antigen-specific mucosal IgA and large numbers of local antigen-reactive B cells, both likely essential for effective mucosal protection.

P08.05

Role of Novel Type I Interferon Epsilon in Mucosal Immunity

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Background: Newly discovered type I interferon-epsilon (IFN-ε) is found to be constitutively expressed in mucosal tissues, i.e lung, reproductive tissue and intestine. Our previous studies have postulated that IFN-ε could play a role in modulating mucosal immunity. As HIV is a disease of the mucosae, we further evaluated the immuno-biology of IFN-ε in the mucosae and tested whether IFN-ε could be used as a mucosal adjuvant to enhance HIV-specific immunity.

Methods: Poxvirus (Vaccinia Virus and Fowl poxvirus) co-expressing HIV-1 gag/pol and interferon epsion (VV-HIV-IFN-ε or FPV-HIV-IFN-ε) were used in this study to evaluate immuno-biology and adjuvant activity of IFN-ε

Results: Firstly, VV-HIV-IFN-ε was utilized to study the immuno-biology of IFN-ε compared to IFN-α4 or IFN-β. Following intranasal (i.n.) VV-HIV-IFN-ε infection, a rapid VV clearance in lung was induced that correlated with 1) an elevated lung VV-specific CD8+CD107a+IFN-γ+, 2) up-regulated activation markers CD69/CD103 on CD8 T cells, 3) enhanced lymphocyte recruitment to lung alveoli with reduced inflammation and 4) heightened functional/cytotoxic CD8+CD4+ T cell subset (CD3hiCCR7hiCD62Llo) in lung lymph nodes. These responses were different to that observed following i.n. VV-HA-IFN-α4 or VV-HA-IFN-β infections. Secondly, intranasal/intramuscular (i.n./i.m.) heterologous prime-boost immunization (FPV-HIV-IFN-ε/VV-HIV-IFN-ε) was used to evaluated adjuvant activity of IFN-ε. Data indicated that IFN-ε induced elevated HIV-specific effector but not memory CD8 T cells responses in spleen, genito-rectal nodes and Peyer's patch compared to the control (i.n. FPV-HIV/i.m. VV-HIV). Interestingly, unlike IFN-β and IFN-α4, IFN-ε uniquely induce elevated frequency of α4β7 and CCR9 expressing HIV-specific CD8 T cells in gut mucosae.

Conclusion: In conclusion, our data indicated that 1) IFN-ε can induced excellent T cell response in the mucosae especially lung and gut, and 2) rather than an vaccine adjuvant IFN-ε has the potential to be used as an anti-microbicide to prevent or reduced mucosal infection such as TB or HIV.

P08.06

Impact of the Innate Environment on Maintaining Memory T-Cell Numbers in the Female Genital Tract: Implications for Mucosal Vaccine Efficacy?

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Background: Preventative HIV vaccines aim to elicit long-lived protective immune responses at the site of HIV transmission, capable of responding quickly to HIV challenge, but which remain stable at effector sites of the genital mucosa. The genital mucosa is, however, commonly confronted with innate immune modifiers and inflammatory agents including sexually-transmitted infections, behavioural and hygiene practices. We investigated the impact of mucosal inflammation and homeostatic cytokines on local T-cell phenotype, proliferation, exhaustion and activation.

Methods: Levels of activation (CD38, HLA-DR), proliferation (Ki67) and senescence (CD57) were measured on T-cells isolated from cervical cytobrushes and blood from 46 HIV-negative women by flow cytometry and inflammatory cytokines and IL-7 in genital secretions were measured by ELISA.

Results: HIV-negative women generally had higher concentrations of inflammatory (IL-1β, IL-6, IL-8) than homeostatic cytokines (IL-15, IL-7) in their genital secretions. Cervical IL-7 correlated positively with inflammatory cytokine concentrations, suggesting that inflammation and homeostatic cytokine production were linked. HIV-negative women with lower cervical $CD4^+$ T-cell frequencies had the highest concentrations of genital IL-7 ($p=0.028$; $rho=-0.23$) suggesting that local IL-7 production increased in response to elevated $CD4^+$ T-cells. In vitro culture of T-cells with IL-7 caused an increase in activation of both $CD4^+$ (CD38 $p=0.002$; HLA-DR $p=0.01$) and $CD8^+$ (CD38 $p=0.01$; HLA-DR $p=0.006$) T-cells compared to cells not stimulated with IL-7. IL-7 also caused an increased Ki67 expression by $CD4^+$ T-cells ($p=0.04$).

Conclusion: In conclusion, local IL-7 in the presence of genital inflammation may favour T-cell activation and turnover of vaccine-induced responses in the genital tract.

Topic 8: Mucosal Immunity

P08.07

Induction of HIV Mucosal Immunity at Distal Sites After Encapsulation of NOD1 and NOD2 Ligands in Biodegradable Nanocarriers

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Background: The use of TLR ligands as mucosal adjuvant for vaccine administration is already largely described; wherease the use of NOD-like receptors ligands is still investigated. As activation of intracytoplasmic NOD like-receptors is able to induce production of pro-inflammatory molecules, we have evaluated if their co-delivery into biodegradable nanocarriers carrying HIV-Gag antigens could amplify the mucosal immune responses in mice at the vaginal and intestinal (HIV replication site)

Methods: We used Poly(Lactic Acid) (PLA) nanoparticles (NPs) (~200 nm) for co-delivery of p24 and NOD ligands. As NOD like-receptors are mainly expressed by antigen presenting cells, we first assessed the capacity of free or encapsulated ligands to induce monocyte derived dendritic cells (MoDCs) maturation. Then, as NOD like-receptors are principally expressed at the intestinal level we compared by oral immunization of BALB/c mice encapsulated ligands co-delivered with PLA-p24 NPs. To assess the adjuvant effect, p24-specific cellular and humoral responses were analyzed on splenocytes and in vaginal washes, faeces and sera.

Results: The state of MoDCs maturation was characterized by the expression of CD80, CD83 and CD86. We showed that encapsulation of NOD1 or NOD2 ligand increases significantly their expression, compared to the effect of free ligands, probably due to a better uptake of encapsulated ligands

By analyzing humoral immune responses, we observed that co-administration of p24 and NOD2 ligand by two different NPs was the most efficient formulation to induce anti-p24 IgG and IgA responses in faeces. By contrast co-formulation of p24 and NOD1 ligand in the same NP induced a better CD8 IFNγ response.

Conclusion: Encapsulation of NOD ligands into PLA nanoparticles seems to favour their action on DCs maturation, their co-administration with PLA-p24 NPs inducing an adjuvant effect. Use of those ligands as mucosal adjuvant deserve further experiments and we are investigating the mechanisms involved and increasing delivery efficacy after co-encapsulations.

P08.08

Anti-gp41 Antibodies Inhibit Infection and Transcytosis of HIV-1 Infectious Molecular Clones Expressing Transmitted/Founder Envelopes

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Background: Prophylactic vaccine strategies against HIV-1 must effectively prevent virus transmission, infection and cell-to-cell spread during the earliest stages of acute infection. Since the genital mucosa is the primary site of entry, mucosal defense is critical for early control of infection. Recent identification of transmitted/founder (T/F) HIV-1 genomes has demonstrated a consistent genetic bottleneck during mucosal transmission and suggests that T/F viruses may exhibit distinct phenotypes. HIV-1 Env gp41-specific responses are among the first to be generated in natural HIV infection.

Methods: Previously, we developed chimeric virus-like particle (VLP) immunogens that elicit potent systemic and mucosal antibodies against two highly conserved regions of gp41, by employing an optimized immunization strategy. The ELDKWA and QARVLAVERY epitopes are found with the membrane proximal external region (MPER) and the coiled coil region of gp41, respectively. Importantly, the epitope-specific IgG and IgA fractions derived from immunized mice were shown to be effective in neutralizing and preventing transcytosis of HIV in vitro. In particular, the QARVLAVERY epitope is remarkably conserved and induced unusually high and early levels of anti-QARV IgA, making it an attractive candidate for generation of broadly reactive mucosal antibodies.

Results: In this study, we assessed the effectiveness of mucosal and systemic mouse antibodies elicited against these gp41 epitopes to inhibit T/F Env function. For this, we employed recombinant infectious molecular clones (Env-IMC) of HIV-1 that encode mucosally transmitted/founder env genes. Our results show that the gp41-specific IgG and IgA fractions effectively prevented the infection of TZM-bl cells and inhibited HIV transcytosis in an assay measuring the passage of infectious virus across an epithelial monolayer. Interestingly, the T/F Env-IMC tested were more sensitive to the antibodies than the R5 lab-adapted strains included as controls.

Conclusion: These results highlight the potential of gp41-based immunogens to impart effective mucosal protection in the earliest stages of HIV transmission and infection.

P08.09

Systemic Administration of a Broadly-Neutralizing IgG Antibody to Generate HIV-Neutralization Responses in Breast Milk

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Background: Postnatal acquisition of HIV during breastfeeding is responsible for almost half of 350,000 pediatric HIV infections occurring yearly. Thus, there is an urgent need to develop immunologic interventions to impede breast milk transmission of HIV, including immunization and passive infusion strategies. We previously observed that functional antibody responses in milk of HIV- infected mothers mirror that in plasma, suggesting that inducing strong systemic IgG responses may lead to virus inhibition in milk. Therefore, we investigated the kinetics of binding and neutralizing antibodies in plasma and milk of passively- infused lactating rhesus monkeys.

Methods: The broadly neutralizing antibody b12 engineered in a rhesus IgG1 backbone was administered intravenously to four hormone-induced, lactating female rhesus monkeys at a dose of 5mg/kg. Milk and blood was collected frequently until 72 h post infusion, then weekly for 4 weeks. Levels of the infused antibody and the neutralizing activity in the milk and systemic compartments were measured at each time-point.

Results: The b12 IgG levels peaked 1 hour post-infusion in plasma and 24 to 72h post-infusion in milk. The median peak b12 antibody levels were 87,503 ng/ml (range 62,548 to 101,525 ng/ml) in plasma and 47 ng/ml (range 16 to 202 ng/ml) in milk. The peak in plasma neutralization was 1 to 6 hours post-infusion and the neutralization titer slowly declined after 24 hours. The peak neutralization titer in milk (median ID50: 70, range: 50-103) was approximately two logs lower than in plasma (median ID50: 2313, range: 1875-3128) and occurred within 24 hours post-infusion in 3 of 4 animals. There was a significant correlation between neutralization titers in milk and plasma (r=0.48, p=0.01).

Conclusion: The neutralizing activity detected in milk following systemic administration of a broadly- neutralizing IgG antibody supports the induction of strong systemic anti-HIV IgG responses to generate HIV inhibitory antibodies in breast milk.

P08.10

The Humanized BLT Mouse to Study HIV Transmission

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Background: Worldwide, the majority of HIV-1 infections are acquired by vaginal transmission. Studies in SIV-1 infected non-human primates have shown that SIV-1 infection takes hold initially in a small population of CCR5+ cells in the in female lower genital tract (FLGT) where the infection expands first locally before disseminating to the draining lymph node (LN) to establish a systemic infection. Our goal is to use humanized BLT mice to address whether the infection paradigm established for SIV-1 in non-human primates holds true during HIV-1 infection in vivo.

Methods: We studied the kinetics of infection during the first two weeks after intravaginal HIV-1 exposure by measuring the presence of virus in the FLGT, LNs and blood after 2, 6, 10 and 12 days post infection (p.i.) by qPCR and flow cytometry.

Results: Our results show that similar to the non-human primate model, the presence of virus is first detected by qRT-PCR in the FLGT as soon as day 2 p.i., followed by the LN at day 6 p.i. and the blood at day 12 p.i.. Similar but delayed kinetics were observed using p24 staining by flow cytometry, with positive staining of T cells located in the FLGT at day 6 p.i., in the draining LN between day 6 and day 10, and in the non draining LN at day 12 p.i..

Conclusion: Our data suggests that HIV-1 transmission and initial replication in BLT mice following intravaginal exposure occurs first locally in the LGT and then disseminates to the draining LN. The virus then spreads to the non-draining LNs and subsequently into the blood, suggesting that BLT mice have an “eclipse phase” following HIV infection similar to what have been described for SIV infection of macaques and HIV infection of humans.

Topic 8: Mucosal Immunity

P08.11

Strong SIV gp120-Specific IgG/IgA Responses in Milk of African Green Monkeys May Contribute to the Rarity of Postnatal Transmission in This Species

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Background: African green monkeys (AGMs), natural SIV hosts, sustain nonpathogenic infections and rarely transmit the virus to their suckling infants, despite exposure to high milk virus RNA loads. Furthermore, we previously reported strong autologous neutralization responses in milk of SIV-infected AGMs which could contribute to impediment of infant virus acquisition. Comparing mucosal B cell populations and responses in milk of AGMs to that of rhesus monkeys (RMs), symptomatic SIV hosts with high rates of postnatal transmission, could elucidate the protection against postnatal virus transmission in natural SIV hosts.

Methods: Six female AGMs and four female RMs were hormonally-induced into lactation prior to intravenous inoculation with SIVsab92018 and SIVmac251, respectively. B cells in milk and blood were phenotypically analyzed by flow cytometry. Total and SIV gp120-specific IgG/IgA responses in milk and plasma were measured using autologous virus-specific ELISA.

Results: SIV gp120-specific IgG responses were approximately one log higher in milk (p=0.02) and plasma (p=0.009) of AGMs compared to that of RMs. Remarkably, the milk SIV gp120-specific IgA response of AGMs was two logs higher than that of RMs (p=0.009). Comparing the milk SIV gp120-specific IgA responses to other mucosal compartments of AGMs, milk responses were higher than rectal (p = 0.03), but similar to vaginal responses. Although there were no significant differences in the milk memory B cells populations of AGMs and RMs, we observed a reduced proportion and absolute number of naive B cells (CD20+, IgD+, CD27-) in milk of RMs (median = 7.9%, 0.16 cells/µl) compared to AGMs (median = 26.5%, 4.2 cells/µl) (p = 0.009 and 0.06, respectively) during chronic infection.

Conclusion: AGMs appear to preserve SIV-specific IgG/IgA responses in milk during chronic infection, potentially due to a lack of immune activation and B cell dysfunction, which may contribute to the rarity of postnatal SIV transmission in this natural host species.

P08.12

Semen Modulates the Differentiation of Monocyte-Derived Dendritic Cells Towards a Tolerogenic Profile

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Background: HIV vaccines have not been able to provide immune protection against sexually-transmitted HIV. We hypothesized that semen has an active role in the transmission of HIV by influencing the early events of the immune response. This study analyzed the ability of spermatozoa and seminal plasma (SP) to modulate in vitro the differentiation profile of human monocyte-derived dendritic cells (DC).

Methods: Spermatozoa were purified by swim-up of semen samples from healthy donors, while SP was obtained by semen centrifugation. DC were obtained by incubating peripheral blood monocytes (>85% purity) with GM-CSF and IL-4 for 5 days, in the absence or presence of spermatozoa (Sp: monocyte ratio 4:1) or SP (dilution 1:5000). DC phenotype was analyzed by flow cytometry while cytokine production was measured by ELISA.

Results: Differentiation of DC performed in the presence of spermatozoa or SP resulted in a marked reduction of CD1a expreession together with increased expression of CD14. The mean fluorescence intensity for CD1a was: 7464 ± 106, 210 ± 25 and 766 ± 35 ($p<0.05$ for controls vs spermatozoa- or SP-treated cells, respectively). We also found that spermatozoa and SP significantly ($p < 0.05$) increased expression of HLA-DR, CD86 and CD80. However, stimulation of treated DC with LPS resulted in reduced production of IL-12p70 or IL-23, but increased secretion of IL-10 ($p < 0.01$). Moreover, DC differentiated in the presence of SP or spermatozoa induced the expansion of CD25+FOXP3+ T lymphocytes: 5±1% vs 12 ±4% and 9 ±2% CD25+FOXP3+ cells ($p<0.05$, control vs SP- or spermatozoa-treated cells respectively). Finally, the effect of SP on DC phenotype and IL-12p70 production was partially abrogated by antgonizing prostaglandin-E2 signalling, suggesting a role of prostaglandins as mediators of semen effects.

Conclusion: Our data support the notion that semen may alter the anti-HIV immune response by inducing a tolerogenic profile in dendritic cells.

P08.13

γδ T-Cells in HIV Infection

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Background: γδ T-cells represent a first line of defense against pathogens in the mucosa. Despite their prevalence in gut associated lymphoid tissue (GALT), little is known about their role in HIV infection. We hypothesize that γδ T-cells are stimulated by viral antigen and demonstrate anti-HIV activity, comprising a critical component of the mucosal response to HIV.

Methods: To assess the role of γδ T-cells, we analyzed peripheral blood and GALT samples from HIV(-) and HIV(+) patients, including elite controllers. γδ T-cells were isolated and assessed in viral inhibition and CD4+ killing assays. The cellular pathway associated with cell killing was also evaluated. An HIV antigen screen was used to stimulate sorted γδ T-cells. Nanostring analysis was used to measure mRNA. High-throughput TCR sequencing was performed in peripheral and mucosal tissue.

Results: The mucosal subtype, Vδ1, exists at higher percentages in HIV(+) peripheral blood, particularly elite controllers (17.1±4.0), relative to HIV(-) subjects (0.3±0.2) (p=0.0001). A 100-fold increase of the Vδ1 subtype was detected in the ileum of HIV controllers. Vδ1 cells in the GALT of HIV(-) patients, unlike those in the periphery, directly kill up to 80%±20% of HIV+CD4+ T-cells in culture and inhibiting virus production by 3 logs. These antiviral effects are expanded to the periphery in the setting of elite control. γδ T-cell mediated killing is correlated to perforin expression (R=0.8088). Nef-specific responses in Vδ1 cells were observed in patients with lower viral loads and higher CD4+ count indicating that antiviral effects may be mediated by an HIV-specific response (p=0.01).

Conclusion: γδ T-cells play a key role in the response to HIV infection. HIV specific γδ T-cells are expanded from mucosal tissue to the periphery where they exert anti-viral effects. Further study may suggest ways to harness this unique subset to stimulate both innate and acquired immunity in response to HIV.

P08.14

Lack of IgA Envelope-Reactive Antibody Producing Cells in Terminal Ileum in Early and Chronic HIV-1 Infection

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Background: HIV-1 vaccines must induce protective antibodies at mucosal surfaces; the role of IgA in protection remains unknown. The HIV-1 Env antibody response begins ~day 17 after transmission, and derives from a polyreactive memory B cell pool of gut flora-reactive IgG1 and IgA B cells. Whereas the IgG Env antibody response persists years after acute HIV-1 infection, the initial IgA response decreases over the first month. There is also selective destruction of terminal ileum germinal centers in early HIV-1 infection (EHI). To determine HIV-1 IgA responses in gut, we isolated Env-reactive antibodies from ileum from patients in EHI and chronic HIV-1 infection (CHI).

Methods: Single plasma cells (PCs) and IgD- memory B cells were sorted from the ileum and/or blood of 7 EHI and 3 CHI. Antibodies were isolated by PCR amplification of Ig heavy chain V(D)J and light chain VJ genes and characterized by ELISA and Luminex.

Results: Whereas CHI blood memory IgA+ B cells reactive with HIV-1 envelope ranged from 0.20-0.79%, only 0-0.07% of ileum IgA+ B cells were Env-reactive. Of 254 mAbs isolated from EHI ileum, only 3 (1.2%) were HIV-1-reactive. In CHI, 9 (5.7%) of 158 mAb were HIV-1 reactive. None of the HIV-1 reactive ileum antibodies were of the IgA isotype.

Conclusion: HIV-1 envelope reactive IgA+ memory B cells and PCs can be found in the blood, but there is a dearth of HIV-1 reactive memory IgA+ B cells and PCs in ileum in EHI and CHI. Loss of IgA in plasma after acute HIV-1 infection is paralleled by the loss of IgA+ B cells in ileum, and is likely a consequence of HIV-1-induced ileum germinal center apoptosis. For vaccine design, it will be important to determine if mucosal IgA+ B cell loss is due to replicating virus or is triggered by soluble HIV-1 envelope.

Topic 8: Mucosal Immunity

P08.15

Human Intestinal Beta Defensins Inhibit Viral Replication and Are Diminished in Chronic Untreated HIV Infection

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Background: One of the hallmarks of HIV infection is an early and dramatic depletion of CD4 T cells in the intestinal lamina propria. This loss of T cells is accompanied by intestinal epithelial cell disruption resulting in the translocation of luminal bacterial products, persistent systemic immune activation and resultant disease progression. Beta defensins are cationic antimicrobial polypeptides produced by intestinal epithelial cells and phagocytes that are a part of the innate immune response. They have a pivotal role in maintenance of immune homeostasis in the gut. Although beta defensins have been reported to inhibit bacterial and viral replication in vitro, their role in HIV infection has been incompletely characterized.

Methods: We investigated anti-viral activity and production of defensins in intestinal mucosal biopsies, stool and plasma from individuals with chronic untreated HIV (chronic progressors), immunologically controlled HIV (elite controllers) and HIV uninfected individuals using ELISA, immunohistochemistry, quantitative PCR and viral inhibition assays.

Results: Plasma levels of beta defensins were increased in the HIV controller group compared to HIV progressors, whereas levels of beta defensins were significantly decreased in biopsies from chronic progressors. Beta defensin release was induced by HIV in ex vivo cultured intestinal cells. Recombinant beta defensins inhibited HIV replication in vitro.

Conclusion: Beta defensins are upregulated by HIV and inhibit viral replication in vitro. Chronic progressors, however, had diminished levels of beta defensins in gut biopsies and in the plasma. We propose that beta defensins are induced in HIV infection and have a role in inhibiting viral replication and preventing intestinal epithelial cell disruption. In chronic infection, however, their release is diminished, likely due to epithelial damage in the setting of persistent viremia and mucosal inflammation.

P08.16

Improved Systemic and Mucosal Antibody Responses with a CCR10 Ligand Adjuvant

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Background: The induction of potent mucosal immune responses will be critical for an effective HIV vaccine, However, a major limitation of current vaccine development is the ability to induce mucosal antibodies by a systemic, non-replicating vector. To address this inadequacy, we have hypothesized that encoding instructions for immune cell targeting to the mucosa in the form of MEC, a mucosal chemokine adjuvant delivered as a plasmid can redirect immune responses in vivo. MEC (CCL28) is normally expressed by epithelium in the skin, lungs, and intestines and it functions to attract CCR10 expressing plasmablasts locally.

Methods: IIndian rhesus macaques were vaccinated using EP delivery with either a pcon SIVmac239 gag, pol, SIVsm unmatched E660 env vaccine delivered IM alone (n=5), with CCL28 (MEC, n=5) or a plasmid expressed H1 HA Influenza vaccine alone (n=4) or with MEC (n=4). SIV Vaccinated animals and 6 naïve controls were challenged vaginally twice weekly for four weeks with 500TCID50 SIVsmE660.

Results: The inclusion of a CCR10 ligand adjuvant enhanced vaginal and serum IgG and IgA titers compared with DNA alone. In Flu vaccinated animals functional HAI antibody titers were significantly elevated and above the 1:40 titer required for protection in humans with just a single dose of H1HA delivered with the MEC adjuvant. Following SIV challenge monkeys vaccinated with a CCR10 adjuvant showed 89% protection from the establishment of infection compared 40% with DNA alone with only 16% of the naïve animals.

Conclusion: Mucosal and systemic antibody responses were enhanced with the inclusion of a CCR10 ligand adjuvant. Dose sparing was also observed. DNA vaccination alone improved challenge outcome, and this was further enhanced by the inclusion of a CCR10 ligand adjuvant. The inclusion of mucosal homing chemokines represents a novel approach to induce improved mucosal immune responses by non-live systemic immunization of relevance to HIV infection.

P08.17

Biodistribution of Neutralizing Monoclonal Antibodies IgG1 b12 and LALA in Mucosal and Lymphatic Tissues of Rhesus Macaques

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Background: HIV-1 transmission occurs predominantly through mucosal surfaces. In macaque models of SHIV mucosal transmission, passive administration of the monoclonal neutralizing antibody IgG1 b12 (b12) can protect against virus infection. However, an Fc variant of b12, deficient in FcγR and complement binding, (LALA) had diminished protective capacity. Concentrations and kinetics of b12 and LALA in serum and in mucosal secretions were determined in the protection studies, but the biodistribution and kinetics of the antibodies in mucosal and lymphatic tissues and around the site of viral challenge have not been assessed.

Methods: We conducted a pilot study to develop protocols to process macaque tissue specimens and to detect and quantify passively transferred neutralizing antibodies (NAbs) in tissue homogenates. To confirm that transport of LALA to the site of challenge was not altered from that of b12, we obtained secretions and tissue specimens from rhesus macaques that had been passively infused with either b12 or LALA at 24 hours prior to necropsy, matching the time of challenge in the protection study.

Results: We quantified passively administered b12 and LALA in a variety of macaque mucosal and lymphoid tissues and assessed the neutralization capacity of the NAbs localized in vaginal and rectal sites typically exposed to virus in challenge studies. We demonstrated that the rapid distribution and broad delivery to lymphoid and mucosal tissues of both b12 and LALA are equivalent.

Conclusion: We developed and utilized a methodology to evaluate the transudation of transferred antibody into mucosal and lymphatic tissues and showed that the reduction in the protective capacity of LALA compared to b12 cannot be attributed to a differential effect of biodistribution. This methodology will be useful to describe the pharmacokinetics of newly discovered MAbs and may inform about the characteristics of therapeutic antibodies or antibodies to elicit by vaccination.

P08.18 LB

T cell Immune Quiescence As A Contributor To Resistance To Infection Among HIV Exposed Seronegative (HESN) Commercial Sex Workers from Nairobi, Kenya

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Background: Participants from the Majengo Commerical Sex Worker Cohort in Nairobi, Kenya have been intensely exposed to HIV for 7-20 years of follow-up, and yet have remained uninfected. Since activated CD4+ T cells are much more susceptible to HIV infection and have been suggested to form the initial focus of mucosal infection, we have conducted a number of studies to characterize the T cell phenotype in the mucosal and systemic compartments of these HIV exposed seronegative women (HESN).

Methods: Representative sampling (n=~30) of HESN women and a similar sized control group of newly enrolled commercial sex workers were compared. Gene expression analysis, immune phenotyping, and in vitro HIV infection assays were performed on peripheral blood mononuclear cells. Mucosal assessment of the female genital tract (FGT) included proteomic analysis by mass spectrometry, flow cytometry and cytokine/chemokines determinations by bead arrays.

Results: Gene expression analysis revealed the HESN women showed reduced gene levels for pathways involved in T cell receptor activation and HIV host dependent factors. Systemic CD4+ T cells showed lower levels of immune activation (CD69) and higher levels of regulatory T cells (T regs). Infection frequency, the number of infected replicate wells, was lower in the HESN women and correlated with ex vivo assessment of reduced T cell activation and elevated T reg levels. Mucosal assessment by proteomics showed higher levels of anti-inflammatory serine proteases and lower levels of the chemokines IP-10 and MIG, which functions are to recruit activated T cells into the mucosal environment.

Conclusion: Together, these data suggest that the HESN women of the Majengo Cohort display a T cell Immune Quiescent phenotype that is characterized by fewer activated T cells, more regulatory T cells and a mucosal environment that favours quiescent cells. The result is an environment that is not favorable for the establishment of HIV infection.

Topic 8: Mucosal Immunity

P08.19 LB

A Novel Mechanism of HIV Transcytosis and Infection

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Background: Female genital tract mucosae are bathed in acidic secretions, which, in the case of HIV-1 infected women, likely contain virus coated with antibody. Thus, uninfected male sexual partners are often exposed to an acidic milieu containing virus in the form of immune complexes. We investigated the impact of low pH and antibody on transcytosis, a potentially critical mechanism by which HIV-1 passes through genital tract epithelia to infect susceptible target cells.

Methods: HIV-1 was incubated with Env-specific monoclonal and polyclonal antibodies at pH 6.0 or pH 7.4 and exposed to the apical surface of tight junction-forming human endometrial carcinoma (HEC-1) cells in transwell plates. The quantity and infectivity of transcytosed virus was measured by RT-PCR and infection of TZMbl cells, respectively.

Results: We found that the combination of acidic pH and Env-specific antibody augmented transcytosis as much as 30-fold compared with Env-specific antibody at neutral pH or compared with non-specific antibody or no antibody at neutral or acidic pH. The pH and antibody dependence of enhanced transcytosis was blocked by antibody specific for the Fc neonatal receptor (FcRn) or by treatment with bafilomycin A1 (which inhibits acidification of endosomes). Non-neutralizing antibodies resulted in a lower quantity of total transcytosed virus, measured by RT-PCR, than did neutralizing antibodies. However, the ratio of total to infectious virus was much higher for neutralizing antibodies, indicating that neutralizing antibodies efficiently allow transcytosis while blocking infectivity of the transcytosed virus; the non-neutralizing antibodies facilitate transcytosis (although to a lesser degree than the neutralizing antibodies) without blocking infectivity.

Conclusion: These results demonstrate that acidity and Env-specific antibody greatly enhance transcytosis of virus across mucosal epithelial cells via FcRn. Since male penile and urethral tissues express FcRn, our results suggest a novel mechanism by which antibody, and in particular, non-neutralizing antibody, might facilitate female-to-male transmission following sexual exposure.

P08.20 LB

Influence of Hormones and HIV Infection on Viral Transport

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Background: Inhibiting transport of virions within the female reproductive tract is an attractive mechanism for transmission prevention. The mucosal environment varies with menstrual cycle and concurrent bacterial vaginosis (BV). Previous studies of transport within mucosal samples have focused on cervico-vaginal samples in exclusion. Severe BV corresponds to increased incidence of female-to-male HIV-1 transmission although the mechanism remains unclear.

Methods: We have established a cohort of HIV +ve and –ve women to be longitudinally studied for correlates of inhibited transport phenotypes. Cervical and cervico-vaginal mucus samples (CM and CVM, respectively) are collected, along with mucosal antibodies, vaginal smears, hormone levels, viral load, T cell monitoring panels as well as medical and behavioral history. Viral transport assays employ a diverse panel of viral isolates, testing clade specific effects. As of this interim analysis, over 60 study subjects have been recruited and repeat sampling is beginning.

Results: The hormone profile demarcating menstrual cycle phase correlates strongly with particle movement. In CVM, the nature of viral interactions with their environment changes; lowest progesterone to estradiol ratio corresponds with hindered diffusion. Non-reactive similarly sized PEGylated beads have freely diffusive behavior throughout the menstrual cycle. Cycle classification into follicular, mid-cycle and luteal periods demonstrates that CVM in the luteal phase is, unexpectedly, most permissive to viral transport. Severe BV has modest effects on pH of CVM and no effect on CM. This is reflected in viral transport characteristics whereby the magnitude and nature of movement is invariant during severe BV relative to healthy flora.

Conclusion: It is unlikely that the mechanisms of increased transmission with BV are related to virus diffusing more freely within mucus. Correlates of hindered diffusion are not restricted to adaptive immune responses. This study begins to reveal the significance of immune correlates and hormone profiles on HIV-1 transmission mechanisms and transport in the female reproductive tract.

P09.01

Trends in Uptake of Couples Voluntary HIV Counseling and Testing (CVCT) in Lusaka and Southern Province, Zambia

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Background: Couples HIV testing and counseling (CVCT) has been proven to decrease transmission of HIV in discordant couples by two-thirds. In order to achieve a snowball effect and establish CVCT as a social norm, the intervention must reach 15-20% of the target population. Considering demographics in Lusaka, Zambia, approximately 40,000 - 50,000 couples must be tested together within a few years to establish norms and set the stage for social diffusion.

Methods: The authors investigated trends in couples seeking first time ZEHRP CVCT services in Lusaka and Southern Province government district clinics from January 2008 – December 2011. Trends in increases or decreases in uptake were compared to historical data such as changes in incentives for couples and CVCT promoters, as well as external events such as couples testing campaigns and government policies.

Results: Over the study period, ZEHRP tested 37,859 couples. Declines in number of couples tested coincided with a discontinuation of performance based pay for District Clinic Promoters (DCPs) and a reduction in couples transport reimbursement. Though six new government clinics began offering ZEHRP CVCT in quarter four of 2008, no performance based pay or transport reimbursement were provided, and numbers were lowest at this time than at any point in the study period.

A sharp increase in CVCT uptake was seen in quarter one of 2010, which coincided with a mass media campaign for CVCT by another Zambian non-governmental organization, though uptake gradually declined once the campaign ended.

Number of couples seen in Southern Province remained stable throughout the study period, while most fluctuations were seen in Lusaka.

Conclusion: Client and promoter incentives as well as mass media have been shown to influence uptake of CVCT. In urban areas where cost of living is high, CVCT programs must consider opportunity costs to encourage uptake.

P09.02

Prior Individual and Couples' Voluntary HIV Counseling and Testing (CVCT) in Couples Seeking CVCT in Lusaka, Zambia

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Background: The majority of new HIV infections in Africa are acquired in marriage, and heterosexual couples represent the largest HIV risk group in sub-Saharan Africa. However, only 15% of Zambians report ever testing for HIV. This study aims to investigate trends in prior individual and couples HIV testing in a cohort of clients who sought couples HIV counseling and testing (CVCT) in Lusaka, Zambia.

Methods: Couples who sought first-time CVCT at Lusaka district government clinics through the Zambia Emory HIV Research Project (ZEHRP) provided information on prior HIV testing. Clients were grouped based on whether: only one of the partners had ever tested (further disaggregated by sex), both had tested individually but never as a couple, or both had tested as a couple.

Results: In a sample of 7,582 discordant and concordant negative couples tested from October 2009 to December 2011, prior testing in the male partner alone was seen in 7%. In 33%, the woman alone had tested previously. In 25%, both partners had tested individually but never as couple, while in another 25% neither partner had ever tested prior to CVCT. Ten percent of couples reported prior testing as a couple.

When disaggregated by sex, woman-only previous testing was predominant throughout the study period, while men-only previous testing, though relatively low, increased by 2.5%. From the beginning of the study period to the end, couples reporting having previously tested together increased by 2%. Couples where both had tested individually but never as a couple increased steadily over the study period.

Conclusion: Both prior individual and couples testing increased over the study period, though this increase was more obvious for individual testing. Despite endorsement of CVCT by the Zambian Ministry of Health since 2008, to date few couples have been jointly tested and counseled in the capital city.

P09.03

Promotion of Couples' Voluntary HIV Counseling and Testing in Lusaka, Zambia by Influence Network Leaders and Agents

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Background: In sub-Saharan Africa, most HIV transmissions occur in stable

heterosexual relationships. Couples' voluntary HIV counseling and testing [CVCT] is an effective strategy targeting this at-risk group. This study identified predictors of successful CVCT promotion in Lusaka, Zambia.

Methods: CVCT promotions were conducted by influential network leaders [INLs] who identified agents [INAs], who in turn delivered CVCT invitations from over an 18-month period, with a mobile unit crossing over from one intervention neighborhood to another at 9 months. INA, couple, and invitation characteristics predictive of couples' testing were evaluated accounting for two-level clustering.

Results: 320 INAs delivered 29,119 invitations resulting in 1727 couples testing (6% success rate). In multivariate analyses, INA characteristics significantly predictive of CVCT uptake included promoting in community-based (adjusted odds ratio [aOR]=1.3) or health (aOR=1.5) networks versus private networks, being employed in the sales/service industry (aOR=1.5) versus unskilled manual labor, owning a home

(aOR=0.7) versus not, and testing for HIV with a partner (aOR=1.4) or alone (aOR=1.3) versus never testing. Cohabiting couples were more likely to test (aOR=1.4) than non-cohabiting couples. Context characteristics predictive of CVCT uptake included inviting couples (aOR=1.2) versus individuals, the woman (aOR=1.6) or couple (aOR=1.4) initiating contact versus the INA, the couple being socially acquainted with the INA (aOR=1.6) versus having just met, home invitation delivery (aOR=1.3) versus elsewhere, and easy invitation delivery (aOR=1.8) versus difficult as reported by the INA. Use of mobile units was very low and did not substantially contribute to CVCT service delivery.

Conclusion: This study demonstrated the ability of influential people to promote CVCT and identified agent, couple, and context-level predictors of CVCT uptake in Lusaka, Zambia. We encourage the development of CVCT promotions in other sub-Saharan African countries to support sustained CVCT dissemination.

P09.04

Effect of an Intervention to Promote Contraceptive Uptake on Incident Pregnancy: A Randomized Controlled Trial Among HIV Positive Couples in Zambia

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Background: Prevention of unintended pregnancy, especially among HIV positive couples and in settings where both HIV and total fertility rates are high, is a critical public health initiative.

Methods: A factorial randomized controlled trial evaluated the effect on incident pregnancy of two interventions (a "Methods" and "Motivational" intervention) to promote long-term contraceptive use among HIV serodiscordant and concordant positive couples (N = 1060) identified from CVCT clinics in Lusaka, Zambia.

Results: Couple baseline serostatus and contraception usage were both individual effect measure modifiers ($p<0.0001$). Among couples in which the woman was not using a contraceptive method at baseline (N=782), there was no significant effect of the interventions overall or when stratifying by couple serostatus on incident pregnancy. Among couples in which the woman was using a contraceptive method at baseline, concordant positive couples (HR = 0.20; 95%CI: 0.08-0.53), and couples in which the woman was HIV positive at baseline (HR = 0.21; 95%CI: 0.09-0.51) who received "Methods + Both" interventions - which combined information on contraceptive methods and motivational messages for future planning behaviors - were at significantly decreased hazard for pregnancy relative to those receiving "Motivational + Control" interventions -- which provided motivational messages for future planning but not information on contraceptive methods.

Conclusion: An educational intervention promoting long-term contraceptive method uptake among HIV positive couples is successful at decreasing time to pregnancy in the context of couples' HIV testing, particularly among women who are HIV+ and already using a contraceptive method. A combination of motivational messages for future planning behaviors and information on long-term contraceptive methods appears to be the best intervention for reducing incident pregnancy among concordant positive and serodiscordant couples. Further work is needed to understand the interventions appropriate for women who are currently not contraceptive users.

P09.05

HIV-Free Children Born to HIV-Seropositive Mothers in Bamako, Mali:A Six-Year Perspective on Providing MTCTP at the Front Line of AIDS

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Background: GAIA Vaccine Foundation (GAIA VF) conducted a six-year retrospective assessment of its Mother To Child Transmission Prevention (MTCTP) program for effectiveness as a non-vaccine HIV prevention tool.

Methods: The MTCTP program at the Sikoro prenatal care center (Chez Rosalie) opened in 2005. We evaluated MTCTP acceptance and HIV test results of babies born in the MTCTP program from 2005-2011. We also surveyed HIV+ mothers at the clinic about MTCTP risk in July 2011.

Results: 10,471 women were counseled about HIV infection during the study period (average 145/month). An overwhelming majority (99.1%) agreed to HIV testing: 202 (2.15%) were HIV+, of whom 125 (61.9%) accepted MTCTP treatment. Ninety-two HIV+ women delivered at Chez Rosalie. Most used the baby formula provided at the clinic, and a minority chose breastfeeding (as per national policy since 2010). Notably, 100% of babies born to MTCTP-adherent mothers were HIV-seronegative. Thirty-five HIV+ mothers were interviewed about MTCTP for their 150 children. Of the seven polygamous women interviewed, none informed their husbands about their HIV+ status; single and monogamous mothers were significantly more likely to communicate their status.

Conclusion: The number of women accepting treatment and remaining in care decreased over the 6-year period. Women moved to another clinic after testing positive and also returned to their home villages to deliver, despite having been educated about risks. Two children of mothers who were followed at Chez Rosalie but not enrolled in MTCTP were born HIV+; risk factors for transmitting HIV included late diagnosis (during pregnancy), breast feeding without concomitant ARV treatment, and single parent status. Lack of disclosure was worrisome, considering the number of polygamous relationships. GAIA is working on improving methods to reduce new HIV infections in Sikoro by destigmatizing HIV and MTCTP, and by scaling-up existing peer-education programs to improve willingness to participate in and adhere to MTCTP.

P09.06

Performance of Self-Reported Adherence to Oral Pre-Exposure Prophylaxis (PrEP) Among HIV Heterosexual Serodiscordant Couples in Rural Uganda

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Background: Adherence is one of the main determinants of PrEP efficacy. Most PrEP studies applied subjective adherence measures, which often produce overestimates and problematic efficacy data interpretation; creating a need for more objective measures. This study examines self-reported adherence to oral PrEP compared to Medical Events Monitoring System (MEMS).

Methods: Seventy-two HIV-uninfected partners (50% women) in Uganda were randomized to daily or intermittent (Monday, Friday and within 2 hours after sex, not exceeding 1 dose/day) oral emtricitabine/tenofovir or placebo in a 2:1:2:1 ratio for four months. Adherence was assessed monthly by MEMS and self-reported taken or missed doses by timeline follow-back calendar. MEMS data was adjusted for extra openings without pill removal and removal of multiple pills. Non-fixed days within intermittent regimen were classified as adherent/non-adherent based on self-reported sex by SMS. Adherence rates by taken/missed doses were compared to raw MEMS data using Spearman correlation.

Results: Treatment and placebo groups were combined since adherence rates were similar. Daily raw MEMS adherence rate was significantly higher than fixed Intermittent rate ($p=0.04$) and post-coital dosing rate ($p<0.0001$). Raw MEMS data for daily and fixed intermittent dosing, poorly correlated with self-reported taken doses ($r=0.14$, $p=0.42$ and $r=0.01$, $p=0.94$, respectively) and missed doses ($r=0.30$, $p=0.08$ and $r=0.07$, $p=0.69$, respectively). Self-reported daily adherence had high sensitivity but only fair positive predictive value (PPV) and very poor specificity. Self-reported adherence to intermittent fixed dosing had fair sensitivity, PPV and negative predictive value (NPV), but poor specificity. Self-reported adherence to post-coital dosing had very good sensitivity and NPV but poor specificity.

Conclusion: Median adherence for daily and intermittent fixed PrEP was high by objective and subjective measures, but poorly correlated. Adherence to post-coital dosing was poor and likely overestimated by self-report (possibly reflecting technical challenges of SMS). Self-reported adherence measures were highly sensitive but poorly specific.

P09.07

Straightforward Selection of Broadly Neutralizing Single-Domain Antibodies Targeting the Conserved CD4 and Co-receptor Binding Sites of HIV-1 gp120

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Background: The interaction of gp120 with CD4 is the first step of HIV cycle. It is the pivotal event allowing the entry of HIV into CD4+ cells. gp120 is the main target for neutralizing antibodies (nAb) but most of its accessible epitopes are highly variable and thus, HIV can bypass these nAbs.

Single domain antibodies (sdAb) derived from llama antibodies bind their antigen without requiring variable domains pairing. They are able to bind unconventional epitopes, like those present in protein cavities. Their small size (13 kDa) allows them to accede to very narrow space, such as the space between virus and cell membranes during infection. SdAbs are highly stable, easy to clone and produce in large amounts.

Methods: Using trimeric gp140, free or bound to a CD4 mimic, as immunogens in llamas, we selected a panel of broadly neutralizing single-domain antibodies that bind either to the CD4 or to the co-receptor binding sites (CD4BS and CoRBS, respectively).

Results: When analyzed as monomers or as homo- or hetero-multimers, the best sdAb candidates could not only neutralize viruses carrying subtype B envelopes, corresponding to the Env molecule used for immunization and selection, but were also efficient in neutralizing a broad panel of envelopes from subtypes A, C, G and CRF01_AE. Interestingly, sdAb multimers exhibited a broader spectrum of neutralizing activity than the parental sdAb monomers.

Conclusion: The extreme stability and high recombinant production yield combined to their broad neutralization capacity make these sdAbs new potential microbicide candidates for HIV-1 transmission prevention.

P09.08

HIV-1 Subtype C Primary Isolates Exhibit High Sensitivity to an Anti-gp120 RNA Aptamer

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Background: Globally, HIV-1 subtype C is the most prevalent subtype, yet most antiretroviral drugs are developed against subtype B. UCLA1 RNA aptamer, which we previously showed neutralizes HIV-1 subtype C Env-pseudotyped viruses was examined for neutralization of subtype C primary isolates in PBMC and monocyte-derived macrophages (MDM). We also assessed the ability of subtype C to develop resistance to UCLA1 inhibition by propagating the isolates in increasing concentrations of the aptamer.

Methods: UCLA1 was tested against clinical isolates in PBMC (6 isolates) and MDM (4 isolates) using a p24 antigen read-out. Three viruses were grown in the presence of increasing aptamer concentrations to select for resistance. The viruses were passaged every 7 days up to 12 weeks in CD8 depleted PBMC. The gp160 was sequenced, analyzed and compared with wildtype viruses.

Results: UCLA1 neutralized 67% and 75% of viruses tested in PBMC and MDM, respectively. Overall, the aptamer neutralized one X4 and six R5 tropic viruses with IC_{80} values in the nanomolar range. Two viruses remained sensitive to the aptamer even in the presence of 4- and 12-fold increased UCLA1 concentrations. One isolate exhibited resistance after 12 weeks of propagation tolerating 12-fold the starting IC_{70}. Fifty-eight amino acid changes and two insertions along the gp160 were observed. The changes observed within the V1/V2 and V3 loops confirmed our previous data shown by truncation and single point mutational analyses to confer resistance to UCLA1.

Conclusion: UCLA1 was able to neutralize infection of primary isolates in PBMC and MDM without tropism restriction. The extensive amino acid sequence changes associated with UCLA1 resistance may indicate a high genetic barrier needed for resistance to UCLA1. This was also suggested by the low rate of resistance (only 1 of 3 isolates) observed in the study suggesting that UCLA1 is a potential anti-HIV-1 subtype C entry inhibitor drug.

P09.09

Are Fishing Communities Another Most-at-Risk-Population? Results of a Community-Based Study Along Lake Victoria, Uganda

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Background: A recent study reported HIV prevalence of 28.8% among high risk persons in fishing communities (FC) of Uganda, indicating that FC may be another most-at-risk-population (MARP). However, these findings do not reflect the population-based HIV prevalence in FC. We conducted a community-based study to determine the population representative HIV prevalence and incidence among FC along Lake Victoria shores, Uganda.

Methods: Community-wide mapping and census of households in 8 fishing communities was conducted. A computer-based random sample of 2200 participants aged 18-49 years was selected for interviewing by same-sex interviewers using a semi-structured questionnaire. Blood was collected for HIV serology using rapid HIV tests as per the national algorithm.

Results: We interviewed 2,192 (99.6%) participants, of whom fifty percent were females, median age 29 years, IQR 24-35 years and 82% had stayed in the communities for at least a year.

HIV prevalence was 26.7%, higher among females than males [32.6% vs. 20.8%, p<0.01,OR=0.5], those who were single [34.7% vs. 27.8%, p<0.01, OR=1.4], those with >5 lifetime sexual partners [32% vs. 21%, p<0.01,OR=1.7], with no formal education [39.2% vs. 25.5%, p<0.01,.OR=0.5], reported alcohol use in previous 3 months [32% vs. 20.8%, p<0.01, OR=1.8], alcohol use before sex [34% vs. 21%, p<0.01, OR=0.5] and used illicit drugs [33% vs. 25.6%, p<0.01, OR=0.7].

HIV prevalence varied by occupation, highest among sex workers (66.7%), boat makers (50%), government employees (43%), bar owners (37.6%) and bar attendants (36.4%), [p<0.01]. Prevalence increased with age [p<0.01] and was not associated with consistent condom use [26.8% vs. 26.4%, p=0.45].

Conclusion: FC in Uganda have a disproportionately high HIV prevalence compared to the national average of 6.7%. Our prevalence estimate was comparable to that reported among high risk FC. Prevention efforts towards reducing HIV prevalence in these communities are needed, and such populations may be considered for future HIV prevention trials.

P09.10

Progress and Challenges in Integrating CVCT into Routine Antenatal Services in Government Clinics in Ndola, Zambia

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Background: In 2008, the Ministry of Health in Zambia recommended that HIV testing be provided to partners of antenatal clients. Herein the Zambia Emory HIV Research Project (ZEHRP) in collaboration with the Arise Program/PATH funded by CIDA examines the transition of Couples' Voluntary HIV Counseling and Testing (CVCT) from NGO-sponsored weekend services to integrated weekday services in government clinics in Ndola, the second largest city in Zambia. We also describe how CVCT data are being recorded and reported.

Methods: Data were extracted from government-issued logbooks in antenatal clinic (ANC), prevention of mother to child (PMTCT), and voluntary counseling and testing (VCT) services in the six largest government clinics for the year 2010, and in15 clinics in the first half of 2011. CVCT procedures and record-keeping were documented through observation and counselor interviews.

Results: In 2010, only one of the six largest clinics tested more than four couples/week. In this clinic, a community promotions campaign in Q2 resulted in an average of 5 couples/day seeking testing, though numbers tapered off over time to 2 couples/day by Q4. In March-May of 2011, 11 of 15 clinics averaged less than 10 couples per month. Four clinics recorded 20 to 60 couples/month: 77% of couples were seen in ANC with the remainder tested in VCT. Obstacles included low participation of men, lack of staff trained to counsel couples jointly, procurement challenges for HIV tests for men in ANC, and non-uniform recording of CVCT in ANC and VCT logbooks.

Conclusion: This study identified several challenges for integrating CVCT into regular clinic services. To address these challenges, we recommend implementing new data recording instruments, increasing training of counselors and nurses in CVCT, prioritizing ANC clients attending with partners and expanding of community sensitization using proven models.

Topic 9: Non-Vaccine Prevention

P09.11

Conformation-Dependent Recognition of HIV Gp120 by DARPins Provides Novel Possibilities to Develop Distinct HIV Entry Inhibitors

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Background: Designed Ankyrin Repeat proteins (DARPins) are a novel type of binding protein scaffold designed as antibody alternative for biomedical applications. DARPins share many properties with antibodies, most noteworthy a high target specificity and affinity, but they differ from antibodies in size, structure and binding pattern and, importantly, favorable biophysical properties such as exceptional stability. This together with high-yield prokaryotic production renders DARPins promising candidates for microbicide development.

Methods: DARPin DNA libraries encoding either two or three internal ankyrin repeats were subjected to ribosome display selections and panned against recombinant gp120 proteins. Wild type, CD4 induced, V1V2 truncated and deglycosylated JR-FL gp120, as well as structural V3 loop mimetics were probed as target. Obtained DARPin clones were sequenced, mapped for reactivity with HIV gp120 by ELISA and probed for inhibitory activity using the TZM-bl pseudotype virus inhibition assay.

Results: DARPin selection proved more successful when conformationally arrested targets were used for panning. Overall, gp120-specific DARPins recognizing a variety of epitopes including the CD4bs, CD4i and the V3 loop were obtained. Gp120 mutant binding analysis revealed that DARPin molecules depended to a higher degree on a structural conservation of the envelope protein than gp120 specific antibodies recognizing overlapping domains. Most noteworthy, V3 loop specific clones were selected, which unlike V3 loop antibodies, recognized the V3 loop in a conformation-dependent manner and thus do not efficiently bind linear V3 loop peptides. In contrast to V3-loop directed antibodies these DARPins proved to bypass the envelope shielding by the V1V2 domain and thus were capable of neutralizing TIER-2 viruses.

Conclusion: Gp120 proved a challenging target for selection of DARPin binders against gp120. Nonetheless, by combining different selection strategies we were able to derive a variety of gp120-specific DARPin molecules, including some with unique HIV entry blocking activity.

P09.12

Alcohol and Illicit Drug Use Among Potential HIV Vaccine Efficacy Trial Volunteers Along Lake Victoria, Uganda

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Background: HIV has adversely affected fishing communities (FC), with prevalence ranging between 25-30% on Lake Victoria. To better characterize the HIV prevalence among FC and explore potential for HIV vaccine efficacy trials, we conducted a pilot study on HIV prevalence, sexual risk behavior, alcohol and drug use in these communities.

Methods: A cross-sectional HIV prevalence survey was conducted in 8 FC over 3 months. Census and mapping to determine the average population size in each community were conducted prior to the study. After obtaining informed consent, 2,200 individuals aged 18-49 years from randomly selected households were tested for HIV and responded to questionnaires on sexual behavior, alcohol consumption and drug use.

Results: Fifty three percent (53.0%) had consumed alcohol in the past three months with males consuming more alcohol than females [59.0% vs. 46.8%, p<0.01].19.2% of alcohol consumers also used illicit drugs [p<0.01]. Alcohol consumption in the previous 3 months was highest among Catholics [p<0.01], those with no formal education [p=0.01], sex workers (100%), loaders/off loaders (79%), bar owners (69.7%), construction workers (66.7%), bar attendants (66.1%) and fishermen [(61.0%), p<0.01]. Daily alcohol consumption was highest among older participants (35-49years) and sex workers [p<0.01]. Alcohol consumption before sex (43.0%) was highest among youths (25-34 years), sex workers, bar owners and attendants [p<0.01]. HIV prevalence was higher amongst alcohol consumers [63.4% vs. 36.6%, p<0.01], with daily and weekly consumers having a higher prevalence [73.0% vs. 27.0%, p=0.05].

Illicit drugs like cocaine, Miraa/Khat were used by 13.8% of participants, mostly amongst sex workers [(33%), p<0.01]. Illicit drugs were used mainly on a daily basis [(44.6%), p<0.01].

Conclusion: Alcohol and illicit drug use is prevalent in FC and associated with higher prevalence of HIV infection. Efforts towards controlling alcohol and illicit drug use might help control HIV in these communities

P09.13

Anti-HIV and Immune Modulating Activities of IND02

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Background: A vaccine that prevents HIV infection should not only induce functional inhibitory, neutralizing antibodies, but also promote Fc-mediated inhibitory antibodies displaying ADCC or phagocytosis. The aim of this study is to analyze the effect of IND02, a cinnamon derived procyanidin polymer on the interaction of HIV-1 gp120 with its co-receptors as well as its adjuvant like activity along with HIV-specific antibodies.

Methods: The ability of IND02 to interact with the HIV envelope glycoprotein gp120 was analyzed by studying binding of IND02 to gp120 envelopes by Biacore. The expressions of FcγRs were evaluated on macrophages and NK primary cells incubated with IND02 by flow cytometry. The inhibitory activity of IND02 was assessed on PBMC using TZM-bl assay for neutralization, Fc-mediated inhibitory activity on macrophages and Antibody Dependant Cellular Cytotoxicity in presence or absence of anti-HIV antibodies.

Results: Binding of IND-02 to gp120 envelopes was dose-dependent within the µM range and was capable to inhibit gp120-CD4 interaction. A moderate decrease in the expression of FcγR I and III was observed on macrophages treated with IND02, while FcγR II expression was unaffected. FcγRIII expression on NK cells was not modified. IND02 demonstrated low inhibitory activity on TZMbL and PBMC, but was able to efficiently inhibit HIV infection on macrophages. Moreover IND02 demonstrated synergistic effect when combined with monoclonal inhibitory antibodies. An enhanced ADCC was detected in presence of IND02 and anti-HIV specific antibodies.

Conclusion: IND02 represents an interesting class of botanical molecule that binds to HIV-1 envelope protein, including the co receptor binding site, impairing HIV interaction with co-receptors on target cells. This interaction could explain the decreased HIV replication observed after IND02 treatment. Besides, an augmentation in the activity of HIV-specific antibodies, involving ADCC and Fc-mediated phagocytosis, was observed in presence of IND02 supporting additional adjuvant mechanisms that could enhance immune responses against HIV.

P09.14

Heme Oxygenase-1 Reverses HIV-1 Tat Activity: Prospects for AIDS Prevention

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Background: Heme oxygenase-1 (HO-1) is a HEME regulator and plays a role in ameliorating HIV-1 infection. In particular, HO-1 inhibits Tat-dependent activation of HIV-1 LTR promoter inhibiting viral gene expression. This suggests that increasing HO-1 activity in HIV-infected cells can reverse Tat action which may contribute to AIDS prevention. However, the correlation between HO-1 and HIV-1 Tat has not been fully elucidated. In order to fully understand how increasing HO-1 activity reverses Tat action and result into the prevention of HIV infection, the mechanism behind the correlation between HIV-1 Tat and HO-1 should first be established.

Methods: Throughout the study we made use of Jurkat T cells (control) and Jurkat-Tat T cells. Whole cell extracts were obtained and mitochondrial extracts were isolated separately. HO-1, HEME, superoxide dismutase (SOD), catalase and hydrogen peroxide (H2O2) levels were measured using commercially available assays. Immunoassays confirmed both the presence of Tat and NADPH oxidase activity via the HEME-activated gp91phox

Results: We found that in Tat-expressing cells, HO-1 and SOD amounts were decreased, HEME and H2O2 levels were increased and catalase concentration was unchanged. In addition, we observed an accumulation of gp91phox and H2O2 amounts. We suspect that Tat activity in Jurkat T cells lead to the following sequence of events: (1) decrease in HO-1 and SOD activities; (2) low SOD amounts leaves catalase amounts unchanged; (3) low HO-1 levels allows HEME to accumulate; (4) high amounts of HEME favors the accumulation of the gp91phox subunit which subseuqently increases NADPH oxidase activity; and (5) ultimately leads to H2O2 accumulation. We hypothesize that by increasing HO-1, as previously reported, HIV-1 infection was ameliorated ascribable to a reversal in Tat activity.

Conclusion: HIV-1 Tat lowers HO-1 activity which consequentially leads to H2O2 accumulation. We suspect, based on a previous report, that increasing HO-1 ameliorated HIV-1 infection by reversing Tat activity.

P09.15

Inhibition of HIV-1 Subtype C by 2'F-RNA Aptamers Isolated Against Enveloped Pseudovirus

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Background: Human immunodeficiency virus type-I (HIV-1) envelope glycoprotein (Env) mediates the first step of entry and represents an attractive target . However, the genetic diversity of Env among HIV-1 subtypes poses a challenge. Although evidence suggest that entry inhibitors in clinical use have comparable efficacy against HIV-1 subtypes, but a subset of anti-Env are resistant to HIV-1 subtype C which is the most globally prevalent virus. In a view to prevent infection of HIV-1 subtype C, we isolated nucleic acids ligands aptamers against HIV-1 subtype C Env pseudovirion and tested their efficacy.

Methods: We isolated 109 unique 2`F-RNA aptamer sequences against HIV-1 subtype C Env pseudovirus using systematic evolution of ligands by exponential enrichment (SELEX) process. After 9 selex cycles, we grouped aptamer clones by binding activity to gp120 then selected one of the aptamer which neutralized infection of parental virus with IC50 values <5 nM. We tested efficacy of a selected aptamer against 31-pseudovirus panel from subtype C using single cycle luciferase assay in TZM-bl cells. In addition, we mapped the epitope on gp120 binding by competition

Results: When screened on a 31-panel of HIV-1 subtype C pseudoviruses, RNA aptamer CSIR 1.1, neutralized 26 /31 (84%) with mean IC50 of 6.7 ± 8.8 nM. Competition ELISA results revealed CSIR 1.1 bind to the epitope overlapping CD4 and VRC01 on monomeric gp120 but not b12 or b6. In contrast to CD4, CSIR 1.1 did not enhance the binding of 17 b to the co-receptor region on monomeric gp120 showing a different binding mode to the soluble CD4

Conclusion: Our data show that we have isolated new RNA aptamers with efficacy against diverse HIV-1 subtype C viruses. The immediate future research would include testing efficacy on primary isolates and precise mapping .

P09.16

HIV Risk Among Men Who Have Sex with Men (MSM) in Nigeria: A Potential Population for HIV Vaccine Trial

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Background: MSM are at a high risk of HIV. Due to their risk, they are potential population for HIV vaccine trials. Nigeria conducted 2010 Integrated Biological Behavioral Surveillance Survey (IBBSS) among the high risk groups. Unfortunately, MSM had the second highest HIV prevalence after female sex workers, and some with female partners. Thus, HIV prevention among MSM is of great national importance. This study evaluates predictors of their risky behavior which will be useful in enrolling them for future HIV vaccine trials.

Methods: Secondary analysis of 2010 IBBSS data involving 1545MSM between 18–49years was done. IBSSS involved HIV testing with information collected on sexual/reproductive health indicators. Risky sexual behavior was defined as sex without condom and with more than one male partner. Multivariate logistic regression was used to model predictors of risky sexual behavior.

Results: Mean age: 25.4±6.0years; HIV prevalence: 17.2%; 12.2% were married to female partners; median anal sex partner was 3. About 31.5% tested and received results for HIV; condom use at last anal sex: 52.1%; sex with girlfriends: 41.9%; 18.3% paid for sex; 12.2% used marijuana; daily alcohol use 26.6% and 33.1% were reached with prevention programs. About 34% MSM engaged in risky sexual behavior. Predictors of risky sexual behavior were use of alcohol OR=2.9 95%CI:1.3-6.1; being away from home for >1month OR=2.5 95%CI:1.3-5.7; anal sex without condom OR=2.9 95%CI:1.6–4.4; sex with ≥3 partners OR=5.6 95%CI:2.1-7.3; while previous STI OR=0.6 95%CI:0.3-0.9; having a female marital partner OR=0.7 95%CI:0.4-0.9; known HIV result prior to survey OR=0.6 95%CI:0.3-0.9 were protective.

Conclusion: High number of MSM practice risky sexual behavior that is driven by alcohol, multiple male partners and is reduced by previous STI or known HIV result. Lastly, there is a need for innovative and result-oriented behavioral interventions among MSM to reduce their HIV risk.

P09.17

Female Sex Workers and Condom Use: Lessons for Future HIV Vaccine Trial in Nigeria

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Background: Condom remains one of the most effective means of HIV prevention in the absence of proven, safe and effective vaccine. Nigeria has a surveillance system that monitors HIV trend among most-at-risk populations. Female sex workers (FSW) have the highest HIV prevalence compared to other groups. Since the ultimate goal is to prevent new infection, there is a need to evaluate condom use among FSW and the lessons will be vital for future HIV vaccine trial.

Methods: Secondary data analysis of 2010 Integrated Bio-Behavioral Surveillance Survey was done with 4559 brothel and non-brothel FSWs in 9 Nigerian states. The survey involved HIV testing. Administered questionnaires had information on socio-demographic, HIV, sexual/reproductive health variables. Predictors of consistent condom use were evaluated using multiple logistic regression models.

Results: The median age was 26years (15-49years); median age at first sex was 17years (11-22years); average clients/day was 4; 38% had ever been married with 8.1% currently married and 3.7% living with their spouse; 74.6% used condom consistently; 47.4% completed at least secondary education; 53.6% had been away from home for more than 1month; 60.2% tested and received HIV result within the last 12months; and 91.2% knew that condom could protect against HIV. Significant predictors of consistent condom use include testing and knowing result for HIV OR=1.9 95%CI:1.4-2.5; being away from home for ≥1month OR=1.4 95%CI:1.1-1.9; engaged in sex trade for 24months or less OR=2.7 95%CI:2.0-3.7; those that had 4 or more clients per day OR=1.7 95%CI:1.3-2.3) and those aged 25years and above OR=1.4 95%CI:1.1-2.1.

Conclusion: Older FSW with shorter duration in sex trade, having more clients, aged above 25years and away from home are more likely to use condom consistently. Lessons from condom uptake among FSW may lead to targeting FSW with these features for inclusion criteria for HIV vaccine trial in Nigeria.

P09.18 LB

Infrequent, Low Magnitude HIV-Specific T Cell Responses In HIV-Uninfected Participants In The 1% Tenofovir Microbicide Gel Trial (CAPRISA004)

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Background: Macaque studies of antiretroviral-containing microbicide gels administered rectally or vaginally followed by SIV challenge have documented priming of SIV-specific T cell responses in the blood of protected animals. This concept has been termed "chemo-vaccination", where aborted viral replication is thought to leave an immune footprint of exposure, which may augment protection provided by microbicides/PrEP. We investigated whether T cell responses were detectable in women participating in CAPRISA004 1% tenofovir microbicide trial, which showed 39% efficacy in reducing HIV acquisition.

Methods: Thirty-eight HIV-uninfected participants were selected based on consistently high gel use and a high number of recorded sex acts over the duration of the trial. Cryopreserved PBMC were stimulated with HIV-1 peptide pools based on the HIV-1 clade C proteome, and IFN-gamma production was measured by the ELISPOT assay. Positive response were defined as >55 SFU/106 PBMC. Samples were tested at the visit at which preceding monthly coital activity was the participant's highest, and at study exit. Assays were conducted blinded to placebo or tenofovir arm.

Results: T cell responses were detected in 1/18 tenofovir and 2/13 placebo participants at the high gel use visit. Responses were of low magnitude (between 60 and 100 SFU/106 PBMC), and directed at peptide pools from HIV Gag, Pol, Nef and Env. T cell responses were not detected at the exit visit. These data suggest that HIV-specific responses are infrequently detected in blood in uninfected participants from a clinical trial of a vaginal microbicide, and where present, are of low magnitude and transient.

Conclusion: Magnitude and timing of viral exposure may account for differences in detecting systemic T cell responses between preclinical studies in non-human primates and a human clinical trial of a vaginal microbicide.

P09.19 LB

Engineering SIV-Resistant Macaque Hematopoietic Stem Cells and CD4+ T Cells with CCR5-Specific Zinc-Finger Nucleases

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Background: CCR5 is the major HIV co-receptor, and individuals homozygous for a 32-bp deletion in CCR5 are resistant to infection by CCR5-tropic HIV-1. Zinc finger nuclease (ZFN) technology is a class of engineered DNA-binding proteins facilitating targeted genome editing by binding to a user-specific locus and causing a double-strand break in the region of interest. As a result, the gene of interest targeted by ZFN cleavage is disrupted. We investigated the ability of a Ccr5 gene-specific ZFN to establish SIV-resistant CD4+ T cells and hematopoietic progenitor cells isolated from macaques.

Methods: Bone marrow and blood samples were obtained from ten naïve uninfected rhesus macaques. Immunomagnetic enrichments were performed to isolate hematopoietic stem/progenitor cells and CD4+ T cells using magnetic-beads. Purified cells were nucleofected with RNAs encoding for a Ccr5-specific ZFNs.

Results: We successfully engineered CCR5-modified macaque CD4+ T lymphocytes that were resistant to in vitro infection with SIVmac239, SIVmac251, and CCR5-tropism SIVagm. CD4+ T lymphocytes that incorporated RNAs encoding for the Ccr5-specific set of ZFNs marked resistance to SIV infection as showed by lack of p27 expression in culture supernatants. Infection of non-modified CD4+ T lymphocytes led to significant p27 production from day 5 post-infection with a pick at day 7 post-infection. We developed and optimized the conditions for proper isolation, expansion and in vitro differentiation of macaque hematopoietic stem cells to be used for the generation of SIV-resistant macaque hematopoietic stem cells using our ZFNs. We successfully engineered CD34+ hematopoietic stem cells using nucleofection of RNAs encoding for a Ccr5-specific ZFNs.

Conclusion: We demonstrated the feasibility of using ZFN technology to establish CD4+ and hematopoietic stem cells resistant to SIV infection in macaque model. The generation of a nonhuman primate model using this modern molecular-based strategy might significantly help in the design of new therapies to prevent viral infection in human.

Topic 10: Social/Ethical/Access/Regulatory Issues

P10.01

Dealing with Research Participants' Complaints in HIV/ AIDS Prevention Studies: Experiences from Zimbabwe

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Background: The Medical Research Council of Zimbabwe (MRCZ) houses the National Ethics Committee which safeguards the rights, safety and well-being of all research participants. Research participants in AIDS Prevention Trials, including HIV/ AIDS vaccine trials, do from time to time have complaints during research implementation. The National Ethics Committee takes these complaints seriously and works tirelessly to address all complaints that research participants report to them in order to continuously improve the ethical conduct of AIDS research. The research community as a whole suffers when even a few investigators ignore the basic principles of ethics.

Methods: We ensured that all research participants in Zimbabwe are well informed of the National Ethics Committee`s complaint procedure. Adequate information on how to lodge a complaint is supplied upon request. Complaints are listed with all relevant details and are investigated fully by the Council. Depending on each complaint, urgent meetings with investigators and study staff are done. Urgent for-cause site inspections, interviews with study participants, impromptu meetings with Community Advisory Board members and the communities are also done. Community Research Ethics Awareness Outreaches are conducted periodically and annual Research Ethics Forums are held for researchers and participants to bridge the gap between them

Results: The MRCZ managed to address all participants complaints reported in 2011. The number of for-cause site inspections for HIV/AIDS Prevention Trials increased from 2 in 2010 to 8 in 2011. Complaints included participants spending longer times at a research site than the time written on the informed consent, insufficient food given at research site, laboratory results not being issued on time as promised, confidentiality not being maintained and insufficient bus fare reimbursements being given

Conclusion: A functional system to handle complaints from research participants helps to improve the ethical conduct of research. Experiences from the Medical Research Council of Zimbabwe will be shared.

P10.02

Protection of Human Research Participants Through Routine Inspection of Ongoing HIV Prevention and Treatment Clinical Trials:Experiences from Zimbabwe

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Background: The Medical Research Council of Zimbabwe (MRCZ) is the National Ethics Committee in Zimbabwe. MRCZ was established in 1974 under the Research Act of 1959 and Government Notice No. 225 of 1974 to promote and co-ordinate health research in Zimbabwe. The main mandate of MRCZ is the scientific review, ethics approval and oversight of all ongoing medical research studies in Zimbabwe inspection of all on going research studies involving humans using an established Human Protection System. Routine inspections and 'for cause' inspections are carried out to ensure utmost protection of research participants and to ensure protocol adherence and ICH-GCP compliance. HIV prevention and HIV drug treatment clinical trials receive more detailed attention during inspections as they are delicate and complicated.

Methods: During the inspection process the following aspects are checked on and thoroughly reviewed: initial and continuing MRCZ approval letters, protocol version at study site, National Drug Regulatory Body approval in case of drug clinical trials, valid continuing review approvals, staff qualifications, staff registration with professional bodies and relevant experience, evidence of research ethics and GCP training, data storage, signed and dated participant consent forms, external monitors reports and recommendations, approved study data collection tools, procedure rooms and interviews study participants. MRCZ adherence to Local and International Research Ethics Guidelines and offers research ethics and GCP training courses to local researchers.

Results: A recent development has been proactive requests from researchers for research ethics and GCP training, an indication of the increased awareness amongst researchers in Zimbabwe of the need for this training.

Conclusion: There has been significant improvement in protection of research participants, protocol adherence and ICH-GCP compliance by researchers as a result of the increased inspection of all ongoing research studies in Zimbabwe. More and more researchers are now aware of international research

Topic 10: Social/Ethical/Access/Regulatory Issues

P10.03

Meta-Analysis of Correlates of HPV Vaccine Acceptability Among Men: Supporting Vaccine Implementation Science

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Background: Understanding ubiquitous research-to-practice gaps in uptake of adult vaccines, particularly those for sexually transmitted infections (e.g., suboptimal human papillomavirus [HPV] vaccine uptake among men in the US), may provide evidence to support the successful dissemination of future HIV vaccines. To this end, we assessed rates of HPV vaccine acceptability and factors correlated with HPV vaccine acceptability among men.

Methods: We used a comprehensive search strategy across multiple electronic databases to locate studies that examined rates and/or correlates of HPV vaccine acceptability. The search strategy had no date or language restrictions. Search keywords included vaccine, acceptability and all terms for human papillomavirus. We calculated mean HPV vaccine acceptability scores across studies. We conducted meta-analysis using a random effects model on cross-sectional studies reporting correlates of HPV vaccine acceptability. All studies were assessed for risk of bias.

Results: Of 301 identified studies, 22 met inclusion criteria. Across 18 studies (n=7787), weighted mean HPV vaccine acceptability = 52.3 (SD 17.5) (100-point scale). HPV vaccine acceptability was significantly higher among gay/bisexual/MSM (65.1, SD 15.1) versus heterosexual men (47.3, SD 14.7). Among 11 studies (n=4064) included in meta-analyses, perceived HPV vaccine benefits and doctor recommendation had medium effect sizes, and the following factors had small effect sizes on HPV vaccine acceptability: anticipatory regret, perceived effectiveness, fear of side effects, perceived partner support for vaccination, perceived susceptibility to HPV, number of lifetime sexual partners, having a current sex partner, Hepatitis B vaccine uptake, smoking cigarettes, HPV awareness, knowledge and non-white ethnicity.

Conclusion: Public health campaigns tailored for men that promote positive HPV vaccine attitudes, HPV knowledge and risk awareness, and healthcare provider education, may support HPV vaccine acceptability for men; these factors may be instructive in planning for future HIV vaccine dissemination. Future investigations employing rigorous designs, including intervention studies, are needed to support effective vaccine promotion among men.

P10.04

Global Implementation of Good Participatory Practice Guidelines for Biomedical HIV Prevention Research: Charting Progress and Setting Milestones

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Background: In 2007, UNAIDS and AVAC developed the Good Participatory Practice (GPP) Guidelines with the aim of ensuring community and stakeholder engagement in HIV prevention research. The second edition, launched in 2011, provides the first standardized framework for GPP compliance. Five years after initial publication, it is possible and necessary to evaluate milestones in adoption of the guidelines at trial site, sponsor, country, and international levels in order to gauge progress and next steps.

Methods: A systematic review of 1) articles citing or mentioning GPP; 2) GPP-specific trainings requested and/or carried out by AVAC partners; 3) national and international forums in which GPP was mentioned, adopted or presented shows that GPP is gaining currency and relevance as key in guiding not only prevention research but all research involving human subjects.

Results: Support for and awareness of GPP have increased dramatically over the past five years. There is, however, a continued need for technical support for implementation. Common gaps in practice included low levels of documentation of engagement activities, planning around possible contentious issues and trial results, and stakeholder input in trial protocols. National level implementation activities provided initial steps in requirement of GPP. For example, GPP was presented to US President Obama's Commission for Bioethics, and subsequently cited as recommendation for community engagement in the Commission's official report. This effort secured international recognition and expanded relevance beyond HIV prevention research. Additional action is necessary for requirement and monitoring of practices.

Conclusion: While stakeholder engagement has long been accepted practice in HIV prevention research, findings from multiple implementation processes suggest the value and power of applying a systematic framework to practices. Awareness of GPP has increased, however meaningful implementation within and outside of HIV prevention research necessitates sustained commitments, new resources and action on the part of national, international and community-level stakeholders.

Topic 10: Social/Ethical/Access/Regulatory Issues

P10.05

Willingness to Participate in Vaccine Trials Among MSM in São Paulo, Brazil

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Background: An effective HIV vaccine still poses a great challenge to research scientists. Among the most vulnerable populations to HIV infection in Europe, United States and Latin America are men who have sex with men (MSM), potential volunteers for HIV vaccine research. We have asked MSM from a large LA city about their willingness to participate (WTP) in HIV vaccine trials.

Methods: A survey of men who attend MSM-identified venues in downtown São Paulo was carried out between November 2011 and January 2012. Time-location sampling approach was used. During sampling events, interviewers approached and asked men to respond a brief eligibility interview and consecutively applied a questionnaire to men who referred having ever anal or oral sex with men or transvestite. Here we analyze WTP in hypothetical HIV preventative vaccine trials.

Results: 1217 MSM were interviewed (missing data=3), 54,6% would accept participating, 35,3% would deny, and 9,9% stated they were unsure/maybe/depending on more information or assurances before deciding whether to participate in vaccine trials. WTP in HIV vaccine trial was similar across age groups (p=.450) and race/ethnicity (p=.534). There was no patterned difference among those who self-identified as homosexual, bisexual or transgender (p=.542) as well as among those who would have sex with an HIV-infected person, the size of their HIV-infected network (p=.251). Although not significant (p=0.06) a trend was suggested with WTP and the level of education of the head of the household, as years of education increased greater WTP.

Conclusion: Findings indicate that, despite the lack of success in recent phase II/III trials, there is substantial WTP in HIV vaccine studies, expressing the hope for an effective vaccine retain a pool of potential volunteers among the most vulnerable population. Subsequent analysis should focus on association with behavioral aspects, as risk behaviors, experience of discrimination due to sexuality and WTP.

P10.06

A Case Study of a National Ethics Committee:- Challenges in Regulating HIV Prevention Research in Zimbabwe

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Background: As efforts in search of effective HIV prevention strategies intensify, studies have become exceedingly complex, posing several regulatory challenges to most African Ethics Committees, the MRCZ included. Challenges faced include: Researchers not being clear on how to operate in compliance to local and sponsor country regulations; i.e. when a host country ethics committee has issued a decision on an issue concerning rights, safety and well being of participants while an overseas ethics committee has not. Prevention studies require frequent assessment, at MRCZ this has been hampered by limited resources. Ascertaining comprehension of these complex studies by participants and ensuring they receive new information promptly has also been a challenge. MRCZ has taken several measures to address regulatory challenges faced.

Methods: Routine, for- cause and passive inspections of ongoing approved research studies have been done. During these, participant comprehension checklists used at enrollment were audited and interviews with participants held to assess study knowledge levels. MRCZ has also set up a database to record types of inspections, number of Serious Adverse Events and protocol deviations

Results: From year 2000, MRCZ has actively and passively inspected about 1000 studies of these 5 were for- cause. MRCZ has also trained 340 research staff and 15 members of Council in Research Ethics, GCP and National Research Guidelines to build capacity. Due to this there has been: - Adoption of a harmonized review process; A decrease in for- cause inspections and an improvement in study documentation. All initial and continuing review submissions now include GCP certificates of study staff as well as participant comprehension checklists. 90% of interviewed participants show understanding of research studies they are involved in.

Conclusion: Regulatory bodies should consolidate efforts in understanding the complex and dynamic HIV research arena and in ensuring that research is carried out in a well regulated arena.

Topic 10: Social/Ethical/Access/Regulatory Issues

P10.07

Human Subject Protection and Ethical Review of Research Protocol

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Background: The purpose of this project was to train health personnel, researchers and IRB members to design protocols with ethical integrity and to approve the conduct of protocols with scientific merit. The burden of TB, HIV and Malaria is heavier in sub - Saharan Africa; hence there are calls for competent review of ethical processes to ensure that research proposals and protocols comply with international standards

Methods: Sixty nine medical personnel including Doctors, Nurses, Biomedical Scientists, and Pharmacists, legal officer, clergy, community representative and Researchers were trained on Human subject protection. Participants were trained on Basic principles and international guidelines on bioethics, Basics of research and clinical trials, Ethical review of research protocols, Risk, benefit and inducement. Other slides that were presented included: Standard of care and prevention, research protocol, standard of care, Good participatory practices, Informed consent, HIV treatment, prevention research and monitoring of research. Pre and Post test was used to evaluation participants' performance.

Results: The 4 day workshop conducted at Mainland Hospital Yaba, Lagos, Nigeria, from 28th June to 1st July, 2011, adopted a participatory approach including brainstorming, group discussion, slide presentations and case studies. The pre and post-test evaluation indicated that participants' knowledge improved significantly in conduct of ethically sound research (45.3% vs 65.0%; P=0.001).

Conclusion: From our experience training and retraining of health personnel and researchers would increase their knowledge and skill to conduct research that is ethically regulated and of international standard. IRB members would improve their ability to understand the operations of research ethics and develop adequate confidence in reviewing research protocols and monitor research activities

P10.08

Knowledge, Attitude and Practice of Safe Sex Among Men Who Have Sex with Men (MSM) in Nigeria

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Background: The success of the antiretroviral drugs in the late 90s resulted in increase sexual risk-taking behaviors among MSM and "business as usual" attitude to sex which has resulted in the high prevalence of HIV infection. We therefore studied the knowledge, Attitude and Practice of safe sexual practices among Men who have sex with men (MSM)

Methods: Self administered open ended questionnaire with four sections: Biodata, knowledge, Attitude and practice of safe sexual practices were administered. 8 MSM communities were selected using stratified sampling method and a key opinion leader was selected per community; the KOLs administered the questionnaire to a total of 1600 MSM (200) per community

Results: 1600 participants were recruited into this survey and all were MSM (100%). 17% were aged 18-20years, 65% were between the age 21-29years while 10% were aged 30-40 years and 8% were over 40years of age. 96% of our respondents had good knowledge of condom use while 39% were informed about the advantage of lubricants for anal sex. 67% admitted to inconsistent use of condom. All our participants have been involved in 3 casual relationships in the last 12months. 78% had unprotected anal sex in the last 3 months with and 88% of them were between the 18-29 years of age. 64% had oral sex in the last 3 months and 36% of them were between 30-40 years.

43% of our participants take alcoholic beverages. 11% smoke cigarette.Only 38% of our participants knew their H.I.V status

Conclusion: MSM have good knowledge of condom use but behavioral change is low. These findings show that more HIV counseling/testing centers need to be created for MSM. Lots of MSM need to be reached with intervention to improve knowledge of risky practices associated with high risk men, reduce high-risk sexual practices, reduce barriers to HIV/STI detection and treatment

Topic 10: Social/Ethical/Access/Regulatory Issues

P10.09

Willingness to Participate in HIV Vaccine Efficacy Trials in a Population of Fishing Communities, Uganda

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Background: With a number of preventive HIV vaccine candidates under pre-clinical and early-phase human studies, it is critical to intensify efforts to identify and prepare populations for potential efficacy trials. Willingness to participate (WTP) in HIV vaccine trials in most at risk populations for HIV infection is unknown especially in Sub-Saharan Africa. We assessed WTP in a potential HIV-1 vaccine efficacy trial population of fishing communities in Uganda.

Methods: A community-representative random sample of 2,200 individuals, 18-49 years was selected at baseline from 8 fishing communities around Lake Victoria. After consent, data on HIV risk behaviors and WTP in future HIV vaccine trials were collected using a semi-structured questionnaire; venous blood was collected for HIV serology using rapid HIV tests as per the Uganda National algorithm.

Results: Fifty percent of respondents were females and a majority (91%) had attained some level of education. Almost all respondents, 89% (1,953/2,192) expressed WTP in future HIV vaccine trials. Males were more willing to participate than females ($p<0.01$). WTP was associated with awareness of ongoing HIV vaccine research ($p<0.01$). Unwillingness to participate was attributed mainly to perceived fear of side effects (42%) and fear that the study vaccine may cause HIV/AIDS (26.3%). The overall HIV prevalence was 26.7%, lower among those who responded positively to WTP (26.8%) than those who did not (27.7%).

Conclusion: WTP in HIV vaccine trials in this population is high but extensive education on HIV vaccine safety particularly on vaccine induced side effects and false positivity will be required in preparation for these trials. Because of the high HIV prevalence, this population should be targeted for future HIV vaccine trials.

P10.10

Funding the Critical Path from Research to Reality: Sustaining HIV Vaccine R&D Amidst Economic Uncertainty

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Background: Since 2004, the HIV Vaccines and Microbicides Resource Tracking Working Group has employed a comprehensive methodology to track trends in R&D investments and expenditures for biomedical HIV prevention options, including HIV vaccines, microbicides, pre-exposure prophylaxis (PrEP), treatment as prevention, adult voluntary medical male circumcision, female condoms, HSV-2 prevention and vertical transmission prevention. Such monitoring is critical to identifying trends in investment and providing a fact base for policy advocacy on R&D spending levels and allocations.

Methods: To estimate annual investment in HIV vaccine R&D, data were collected from government agencies, nonprofit research organizations, foundations and pharmaceutical/biotechnology companies on annual disbursements for product development, clinical trials and trial preparation, community education and policy advocacy efforts.

Results: Preliminary estimates suggest that public and philanthropic funding for HIV vaccine research has been experiencing increased stress. As funding priorities shifted within governments and major donors revised their R&D grant strategies, disbursements for research in 2011 were often flat-lined or decreased. While the biotechnology industry continued their R&D efforts, companies increasingly focused on forming product development partnerships to ensure future funding and relied on grants from public sector sources to continue their clinical trials.

Conclusion: 2011 saw continued progress toward the development of a safe and effective HIV vaccine. With the discovery of antibodies predicting the risk of HIV infection, the start of large-scale follow-on trials and the initiation of HIV vaccine R&D programs in more countries around the world, there has been real progress on the path from research to reality. However, funding to pursue all this promise is highly uncertain. Sustained resources will be essential to assure that trials now underway can proceed, and increased expenditures will be similarly essential for the successive clinical phases of the most promising vaccine candidates.

Topic 10: Social/Ethical/Access/Regulatory Issues

P10.11

Training Trial Staff in the Use of a Mixed Method Assessment of Understanding Tool in HIV Vaccine Trials

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Background: Volunteer understanding of trial concepts is a critical part of informed consent in clinical trials. Standard practice is to use closed-ended true/false forms to assess understanding; however such tools may overestimate understanding and most likely measure memory only. Open ended tools, such as scenarios, may be preferable. Following pilot studies that compared various methods of assessing understanding, a mixed method tool was developed by HAVEG, IAVI and partners. The tool employs both True/False questions for simpler concepts and open-ended scenarios for complex concepts.

Methods: Trainings on use and implementation of the mixed method tool were conducted with doctors and counselors from IAVI partner research centers in Kenya, Uganda and Rwanda. Their understanding of all informed consent concepts was assessed, and practice sessions with past volunteers and other community staff with similar profiles to actual trial volunteers were conducted.

Results: Initially the training participants did not see the benefit of the new tool, and expressed reservations about cost, skill, screen outs and time required to implement it.

However, following exposure to the tool, the participants felt the tool improves interaction between staff and volunteers, better evaluates understanding and identifies challenging concepts. They reported that scenarios bring to light unfounded assumptions about volunteer understanding or lack thereof.

It also became apparent that the mixed methods tool requires staff to have much better understanding of trial concepts themselves.

"Volunteers" also seemed to prefer the tool as it offered them an opportunity to demonstrate their understanding.

Conclusion: Training significantly increases support for the mixed method tool reflecting the importance capacity building. Further, staff responses suggest mixed method tools of assessing understanding, not only provide better assessment of concepts, but may be preferred by trial staff and volunteers.

P10.12

Exploring Research Participants' Perceptions and Comprehension of the Informed Consent Process in a Pre-exposure HIV Prevention Study: A Case Study

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Background: Ensuring informed consent is a complicated component of clinical trials particularly with HIV prevention trials conducted in resource-limited settings. An inherent challenge of the informed consent process for HIV prevention studies is making sure trial participants understand that their participation does not increase exposure to HIV. Participants need to comprehend that partaking in such trials does not necessarily protect them from HIV. It is important to continuously monitor the informed consent process for clinical trials with view to improving the procedure.

Methods: Between June and September 2011, gender-specific in-depth interviews were held with interviewees who had been purposively selected from female participants who had exited a vaginal HIV prevention study. An interview guide was used to elicit views around the informed consent process. Discussions were conducted and audio-recorded. Audio-recorded data were transcribed, translated verbatim into English, coded using NVivo 8 and analysed using grounded theory principles.

Results: Twenty interviewees were held. Key information about the study was given as participants articulated study aims well. The informed consent process had been rushed and participants had not had enough time to decide and consult. Due to both excitement and anxiety, participants felt pressured to sign consent forms before comprehending some aspects of the study. Some found it difficult to ask questions. Data suggested that both the study procedure and duration had not been fully explained. Mixed feelings on male partner involvement in decision-making around study participation existed, with some feeling that spouses should have been involved and others stating that partner consultation did not matter.

Conclusion: This study elicited some of the issues that characterise the informed consent process for clinical trials conducted in resource-limited settings. It highlighted the need for researchers' ingenuity in order to come up with strategies that tailor the informed process to suit specific needs and circumstances of individual participants.

Topic 10: Social/Ethical/Access/Regulatory Issues

P10.13 LB

Update on Policy Change Proposal to End Institutional Biosafety Committee (IBC) Review of rDNA Vaccine Clinical Trials

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Background: The HIV vaccine regimen showing partial efficacy in the RV 144 study included a canarypox-vectored vaccine (ALVAC). The ongoing HIV vaccine efficacy trial, HVTN 505, involves two gene-based vaccines developed by VRC/NIAID/NIH: plasmid DNA and adenoviral-vectored vaccines. The "NIH Guidelines for Research Involving Recombinant DNA Molecules," established in 1976, have evolved over time. Current NIH policy is that every clinical trial of rDNA vaccines must have IBC reviews. The primary role of the IBC is to assess risk to public health and the environment.

Methods: The NIAID Barriers to Clinical Research project identified repetitive IBC review of gene-based vaccines as a barrier. For example, in the last 10 years >100 local IBC reviews have been conducted for the VRC HIV vaccines. IBC reviews for ALVAC vaccines remain a requirement for NIH funding despite completion of a Phase III clinical trial, >20 years of human experience with ALVAC vaccines and widespread community-based use of USDA-approved canarypox-vectored veterinary vaccines, which have not indicated a risk to public health or the environment.

Results: The NIAID policy change proposal has been considered by a Recombinant DNA Advisory Committee (RAC) working group. Policy change options were discussed at the September 2011, December 2011 and March 2012 RAC meetings. The RAC proposal will be published in the Federal Register for public comments.

Conclusion: Repetitive IBC reviews of rDNA vaccine clinical trials divert time and attention of IBC expertise from truly novel agents and incur a cost to NIH with no added safety benefit. NIAID proposes that IBC review is no longer needed for clinical trials of non-transmissible rDNA vaccines because there are no unique biosafety concerns for this class of vaccines based on their recombinant DNA nature. The IBC specific roles are not applicable for rDNA vaccine protocols and are fulfilled through other regulatory oversight including the FDA.

P10.14 LB

Does AID Vaccine Research Offer Fresh Messages for Public Health Campaigns? A Review of Public Health Communication Related to HIV AIDS In Uganda

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Background: HIV AIDS is a complex scientific phenomenon of our time. Ultimately however, HIV AIDS is a public health matter of unparalleled concern . This is due to the social and economic dimension of the HIV AIDS epidemic in the public domain. Public health communication is an integral part and key instrument employed by national health systems worldwide, in the management of public health.

Methods: By quantitative and qualitative method of data analysis. This paper investigates t he evolution of public health communication in Uganda related to HIV AIDS in a period covering over two decades. A period that initially placed Uganda as model country in the fight against HIV AIDS globally, as result of the country's success in decreasing HIV AIDS prevalence rates at national level. It also examines the likely factors behind the recent resurgence in new infections and prevalence rates.

Results: This paper postulate, giving empirical evidence, that key to the initial success of the fight against HIV AIDS in Uganda were the novelty of public health messages in the face of a fatal disease hitherto unknown by the public, and the means of their delivery. It argues that with the passage of time and reduction in stigma associated with being an HIV AIDS patient, public health messages became over used and ineffective.

Conclusion: The paper explores possible sources of new public health messages and techniques of dissemination using traditional and new media. It probes whether the on-going research on AIDS Vaccine could generate new messages vital for public health campaigns, without giving a false impression of cure, a tendency that experts concur could lead to the rise in risky behavior in the public in some countries, as has been the case with the introduction of antiretroviral drugs and medical male circumcision.

Topic 11: T Cell Immunity

P11.01

Evaluation of the Dual IFNγ/IL-2 Fluorospot-Assay with Flow Cytometry For Detection of HLA-Restricted HIV-Specific T-Cell Responses in HIV Controllers

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Background: The IFNγ ELISpot assay is used widely for high through-put screening of HIV-specific responses in studies of HIV infection and vaccine studies. However, dual production of IFN/IL-2 and increased proliferative capacity may be associated with better natural control of HIV infection. We evaluated a novel fluorospot assay enabling the identification of dual IFNγ/IL-2 producing antigen-specific cells and compared it with intracellular cytokine staining by standard flow cytometry in individuals with natural control of HIV-infection.

Methods: PBMC from five untreated HIV-infected individuals were stimulated overnight with HIV peptides or controls in dual IFN/IL-2 pre-coated plates. Peptide arrays were specifically selected based on the individual HLA type. Secreted IFNγ and IL-2 were detected using fluorescent-conjugated antibodies. Fluorescent spots were enumerated on the iSpot AID reader. Responses were considered positive if >50 spots/million cells SFU after background subtraction.Positive responses were then evaluated by flow cytometry using the Gallios flow cytometer.

Results: Dual IL-2/IFNγproducing cells were detected to anti-CD3-stimulated PBMC from all patients. IFNγ responses alone were detected to 35 of 136 HLA-restricted peptides tested (median =73, 52-4190 SFU) across the 5 patients (1/19, 5/19, 7/28, 9/15 and 13/55 for each patient), while IL-2 responses were either low grade or undetectable for the majority of HIV peptides tested. Dual IFNγ/IL-2 producing HIV-specific T cells were not detected using the fluorospot assay. 24/35 peptides induced CD8 T cell-IFNγ production by flow cytometry.

Conclusion: HIV-specific mono-IFNγ responses were detected using the novel fluorospot assay. However limited HIV-specific dual IFNγ/IL-2 responses were detected in this patient group. A greater number of epitope-specific positive responses were detected in the fluorospot compared with flow cytometry suggesting the fluorospot may be more sensitive in detecting a greater breadth of epitope-specific T cell responses, and therefore better for screening purposes than flow cytometric methods.

P11.02

Early Changes in the CD8 T Cell Immunodominance Hierarchy in Primary HIV Infection Prior to Seroconversion

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Background: Identification of the earliest CD8 T-cell responses against HIV may help select critical viral targets for inclusion in an HIV vaccine. We describe changes to the earliest detected CD8 T-cell responses and changes in genetic sequence encoding targeted epitopes in an individual who presented with Fiebig stage II, clade C acute HIV-infection, 27 days after sexual transmission.

Methods: We examined HLA-restricted CD8 T-cell responses by IFNγ ELISpot and HIV Gag, Pol, Nef and Env sequence by 454 deep sequencing over 6 timepoints, from days 27-118 after HIV-transmission. HIV-specific IFNγ responses were detected against ten HIV epitopes in Gag, Nef and Env at day 27 post HIV-transmission when the patient's CD4 T-cell count was at a nadir of 224 cells/_l, the plasma HIV RNA >106 copies/ml and prior to any detectable p24 antibody.

Results: Immunodominant responses were detected to the HLA-B*07:02 restricted Env IIRRIRQGL (IL9) epitope and the B*07:02 Gag GPGHKARVL (GL9) epitope (>4000SFU). A detectable but weaker response was observed to HLA-B*08 Nef FLKEKGGL (FL8) epitope (1010SFU). These responses declined over subsequent timepoints to 470 and 1630SFU on day 118 coincident with a 2 log fall in the plasma viral load, a rise in the CD4 T cell count to 533 cells/µL and antibody seroconversion. The Nef FL8 became the immunodominant response after day 34. IFN_ responses broadened from 10 responses at day 27 to 23 responses by day 118. Analysis of Gag, Nef and Pol genes by 454 deep sequencing showed no evidence of escape within the targeted epitopes as a cause of their decline over time.

Conclusion: Early changes in the CD8 T-cell immunodominance hierarchy are apparent in acute HIV-infection prior to seroconversion, including early immunodominant targeting of Env epitopes. Subsequent broadening of the CD8 T-cell response was not associated with CD8 T-cell escape in this case.

P11.03

Gag-Specific Cellular Immunity Determines In Vitro Viral Inhibition and In Vivo Virologic Control Following SIV Challenges of Vaccinated Monkeys

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Background: A vaccine for HIV-1 would ideally block HIV-1 acquisition as well as durably control viral replication in breakthrough infections. A recently published study showed that optimal SIV vaccines can reduce SIV infection risk and setpoint viral loads following SIV challenges in rhesus monkeys, and that immunization with SIV Env was required for blocking acquisition of infection (Nature 2012 482:89-93). Here we investigate whether CD8+ T lymphocytes from these vaccinated rhesus monkeys mediate viral inhibition in vitro and whether these responses predict virologic control following SIV challenge.

Methods: PBMC from 23 monkeys that received DNA/MVA, MVA/MVA, Ad26/MVA, or MVA/Ad26 vaccines expressing SIVsmE543 Gag/Pol/Env were used in CD8+ T-cell-mediated in vitro viral inhibition assays. CD8-depleted PBMC were infected with SIVmac251, and viral inhibition was defined as the log reduction in p27 of cultures of CD8-depleted PBMC with and without CD8+ T lymphocytes. Viral inhibition was correlated with cellular immune responses and setpoint viral loads using Spearman rank-correlation tests.

Results: In vitro CD8+ T-cell-mediated viral inhibition prior to challenge correlated with Gag-specific ELISPOT (P=.002), total and central memory CD8+ (P<.001 for both), and total and central memory CD4+ responses (P=.002 and P=.001, respectively). A trend was observed with Gag-specific effector memory CD8+ T-cell responses (P=.014; P<.006 required for significance after multiple comparison adjustments). Viral inhibition did not correlate with Pol- or Env-specific cellular immune responses. Moreover, in vitro viral inhibition prior to challenge inversely correlated with in vivo setpoint viral loads following challenge (P=.014).

Conclusion: These data demonstrate for the first time that the in vitro viral inhibition assay following vaccination is a predictor of in vivo virologic control following infection. Furthermore, in vitro viral inhibition correlated with Gag-specific, but not Pol- or Env-specific, cellular immune responses. These data suggest the importance of including Gag in an HIV-1 vaccine in which virologic control is desired.

P11.04

Different Memory T Cell Phenotypes Are Elicited by Ad5 and Rare Adenoviruses

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Background: The anamnestic potential of memory T cells are pivotal components of the adaptive immune response. Analyzing memory T cells elicited by different vaccine vectors is crucial for the identification of novel vaccine modalities against HIV.

Methods: 6-8 week old C57BL6 mice were used for these experiments. Mice were immunized intramuscularly with 10^10 VP in PBS. The different adenoviral vaccines (Ad5, Ad26, Ad35, Ad48) expressed the glycoprotein (GP) derived from lymphocytic choriomeningitis virus (LCMV). For challenge studies, the mice were infected with 2x10^6 PFU of LCMV Cl-13 intravenously. Early and late CD4 and CD8 T cell recall was measured by flow cytometry. Viral control was assessed by standard plaque assay on VERO cells.

Results: We have compared T cell memory phenotypes and immune protection elicited by common adenoviruses serotype 5 (Ad5) versus rare adenoviruses serotypes (Ad26, Ad35, and Ad48). For these comparative studies, we immunized mice with non-replicating adenovirus vectors expressing the lymphocytic choriomeningitis virus (LCMV) GP, and challenged with chronic LCMV Cl-13. Our comparative data show that Ad5-GP generates an increased magnitude of LCMV GP-specific CD8 T cell responses (compared to that generated by rare adenoviruses). However, GP-specific CD8 T cells elicited by Ad5-GP immunization express inhibitory PD-1, and produce reduced amounts of cytokines, suggesting qualitative defects in memory CD8 T cells. This unexpected expression of PD-1 may also reflect antigen-persistence of Ad5.

Conclusion: Memory T cells elicited by rare adenovirus-based vaccines results in greater T cell recall potential after viral challenge, and higher functionality compared to vaccination with Ad5. These data suggest that alternative serotype Ad vectors may offer substantial benefits over Ad5 as vaccine vectors in eliciting optimal T cell memory to chronic viruses such as HIV, HBV or HCV.

Topic 11: T Cell Immunity

P11.05

Loading Dendritic Cells from HIV-1 Infected Patients with PLA-p24 Nanoparticles or MVA Expressing HIV Genes Induces HIV-1-Specific T Cell Responses

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Background: The evaluation of the interaction of new immunogens with dendritic cells is important for new vaccine strategies. We used polylactic acid (PLA) colloidal biodegradable particles, coated with HIV Gag antigens (p24), and Modified Vaccinia Ankara (MVA) expressing Gag or Tat, Nef and Rev in an ex vivo model of human monocyte-derived dendritic cells (MDDC) to compare two different loading strategies,either viral or synthetic derived.

Methods: We have assessed the interaction of PLA nanoparticles bearing p24 (PLA-p24) or MVA expressing Gag or Tat, Nef and Rev with MDDC of HIV-1 infected patients and the capacity of these cells to process and present the HIV-1 antigens to autologous human T cells from HIV-1-infected patients.

Results: PLA-nanoparticles were captured by 98% of MDDC from HIV-1 infected patients, without deleterious effects for the cells. Capture of PLA-p24 induced a slight degree of MDDC maturation, cytokine and chemokine secretion and migration towards a gradient of CCL19 chemokine. After complete maturation induction of PLA-p24-pulsed MDDC, maximal expression of maturation markers was observed, together with a strong migration towards a gradient of CCL19 chemokine. PLA-p24-loaded MDDC were able to induce HIV-specific T cell proliferation (two-fold higher for CD4+ than CD8+) and cytokine secretion (IFN-γ and IL-2). Upon exposure to MVA-gag, MDDC produced cytokines and chemokines and maintained their capacity to migrate to a gradient of CCL19. MDDC infected with MVA-gag, MVA-gag trans-membrane and MVA-nef were able to induce HIV-specific CD8+ proliferation and secretion of IFN-γ, IL-2, IL-6 and TNF-α.

Conclusion: These findings support the use of both HIV antigens (PLA particles carrying HIV antigens or MVA expressing HIV genes) as anti-HIV vaccines.

P11.06

The Roles of HIV-1 Specific CD8+ T Cell Responses and HLA Class I Alleles on Viral Control and Viral Escape in HIV-1 Infected Thai Individuals

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Background: Knowledge about the role of specific HLA class I alleles, CD8+ T cell responses, and viral escape on viral control have not been well characterized in clade CRF01_AE and Asian ethnics.

Methods: 195 naïve HIV-1 CRF01_AE infected Thai individuals were screened for HIV-1 specific CD8+ T cell responses with a set of 413 OLPs of the HIV-1 proteome by IFN-gamma ELISpot assay. Novel epitopes were characterized by HLA restriction and fine epitope mapping. The association of epitopes and/or HLA alleles with low VL level and/or viral escape was analyzed.

Results: Thirty-three OLPs were identified as potential novel epitopes. A viral control epitope, RI10 (HIV-protease, previously described in HIV-1 B clade as -B*13 restricted) was found restricted by HLA-A*0203 in Thais. Interestingly, HLA-A*0203+ve patients with RI10 responders had a significantly lower VL than non-responders (p = 0.0167). This data may support the low VL from loss of viral fitness of RI10. Moreover, the patients exhibiting mutations in RI10 showed no ELISpot responses. Another known HLA-A*1101 epitope, AK11 (in Gag-p24) was also identified as a viral control epitope in this study. Of note, there was no significant mutation found in patients expressing A*1101. We also found a novel immunodominant epitope (29% response rate) restricted by HLA-Cw*0102: YI9 (in Gag-p24) which was associated with viral escape. Mutations at P2 (S278X), P4 (V280X) and P5 (S281G) impaired the Elispot responses, however the P2 anchor S278K mutation had the highest negative impact (p = 0.0002).

Conclusion: In HIV-1 CRF01_AE infected Thais we characterized three CD8 epitopes (RI10, AK11 and YI9) restricted by HLA-A*0203, -A*1101 and -Cw*0102, respectively. RI10 and AK11, but not YI9, were associated with lower VL and possible control of HIV. Further characterization of those possible novel epitopes is warranted.

P11.07

Rapid Evolution of HIV-1 to Functional CD8+ T-Cell Responses in Humanized BLT Mice

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Background: The newly developed humanized BLT (bone marrow, liver, thymus) mouse model holds great promise to facilitate the in vivo study of human immune responses. However, little data exists regarding the extent to which cellular immune responses in humanized BLT mice accurately reflect those seen in humans.

Methods: Multiple sets of humanized BLT mice reconstituted with distinct human tissues were infected with the HIV-1 molecular clone JR-CSF. Mice were then bled every two weeks to measure plasma viral loads, sequence evolution and cellular immune responses.

Results: During the acute phase of infection BLT mice rapidly mounted multiple HIV-1-specific CD8+ cellular immune responses against normally immunodominant human CD8 epitopes, with rapid and reproducible viral escape observed within epitopes that similarly tend to escape early in humans. CD8+ T cell responses and viral escape to these same epitopes was confirmed in mice reconstituted with distinct human tissue but expressing the same restricting HLA alleles. Importantly, in two independent groups of mice expressing HLA-B*57 we observed the rapid induction of CD8+ T-cell responses against a number of B*57-restricted responses at frequencies similar to those seen in humans, including the normally immunodominant B*57-IW9, -KF11 and -TW10 epitopes in Gag from which the virus failed to rapidly escape. As in humans, the presence of these conserved responses correlated with significantly greater control over early viral replication. Preliminary vaccine studies in BLT mice support the ability of conventional approaches to induce CD8+ T cell responses and suppress viral loads.

Conclusion: These studies indicate that the specificity, magnitude, and immunodominance patterns of human CD8+ T-cell responses in humanized BLT mice appear to closely reflect those of humans. These data support the potential of humanized BLT mice to significantly advance HIV-1 vaccine development, providing a critical new tool to complement the SIV infected macaque model.

P11.08

Increased Differentiation Associates with Decreased Polyfunctionality for HIV but Not CMV-Specific CD8+ T Cell Responses

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Background: The generation of polyfunctional CD8+ T cells, in response to vaccination or natural infection, has been associated with improved protective immunity. However, it remains unclear whether the maintenance of polyfunctionality is linked to particular phenotypic characteristics of the cell, such as the differentiation stage of memory T cells. The goal of this study was to investigate the relationship between the memory maturation stage and polyfunctional profiles of antigen-specific CD8+ T cells.

Methods: We analyzed the polyfunctionality of HIV-specific CD8+ T cells within different memory subpopulations in 20 ART-naïve HIV-1 infected individuals at approximately 34 weeks post-infection and compared them to CMV-specific CD8+ T cell responses. Memory subsets were distinguished based on CD45RO and CD27 expression levels, and four functions were assessed (CD107a, MIP-1β, TNFα and IFNγ).

Results: Our results show that polyfunctional abilities of HIV-specific CD8+ T cells differ according to their memory phenotype, where terminally-differentiated HIV-specific CD8+ T cells (CD45RO-CD27-) were mostly mono-functional (median 69% [IQR: 57-83]), producing predominantly CD107a or MIP-1β. Moreover, the proportion of HIV-specific mono-functional CD8+ T cells associated positively with the proportion of terminally-differentiated HIV-specific CD8+ T cells ($p=0.019$, $r=0.54$). On the other hand, HIV-specific early-differentiated cells of a central memory-like phenotype (CD45RO+CD27+) exhibited a higher proportion of cells positive for three or four functions ($p<0.001$) and a lower proportion of mono-functional cells (median 27% [IQR: 16-38], $p<0.001$) compared to terminally-differentiated cells. In contrast, CMV-specific CD8+ T cell polyfunctional capacities were similar across all memory subpopulations, with terminally and early-differentiated cells endowed with comparable polyfunctionality.

Conclusion: Overall, these data show that for HIV-specific responses, the memory differentiation stage can influence the polyfunctional properties of CD8+ T cells, suggesting that terminal differentiation of HIV-specific CD8+ T cells might be detrimental for viral control. These results may help in understanding phenotypic attributes related to effective T cell responses against HIV-1.

Topic 11: T Cell Immunity

P11.09

Cross-Clade CTL Recognitions for Clade B and A/E Viruses in A/E Virus-Infected Japanese Individuals

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Background: Cytotoxic T lymphocytes (CTLs) play an important role in the control of HIV-1. CTL responses to HIV-1 have been well studied in HIV-1 clade B-infected and clade C-infected individuals. However cross-clade CTL recognitions have not been well analyzed. In this study, we analyzed cross-clade CTL recognition for clade B and A/E viruses in A/E virus-infected Japanese individuals

Methods: PBMC samples were collected from chronically HIV-1 infected Japanese cohort in NCGM. Twenty-six clade A/E-infected individuals were analyzed by ELISPOT assay using the 11-mer overlapping peptides and then the responses of CTLs to these peptides was compared to those from 402 clade B-infected Japanese individuals. Thereafter CTL responses to each single peptide and to truncated peptides were evaluated by ELISPOT assay and intracellular cytokine staining (ICC) assay, respectively.

Results: Similar level of CTL responses to Gag, Pol and Nef were found in clade A/E-infected individuals as compared to that in clade B-infected ones. We identified 15 cross-clade CTL epitopes from 14 cocktails where the frequency of responders was high in clade A/E infected samples. The sequences of 7 epitopes were conserved between clade B and clade A/E viruses, whereas 8 epitopes showed different amino acid sequences between two viruses. In these 8 epitope regions, we confirmed cross-clade CTL recognition by ICC assay using clade A/E consensus sequence peptide.

Conclusion: Cross-clade CTLs were predominantly induced in clade A/E-infected individuals by clade B consensus sequence peptides in this study. Moreover, CTL responses were induced not only in conserved region but also in different sequence region between the 2 viruses, indicating that polymorphic sequence epitopes among clades can be also candidate for the target of CTL-based vaccines. Further analysis of cross-clade CTL recognition is needed for the widely applicable vaccine development.

P11.10

The Increased Sensitivity of CTLs Induced by Vaccinia Vector Is Developed as Intrinsic Feature In Vivo and Independent of Microenvironment In Vitro

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Background: DNA prime-vaccinia viral vector (rVV) boost has been widely used as an effective regimen for T cell vaccine. This regimen could efficiently enhance the potency of immunogen-specific T cell responses with minimizing responses to vectors. In addition, we and others found that the sensitivity for T cells responding to antigenic stimulation is elevated by this vaccination regimen. However, it remains unknown whether the increased sensitivity of CD8+ T cells is gained during the activation and differentiation as intrinsic property or is resulted from the influence of microenvironment

Methods: The magnitude, potency and sensitivity of T cell responses induced by different vaccine regimens were compared by using ELISpot and ICS. Purified CD8+ T cells from each vaccination regimen were mixed with splenocytes from naïve or mock rVV immunized mouse and determined for their sensitivities. Statistical analysis was performed by using Prism5.0 software

Results: As expected, the magnitude, potency and sensitivity of T cell responses induced by DNA prime-rVV boost is 20-, 15- and 100- fold higher than that in group with repeated DNA boost, respectively. Interestingly, the sensitivity of CD8+ T cells purified from each vaccination regimen group is comparable before and after the mixture with splenocytes from either naïve or mock rVV immunized mouse, suggesting that the removal of inflammatory microenvironment did not decrease the sensitivity of CD8+ T cell responses. Accordingly, the addition of vaccinia induced microenvironment to CD8+ T cells purified from DNA group does not enhance their sensitivity

Conclusion: Our results demonstrated that the increased sensitivity of CD8+ T cells by DNA prime-rVV boost regimen is intrinsic property and likely to be developed and gained during the activation and differentiation in vivo by vaccine, this effect is independent with inflammatory microenvironment in memory status. These findings have important implications for the design of new vaccine and immunization strategies

P11.11

The Recognition of HIV-1 Consensus Group M Gag and Nef Peptide Reagents in Mono- and Multi-clade Epidemics: Implications for HIV Vaccine Design

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Background: The high level of genetic diversity of HIV-1 poses a major challenge for global vaccine development. Vaccines based on centralized sequences would minimize genetic distances to multiple clades and potentially maximize cross-reactivity. Whether reactivity of these centralized peptide reagents differs in mono- and multi-clade epidemic is unknown.

Methods: In this study, full-length gag and nef gene sequences (from Cameroon, 50 and 54, respectively and South Africa, 23 and 19, respectively) were characterized. HIV-specific T-cell responses to group M consensus Gag and Nef peptide reagents were characterized at the peptide level using the IFN-γ ELISpot assay.

Results: Viruses from Cameron exhibited a large degree of genetic diversity; all subtypes were present excluding subtype C, with CRF02_AG being dominating (49%). Contrary, sequenced viruses from South Africa were all pure subtype C viruses. Despite the greater diversity of viral clades in the Cameroonian cohort, the genetic distances to the consensus M reagents was similar for both cohorts (11% and 15% for Gag and Nef, respectively). Whilst the magnitude and breadth of responses to Con M Gag and Nef did not differ significantly between the two cohorts, there was a greater frequency of responders in the Cameroonian cohort compared to South Africans (95% versus 82%, respectively). For the Cameroonian cohort, 75/182 (41%) of the consensus M peptides were targeted, while for the South African cohort 66/182 (36%) of the peptides were targeted, with the majority being recognized in only 1-2 participants (69% and 76%, respectively). Patterns of immunodominance and targeting, however, differed dramatically between the two cohorts, with only 36% and 39% of Gag and Nef peptides commonly recognized between the two cohorts.

Conclusion: Although similarities in total magnitude and breadth may be observed between different epidemics, patterns of immunodominance of centralized immunogens may be different which may have implications for vaccine development.

P11.12

Programmed Death-1(PD-1), a Correlate of Protection Against Disease Progression in HIV-1 Infected Long-Term Non-Progressors

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Background: Long-Term Non-Progressors (LTNP) control HIV-1 disease progression but correlates of control are not clear. T-cell activation and expression of Programmed Death-1 (PD-1), a marker of T-cell inhibition/exhaustion, have been suggested as markers of progression to AIDS. We assessed levels of T-cell activation and PD-1 in LTNP and Rapid progressors (RP).

Methods: We recruited 15 LTNP and 15 RP originally enrolled in the Entebbe Cohort in Uganda. All were ART naïve and 29 were women. HLADR, CD38 and PD-1 levels were assessed in CD4, CD8 and CD45RA T-cells by flow cytometry. HIV-1 disease progression markers: plasma lipopolysaccharide (LPS) levels, HIV-1 RNA viral load (VL), HIV-1 pro-viral DNA load (PVL) and CD4 counts at enrollment were quantified. Comparisons between groups were performed using the Mann-Whitney U test and correlations by Spearman's linear correlation coefficients.

Results: Activated (HLADR+CD38+) CD4+CD45RA+ were higher in the LTNP (median 0.64% for LTNP and 0.18% for RP, p=0.03). PD-1 expression in the CD4 and CD8 T-cell subsets was higher in the LTNP (CD4+PD-1+ median 39.6% for LTNP and 1.0% for RP, p=0.001; CD8+PD-1+ median 60.8% for LTNP and 13.5% for RP, p=0.003). VL (p=0.05), PVL (p=0.03), LPS (p=0.005) were higher in the RP and enrollment CD4 count (p=0.0002) was higher in the LTNP. VL, PVL and LPS positively associated with each other and all negatively associated with enrollment CD4 count. CD4+PD-1+ correlated negatively with VL (rs=-0.40, p=0.03), LPS (rs=-0.38, p=0.04) and positively with enrollment CD4 count (rs=0.36, p=0.05). CD4+CD45RA+HLADR+CD38+ correlated positively with enrollment CD4 counts (rs=0.38, p=0.04). Positive correlations were observed between CD4+CD45RA+/-HLADR+CD38+, CD8+CD45RA+HLADR+CD38+ and CD4+PD-1+ and CD8+PD-1+ T-cells.

Conclusion: Co-expression of PD-1 and activation markers was higher in the LTNP compared to the RP, contrary to other studies. PD-1 correlated with markers of protection against HIV-1 disease progression, suggesting a beneficial role for PD-1.

Topic 11: T Cell Immunity

P11.13

Early Presentation of HIV-1 KF11Gag and KK10Gag Protective Epitopes Facilitate Rapid CD8+ T Cell Activation and Killing of Virus Infected Cells

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Background: CD8+ T cells are major players for the antiviral immunity against HIV-1 through recognition of viral epitopes presented on the surface of infected cells. However, the kinetics and timing of HIV-1 epitope presentation remains poorly understood but nonetheless crucial for development of a successful CD8+ T cell based vaccine.

Methods: Epitope presentation and killing of virus infected cells was assessed in HIV-1 susceptible T cell lines, H9, U937, primary CD4+, and B cell lines expressing HLA-B*5701 or B*2705 by synchronized infection with HIV-1 or VSV-HIV-1 to determined the contribution of incoming particles to epitope presentation. Infected cells were co-cultured with CD8+ T cell lines specific for the epitopes KF11Gag, KK10Gag, KY9Pol and VL9Vpr. Kinetics of epitope presentation were monitored by the production of CD107b and MiP1ß in the co-culture at 0, 3, 6, 18 and 24 hours post-infection. In addition, killing of infected cells was determined in paralleled by the decrease of CD4+/p24 Gag+ cells

Results: We comprehensively studied the kinetics of antigen presentation of the KF11Gag and KK10Gag epitopes, restricted by protective HLA alleles B*5701 and B*2705, and compared these to KY9Pol and VL9Vpr epitopes, in a single cycle of virus replication. We observed differences in epitope presentation kinetics with early presentation within 3 hours post-infection, for KF11Gag, KK10Gag and KY9Pol epitopes, but only late presentation for VL9Vpr. In addition, we illustrate how early presentation relies on antigen processing from incoming virus, which correlates with rapid CD8+ T cell activation and clearance of virus-infected cells.

Conclusion: Our data strongly support the importance of identifying early-presented HIV-1 epitopes to eliminate infected cells before the release of new infectious viral particles.

P11.14

Control of HIV-1 by Multiple Immunodominant HIV-1-Specific CD8+ T Cells in HIV-1-Infected Japanese Individuals

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Background: Previous studies of the comprehensive analysis of HIV-1-specific CTL responses in Caucasian and African cohorts demonstrated the association of the CTL responses to HIV-1 Gag protein with the control of HIV-1 replication. However, such analysis in Asian cohorts has not been reported. In the present study, we performed the comprehensive analysis of $CD8^+$ T cell responses against 11-mer overlapping HIV-1 Nef, Gag, and Pol peptides in 401 chronically HIV-1 clade B-infected treatment-naive Japanese individuals.

Methods: The $CD8^+$ T cell responses to cocktails of the peptides were evaluated by measuring IFN-g-producing $CD8^+$T cells by using ELISPOT assay.

Results: To clarify CTLs which control HIV-1 infection in this cohort, we statistically analyzed differences of viral load and CD4 counts between responders to each peptide cocktail in each HLA^+ individuals and non-responders using two-tailed Mann-Whitney's test. We found that several HLA alleles were significantly correlated with low viral load and high CD4 counts in the responses to 5 Nef, 10 Gag, or 16 Pol cocktails. In these cocktails, we identified 2 Nef, 12 Gag and 7 Pol CTL epitopes restricted by 9 HLA alleles. The breadth of CTL responses to these epitopes was significantly associated with low viral load ($p=1.7x10^{-10}$) and high CD4 counts ($p=4.1x10^{-13}$). The total magnitude of responses to the epitopes was also significantly correlated with low viral load ($r=-0.30$, $p=1.8x10^{-9}$) and high CD4 counts ($r=0.37$, $p=5.0x10^{-14}$).

Conclusion: These results suggest that the CTL responses to these epitopes play an important role in the control of HIV-1 infection in chronically HIV-1-infected Japanese individuals.

POSTERS

P11.15

The Impact of HLA-Cw*12:02 on Control of HIV-1 Infection

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Background: Previous studies have demonstrated that higher HLA-C expression, which is determined through a single nucleotide polymorphism 35 kb upstream and the variation within the 3' untranslated region of the HLA-C locus, associate with slow progression of HIV-1-infected disease. Although HLA-C plays important roles in presenting antigens to CTLs or a ligand for inhibitory killer cell Ig-like receptors (KIR), the role of HLA-C-restricted CTL and NK cells in the control of HIV-1 is still unclear. Our recent study of chronically HIV-1 infected Japanese cohort showed that the HLA-B*52:01-Cw*12:02 haplotype was significantly associated with lower viral load. In this study, we investigated whether HLA-Cw*12:02-restricted CTLs or NK cells via KIR have a significant impact on viraemic control.

Methods: We sequenced Pol, Gag and Nef from 400 chronically HIV-1 clade B-infected treatment-naïve Japanese individuals and then analyzed amino acid polymorphisms associated with HLA-B*52:01-Cw*12:02 haplotype using Fisher's exact test. Next we performed intracellular IFNg staining or IFNg ELISPOT assay to detect CTL responses to the peptides including those polymorphisms.

Results: We found 9 amino acid polymorphisms significantly associated with HLA-B*52:01-Cw*12:02 haplotype ($p<0.002$ $q<0.2$). By using ICC assay, we identified 2 Cw*12:02-restricted CTL epitopes and 4 B*52:01-restricted ones. Four Cw*12:02-restricted CTL epitopes including previously reported ones were analyzed to investigate the effect of Cw*12:02-restricted CTLs on control of HIV-1. No significant correlation between the responses to these Cw*12:02-restricted epitopes and viral load was found in chronically HIV-1 infected Cw*12:02 positive Japanese individuals.

Conclusion: Those results showed that HLA-Cw*12:02-restrected CTLs have no effect on control of HIV-1 and suggested that HLA-B*52:01-restrected CTLs or Cw*12:02-resticted NK cells control HIV-1 viraemia in Japanese cohort.

P11.16

Detection of HIV T-Cell Responses with Polifunctionality and High Plasma Levels of the B-Chemokine MDC in Exposed HIV-Seronegative Individuals (ESN)

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Background: The mechanisms of protection from infection in ESN individuals are largely undefined and likely multifactorial. Our aim was to analyze HIV-specific T-cell responses and plasma cytokine levels in ESN and their respective HIV partners (HIV-P) in comparison with HIV chronically infected subjects (C) and healthy donors (HD).

Methods: Plasma levels of 37 cytokines were measured by Luminex technology in: 10 HD, 9 ESN and their HIV-P, and 12 C. All HIV patients were off-HAART. Frozen PBMCs from the same individuals were used to determine HIV-specific T-cell responses by IFN-g ELISPOT. Also, production of different cytokines was evaluated in certain samples by intracellular staining (ICS). Data was compared inter- and intra-groups and correlated to viral load (VL) and CD4 T cell counts, using parametric and non-parametric statistics.

Results: Elispot responses were evaluated in 6 of the serodiscordant couples. Two ESN showed T-cell responses against Env or Gag. ESN had a higher proportion of HIV-specific bifunctional and trifunctional cells in comparison with their HIV-P partners. HIV-P had Nef and Env T-cell responses of significant higher magnitudes compared to C. Macrophage-derived chemokine (MDC), characterized to have HIV-suppressive activities, was the only soluble marker significantly elevated in plasma of ESN in comparison with HD. Interestingly, MDC showed a positive correlation with CD4 T-cell count among HIV-P. HIV-P compared to C showed minor levels of the inflammation biomarker sCD40L associated with AIDS defining illness, and in this group VL was positively correlated with IP-10 and IL-10, whereas TNF-a correlated with VL in C.

Conclusion: Both innate and specific immune components may be acting in the resistance to HIV infection in ESN. On the other hand, the characteristics of the immune response generated in their partners can also play a role in the viral pressure generated affecting the viral population to be transmitted.

Topic 11: T Cell Immunity

P11.17

Recombinant DNA/MVA/ChAdV-63-Elicited T Cells Specific for Conserved Regions of the HIV-1 Proteome Recognize HIV-1 Infected Cells and Suppress HIV-1

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Background: Currently, immunogenicity assessments of candidate HIV-1 vaccines in human clinical trials rely on the use of peptides for sensitization of target cells. However, employment of HIV-1-infected cells provides more informative and relevant in vitro readouts of HIV-1 recognition.

Methods: A viral suppression assay (VSA) was developed to assess CD8+ T cell responses to infected autologous CD4+ targets using HIV-1 p24 levels as measured by flow cytometry and ELISA. In parallel, we also investigated live virus infected cells for re-stimulation of vaccine elicited T cells in an IFN-γ ELISpot assay.

Results: HIV-1 infection kinetics is influenced by the multiplicity of infection (MOI) used to infect autologous CD4+ cells, with rapid kinetics observed at higher MOIs. For HIV-1 BaL optimal infection of autologous CD4+ cells was achieved at MOI of 0.05. In the VSA, HIV-1 BaL virus replication was shown to be sensitive to the chemokines MIP-1α and RANTES added in the absence of effector cells. Pre-activated effector cells indicated increased non-specific background suppression in healthy controls, which was reduced following a prolonged rest before co-culture with autologous CD4+ targets, but led to marked proliferation of the CD8+ T cell effectors. Preliminary investigations in vaccinees show that HIV-1 suppression mediated by CD8+ T cells can be detected in vitro following vaccination and that P24 ELISA has higher sensitivity than flow cytometry. As an alternative to the VSA, an IFN-γ ELISpot assay has also been optimized for the use of autologous HIV-1-infected CD4+ cells. Using both of the above assays, preliminary characterization of T cell responses induced in volunteers receiving pSG2.HIVconsv DNA, MVA.HIVconsv and ChAdV63.HIVconsv vaccines will be shown.

Conclusion: Two assays employing HIV-1 infected target cells were standardised and employed to characterize responses elicited in participants of the HIV-1 vaccine trial HIVCORE002 in Oxford, UK.

P11.18

Breadth or Conservation Score (CS): Which Is More Important for HIV-1 T Cell Based Vaccine Immunogen Design?

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Background: One of the greatest challenges to develop an efficacious HIV vaccine is the enormous diversity of HIV-1. To tackle this problem, T cells based vaccine approaches have come up with two main camps: the mosaic immunogen camp: increasing the breadth of vaccine-induced responses, and the conserved immunogen camp: targeting vaccine-induced T-cell responses only to highly conserved viral regions. While both approaches are theoretically sound, there is no current data suggesting that either approach will be successful in inducing T cells with superior antiviral efficacy. Here we analyzed T cell responses elicited during early HIV-1 infection, to address the question whether CS of targeted epitopes and breadth of T cell responses play an important role in viral control.

Methods: Using IFN-γ ELISpot, we comprehensively mapped T cell epitope specificities recognized by 24 ART-naïve individuals during early infection. We identified CS of targeted epitopes, where the CS is defined as the proportion of random HIV-1 group M amino acid sequences in the LANL database that include the epitope. We used a prediction model to impute the viral load (VL) set-point using the first available VL as a predictor for subjects lacking VL set-point. We further evaluated the association between the CS of the targeted epitopes and breadth of T cell responses to the individuals' VL set-point.

Results: The breadth of CD8+ T cell responses inversely correlated with VL set-point (r=-0.46, p=0.025). Subjects possessing CD8+ T cells recognizing at least one conserved epitope had a lower VL set-point compared to those recognizing only variable epitopes (p=0.093).

Conclusion: Breadth and CS of HIV-specific CD8+ T cells elicited during early infection are both important for controlling viral replication in vivo. Rationale design of immunization approaches should aim at eliciting a greater breadth of CD8+ T cell to conserved epitopes.

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POSTERS

P11.19

Downregulation of the 5'-Ectonucleotidase CD73 of CD8+ CTL of HIV Infected Patients Correlates with Immune Activation and Diminished IL-2 Production

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Background: Chronic untreated HIV infection is immunologically characterized by irreversible loss of CD4+ T cells, general immune activation and CD4+ and CD8+ T cell dysfunction with diminished proliferative capacity. CD73 is an ectoenzyme (5'-ectonucleotidase) expressed on T cells converting 5'-AMP to adenosine, but there is additional evidence of ectonucleotidase-independent CD73 function. Altogether CD73 seems to play a role as a co-stimulatory molecule for T cell differentiation.

Methods: Peripheral blood of a large cohort of 103 HIV infected patients at different stages of HIV disease, including long-term nonprogressors and elite controllers, was analyzed by multicolour flow cytometry to determine the expression of CD73+ on CD8+ CTL, CD4+ T effector cells and Tregs.

Results: Surprisingly CD73 was not expressed on human regulatory T cells regardless of the infection status. However we find high expression of CD8+ in healthy controls. In HIV infection, CD73 seems to be generally suppressed on CD8+ T cells, independent of their naive or memory subtype. We find significant correlation between downregulation of CD73 and viremia, and there is an inverse correlation with CD8+ immune activation. Elite controllers show comparable CD73 expression to healthy controls. First proliferation studies show that that HIV-specific CD8+ CD73- T cell population produces less IL-2 than their CD73+ counterparts.

Conclusion: CD73 is not expressed on human Tregs. CD73 downregulation of CD8+ T cells correlates with HIV disease progression. Further functional studies should look into the exact role of CD73 in HIV.

P11.20

Effect of HIV Infection on the Expression and the Activity of the Proteasome in Primary CD4 T Cells

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Background: HIV-specific CD8 T cells responses rely on the recognition of peptide-MHC-I complexes by cognate T cell receptors. HIV-derived MHC-I epitopes result from the degradation of viral proteins by the cellular processing machinery including proteasomes and aminopeptidases. Interferon gamma changes proteasome composition and peptidase activities. We hypothesize that HIV infection might affect the expression or activities of the antigen processing machinery, either through a direct effect of the virus or indirectly through cellular activation or from the release of cytokines by surrounding infected cells.

Methods: Primary CD4 T cells were selected from peripheral blood mononuclear cells of HIV seronegative individuals, and then activated with phytohemagglutinin or anti-CD3/CD28 beads prior to infection with HIV NL4-3. Cell activation was assessed by flow cytometry. The expression of proteasomal subunits was quantified by western blot at 4 days post activation or at 12 to 18 days post infection. Peptidase activities of CD4 T cells were characterized using a fluorescence-based assay.

Results: In CD4 T cells isolated from 15 donors, the expression of 2 proteasome catalytic, 2 non-catalytic and 2 19S lid subunits significantly decreased with increasing percentage of HIV-infected cells ($p<0.05$). Accordingly, the chymotryptic activity of the proteasome decreased upon HIV-infection ($p=0.027$). After PHA activation, peptidase activities increased and the fold change in aminopeptidase and caspase-like peptidase activities between PHA-activated and non-activated samples correlated with the percentage of CD25-positive cells ($p<0.01$). The in vitro degradation of HIV peptide in extracts from T cells analyzed by mass spectrometry shows differences in epitope production between non-activated and PHA-activated cells.

Conclusion: Both HIV infection and cellular activation affect the expression and peptidase activities of the antigen processing machinery of primary CD4 T cells which may impact the presentation of epitopes by both uninfected and infected cells during HIV infection.

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Topic 11: T Cell Immunity

P11.21

Recombinant Attenuated M.Tuberculosis-SIVgag(rAMtb-gag) Vaccination Primes for SIV-Specific CD8 T Cell Response That Are Boosted by Ad5-SIVgag in Mice

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Background: BCG vaccination is no longer advised in children with HIV infection due to the risk of disseminated disease. As a safer alternative to BCG and as a vector for HIV vaccination, we developed a novel vaccine based on an M. tb mutant that contains genetic deletions in essential nutrients (pantothenate and Leucine) and immune modulating pathways (ΔSecA2)

Methods: To optimize T cell responses against SIV after vaccine priming with a candidate ΔPanCD ΔLeuCD ΔSecA2 M. tuberculosis ($mc^2$6208) strain expressing the codon optimized SIVmac239 gag (designated $mc^2$6435), we boosted with Adenovirus 5 expressing SIV gag in 5-6 week old C57/BL6 mice. Lymphocytes from blood, lung and spleen cells were collected 2 and 6 weeks after boosting to detect SIV gag-specific CD8 T cells by tetrameric staining for the H-2Db haplotype AL11 tetramer (AAVKNWMTQTL) and flow cytometric analysis.

Results: To determine the relative priming ability of rAMtb, rBCG, DNA vaccine, and rAd expressing the relevant Gag sequence, several groups of mice (5 per group) were immunized according to various prime/boost schedules. In general, Ad5gag boosting enhanced T cell responses after either $mc^2$6435 or BCG-SIV priming compare to mycobacterial priming alone.

Conclusion: $mc^2$6435, $mc^2$6206 (ΔPanCD ΔLeuCD) expressing SIV gag and rBCG boosted with Ad5gag had comparable response in the lung at 2 wks (2-6%). Although the most frequent SIV-specific CD8+ T cell responses were observed after Ad5gag boosting among splenocytes (7%) in BCG primed mice, significant responses (3-6% of all CD8+ T cells) were observed after Ad5gag boosting in mice primed with either rAMtb-gag strain $mc^2$6435 or $mc^2$6206. These results suggest that SIV-specific T cell responses after rAMtb-gag priming can be boosted by the Ad5gag. rAMtb-HIV is a safe candidate vaccine for infants that is likely to prime for T cell responses against both HIV and TB.

P11.22

T Cell Receptor Clonotypes Modulate the Protective Effect of Human Leukocyte Antigen Class I Alleles in HIV-1 Infection

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Background: Human leukocyte antigen (HLA) class I alleles B*27 and B*57 are associated with protection against HIV-1 disease progression, but factors modulating the HLA protective effect remain unclear.

Methods: Clonality of tetramer-sorted HIV-1 epitope-specific CD8 T cells from HIV-1 elite controllers and chronic progressors expressing HLA B*27 or B*57 were determined by T cell receptor (TCR) gene sequencing. Polyfunctionality, proliferation, avidity and differentiation phenotypes were analyzed by flow cytometry. Recognition of a GFP reporter cell line or autologous CD4 T cells infected with HIV-1 containing known mutations by HIV-1 epitope-specific bulk CD8 T cells or clonotypic CD8 T cell clones was analyzed by flow cytometry or analysis of p24 production. Immunological synapses between different CD8 T cell clones and infected target cells and lytic granule loading and delivery on a per cell basis were examined using three-dimensional confocal microscopy.

Results: HLA-B*27-restricted CD8 T cells in controllers and progressors are quantitatively similar but clearly differentiate based on the ability to inhibit virus replication through targeting of the immunodominant Gag epitope. This in turn is associated with distinct TCR clonotypes, which are characterized by superior control of HIV-1 replication in vitro, greater cross-reactivity against epitope variants, and enhanced perforin expression and delivery at the immunological synapse. Clonotype-specific differences in antiviral efficacy were also observed for an immunodominant HLA-B*57 restricted response in controllers and progressors.

Conclusion: These data indicate that the efficacy of protective alleles is modulated by specific TCR clonotypes that are selected in natural infection, and provide a functional explanation for divergent HIV-1 outcomes in persons with protective HLA alleles. Efforts to define the factors that contribute to junctional rearrangement of more effective TCR may be of critical importance for T cell vaccine design and therapeutic strategies for highly variable pathogens like HIV-1.

P11.23

Plasma Cytokine Levels and HIV-Specific Immune Responses During Acute/Early HIV Infection

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Background: It is believed that initial encounter between HIV and the human host triggers a complex series of events that dictate future disease course. Inter-individual differences among the host-players involved in these processes seem to early determine different rates of disease progression. Here we were aimed at studying the relationship between innate and adaptive soluble immune mediators, HIV-specific T-cell response and the course of acute infection.

Methods: Plasma levels of 37 cytokines were measured by Luminex technology in different groups of volunteers: 10 healthy donors (HD) and 50 HIV infected-subjects: 10 chronics, 12 aviremic controllers (EC) and 28 subjects enrolled during acute infection (AI). All HIV patients were off-HAART. Frozen PBMCs from the same individuals were used to determine HIV-specific T-cell responses by IFN-gamma ELISPOT. Data was compared inter- and intra-groups and correlated to viral load (VL), CD4 T cell counts and both virological (VL) and immunological (CD4 count) set-points (in AI), using parametric and non-parametric statistics.

Results: Compared to HD, cytokines significantly elevated during acute and chronic infection included IL-1alfa, IL-10, IP-10 and TNF-alfa. Conversely, IL-12p40 and the macrophage-derived chemokine (MDC) were only significantly elevated in chronics and not in AI subjects who showed similar levels to HD and even EC. Moreover, levels of IL-12p40, IL-12p70 and MDC directly correlated with CD4 T-cell count among chronics and both CD4 T-cell count and immunological set point in AI. Regarding HIV-specific T-cell response during AI, proportion of Gag-specific and Nef-specific cells significantly correlated (directly and inversely, respectively) with immunological set point

Conclusion: Both early and late components of the immune system help preserve CD4 T-cell subset in HIV+ subjects: key cytokines involved in the initiation and regulation of cellular immune response and anti-Gag specificity of effector T-cells. These features should be taken into account during vaccine formulation design to boost favorable results.

P11.24

Variable Processing and Presentation of HIV Epitopes in Dendritic Cells and Macrophages to CD8 T Cells

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Background: Whether HIV-infectable subsets, such as CD4 T cells, monocytes, macrophages and dendritic cells (DCs), have equivalent capacity to produce and present MHC-I restricted epitopes to HIV-specific CD8 T cells is unknown. MHC-I epitopes are processed by an intracellular degradation pathway involving multiple proteases. In this study we analyzed the effect of toll-like receptor (TLR) agonist-mediated maturation on the processing and presentation of HIV antigens in monocyte-derived DCs and macrophages.

Methods: Proteolytic activities were measured with a fluorescence-based activity assay. Cytosolic extracts were used as a source of peptidases to degrade extended epitopes in vitro. Degradation products were then analyzed by mass spectrometry and antigenicity was tested by a ^{51}Cr release assay and a real-time killing assay.

Results: Upon maturation with LPS or R848, the proteasomal and lysosomal activities in matured macrophages are significantly higher compared to matured DCs. The proteasomal tryptic and caspase-like activities as well as the lysosomal activities decreased approximately 1.5-fold in matured DCs. The degradation of the N-terminal extended epitope 3-ISW9 in LPS-matured DCs yielded 2-fold more optimal epitope ISW9 than in immature DCs, which resulted in a 3-fold higher cell lysis in a ^{51}Cr release assay. In addition, the cross-presentation of exogenously added p24 protein by DCs or macrophages showed reduced killing of APCs by ISW9-specific CTLs compared to CTLs specific for TW10 or KF11. This lower ISW9-specific killing was partly rescued by preincubation with protease inhibitor.

Conclusion: We showed that differences in antigen processing activities in DCs and macrophages upon maturation, and differences in HIV sequences sensitivity to intracellular degradation may affect the production and presentation of epitopes and thus the capacity of HIV-specific CTLs to recognize and kill infected cells. For the design of a vaccine immunogen it is critical to identify factors regulating the processing and presentation of epitopes.

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Topic 11: T Cell Immunity

P11.25

Increased Mucosal CD4+ T-Cell Activation Following Vaccination with an Adenoviral Vector in Rhesus Macaques

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Background: The possibility that vaccination with Adenoviral vectors increased mucosal T-cell activation remains a central hypothesis to explain the potential enhancement of HIV acquisition within the STEP trial. Modeling this within rhesus macaques is complicated because human Adenoviruses, including Adenovirus type 5 (HAd5), do not productively infect macaques. We created a vector based upon a naturally occurring rhesus macaque Adenovirus (SAdV7) to test whether vaccination with a species-specific Adenoviral vector enhances mucosal T-cell activation within the natural host.

Methods: Twelve rhesus macaques were vaccinated 3x intramuscularly with SAdV7 vector. Five HAd5-vaccinated animals were included as controls. PBMC and rectal biopsies were obtained at baseline, multiple times post-prime and post-17 week boost (8x/animal), and post-31 week second boost (1x/animal). We assessed rectal mucosal lamina propria and blood for frequency changes of Ad-specific T-cell responses and T-cell activation levels by measuring IFNg, TNFa, IL2, CD25, Ki67, CD69, and HLA-DR.

Results: Naturally acquired pre-existing SAdV7-specific CD4+ T-cells were identified in 10/13 macaques within blood and/or rectal mucosa. Following intramuscular SAdV7 vaccination, rectal SAdV7-specific CD4+ T-cell responses increased above baseline in 9/9 animals 2-5 weeks post-prime, and subsequently contracted. Five weeks post-prime, 10/12 animals had rectal SAdV7-specific CD4+ T-cell responses ranging from 0.1-16.84%. As expected, SAdV7-specific CD4+ T-cells expressed CD69 and other activation markers (but not Ki67). Heightened expression of CD25, CD69, and HLA-DR was observed on total rectal memory CD4+ T-cells in SAdV7-vaccinated animals, and maintained 15 weeks after the prime. Interestingly, upregulation of activation markers in rectal mucosa also occurred in HAd5-vaccinated animals. No change in activation was observed in the blood throughout the entire study.

Conclusion: These results indicate that peripheral vaccination with an Adenovirus vector can increase the activation of mucosal CD4+ T-cells providing an experimental model to further evaluate the role of host-vector interactions on increased HIV acquisition.

P11.26

Different Abilities of CTL Specific for Two HLA-A*24:02-Restricted Overlapping Optimal Epitopes to Select Same HIV-1 Escape Mutant Virus

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Background: Cytotoxic T lymphocytes (CTLs) are thought to exert immunologic selection pressure of escape mutation. Previous reports have showen that some overlapping peptide epitopes were presented by same HLA molecules. However, the abilities and properties of those CTLs to selection of same escape mutation are not well studied.

Methods: CTL clones were established by stimulation of PBMC with a synthetic peptide (Nef138-8: RYPLTFGW or Nef138-10: RYPLTFGWCF) by limiting dilution method. Cytotoxic activity toward peptide-loaded cells was performed by 51Cr releasing assay. CTL suppression ability was tested by HIV-1 replication assay toward primary CD4+ cell. In vitro selection of escape mutation was performed by competitive HIV-1 replication assay. The frequency of tetramer positive cells in PBMC of HLA-A*24:02+ patients was detected by flow cytometry analysis.

Results: Both 8-mer and 10-mer epitopes specific CTLs were established from PBMC of the patients. The ability of Nef138-10-specific CTLs to suppression HIV-1 replication in vitro was much higher than that of Nef138-8-specific CTLs. In addition, at the early stage of infection, Nef138-10-specific CTLs was predominantly elicited in the patients more than the latter ones. Cross-reactive Nef138-8-specific CTLs recognizing both WT and 2F epitopes were detected in some patients. Moreover, in vitro competitive HIV-1 assay showed that both CTLs can select escape mutants, though the ability of Nef138-10-specific CTLs was stronger than that of Nef138-8-specific ones.

Conclusion: The present study demonstrated that Nef138-10-specific CTLs play a major role in the selection of the escape mutation, and that Nef138-8-specific CTLs also have ability to select it. We showed selection of the same escape mutants by CTL specific for same HLA-restricted overlapping epitopes.

P11.27

HLA-Associated Viral Polymorphism in Chronically HIV-1-Infected Japanese Cohort: Analysis of Four-Digit HLA Allele Level

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Background: It is assumed that the difference of HLA class I distribution among ethnic populations influences HIV evolution because HLA-restricted immune pressure selects escape mutations. Approximately 50% of HLA class I alleles are shared between Japanese and Caucasians. The analysis of HLA-associated polymorphism (HLA-AP) in both Japanese and Caucasian infected with clade B virus is expected to clarify the difference of HIV-1 evolution between both populations.

Methods: We sequenced Gag, Pol, and Nef genes in 430 treatment-naïve Japanese chronically infected with HIV-1 clade B and then identified HLA-associated amino acids at each codon using a phylogenetically corrected logistic regression model and false discovery rates to correct for multiple tests.

Results: We completely determined 400, 366, and 309 sequences of Gag, Pol, and Nef, respectively, and then analyzed polymorphisms associated with 78 four-digit HLA alleles (21 HLA-A, 38 HLA-B, and 19 HLA-C alleles). At the threshold of $q<0.2$, we found 195 HLA-APs (67 in Gag, 61 in Pol, and 67 in Nef). These polymorphisms were observed at 39 of 501 (7.8%) Gag, 42 of 1004 (4.2%) Pol, and 33 of 207 (16.0%) Nef codons. Ninety-six HLA-APs associated with more than one HLA subtype allele were detected in 4-digital HLA allele analysis. Approximately 40% of HLA-APs were associated with HLA alleles predominantly found in Asia. Out of HLA-APs associated with common HLA alleles, approximately 50% were found in Caucasian population (IHAC cohort).

Conclusion: This study demonstrated that only 30% of HLA-APs were shared between Caucasians and Japanese, indicating that the difference in HLA allele distributions resulted in distinct HIV-1 evolution.

P11.28

Highly Expression of Tim-3 on HIV-Specific T Cells Associated with Disease Progression and T-Cell Exhaustion in HIV-1 Infected Chinese

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Background: The exact mechanism of T-cell exhaustion remains to be defined during HIV-1 infection. Recent studies suggest that the inhibitory receptor T-cell immunoglobulin domain and mucin domain 3(Tim-3) may play an important role in the exhaustion of HIV-specific T cells.

Methods: 72 HIV-1 infected individuals with different disease outcomes were recruited in this study. Tim-3 expression and the profile of cellular immune response were measured by using Multicolor Intracellular Cytokine Staining (ICS) assay. Association between Tim-3 expression levels and disease progression was analyzed. And the potential role of Tim-3 on immune regulation during HIV-1 infection was investigated through assessment of CTL response with frequencies of Tim-3 expression and blocking effect.

Results: Tim-3 was found to be highly expressed on HIV-specific CD4+ T cells and CD8+ T cells, especially in primary infectors and AIDS patients. The frequencies of Tim-3 expression correlate with disease progression. The level of Tim-3 expression was related with cytokines production and blockade of Gal-9/TIM-3 signaling partially restored the ability of HIV-specific T cells to secrete cytokines in vitro.

Conclusion: The frequencies of Tim-3 expression correlate with disease progression in HIV-1 infected individuals and manipulating Gal-9/TIM-3 signaling pathway may provide a novel therapeutic measure to control HIV-1 replication.

P11.29

Changes in gd T Cell Function and Gut Homing Receptors Following SIV Infection of Rhesus Macaques

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Background: During simian immunodeficiency virus (SIV) infection changes occur in the gd TCR T cell population. The major subset in mucosal tissue, Vd1, becomes prevalent in peripheral blood relative to the Vd2 subset. gd T cells have the ability to expand in vitro and in vivo making them attractive as cytotoxic effectors against infections like SIV and HIV.

Methods: We studied gut homing receptors and functionality of Vd1 and Vd2 T cells in blood and jejunum of naïve (8 and 3 respectively) and SIV infected (13 and 9 respectively) animals. Vd1+ and Vd2+ T cells were identified by flow cytometry using pan gd TCR-PE and Vd2-FITC mAbs. Intracellular staining for IFN-g and perforin was performed after Phorbol-Myristate-Acetate/ Ionomycin stimulation.

Results: Here, the Vd1 subset in SIV infected macaques was not significantly increased in blood, but in jejunum, Vd1 T cells became predominant compared to Vd2 T cells (p=0.0044) and to the level in blood (p=0.0014). Expansion of the Vd1 subset in jejunum was linked to increased expression of the a4b7 gut homing marker on peripheral blood Vd1 T cells (p=0.033). After stimulation, Vd1 and Vd2 T cells in blood produced both IFN-g and perforin. Higher frequencies of Vd1 T cells produced perforin in SIV+ animals compared to naïve (p=0.0186), while IFN-g producing cell frequencies decreased (p=0.0066). Most Vd1 T cells were CD4-CD8- (DN). Those producing perforin were also mainly DN compared to CD4+ and CD8+ Vd1 T cells (p=0.0156).

Conclusion: Expansion of Vd1 T cells in the jejunum is associated with increased trafficking from peripheral blood to the mucosal site. This expanded population with cytotoxic potential could contribute to viremia control.

P11.30

Longitudinal Assessment of HIV-1-Specific T-Cell Responses Generated During Acute Subtype C HIV-1 Infection and Associations with Viral Set Point

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Background: HIV-1-specific CD8+ T-cell responses generated during acute infection play a key role in determining the course of disease. However, these early responses may disappear as the infection progresses. Tracking the phenotype, functional ability and fate of these early responses may help elucidate features of CD8+ T-cells that contribute to viral control.

Methods: We characterized the magnitude and breadth of HIV-1-specific CD8+T-cell responses using the overnight IFN-γ ELISPOT assay in 20 HIV-1 subtype C acutely infected, antiretroviral naive individuals as early as 28 days post initial exposure and up to 12 months post-infection. Cultured IFN-γ ELISPOT assays were used to assess early epitope-specific CD8+ T-cell responses that were below the limit of detection by overnight ELISPOT assays at 12 months post-infection.

Results: The initial CD8+ T-cell responses detected during the decline in acute phase peak viremia were narrowly directed: T-cell responses were directed against an average of three (range 0-6) of the 410 peptides tested. At 12 months post-infection, immune responses had broadened with an average of seven peptides targeted (range 2-11). The majority of persistent T-cell responses targeted epitopes within Gag and Pol (50% and 30%, respectively). The breadth of persistent CD8+T-cell responses correlated negatively with viral set point (P=0.02). An average of 65% of earlier CD8+ T-cell responses which where not detected by the overnight ELISPOT at later time points, were detected by the cultured ELISPOT assay. There was no correlation between the magnitude or breadth of responses measured using the cultured ELISPOT assay and viral set point.

Conclusion: These data suggest that persistent CD8+ T-cell responses generated during the earliest stages of HIV-1C infection may play a role in viral control. Furthermore, effector T-cell responses that disappear following acute infection are maintained as memory T-cell responses and our preliminary data suggest that they have no significant impact on viral control.

P11.31

Breadth, Phenotype and Functionality of Gag-Specific T Cell Responses Induced by a Heterologous DNA/MVA Prime-Boost HIV-1 Vaccine Regimen

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Background: Broad Gag recognition and polyfunctionality of vaccine-induced AIDS virus-specific T cell responses correlate with better viral control in non-human primates and also in chronically HIV-infected individuals.

Methods: Breadth and polyfunctionality of HIV-vaccine induced Gag-specific T cell responses were investigated in healthy Tanzanian volunteers who participated in the Tanzania Mozambique HIV vaccine trial (TaMoVac 01). Vaccine recipients received 3x 0.6 or 1.0mg intradermal injections of multiclade, multigene HIV-DNA vaccine boosted with 2x heterologous Modified Vaccinia Ankara (MVA)-CMDR. Using fresh peripheral blood mononuclear cells, the breadth of response was determined after the first MVA-CMDR boost using peptide pools for 9 successive Gag regions with an IFN-gamma ELISpot assay. Functionality (IFN-gamma, IL-2, TNF-alpha, Mip-1beta and the degranulation marker CD107) and phenotype (CD3, CD4, CD8) of HIV-specific T cells were assessed using flow cytometry in 52 participants after stimulation with peptide pools covering whole Gag-CMDR protein.

Results: Two weeks after the first MVA-CMDR boost, a median of 2 Gag regions were recognized (range: 0-9, Placebos not excluded) by 45 participants. There was a strong linear correlation between the magnitude and the breadth of vaccine-induced Gag recognition ($p<0.0001$, $r2=0.44$). 13 (25%), 13 (25%), 3 (6%) and 29 (56%) of 52 subjects mounted IFN-gamma+ Gag-specific T cells that were either only CD4+, CD4+ & CD8+, only CD8+, or CD4+ and/or CD8+, respectively. Fifty percent of IFN-gamma+ Gag-specific CD4 T cells co-expressed TNF-alpha and/or IL-2. Co-expression of Mip-1beta or CD107 was reduced compared to CMVpp65-specific CD4 T-cells, which were measured simultaneously. More than 50% of IFN-gamma+ Gag-specific CD8 T-cells co-expressed CCR5 ligand Mip-1beta and a large proportion of these had degranulated.

Conclusion: The TaMoVac-01 HIV-1 vaccine regimen induces a relatively broad Gag-specific response frequently dominated by CD4 T cells, many of which co-express IL-2 and/or TNF-alpha, but also induces detectable Gag-specific CD8 T-cells in a third of vaccine recipients.

P11.32

HIV-Specific Cytolytic CD4 T-Cell Responses Effectively Control HIV Infection in Macrophages

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Background: HIV-specific cytolytic CD4 T-cell responses expand during acute HIV infection in individuals who control viremia and are associated with better disease outcome. Up to 75% of the HIV-specific CD4 T-cells exhibit a cytolytic phenotype during acute infection, but it is not understood how cytolytic CD4 T-cells contribute to viral control or what their primary target cells are.

Methods: Using a novel, fluorescence-based single-round viral suppression assay, we assessed the ability of CD4 T-cells from HIV infected subjects to lyse infected macrophages. Elimination of infected macrophages and CD4 cytolytic phenotype were determined by flow cytometry. In addition, HIV-specific CD4 T-cell clones were generated and their cytolytic ability examined by Cr51-release and viral inhibition assays.

Results: We observed significantly higher degranulatory HIV-specific CD4 T-cell responses in HIV controllers compared to progressors ($p=0.015$). Moreover, about 1/4 of all HIV-specific CD4 T-cell clones showed cytolytic activity by Cr51-release. Using a single-round viral suppression assay, we additionally observed that HIV-specific CD4 T-cells from chronically infected subjects were able to significantly lyse HIV infected macrophages ($p=0.004$). Elimination of HIV-infected macrophages was dose-dependent, up to 37% at E:T=5:1. Lytic ability could be observed ex vivo, and was enhanced after short term Gag-specific expansion in culture (11% to 32%, $p=0.014$). Furthermore, we observed that the inhibitory capacity of CD4 T cells could be abrogated using an HLA-DR blocking antibody. CD4 T-cell-mediated macrophage lysis was associated with strong HIV-specific cytolytic activity by intracellular cytokine staining and high expression of granzymes/perforin within HIV-specific CD4 T-cells.

Conclusion: Our data demonstrate that HIV-specific CD4 T-cells derived from infected individuals have the ability to eliminate infected macrophages. These data suggest a role for HIV-specific cytolytic CD4 T-cell responses, in the absence of CD8 T cell responses, in the lysis of HIV-infected macrophages, which represent important reservoirs for viral infection and viral dissemination.

P11.33

Alteration of HIV Epitope Processing and Presentation by HIV Protease Inhibitors

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Background: Epitopes displayed by MHC-I come from the multistep degradation of proteins by intracellular peptidases such as proteasome and aminopeptidases or cathepsins in the exogenous pathway. We hypothesize that due to structural homologies HIV protease inhibitors (PIs) used in antiretroviral therapies may affect activities of cellular peptidases involved in epitope processing and may affect epitope presentation to immune cells.

Methods: Using a fluorogenic assay the effect of 5 HIV-1 PIs (Ritonavir, Saquinavir, Nelfinavir, Indinavir, Atazanavir) on proteasome, aminopeptidase and cathepsin activities was tested in PBMCs from at least 6 healthy donors. Using PBMC cytosol as a source of peptidases and HPLC and mass spectrometry to define and quantify the degradation products, the effect of HIV PIs on HIV peptide processing kinetics and HIV epitope half-life was assessed. Finally we assessed the impact of PIs on the endogenous processing and presentation of epitopes by infected cells to CD8 T cells using a fluorescence-based cytotoxicity assay.

Results: HIV PIs variably altered proteasome, post-proteasomal aminopeptidases and cathepsin activities. Depending on the PI, some activities were inhibited (from 1.1 to 5 folds, $p<0.001$), enhanced (1.2 to 9 folds, $p<0.001$), and others not changed. These PI-induced changes in protease activities modified HIV peptide processing patterns and HIV epitope intracellular half-life prior to MHC-I loading. Depending on the PI and the epitope the half-life was increased 1.5 fold ($p<0.01$) or decreased 1.3 fold ($p<0.05$). Furthermore HIV PI altered (from 2.2 fold decrease to 1.3 fold increase, $p<0.01$) the presentation of HIV epitopes and recognition by epitope-specific CD8 T cells.

Conclusion: These findings suggest that in HIV-infected patients an antiretroviral therapy including PIs might -by altering host proteases function- modify the pattern of epitope presentation, leading to the elicitation of additional CTL responses against HIV and potentially against other pathogens co-infecting HIV+ persons.

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P11.34

Potent Cellular Immune Responses After Therapeutic Immunization of HIV-Positive Patients with the PENNVAX®-B DNA Vaccine in a Phase I Trial

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Background: Although highly active antiretroviral therapy (HAART) regimens have dramatically transformed treatment of HIV infection, achieving 95% adherence to HAART regimens is notoriously difficult. The successful creation of an immunotherapy for infection could eliminate the potential pitfalls associated with the necessity for long-term adherence to drug therapy. To that end, we evaluated the safety and immunogenicity of the PENNVAX®-B vaccine, delivered with in vivo electroporation (EP) in HIV-infected volunteers on HAART in a Phase I open-label study.

Methods: Enrollment criteria included HIV RNA<75copies/mL, CD4>400/µL with nadir >200/µL. Twelve eligible subjects received a 4 dose series (day 0, weeks 4, 8 and 16) of 3 mg PENNVAX®-B (consisting of SynCon® HIV Gag, Pol, and Env immunogens) intramuscularly followed by in vivo EP with the CELLECTRA®-5P device.

Results: All the enrolled subjects completed the immunization schedule. The vaccine demonstrated an acceptable safety profile and was generally well tolerated. Overall, 9 out of 12 subjects (75%) showed significant vaccine-specific T-cell responses in the form of IFN-γ ELISpot against at least one of the three vaccine antigens (Gag, Pol, or Env) following vaccination. Furthermore, responses were not dominated by a single antigen, as 50% of subjects had strong vaccine induced responses to at least 2 of the 3 antigens and 3 showed vaccine-induced responses to all 3 antigens. Importantly, the ELISpot responses induced by vaccination were predominantly CD8+T-cells, which are considered to be paramount in clearing chronic viral infections and an important measure of the performance of a therapeutic vaccine. Additionally, HIV-specific immune responses were assayed by flow cytometry to measure IFN-γ production by both CD4+ and CD8+T cells as well as co-expression of the CTL-related markers CD107a, GranzymeB and Perforin.

Conclusion: Analysis of these data should provide more definitive evidence of HIV-specific CTL function, which has been implicated in control of viral replication in HIV-infected patients.

P11.35

Peripheral T Follicular Helper Cells from H1N1/09 Vaccine Nonresponders Fail to Induce Antigen-Specific Antibody Production In Vitro

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Background: Mechanisms underlying poor Ab responses to vaccines in well controlled HIV infected patients are not fully understood. In this study we have examined the role of a novel subset, the peripheral T follicular helper cells (pTFH) in Ab production.

Methods: The study was conducted in cryopreserved cells of 16 HIV-infected, ART-treated individuals and 8 healthy donors (HD) who had been given a single dose of H1N1/09 influenza vaccine in 2009. Only 8 of the 16 patients and all HD had a flu-Ab response at 4 wks post vaccination. Vaccine responder (VR) and non responder (VNR) patients were equivalent in mean age, viral load, CD4 and CD8 T and B cells. B (CD20+) and TFH (CD3+ CD4+ CD45RA– CXCR5+) cells were purified by cell sorting on a FACS ARIA and co-cultured. IgG levels in culture supernatants were determined by ELISA. B and T cell phenotypic analysis was performed by multicolor flow cytometry. Differences between groups were analyzed by Student t-test or the 2-sample Wilcoxon rank-sum (Mann-Whitney) test.

Results: Frequency of pTFH was equivalent in HIV+ patients and HD before vaccination. pTFH cells underwent significant expansion at wk4 compared to baseline in VR patients (p= 0.003) and HD (p= 0.001) with increased frequency of Ki67+ cells. In VR, H1N1-specific IgG production was evident in CD4+ CXCR5+/B cell co-cultures but not in CD4+ CXCR5–/B cell co-cultures [HIV+ n = 3; p = 0.043] and [HD n=3; p = 0.014], concurrently with increase in frequencies of plasmablasts. These changes were not seen in vaccine nonresponders.

Conclusion: pTFH cells promote antigen-specific Ab production by B cells in vitro. Although their relationship to lymph node germinal center TFH has not been clarified, analysis of pTFH function represents a novel and easily accessible surrogate marker for vaccine responsiveness in stable HIV infected patients with equivalent CD4 T cells.

P11.36

Preferential Targeting Of Co-evolving Gag Residues in Long-Term Non Progressors

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Background: A recent analysis of mutational patterns within Gag revealed independently evolving groups of residues (termed sectors) whose mutations are collectively coordinated. Of these sectors, sector 3 is the least tolerant of multiple simultaneous mutations and therefore is proposed to be the most vulnerable to a targeted immune attack. We hypothesized that coordinated CTL targeting of sector 3 residues is associated with immune control.

Methods: We completed a comprehensive evaluation of Gag-specific responses in a cohort of 9 Long-term non-progressors (LTNPs, VL <2000 RNA copies/ml, untreated) and 9 HIV progressors (VL>10,000 RNA copies/ml, untreated). A Gag peptide set of 11-mer peptides overlapping by 10 amino acids was generated to reflect all variants found in at least 5% of clade B sequences in the LANL HIV Sequence Database. This peptide set includes 1300 peptides and covers all 500 amino acids of Gag. All study subjects were screened for responses to all peptides by IFN-γ/IL-2 FluoroSpot.

Results: We observed a trend in the preferential targeting of sector 3 residues by LTNPs (p=0.07). This trend was not observed for any other sector or in total breadth of responses. Supporting the importance of sector 3 targeting, we found a significant positive correlation in our cohort between the relative proportion of sector 3 responses and CD4 count (r=0.49, p=0.04). We found no significant differences between LTNPs and HIV-Progressors in either the targeting of conserved 11-mers or overall Gag epitope variant recognition. Interestingly, LTNPs demonstrated higher levels of variant recognition than HIV-progressors when considering only the variable regions containing sector 3 residues.

Conclusion: We found that preferential targeting of sector 3 residues distinguished Gag-specific responses between LTNPs and HIV-progressors, and that coordinated targeting of sector 3 residues may require cross-reactive responses. Additional investigations are ongoing to elucidate the role of sector 3 targeting in immune control of HIV.

P11.37

Identification of HIV-1-Specific Regulatory T Cells Using HLA-Class-II Tetramers

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Background: Regulatory T cells (Tregs) are potent immune modulators whose role in HIV-1 immuno-pathogenesis remains inadequately understood. While "bulk" Treg populations have been studied extensively in the context of HIV-1 infection, no reliable data is available on HIV-1 specificity of Tregs and induction of these cells in infected individuals or vaccine recipients.

Part of the challenge in detecting antigen-specific Treg populations relates to the limited availability of visualization tools and the scarcity of the overall human Treg population -representing roughly 5% of CD4+ T cells with absolute Treg numbers further decreasing during HIV-1 disease progression.

Methods: In order to screen for HIV-1 specific regulatory T cell populations, we first flow-sorted and expanded CD4+CD25+CD127low Tregs ex vivo from HLA DRB1*0401 expressing HIV-1 infected individuals. Expanded Tregs underwent analysis of function, phenotype, deep TCR sequencing and epigenetic analysis. Treg lines were then stained with HLA class II tetramers specific for HIV-p24-gag and appropriate controls.

Results: Expanded Tregs were highly suppressive and displayed the phenotype of "activated" Tregs. Tregs were highly demethylated at the TSDR as shown by epigenetic analysis of the FOXP3 gene and showed an unskewed TCR repertoire compared to unexpanded ex vivo-sorted Tregs. Staining with HLA-class-II-tetramers specific for the HIV-p24-Gag epitope DRFYKTLRAEQASQ revealed a detectable response in one subject (an individual with chronic untreated progressive HIV-1 infection), at a frequency of 0.19% of CD4+ T cells in the non-enriched Treg culture. After tetramer-positive T cell enrichment, this frequency was increased to 6.14%.

Conclusion: Our data represent the first identification of HIV-1-epitope-specific Tregs in HIV-1 infected individuals. Identification and further functional characterization of HIV-1 specific Tregs will provide important insight for the evaluation of vaccine strategies as these may lead to induction of not only HIV-1-specific effector populations but also HIV-1-specific regulatory T cells, which may negatively impact vaccine immunogenicity.

P11.38

A High-Dimensional Immune Monitoring Model of HIV-1-Specific CD8 T Cell Responses Accurately Identifies Subjects Achieving Spontaneous Viral Control

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Background: The development of immune monitoring models to determine HIV-1 vaccine efficacy is a major challenge. HIV-1-specific CD8 T cells likely play a critical role in individuals achieving spontaneous viral control (HIV-1 controllers) and will be important in immune interventions. However, no single CD8 T cell function is uniquely associated with controller status.

Methods: Here we describe the building of immune monitoring models based on inter- and intra-donor analysis of HIV-1-specific CD8 T cell proliferation and cytokine secretion assessed at different time points after antigen stimulation. The discovery data set used to build a palette of immune monitoring models of HIV-1-specific CD8 T cell functions was generated on 26 controllers, 15 progressors and 23 ART-treated subjects. An independent cohort of 10 controllers and 10 progressors was investigated to validate our results. We used Area Under the Receiving Operating Characteristic curves (AUC) to assess the ability of individual variables to differentiate between controllers and non-controllers.

Results: Our analyses identified links between HIV-1-specific CD8 T functions, HLA-I alleles, and disease stage. The best accuracy (AUC) values were observed for proliferation. Early (6h) IL-2 secretion and slopes of TNF-α, IL-2 and IFN-γ production also contributed to the models whereas gender and age had no discriminatory value. A model incorporating five HIV-1-specific CD8 T cell functions achieved 90% accuracy in the discovery cohort on which it was trained, and was able to accurately discriminate controllers from non-controllers in the validation cohort.

Conclusion: Our multidimensional modeling approach shows that integration of different dimensions of data leverages independent associations and discriminates much better than any one measure. This modeling approach is amenable to incremental incorporation of new knowledge to build evolving flexible tools that are usable in translational clinical research and thus, has important applications in predictive model development and immune monitoring of HIV-1 vaccine trials.

P11.39

The HIV-1 Protective -35SNP Effect in Caucasians Is CD8 T Cell Mediated

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Background: Previous studies in Caucasians have observed that a single nucleotide polymorphism 35kb upstream of the HLA-C gene (-35SNP) associates with control of HIV-1 viral load set-point and cell surface expression of HLA-C. HIV-1 selectively downregulates HLA-A and HLA-B but not HLA-C, via the action of the Nef protein. Thus it has been speculated that higher cell surface HLA-C expression results in a stronger HLA-C-restricted T cell response which might play a role in the control of HIV-1 replication in individuals with the protective -35C variant. However, HLA-C-restricted CD8 T cell responses are relatively weak and we could find no difference in functional HLA-C-restricted CD8 T cell activity measured by IFN-γ ELISPOT assay, according to -35SNP genotype. Therefore, we aimed to examine if there is any correlation between total CD8 T cell function and the -35SNP.

Methods: The viral suppression assay, which involves directly infecting autologous CD4 T cells with primary HIV-1 strains and co-culturing with autologous CD8 T cells, was used as a surrogate for immune control in vivo. The CD8 T cells from 46 antiretroviral therapy naïve HIV-1 infected Caucasians were assessed using this assay.

Results: When CD8 T cell antiviral activity was grouped according to -35SNP genotype, the -35CC group possessed significantly higher CD8 T cell antiviral activity than the -35TT group (p=0.0151; Mann-Whitney). Protective HLA-B alleles were always in linkage disequilibrium with HLA-C alleles that are in linkage disequilibrium with the -35C allele. Similarly risk HLA-B alleles were in linkage disequilibrium with HLA-C alleles that are in linkage disequilibrium with the -35T allele.

Conclusion: In conclusion, the protective -35SNP effect in HIV-1 disease is mediated through CD8 T cells. However, the -35SNP may simply be a marker for protective and risk HLA-B alleles.

P11.40 LB

ABSTRACT WITHDRAWN

P11.41 LB

Chronic Progressive HIV-1 Infection Is Associated With Elevated Levels Of Myeloid-Derived Suppressor Cells

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Background: Myeloid-derived suppressor cells (MDSC) have been described as suppressors of T cell functions in many tumor models. However MDSC in HIV-1 infection have not been studied to date. As impaired T cell function is a hallmark of chronic progressive HIV-1 infection, we hypothesized that MDSC also play a role here.

Methods: Surface staining and FACS analysis were performed on freshly isolated PBMC of HIV-infected individuals and compared to healthy controls and individuals with lung carcinoma. MDSC of late-stage HIV-infected subjects were isolated using magnetic beads and co-cultured with the respective CD8 T cells for evaluation of proliferative capacity.

Results: We found that chronically HIV-infected HAART-naïve individuals had significantly higher CD11b+CD14-CD33+CD15+ MDSC levels than healthy controls (p=0.01). MDSC frequencies showed a positive correlation with viral load (r2=0.24, p=0.0002) and a negative correlation with CD4 count (r2=0.29, p<0.0001). Initiation of HAART led to a rapid drop in MDSC levels. MDSC from HIV-infected progressors restricted the proliferative capacity of CD8 T cells from healthy donors and of Gag/Nef-specific CD8 T cells from HIV-controllers in vitro. Furthermore CD11b+CD14-CD33+CD15+ MDSC induced the expansion of CD4+CD25+FoxP3+ regulatory T cells when co-incubated with PBMC from controllers in vitro.

Conclusion: We conclude that chronic uncontrolled HIV-infection is associated with elevated levels of MDSC which potentially contribute to the impaired T cell responses characteristic for the progressive disease stage.

P11.42 LB

Plasticity of HIV-Specific CD8 T Cell Responses In Untreated HIV-1 Infection- A Step Towards A Therapeutic Vaccine Against Drug Resistance Mutations

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Background: For a therapeutic HIV-1 vaccine, it should be considered that the immune system has been confronted with a certain viral sequence and mounted CD8 T cell responses specifically towards the infecting virus. One question is whether the immune system of HIV-infected individuals can create a new response towards a variant epitope in the case of vaccination. To study this in a comparable setting, we addressed the question how frequently a new CD8 T cell response can be generated after the occurrence of a viral escape mutation in its recognized epitope in a population not selected for a certain HLA allele.

Methods: 19 HIV-infected untreated subjects were sampled longitudinally (>6 months. We searched for CD8 T cell responses that declined over the course of untreated infection and sequenced the autologous virus of the early and late time point by RT-PCR. Recognition of wildtype and newly arising sequences was compared in peptide titration assays.

Results: A total of 30 declining CD8 T cell responses were studied in detail and viral sequence analyses showed amino acid changes in 25 (83%) of these. Peptide titration assays revealed 12 (48%) viral escape mutations with 2 de-novo responses (17%). Here the de-novo response showed less effector functions than the original CD8 T cell response. In addition we identified 5 (20%) shifts in immunodominance. None of the subjects with adaptation to the changing virus carried the HLA alleles B27, B57 or B*5801.

Conclusion: Our results show that CD8 T cell responses can adapt to the mutations of HIV. However it was limited to only 28% (7 out of 25) of cases in a cohort not expressing protective HLA alleles.

P11.43 LB

The Early Th17/Treg Ratio Predicts The Immune Activation Set Point In Patients With Primary HIV Infection

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Background: Persistent systemic immune activation plays a central role in the pathogenesis of HIV disease. Impairment of the intestinal barrier and subsequent microbial translocation might be involved in chronic immune activation. Th17 cells are important in the maintenance of intact epithelium and host defense against extracellular pathogens. The ratio between the two closely related CD4 subsets Th17 and Tregs has been recently found to shrink with HIV/SIV disease progression. The aim of the study was to analyze, in patients with early primary HIV infection (PHI), the relationship between Th17/Treg ratio and the immune activation set point, known to predict disease progression.

Methods: 27 patients with early PHI were included in a prospective longitudinal study and followed-up for 6 months. T-cell activation and $CD4^+CD25^+CD127^{low}Foxp3^+$ Treg frequency were assessed on fresh PBMC. Th17 cells were quantified by intracellular cytokine staining on sorted peripheral CD4 T cells stimulated with PMA/ionomycin for 5h. Correlations were assessed using spearman non-parametric tests. Plasma I-FABP, a marker of mucosal damages and soluble CD14 (sCD14) were measured by ELISA.

Results: A strong negative relationship was found at baseline between the Th17/Treg ratio and the proportion of activated CD8 T cells expressing CD38/HLA-DR (p=0.008) or Ki-67 (P=0.001). At baseline, Th17/Treg ratios also negatively correlated with sCD14 plasma levels (p=0.003). I-FABP levels, which were similar to controls at baseline, increased at month 6. The Th17/Treg ratio at baseline (but not the proportion of Th17 cells) negatively correlated with the frequency of $HLA\text{-}DR^+CD38^+$ or $Ki\text{-}67^+$ CD8 T cells at month 6, defining the immune activation set point (p=0.02 and p=0.0005 respectively). sCD14 plasma levels were also found to predict the immune set point (p=0.02).

Conclusion: Our data do not support early mucosal damages in PHI. However, the early Th17/Treg balance correlates with sCD14 levels and predicts the immune activation set point.

P11.44 LB

Comparable Antiviral Capacity but Favorable In Vitro Survival of CD8s from Elite Controllers Compared to Untreated Progressors

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Background: CD8s inhibition of viral replication in vitro was demonstrated as one of the best laboratory correlates to HIV control. However, past studies invariably used CD8s which were rested in vitro for few days before antiviral capacity examined. This might suggest that the difference between CD8s from elite controllers (EC) and chronic progressors (CP) is not in their inhibition capacity but in their ability to retain cytotoxicity during prolonged incubation. Here we compared cytotoxicity and apoptosis of CD8s immediately after their purification ("fresh CD8s") or 3 days after in vitro rest ("old CD8s")

Methods: Samples from 10 EC, 10 CP, 5 HAART treated and 5 HIV negative patients were examined. "Fresh" and "old" CD8s were used as effectors in viral inhibition assays. HIV-specific CD8s were quantified using tetramer staining. Annexin V binding was used to evaluate apoptosis.

Results: Using "old" CD8s inhibition capacity was higher among EC compared to CP (logP24 reduction 1.225 vs 0.238, p=0.044). For both EC and CP inhibition was much stronger using "fresh" CD8s, but no significant difference was found between EC and CP (logP24 reduction: 3.13 vs 3.85, p=0.29). HIV negative subjects showed no inhibition using "fresh" or "old" CD8s. IL-2 partially rescued antiviral capacity of rested CD8s. Loss of HIV-specific CD8s measured by tetramer staining was higher in CP compared to EC with up to 10-fold increase in Annexin V binding

Conclusion: HIV-specific CD8s from CP are endowed with an unexpectedly strong viral inhibition capacity when examined directly ex vivo. CD8s from EC and CP mediated similar HIV suppression directly ex vivo, while the superior antiviral activity of CD8s from EC after a 3d incubation was associated with better survival of HIV-specific CD8s. The capacity to survive and exert effector functions over extended periods, rather than the intrinsic antiviral capacity, best distinguishes CD8s from EC and CP.

Topic 11: T Cell Immunity

P11.45 LB

Loss and Regain of SIV Control Upon CD8+ Cell Depletion In Vivo In SIV-Controller Macaques Is Not Associated With Efficient SIV Specific CD8+ T-Cells

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Background: Spontaneous long-term HIV/SIV control in HIV-controller patients and SIV-controller macaques (SIC) is usually associated to protective MHC-class-I alleles and efficient CD8 T-cell responses. However, many HIV-controllers efficiently control HIV-infection despite of non-protective MHC background and weak CD8 T-cell responses, raising the question of real contribution of this response in maintaining viral control. We addressed this question by depleting in vivo CD8+ cells in SIC bearing or not protective MHC and weak CD8 T-cell responses.

Methods: We studied five SIVmac$_{251}$</sub>-infected cynomolgous macaques which maintained viremia <400 RNA copies/ml during 5 years. We transiently depleted CD8+ cells in vivo in these animals by injection of anti-CD8 mAb. We analysed, in blood and tissues, the viral load evolution, T-cell frequency and activation, SIV-specific T-cell functionality and plasma cytokine levels.

Results: CD8+ -depletion in blood and tissues lasted between 10-21 days. One SIC kept undetectable viremia during depletion despite carrying infectious and in vitro inducible SIVmac$_{251}$ in CD4 T-cells. Four SIC experienced a viral rebound (3.10^3 -7.10^4 RNA copies/ml) but were able to subsequently re-control viremia to baseline levels (17-21 days post-depletion). In two SIC, regain of viral control started despite CD8+ T-cells being still undetectable. In the two other SIC, regain of viral control coincided temporally with the recovery of CD8 T-cells. However, CD8 T-cell recovery was accompanied by relatively weak expansion of SIV-specific CD8 T-cells with no acquisition of effector antiviral functions. Depletion provoked induction of pro-inflammatory cytokines and homeostatic activation/expansion of CD4+ T-cells, which correlated with plasma viremia (p=0.0006).

Conclusion: Our results suggest that SIV-specific CD8 T-cell responses are likely not the major contributor for the long-term maintainance of SIV-control in this SIC model. Other mechanisms, including weak viral reservoirs and control of activation, may contribute to viral control. Our SIC model will be useful to investigate mechanisms contributing to natural HIV-control in humans.

P11.46 LB

Comparison of the Depth of Vaccine-Elicited HIV-1 Env Epitope-Specific CD8+ T Lymphocyte Responses

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Background: One of the major challenges in the development of an effective HIV-1 vaccine is the extraordinary genetic diversity of the virus. Immunizations of nonhuman primates using consensus and mosaic immunogens have been shown to elicit cross-reactive CD8+T lymphocyte responses that increase the depth of epitope recognition. However, one of the limitations of vaccine-induced epitope-specific CD8+T lymphocytes includes lack of protection against diverse strains and emergent forms of HIV-1 due to altered T cell receptor (TCR) affinity for variant peptide:MHC class I complexes.

Methods: In this study, we immunized a cohort of fifteen Mamu-A*01+ rhesus monkeys with either a 3-valent mosaic Env, group M consensus Env, or single clade B Env vaccine and compared the ability of the CD8+T lymphocyte populations elicited by each immunogen to recognize variants of an HIV-1 envelope epitope sequence p41A (YI9). We identified vaccine-induced CD8+T lymphocytes populations using tetramers constructed with 9 variants of p41A epitope. We assessed the ability of those variant peptides to activate CTL by measuring cytokine production and CD107a expression. We evaluated proliferation using carboxyfluorescein succinimidyl ester. Finally, we investigated the functional avidity of these CD8+T lymphocytes for the variant peptide:Mamu-A*01 complexes using surface plasmon resonance technology.

Results: Our data show that Env immunizations can generate cross-reactive CD8+T lymphocytes that recognize 2 of 9 (22%) of the variants of p41A epitope, with higher responses induced by the consensus and the 3-valent mosaic immunogens (variant from clade C and variant from clade A/E) compared to the single clade Env immunogen. Tetramer-binding data also show that CD8+ T lymphocytes from monkeys immunized with mosaic immunogen have a trend of higher binding to majority of the variant peptides tested.

Conclusion: This underscores the potential of mosaic immunogens for generating cellular immune responses with greater depth.

P11.47 LB

Understanding The Precursor Frequencies of HIV-1 Specific CD4+ T Cells In Seronegative Donors

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Background: HIV-1 specific T cell responses are detectable amongst a proportion of HIV-1 exposed, seronegative individuals. Previous studies from our group have demonstrated these responses are predominately mediated by CD4+ T cells and can be mapped and titrated at the peptide level. Curiously, approximately 1 in 4 HIV-1 un-exposed seronegative donors also have demonstrable HIV-1 specific T cell responses. This observation raises a number of questions regarding the ontogeny of pre-existing HIV-1 specific T cells and their potential role in the acquisition of HIV-1

Methods: A highly sensitive T cell library method was used to screen the naïve, central and effector memory CD4+ T cell subsets from 10 healthy, HIV-1 seronegative, leukapharesis donors. 192 cell lines per subset were screened against pools of overlapping 18mer peptides, spanning the entire HIV-1 proteome and proliferative responses quantified using tritiated thymidine incorporation.

Results: HIV-1 specific CD4+ T cell response were detectable within the CD4+ T cell memory compartments of all 10 subjects tested, albeit at low frequency. HIV-1 specific CD4+ T cell responses spanned the entire HIV-1 proteome and were typically of low avidity. There was considerable variability between donors both in the proteins recognized and precursor frequencies of HIV-1 specific T cell responses. However, across all subsets tested CD4+ T cells specific for HIV-1 envelope appeared to exist at the highest precursor frequency, with Pol seemingly the least frequently targeted.

Conclusion: We show HIV-1 specific CD4+ T cells to be detectable within the memory compartment of all 10 donors tested. In the absence of known prior exposure to HIV-1 these observations are indicative of low level cross reactivity within the immune system. The use of the T cell library technique to interrogate the naïve and memory precursor frequencies of HIV-1 specific T cells should prove beneficial in the design of novel therapeutic vaccines.

P11.48 LB

IL-7 Abrogates Memory T Regulatory Cell Functions By Modulation of CD39/ATP Axis In Vitro And In Vivo In HIV Infected And Non-Infected Patients

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Background: IL-7 cytokine regulate the expansion and maturation of T cells. The good safety profile of IL-7 raises the opportunity of its use as a vaccine adjuvant. However, its role on the regulation of T cell responses has not been explored. We investigated the effects of IL-7 on regulatory T cell (Treg) functions invitro and invivo.

Methods: Treg and CD8+ T cells were isolated from healthy donors (n=10) (HD) and chronically HAART treated HIV+ pt enrolled in a phase I/II IL-7 INSPIRE study (n=6X) (Levy et al, CID, in press). Treg subpopulations (naïve CD45RA+FoxP3++CD 45RA+CD25++CD127+/-, memory (mTreg) Foxp3highCD45RA-CD25highCD127low, FoxP3++CD45RA-CD25++CD127+/-) were cultured with IL-7 (10 ng/ml). Phenotype (CD39, Bcl-2, Stat5P, purinergic receptor P2X7R), suppression of the proliferation of autologous antiCD3 activated CD8+ T cells (CFSE stained) and cytokine profile (IL-17 production) of Treg were analyzed.

Results: In HD, IL-7 induces expression of STAT5 and BCL-2 on all Treg populations. IL-7 reduces the suppressive effects of mTreg on CD8+ proliferation (% CFSE low w/wo IL7 was 15% and 40%, respectively, n=4, P=0.01). This effect was associated with a down-modulation of CD39 enzyme (MFI 65 vs 90 w/wo IL7, P=0.01) and an increase of P2X7R expression. IL-7 effect was reproduced using anti-CD39 blocking antibody and PPAD (inhibitor of P2X7R). IL-7 incubated Treg switched to a Th17 phenotype as assessed by the increase of Th17 production and RORgC expression. IL-7 treated patients exhibited a decrease of the frequency of Treg/ CD39+ and an increase of RORgC mRNA in PBMCs as compared to pre-IL-7 therapy.

Conclusion: IL-7 relieves the suppressive effect of mTreg through a modulation of the CD39/ATP axis. By increasing P2X7R expression, IL-7 increases the susceptibility of these cells to ATP, a trigger of Th17 differentiation. An effect also observed in IL-7 treated patients. These results suggest that IL-7 could be used as adjuvant to reinforce T cell responses.

Topic 12: Vaccine Concepts and Design

P12.01

Construction of Site Selected Phage Library and Characterization of Anti-V3 scFvs from Indian Clade C HIV-1 Infected Patient

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Background: Till date, few broadly neutralizing antibodies are generated against HIV-1 and they have limited breadth and potency against clade C viruses, which are predominant worldwide and in India. Here we have produced nine different human scFvs against the V3 region of HIV-1 envelope.

Methods: A V3 specific phage library was constructed from EBV transformed B cells of a clade C HIV-1 infected Indian patient, whose plasma exhibited good neutralization potential against a panel of viruses. Diversity of the constructed phage library was analysed by DNA fingerprinting of 10 randomly selected clones from the unselected library using BstN1 enzyme. One round of biopanning was done against HIV-1 consensus V3C and V3B peptides. scFvs were than characterised for their binding, specifity and expression profile. VH and VL genes of anti-V3 scFvs were sequenced for their preferential gene usage.

Results: DNA fingerprinting analysis of clones from unselected library showed that 90% of clones in the library were distinct. Thirty clones were randomly selected after biopanning. Nine clones showed binding in phage ELISA and exhibited a unique DNA fingerprint. Soluble expression of the selected scFvs was checked by SDS-PAGE and confirmed by Western blot. All the nine anti-V3 scFvs showed cross-reactivity against both the V3 peptides and did not bind to unrelated peptides. Distribution of VH gene segments of these anti-V3 scFvs were different, 56% (5/9) of scFvs used VH4, thirty 33% (3/9) VH5 and 11% (1/9) showed VH3 gene usage. Among the light chains, IGKV1 and IGKV3 were most preferentially used gene segments. Further these scFvs displayed a stable binding to V3 peptides in different denaturing agents.

Conclusion: This is the first study to generate human anti-V3 scFvs against HIV-1 clade C. Further characterization of these scFvs for their neutralization potential and epitope mapping will provide useful information for immunogen design.

P12.02

Improving Immunogenicity of HIV-1 Envelope gp120 by Glycan Removal and Immune Complex Formation

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Background: HIV-1 envelope (Env) gp120 is an important target for neutralizing antibody (Ab) responses against the virus. However, developing gp120 vaccines that elicit potent and broad neutralizing Abs has proven to be a formidable challenge. The envelope gp120 is highly glycosylated and carbohydrate moieties play an important role in modulation of immunobiological property of HIV-1 Env gp120. Previously, removal of a specific N-linked glycan at residue 448 by N to Q mutation (N448Q) has been found to enhance in vitro antigenicity of neutralizing epitopes in the V3 loop. In the present study we examined immunogenicity of mutant gp120 in mice.

Methods: Two immunization protocols were tested. First, using plasmid DNA expressing gp120BH10 followed by protein boost with QS-21 adjuvant. Second, gp120 was administered as an immune complex with antibody in DDA/MPL adjuvant. Cellular responses were measured using spleen cell proliferation and cytokine production by Bio-Plex multiplex assay. Sera were evaluated for antibody binding via ELISA and neutralization activity via TZM-bl assay

Results: With the DNA prime/protein boost protocol, N448Q gp120 mutant induced higher levels of gp120 specific lymphoproliferation and cytokine production as compared to wild type. However, both mutant and wild type gp120s failed to generate anti-V3 Abs and virus-neutralizing Ab response. In contrast, immunization with mutant gp120 in complex with mAb 654 elicited higher titers of neutralizing Abs activity than the wild type counterpart. Neutralizing activity was directed to V3 and other undefined neutralizing epitopes. Improved immunogenicity of immune complexes correlated with increased reactivity and proteolytic resistance of V3 and other Ab epitopes

Conclusion: These data demonstrate the advantage of combining site-specific N-glycan removal and immune complex formation as a novel vaccine strategy to improve immunogenicity of Ab epitopes on critical regions of HIV-1 gp120. Importantly, epitope immunogenicity is governed not only by its antigenicity but also by its stability against proteolytic degradation

P12.03

ABSTRACT WITHDRAWN

P12.04

Simian Immunodeficiency Virus-Vpx as an Adjuvant for Integrase Defective Lentiviral Vector-Based Vaccines

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Background: Integrase defective lentiviral vectors (IDLV) represent a promising delivery system for immunization purposes. Human dendritic cells (DC) are the main cell types mediating the immune response and are readily transduced by IDLV, allowing effective triggering of in vitro expansion of antigen-specific primed CD8+ T cells. However, DC transduction efficiency is hindered by the presence of SAMHD1 restriction factor, which inhibits viral DNA synthesis.

Methods: IDLV expressing Flu-M1 containing simian immunodeficiency virus (SIV)-Vpx was produced and titred on 293T by standard methods. Monocytes from HLA-A*0201 and M1-positive selected donors were differentiated into DC and transduced with IDLV-M1/Vpx and control IDLV/M1 or left untreated. IDLV-transduced DC were co-cultured with autologous PBMC and the expansion of M1-specific CD8+ T cells was analysed by pentamer staining and IFN_ ELISPOT

Results: The addition of the SIV-Vpx protein during IDLV preparation resulted in a striking improvement of IDLV transduction of human DC, thus increasing the ability of IDLV-transduced DC to act as functional antigen presenting cells, as evaluated by pentamer staining and IFN_ ELISPOT, in the absence of vector integration. Importantly, the presence of SIV-Vpx allows for the use of lower amount of input vector preparation, further improving the safety profile of IDLV.

Conclusion: These results have important implications for the development of vaccine strategies based on the use of IDLV as a novel, safe and efficient delivery system.

Topic 12: Vaccine Concepts and Design

P12.05

Conservation of HIV-1 T Cell Epitopes Across Time and Clades: Validation of Immunogenic HLA-A2 Epitopes Selected for the GAIA HIV Vaccine

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Background: HIV genomic sequence variability has complicated efforts to generate an effective globally relevant vaccine. Our strategy for HIV-1 vaccine design is to select epitopes that can induce broad and dominant HLA-restricted immune responses targeted to the regions of the viral genome conserved in sequence, three-dimensional configuration, and across time which may represent regions that are constrained due to functional or structural limitations. These "Achilles' Heel" epitopes would be ideal candidates for inclusion in an epitope-based HIV vaccine.

Methods: Highly conserved T-cell epitopes were selected using the EpiMatrix suite of immunoinformatic tools. This analysis was first performed in 2002 on 10,803 HIV-1 sequences available at that time and again in 2009 on an expanded 43,822 sequences. Selected epitopes were validated for binding and immunogenicity with PBMCs from HIV-infected donors in Providence, RI and Bamako, Mali.

Results: 38 highly conserved HLA-A2 candidate epitopes were selected. Analysis done in 2009 revealed surprising stability of 25 of the epitopes selected in 2002 and identified an additional 13 highly conserved HLA-A2 candidates. Thirty-five (92%) of the 38 selected epitopes stimulated IFNg response in PBMC from at least one subject. Twenty-one of 25 peptides selected in 2002 were validated in assays performed in Providence. Eleven (85%) of the 13 peptides selected in 2009 were confirmed in assays performed in Mali. Twelve of 18 peptides assayed in both Providence and Mali were confirmed in both locations.

Conclusion: The validation of these selected HLA-A2 epitopes conserved across time (2002-to-2009), geography (Providence and Mali) and clades supports the hypothesis that these epitopes could provide effective coverage of virus diversity and would be appropriate candidates for inclusion in a globally relevant HIV vaccine.

P12.06

Further Confirmation of Broadly Conserved, Highly Immunogenic Cross-Clade HIV CTL Epitopes for Inclusion in the GAIA HIV Vaccine

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Background: One of the biggest challenges for HIV vaccine design is identifying conserved HLA Class I- and Class II-restricted epitopes that would impart substantial fitness cost to the virus, thereby controlling or preventing infection. Here, we set out to develop an epitope-driven, DNA-prime/pseudoprotein-boost HIV vaccine (GAIA vaccine) composed of such epitopes.

Methods: Analysis was performed in 2003 on 10,803 HIV-1 sequences, and again in 2009, on an expanded set of 43,822 sequences. These were searched for conserved 9-10-mer segments with the EpiMatrix suite of immunoinformatic algorithms. From the most highly conserved (>5% isolates) or top 1,000 scoring 9-mers, HLA Class I binding sequences and Class II immunogenic consensus sequences (ICS) were identified for vaccine design. Validation was performed in Thailand, the USA, and Mali via in vitro binding and IFN-γ ELISpot assays, using HIV-infected donor peripheral blood. HLA transgenic mice were immunized in DNA-prime/peptide-boost vaccine studies including these epitopes.

Results: Epitopes selected as described are more broadly conserved than those selected for other epitope-based vaccines (>70%, compared to Epimmune's 40%). Antigenicity of 98% of ICS epitopes and Class I epitopes (87% A2, 29% A3, 67% B7, 20% A24) was confirmed in HIV-infected subjects. Fifteen ICS peptides and 12 A2 peptides were confirmed in both Mali and the USA. Sixteen A3 peptides were confirmed weighting sequences for conservation and immunogenicity (as determined by EpiMatrix score), in vitro binding, and positive ELISpot responses. Epitope immunogenicity was validated in HLA transgenic mouse immunization studies.

Conclusion: The GAIA vaccine approach is an effective means of triaging HIV epitope sequences to identify the most immunogenic and conserved epitope candidates across HLA allotypes. HLA transgenic mouse studies showed prototype DNA vaccines including these epitopes to be immunogenic, suggesting the epitope-rich GAIA vaccine would induce greater immunogenicity than other DNA/viral vector prime-boost vaccines in humans.

P12.07

Extensive Glycoform Heterogeneity in the gp120 Envelope Proteins Used in the RV144 Trial

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Background: The AIDSVAX B/E vaccine used in the RV144 trial consisted of recombinant gp120s derived from the MN and A244 strains of HIV-1, both produced in CHO cells. In order to understand the correlates of protection and to design RV144 follow-up studies, it is important to characterize the vaccine immunogens and to know the extent to which newly manufactured gp120 subunit vaccines replicate the glycosylation of the AIDSVAX B/E vaccine immunogens. It has long been known that glycosylation affects antigenicity and immunogenicity. Recent data suggest that several epitopes recognized by broadly neutralizing antibodies are critically dependent on glycosylation in the gp120 V2 domain. In these studies we investigate the heterogeneity in net charge attributed to glycoform variation.

Methods: Isoelectric focusing was used to analyze recombinant MN and A244 rgp120 proteins from the unformulated bulk used to manufacture the AIDSVAX B/E vaccine. We compared these proteins with MN and A244 rgp120 proteins freshly produced in 293 cells. Differences in the binding of monoclonal antibodies and soluble CD4 were measured by ELISA.

Results: MN and A244 rgp120 proteins produced in CHO cells and 293 cells exhibit extensive heterogeneity in net charge due to glycosylation. More than 16 species of MN-rgp120 and 24 species of A244-rgp120 were identified. Proteins produced in CHO cells were distinctly more acidic than proteins produced in 293 cells. These differences affected the binding of ligands that targeted the CD4 binding site but not other regions of gp120.

Conclusion: The rgp120 proteins used in the RV144 trial exhibited remarkable variation in net charge attributable to differences in glycosylation. The extent of gp120 glycosylation is cell-line dependent. Differences in glycosylation affect antibody binding and may represent a significant variable in the development of new antigens for RV144 follow-up studies and in neutralization assays that depend on pseudoviruses produced in 293 cells.

P12.08

Antigen Processing Sites in gp120 Are Conserved Across HIV Virus Clades

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Background: A puzzling observation in HIV vaccine research is the fact that recombinant gp120 is able to adsorb bNAbs from HIV+ patient sera, but unable to elicit such bNAbs. To account for its poor immunogenicity, we wondered if the epitopes recognized by bNAbs might be proteolyzed in vivo. Cathepsins L, S, and D are the major proteases responsible for antigen processing and presentation. Previously, we defined the cathepsin cleavage sites on MN rgp120 and found that they co-localized with epitopes recognized by bNAbs. Although examination of gp120 sequences suggested that these sites were conserved, the recognition motifs for cathepsins are poorly defined and it was important to verify their presence by protease digestion studies.

Methods: Purified gp120s from three clades of HIV were digested with cathepsins L, S, and D. These included envelope proteins from the from the 108060 (clade B), A244 (CRF01_AE) and 97001 (clade C) isolates. N-terminal sequencing was used to identify the cathepsin cleavage sites.

Results: When combined with the previous MN-rgp120 results, we found that 6 out of 10 cathepsin cleavage sites were conserved in four viruses from three different clades of HIV. We found that polymorphisms that inactivate cleavage sites often result in the formation of an alternate nearby site. Although cleavage is an ordered processing beginning in the V3 domain, the cleavage sites in this domain are more polymorphic than other sites.

Conclusion: Our results suggest that cathepsin cleavage sites are highly conserved in gp120 and co-localize to regions recognized by bNAbs. These results are consistent with our hypothesis that the poor immunogenicity of gp120 epitopes results from protease digestion in vivo. Current studies are in progress to determine whether inactivation of these sites or enzymes may provide a new approach to improving the immunogenicity of epitopes recognized by bNAbs.

Topic 12: Vaccine Concepts and Design

P12.09

Development of Chimeric HIV Env Immunogens for Mucosal Delivery with Attenuated Canine Distemper Virus (CDV) Vaccine Vectors

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Background: Our aim is to develop replication-competent viral vectors for mucosal delivery, which express HIV-Env immunogens that closely mimic the trimeric glycoprotein spike found on HIV particles. We have developed attenuated recombinant CDV (rCDV) expressing SIVmac239-Env and shown that this vector can be used safely to elicit Env-specific immune responses in ferrets and non-human primates through intranasal administration.

Methods: To augment the cell surface expression of trimeric HIV-Env and increase the replicative capacity of rCDV vectors encoding the HIV glycoprotein, we have constructed chimeric immunogens in which signal peptide (SP), transmembrane domain (TM), or cytoplasmic tail (CT) domains in HIV-Env have been replaced with analogous sequences from the vesicular stomatitis virus (VSV)-G or CDV-F glycoproteins and compared cell surface protein expression, antibody binding profiles and Env function in transient expression assays.

Results: Chimeric glycoprotein based on subtype C Env proteins were expressed on the surface of transfected cells in conformations recognized by various broadly neutralizing antibodies (bnAb) targeting distinct Env regions including VRC01, b12, and b6 specific for the CD4 binding site, PG9 and PG16 (V1/V2), 4e10 (gp41) but not by PGT-126 (V3) and 2G12 (glycans). Moreover, treatment of transfected cells with soluble CD4 induced conformational changes needed to expose epitopes for CD4 binding-dependent antibodies (17b, 48d and F425-A1g8). Recombinant CDV vectors encoding two chimeric Envs have been created. One is expressed most abundantly in transfected cells and contained the VSV SP and CDV TM-CT. The second is a highly fusogenic Env that contained the CDV SP and CDV CT. Both vectors expressed Env and are being further characterized in vitro and in vivo.

Conclusion: Collectively, we have shown CDV can be used as a mucosal delivery vector for SIV-Env and that HIV-Env modifications can be made that improve cell surface expression of the trimeric glycoprotein containing structural determinants recognized by bnAbs.

P12.10

Stability and Neutralization Capacity of a Novel Mosaic HIV-1 gp140 Trimer in a Guinea Pig Model

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Background: The generation of globally relevant HIV-1 immunogens mimicking native, trimeric Envelope (Env) structure, remains a major challenge for HIV-1 vaccine development. We identified a mosaic Env sequence, originally designed to optimize cellular immunologic coverage of global HIV-1 sequence diversity, which was capable of forming biochemically stable trimers. We assessed this mosaic Env gp140 trimer in guinea pig immunogenicity studies compared to our previously reported biochemically stable C97ZA012 (clade C) trimer.

Methods: Stable Env gp140 trimer derived from a synthetic mosaic sequence was stabilized with the T4-fibritin C-terminal trimerization tag and produced in 293T cells via PEI transfection. Characterization was performed by Western blotting, size-exclusion chromatography and surface plasmon resonance (SPR). Guinea pigs were immunized three times with 100 µg of mosaic or clade C gp140 protein trimer in CpG/Emulsigen adjuvants. Antibody responses were determined by ELISA and TZM.bl neutralizing antibody assays.

Results: Stabilized mosaic gp140 trimer exhibited a single band by Western blotting, single peak by size-exclusion chromatography both after production, a freeze-thaw cycle and 7-day incubation at 4ºC. SPR analyses revealed mosaic gp140 binding with bNAb VRC01. The trimeric mosaic gp140 immunogen elicited high-titer antibodies in guinea pigs by ELISA and high-titer, cross-clade neutralization activity against tier 1 viruses. When compared to clade C gp140 trimer, NAb responses generated by mosaic gp140 trimer were 8.2- and 3.7-fold higher against clade B viruses (SF162.LS and Bal.26, respectively) but were 10.3- and 46.7-fold lower against clade A and C viruses (DJ263.8 and MW965.26, respectively).

Conclusion: A novel, foldon-stabilized mosaic gp140 trimer elicits high-titer binding antibodies as well as high-titer, cross-clade neutralization of tier 1 viruses. The profile of the NAbs elicited by the mosaic trimer differed from that elicited by the clade C trimer. Further exploration and refinement of this concept may contribute to the development of a globally relevant Env immunogen.

Topic 12: Vaccine Concepts and Design

P12.11

Targeting HIV-1 Envelope Glycoprotein Trimers to B Cells Using APRIL Improves Antibody Responses

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Background: An HIV-1 vaccine remains elusive, in part because various factors limit the quantity and quality of the antibodies raised against the viral envelope glycoprotein complex (Env). We hypothesized that targeting Env vaccines directly to B cells, by fusing them to molecules that bind and activate these cells, would improve Env-specific antibody responses.

Methods: We fused trimeric Env gp140 to A PRoliferation-Inducing Ligand (APRIL), B-cell Activating Factor (BAFF), and CD40 Ligand (CD40L).

Results: The Env-APRIL, Env-BAFF and Env-CD40L gp140 trimers all enhanced the expression of activation-induced cytidine deaminase (AID) expression, the enzyme responsible for inducing somatic hypermutation, antibody affinity maturation and antibody class-switching. They also triggered IgM, IgG and IgA secretion from human B cells in vitro. The Env-APRIL trimers induced higher anti-Env antibody responses in rabbits, including neutralizing antibodies against Tier 1 viruses. The enhanced Env-specific responses were not associated with a general increase in total plasma antibody concentrations, indicating that the effect of APRIL was Env-specific. All the rabbit sera raised against gp140 trimers, irrespective of the presence of CD40L, BAFF or APRIL, recognized trimeric Env efficiently, while sera raised against gp120 monomers did not. The levels of trimer-binding and virus-neutralizing antibodies were strongly correlated, suggesting that gp140 trimers are superior immunogens to gp120 monomers.

Conclusion: Targeting and activating B cells with a trimeric HIV-1 Env-APRIL fusion protein may improve the induction of humoral immunity against HIV-1. Targeting B cells directly may also be useful for other vaccines.

P12.12

The Viral Vector Vaccine VSV-GP Boosts Immune Response upon Repeated Applications

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Background: Vesicular stomatitis virus (VSV) is a potent candidate vaccine vector for various viral diseases (e.g. HIV, HCV, RSV). The biggest limitation of VSV, however, is its neurotoxicity, which limits application in humans. The second drawback is that VSV induces neutralizing antibodies rapidly and is thus ineffective as a vaccine vector upon repeated applications. Our group has recently shown that VSV pseudotyped with the glycoprotein (GP) of the lymphocytic choriomeningitis virus (LCMV), VSV-GP, is not neurotoxic. The aim of this project was to evaluate the potential of VSV-GP as a vaccine vector.

Methods: For this purpose, we used Ovalbumin (OVA) as a model antigen and analyzed immunogenicity of GP-pseudotyped and wildtype VSV containing OVA (VSV-GP-OVA and VSV-OVA) in vitro and in vivo in mouse models.

Results: We showed that both vectors infected murine bone marrow-derived dendritic cells (bmDCs) in vitro. These bmDCs were able to activate OVA specific CD8+ and CD4+ T cells. Immunization experiments in mice revealed that both VSV-OVA and VSV-GP-OVA induced functional OVA-specific cytotoxic T cells (CTLs) after a single immunization. In addition, with both viruses, mice generated antibodies against OVA. However, boosting with the same virus was only possible for the GP-pseudotyped virus but not for wild type VSV. The efficacy of repeated immunization with VSV-OVA was most likely limited by high levels of neutralizing antibodies, which we detected after the first immunization. In contrast, no neutralizing antibodies against VSV-GP were induced even after boosting.

Conclusion: Taken together, we showed that the non-neurotoxic VSV-GP is able to induce specific T cell and B cell responses against the model antigen OVA to the same level as the wild type VSV vector. However, in contrast to wild type VSV, VSV-GP-OVA boosted the immune response upon repeated applications. Thus, VSV-GP is a promising novel vaccine vector.

Topic 12: Vaccine Concepts and Design

P12.13

Immune Responses Triggered by HIV/AIDS Vaccine Candidates, Derived from MVA-B, with Deletions in Several Immune Regulatory Genes

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Background: Poxvirus vector Modified Vaccinia Virus Ankara (MVA) expressing HIV-1 Env, Gag, Pol and Nef antigens from clade B (termed MVA-B) is a promising HIV/AIDS vaccine candidate, as it was shown in the results obtained from a phase I clinical trial.

Methods: To try to improve the immunogenicity elicited by MVA-B we have generated and characterized the innate immune sensing and the in vivo immunogenicity profile of new optimizing MVA-B vaccine candidates, which contains deletions in one, two or three different immunomodulatory vaccinia virus (VACV) genes blocking the same signaling pathway, involved in the induction of type I IFN.

Results: The innate immune signals elicited by these MVA-B deletion mutants in human macrophages showed an up-regulation of the expression of IFN-β and IFN-α/β-inducible genes. A DNA prime/MVA boost immunization protocol in mice revealed that these MVA-B deletion mutants were able to induce strong and polyfunctional HIV-1-specific CD4+ and CD8+ T-cell adaptive and memory immune responses, which were mostly mediated by CD8+ T cells with an effector phenotype. CD4+ T-cell responses were mainly directed against Env in MVA-B and all the MVA-B deletion mutants. However and interestingly, while MVA-B induced preferentially Env- and Gag-specific CD8+ T-cell responses, MVA-B deletion mutants induced more GPN-specific CD8+ T-cell responses. Moreover, an enhanced HIV-1-specific lymphoproliferative response was observed with the MVA-B deletion mutants. Furthermore, MVA-B and MVA-B deletion mutants were also able to induce antibodies against Env.

Conclusion: These findings revealed that deletion in MVA-B of VACV genes that act blocking the same signaling pathway confers an immunological benefit by inducing innate immune responses and increasing the magnitude, quality and durability of the HIV-1-specific T-cell immune responses. Our observations focused the use of highly optimizing MVA-based vectors as more potent HIV-1 vaccines.

P12.14

Systems Analysis of MVA-C Induced Immune Response Reveals Its Significance as a Vaccine Candidate Against HIV/AIDS of Clade C

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Background: Based on the partial efficacy of the HIV/AIDS Thai trial (RV144) with a canarypox vector prime and protein boost, attenuated poxvirus recombinants expressing HIV-1 antigens are increasingly sought as vaccine candidates against HIV/AIDS.

Methods: Here we describe using systems analysis the biological and immunological characteristics of the attenuated vaccinia virus Ankara strain expressing the HIV-1 antigens Env/Gag-Pol-Nef of HIV-1 of clade C (referred as MVA-C).

Results: MVA-C infection of human monocyte derived dendritic cells (moDCs) induced the expression of HIV-1 antigens at high levels from 2 to 8 hpi and triggered moDCs maturation as revealed by enhanced expression of HLA-DR, CD86, CD40, HLA-A2 and CD80 molecules. Infection ex vivo of purified mDC and pDC with MVA-C induced the expression of immunoregulatory pathways associated with antiviral responses, antigen presentation, T cell and B cell responses. Similarly, human whole blood or primary macrophages infected with MVA-C express high levels of proinflammatory cytokines and chemokines involved with T cell activation. The vector MVA-C has the ability to cross-present antigens to HIV-specific CD8 T cells in vitro and to increase CD8 T cell proliferation in a dose-dependent manner. The immunogenic profiling in mice after DNA-C prime/MVA-C boost combination revealed activation of HIV-1-specific CD4 and CD8 T cell memory responses, that are polyfunctional and with effector memory phenotype. Env-specific IgG binding antibodies were also produced in animals receiving DNA-C prime/MVA-C boost.

Conclusion: Our systems analysis of profiling immune response to MVA-C infection highlights the potential benefit of MVA-C as vaccine candidate against HIV/AIDS for clade C, the prevalent subtype virus in the most affected areas of the world.

P12.15

A Novel HIV Vaccine Targets the 12 Protease Cleavage Sites

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Background: The protease of HIV-1 is a small 99-amino acid aspartic enzyme mediating the cleavage of Gag, Gag-Pol and Nef precursor polyproteins. The process is highly specific, temporally regulated and essential for the production of infectious virions. A total of 12 proteolytic reactions are required to generate a viable virion. Therefore, a vaccine targeting the 12 protease cleavage sites(PCS) could be effective. The PCS of HIV-1 are highly conserved among major subtypes, direct immune responses against these sites would yield several advantages. First, the immune response could destroy the virus before its establishment in the host. Second, the vaccine could force the virus to accumulate mutations eliminating the normal function of the HIV protease. Third, restricting the immune responses to these sites can avoid distracting immune responses that often generate unwanted inflammatory responses, induce excess immune activation, and attract more targets for HIV-1 infection, establishment and spread.

Methods: We have conducted a pilot study to investigate the feasibility and effectiveness of this approach. The recombinant VSV-peptides were used to immunize cynomolgus macaques and nanopackaged peptides were used to boost the immune response to the 12 PCS of SIVmac239. The controls and immunized macaques were repeatedly challenged intrarectally with an increased dosage of SIVmac239.

Results: Results showed that antibody and T cell responses to the 12 PCS can protect macaques against higher dosage of SIVmac239 challenge (p=0.0005, R=0.8005) and the vaccine group maintains significantly higher CD4+ counts (p=0.0002) than the controls weeks after being infected. Population coverage analysis showed that this approach can be applied to >95% populations in the world.

Conclusion: A vaccine targets the 12 protease cleavage sites is a viable approach for HIV prevention and treatment.

P12.16

A Minimal T-Cell Immunogen Designed to Cover HIV-1 Specificities Associated with Control Is Immunogenic in Mice and Breaks CTL Immunodominance

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Background: Few T-cell immunogen vaccine designs exist that are based on large human immunogenicity data and which avoid inducing responses to mutable epitopes that may serve as immunodominant decoys. We have developed and tested a rationally designed T cell immunogen sequence which overcomes these limitations and which is currently undergoing pre-clinical testing.

Methods: 250 HIV-1 clade B infected individuals were screened for T cell responses to the entire HIV proteome. This yielded 26 regions in HIV-1 Gag, Pol, Vif and Nef proteins that were i) preferentially targeted by individuals with low viral loads, ii) more conserved and iii) elicited responses of higher functional avidity and broader cross-reactivity than responses to other, less-beneficial regions. The 'beneficial' segments were linked by triple alanines, translated into an expression-optimized nucleotide sequence and cloned into a CMV plasmid harboring a GM-CSF signal peptide. Immunogenicity was evaluated in C57BL/6 mice two weeks after a second DNA vaccination. Cellular immune responses were characterized using intracellular cytokine staining and IFN-γ ELISPOT using overlapping peptide pools covering the segments included in the T-cell immunogen.

Results: Vaccination with 20 μg of DNA generated both CD4 and CD8 IFN-γ+ responses to the immunogen sequence. The T-cell immunogen elicited a more balanced, broad T cell response to all protein components (Gag, Pol, Vif and Nef) contained in the immunogen than immunizations using plasmids encoding for the entire Gag, Pol, Nef, Tat and Vif proteins, which induced a strong Gag dominance.

Conclusion: Despite lower in vitro expression, the DNA vaccine was strongly immunogenic in C57BL/6 mice, induced broad CD4 and CD8 T cell responses and was able to break the immunodominance of responses to targets that do not emerge as particularly beneficial in large cohort screenings. Experiments in humanized BLT mice are currently ongoing to map induced responses in the context of different HLA genotypes.

Topic 12: Vaccine Concepts and Design

P12.17

Control of HIV Replication By a Novel Constitutively Active 'Super-PRR'

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Background: Innate responses are key determinants of the outcome of HIV infection, influencing critical events in the earliest stages of infection. Innate antiviral immune defenses are triggered through the recognition of conserved pathogen associated molecular pattern (PAMP) motifs within viral products by intracellular pattern recognition receptor (PRR) proteins in infected cells. Type I interferons (IFN α and beta) are induced directly in response to viral infection, resulting in an antiviral state for the cell.

Methods: A ligand independent constitutively active form of PRR adaptor protein ('super-PRR') was constructed and tested for antiviral properties. NF-kb and IFN production by construct was tested by Luciferase reporter assays. The construct was transfected into RAW 264.7 cells, analyzed for surface markers and inflammatory cytokines. RT-PCR was performed to analyze the expression of cytokine/chemokine genes. TZM-bl cells were transfected with plasmid expressing 'super-PRR', infected with HIV-BaL virus and beta-galactosidase activity was measured.

Results: The constitutively active 'super-PRR' generated 50-fold and 10-fold increase in NF-kb and IFN-beta production respectively as compared to vector alone. There was significant increased expression of CD80, CD86, CD40, CCR7, HLA-DR, secretion of IL-1beta, IL-6, TNF-α and expression of RANTES, MIP-1beta, IP-10 on transfected RAW cells. TZM-bl cells transfected with 'super-PRR' after infection with HIV-Bal virus showed significantly reduced replication of virus. In a transwell experiment, 293T cells transfected with the 'super-PRR' was sufficient to significantly reduce HIV-1 infection of TZM-bl cells, suggesting soluble factors are involved.

Conclusion: This constitutively active form of PRR adapter protein induced potent anti-viral innate immune response and prevented infection with HIV. The modulation of innate immunity has potential as a powerful strategy to complement traditional approaches to HIV therapy by protecting cells from viral infection. We are currently constructing lentiviral vector vaccines encoding this 'super-PRR' as a method to target this anti-viral response to the site of HIV-1 Infection.

P12.18

Conformational Heterogeneity in V1/V2 Domain Affects the Immunological Properties of This Region

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Background: Recent information has shown the importance of the V1/V2 domain as a target in HIV-1 vaccines. This includes the localization of epitopes to potently neutralizing antibodies to this region, the demonstration that binding between a site in V1/V2 and α4β7 receptors facilitates infection in primary CD4-positive T cells, and the demonstration that the presence of antibodies binding to the native form of the V1/V2 domain of HIV-1 gp120 correlated with protection in the RV144 vaccine trial. These results highlight the need to better understand the structure and immunological properties of this region, in order to optimally express the relevant targets in HIV-1 vaccines.

Methods: The native V1/V2 domain of a clade B sequence (CaseA2) was expressed by fusion to the C-terminus of a 273 aa fragment of the MuLV gp70 domain. This fusion glycoprotein was characterized by SDS-PAGE under various conditions, by radioimmunoprecipitation experiments with a panel of mAbs directed against V1/V2-specific epitopes, and by MALDI-TOF analysis of immunologically fractionated forms.

Results: SDS-PAGE analysis of this protein after deglycosylation under non-reducing conditions revealed the presence of a closely migrating doublet, and mass-spec analysis of the immunologically fractionated forms suggested that these consisted of different disulfide-linked conformers. The two forms reacted differentially with a panel of V1/V2-specific antibodies and possessed variable reactivity with different polyclonal sera.

Conclusion: These studies identify a conformational heterogeneity in the V1/V2 domain that is due to alternative disulfide bonding patterns. These conformations profoundly affect the immunoreactivity of this region, and will presumably influence their immunogenicity as well. Due to the importance of this region as a target for neutralizing and otherwise protective antibodies, it is likely that methods for resolving this heterogeneity would improve the efficacy of induction of relevant antibodies to this region by HIV Env vaccines.

P12.19

Immunisation with the Membrane Proximal External Region of gp41 of HIV-1 Grafted into the Transmembrane Envelope Protein of a Gammaretrovirus

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Background: Immunisation with the transmembrane envelope (TM) proteins p15E of different gammaretroviruses (e.g., porcine endogenous retrovirus, feline leukaemia virus, Koala retrovirus) resulted in strong neutralising activity, the antibodies recognised epitopes in the fusion peptide proximal region (FPPR) and in the membrane proximal external region (MPER). The MPER epitopes were localised similarly as the epitopes recognised by the broadly neutralising antibodies 2F5 and 4E10 in gp41 of HIV-1. Despite the evolutionary difference between HIV-1 and the gammaretroviruses, the MPER epitope of antibodies neutralising PERV (FEGWFN) showed partial homology to the epitope of the 4E10 (NWFNIT, note three identical amino acids). To generate hybrid antigens able to induce 2F5/4E10-like antibodies, sequences of the MPER and FPPR of gp41 were grafted into the p15E backbone of a gammaretrovirus.

Methods: Different hybrid antigens were cloned, expressed in E. coli and purified. Immunisation studies in rats and guinea pigs were performed and the antisera were characterised by ELISA, Western blot analysis, epitope mapping using microarray chips with overlapping peptides and a neutralisation assay based on TZM-bl cells.

Results: Antibodies against gp41 of HIV-1 were induced, recognising epitopes in the FPPR, but also the 2F5 epitope (ELDKWA) in the MPER. Step by step changes in the sequence of the hybrids resulted in improved binding of the antibodies to this epitope. However, none of the immune sera or purified IgG neutralised HIV-1 more that 50%.

Conclusion: Since modifications in the hybrid proteins led to an increased anti-MPER response, it may be expected that further modifications increase neutralisation efficacy and that these hybrids may be the basis for candidate vaccines against HIV-1.

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P12.20

Development of a Novel Simian Adenovirus 24 Based Vaccine Vector

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Background: Human adenovirus serotype 5 is a potent vaccine vector, but its use has been hampered by high seroprevalence amongst people in sub-Sahara Africa. Novel adenoviral vaccine vectors from strains with lower seroprevalence worldwide are being developed that can evade pre-existing immunity. Here we describe the development of a simian Ad24 (sAd24)-based vaccine vector.

Methods: Neutralizing antibodies against sAd24 were determined using a panel of 106 rhesus macaque sera and 128 human sera from Rwanda and South Africa using a luciferase-based adenovirus neutralization assay.

The immunogenicity of a single dose of 10E7, 10E8 or 10E9 virus particles of sAd24-SIV Gag based vector was determined in C57BL/6 mice. SIV-Gag-specific immune responses were assessed by Db/AL11 tetramer binding assays, IFN-γ ELISPOT assays and ICS assays.

Results: Neutralizing antibodies were found in 7% of monkeys, all with titers <200. In humans from sub-Saharan Africa, 45% was positive for sAd24 neutralizing antibodies, but titers remained low and 90% had titers <200. In comparison, seroprevalence of Ad5 in sub-Saharan Africa is 86.4-89.5% with 61.1-78.7% of this population showing titers >200 and 25.1-46.8% showing titers >1000. Gag specific cellular immune responses elicited by sAd24-SIV Gag in mice are comparable to those seen with the human Ad26 and Ad28 vectors currently in development.

Conclusion: These data suggest that sAd24 is promising for further studies as a candidate vaccine vector.

Topic 12: Vaccine Concepts and Design

P12.21

Development of Replication-Competent Adenovirus Based Vaccine Vectors

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Background: Replication-incompetent adenovirus vectors have shown promise as vaccine candidates. We are developing replication-competent adenovirus vectors to increase antigen expression and duration and to facilitate mucosal routes of vaccine delivery. We have developed replication-competent Ad5 (rcAd5) and Ad26 (rcAd26) based vectors, tested their growth in human and simian cell lines, and determined the dynamics of virus shedding after inoculation of rhesus monkeys.

Methods: Replication-competent Ad5 and Ad26 vectors were produced by adding the E1 region back into the vector. To facilitate rcAd5 growth in rhesus monkey cells, 2 host range mutations were also introduced into the DNA binding protein. The growth of rcAd5 and rcAd26 was tested in human (Per55K, 293, and A549) and simian (CV-1 and Cos7) cell lines. In addition, rcAd5 was administered intranasally to rhesus monkeys, and the kinetics of viral shedding was determined by qPCR on nasal, oral, and rectal swabs, and serum.

Results: As expected, replication-incompetent Ad5 and Ad26 grew in the E1-complementing cell lines Per55K and 293, but not in A549 or CV-1 cells. In contrast, rcAd5 and rcAd26 grew in all human and simian cell lines tested, although rcAd26 growth was suboptimal in simian cells. After intranasal inoculation of rhesus monkeys, rcAd5 viral sequences could be detected by qPCR in nasal swabs for 5 weeks post-inoculation.

Conclusion: Replication-competent Ad vectors can be produced efficiently by the re-introduction of E1 into standard replication-incompetent vector backbones. However, the extent of replication in simian cells appears to vary based on Ad serotype. Future studies will compare the immunogenicity of replication-competent vs. replication-incompetent Ad vectors.

P12.22

IFN-γ Secreting Capacity of CD8+T Cell Is Compromised with the Increased Copies of Epitope Encoding Sequences in DNA Vaccine Design

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Background: Epitope based vaccines are widely used in vaccine development against HIV-1, cancer and other diseases. One of the key components is how to enhance its immunogenicity. Previous studies suggested that the magnitudes of both T cell and antibody responses could be improved through repeating epitope in design, however, it remains unknown how this could influence the functional features of T cell responses and its optimization.

Methods: A previously indentified HIV-1_{RL42} envelope T cell epitope GIRKNYQHLWRWGTM (Env2) was employed, mini-genes encoding a single, triplicated or sextuplicated copies of this epitope were synthesized and inserted into a DNA vector. To enhance their expression efficiency and immunogenicity, Kozak sequence, ER signal sequence and an universal Th2 epitope were introduced, His tag was added to detect its expression. In vitro expression was confirmed by transfection and immunoprecipitation. C57B/C mice were inoculated i.m. and sacrificed to do in vivo assessment. ICS assays were used to read out Env2-specific immune responses. Statistical analysis was done with Prism5.0 software.

Results: It's showed that all three mini-gene DNA vaccines could elicited appreciable IFN-γresponses in CD8+ T cells, no significant IL-2 secretion was observed. One way ANOVA analysis showed that the frequencies of IFN-γ+CD8+T cells induced ranked as single copy of Env2< triplicated < sextuplicated (P=0.02). Further analysis indicated that MFI of IFN-γ+CD8+T cells decreased along with the increasing of epitope copy number, which was single-Env2 group > triplicated > sextuplicated (p=0.09). When a single copy Env2 and the combined data from triplicated- and sextuplicated-Env2 were compared, we observed MFI in single copy Env2 > multiple copy (p=0.004).

Conclusion: Our data confirmed previous observation that repeated epitope design could improve the frequencies of specific T cells. Interestingly, we demonstrated that the IFN-γ secreting capacity for individual T cell might be compromised along with the increased responding frequencies, which should be taken into consideration in vaccine design.

P12.23

Potency Of An HIV-SAM™ Vaccine In A Heterologous Prime-Boost Vaccination Regimen

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Background: Recombinant alphavirus replicon particles (VRP), carrying self-amplifying RNA, protects rhesus macaques against SHIVSF162P4 challenge when used in prime-boost regimen.

Methods: Novartis has developed a synthetic self-amplifying mRNA (SAM™) vaccine platform that avoids limitations of cell culture production and employs synthetic non-viral vaccine delivery systems.

Results: We evaluated systemic and mucosal immune responses in mice and rabbits using the SAM™ platform expressing HIV-1 gp140 (HIV-SAM™ vaccine) prime, protein/MF59 vaccine boost regimen for both HIV-1 Clade B and C Env antigens. In mice, the primed Env-specific IgG response to 1 µg of the HIV-SAM™ vaccine was comparable to a 10 µg dose of an identically formulated DNA vaccine, 10(7) IU of VRP, and 10 µg protein/MF59 vaccines. The HIV-SAM™ vaccine primed response could be boosted robustly by a protein/MF59 vaccine and resulted in a balanced IgG1, IgG2a subclass response, similar to that seen with the VRP vaccine, but unlike the dominant IgG1 response to protein/MF59 only vaccinations. Both Env-specific CD4+ and CD8+ T-cell responses were detectable after two HIV-SAM™ vaccinations. A TH1 type (IFNγ+, IL-5-) profile was demonstrable for the HIV-SAM™ vaccine primed, protein boosted CD4+ T-cell response, similar to that seen with the DNA or VRP primed protein boosted responses, in contrast to a TH2 type (IFNγlow, IL-5+) response seen with protein/MF59 vaccination. In rabbits, priming with the 25 or 50 µg of the formulated HIV-SAM™ vaccine induced robust and avid Env-binding IgG and HIV neutralizing antibodies that were superior to 500 µg of an unformulated DNA vaccine and comparable to VRP and protein/MF59 vaccines. In addition, protein/MF59 boostable Env-specific vaginal wash Ig was consistently demonstrable in both mice and rabbits immunized with the HIV-SAM™

Conclusion: Together, these results suggest that HIV-SAM™ vaccine is potent and versatile and offers potential as a novel immune priming strategy. NIAID-NIH Grant 5P01AI066287.

P12.24

Antigenicity of Soluble HIV gp140 Trimers Reveals Differences in Solution Conformation for Differing HIV Strains

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Background: Eliciting effective antibody responses are key to the design of HIV vaccines. While the HIV envelope protein is highly immunogenic and provokes a high-titer antibody response during viral infection and experimental immunization, affinity-matured antibodies capable of neutralizing diverse HIV isolates are rarely elicited. While recent vaccine regimens have focused on DNA, viral vector, VLP, or attenuated virus vaccines, of increasing interest are improved recombinant envelope protein immunogens. Immunizations with trimeric gp140 proteins induce higher-titer neutralizing antibody responses and have structural benefits over monomeric gp120 immunizations. Novel approaches being taken for recombinant trimeric gp140 immunogens include the use of consensus and multi-clade gp140 trimers. A consideration for these synthetic proteins that is key to vaccine efficacy: do these proteins structurally represent a native trimeric envelope?

Methods: To address potential differences in the functional conformation of gp140 trimers, we evaluated the conformational characteristics of trimeric gp140 proteins from varying HIV-1 strains as well as engineered consensus gp140s via binding studies using surface plasmon resonance with a panel of well-characterized monoclonal antibodies (MAbs).

Results: Consensus trimers were recognized by more monoclonal antibodies than the primary strain gp140s, suggestive of the benefits of engineered trimers. However, analysis revealed a low trimer concentration that is competent to bind the quaternary-recognizing MAb PG9. Considering the binding to other conformational MAbs, this suggests that while the consensus gp140s contain appropriately conformational monomers and are trimeric, the vast majority of the protein is not in a proper quaternary structure.

Conclusion: These data suggest that it is important to fully assess structural differences of immunogens even though obvious phenotypic differences may not be present. Taken together, these observations demonstrate the need to evaluate immunogens in a manner that allows the measurement of functional epitope exposure and solution conformation to assess the potential to elicit a potent, broadly-neutralizing antibody response.

Topic 12: Vaccine Concepts and Design

P12.25

Priming with DNA Encoding Functional Trimers and Boosting with Soluble Trimeric Protein Elicited Tier 2 Neutralizing Antibodies in Non-human Primates

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Background: All known HIV-1 gp120-directed broadly neutralizing antibodies efficiently recognize fully cleaved JR-FL spikes, however, the non-neutralizing gp120-directed antibodies only recognize the uncleaved JR-FL spikes. Therefore, as an immunogen, cleaved, functional spikes may selectively present neutralizing epitopes to B cells, perhaps more efficiently eliciting neutralizing antibodies. However, attempts to make soluble versions of Env that fully mimic the viral spike are yet to be successful. In vitro, this plasmid encodes for fully cleaved, cell-surface, trimeric JR-FL Env spikes, but only when expressed from a mini-LTR in a non-codon optimized state. In vivo, the LTR-driven, non-codon-optimized Env plasmid DNA, likely requires tat co-transfection in trans, and rev expression in cis, for efficient Env expression.

Methods: To present functional, cleaved spikes to the immune system, we inoculated non-human primates (NHPs) with non-codon optimized JR-FL Env expressing plasmid DNA along with plasmid expressing Tat protein by electroporation. DNA priming was followed by boosts with soluble JR-FL gp140 trimers in adjuvant. As controls codon-optimized and CMV-driven Env plasmids were used to prime and three animals received protein in adjuvant only.

Results: We report that the DNA priming elicited reasonable ELISA binding titers in NHPs and that the elicitation of neutralizing antibodies against Tier 1 viruses. Following trimer protein boosting of the DNA-primed animals, reasonable titers of Tier 2 neutralizing antibodies in the A3R5 assay were elicited. For the Tier 2 isolates tested to date, much of the neutralizing activity in sera maps to the CD4 binding site.

Conclusion: We conclude that DNA priming by electroporation is an interesting and means to present functional, cleaved HIV-1 Env spikes to the B cell immune system to prime or initiate functional trimer-elicited neutralizing antibodies in primates.

P12.26

A Pantothenate Auxotroph of BCG Expressing Gag Confers Enhanced HIV-Specific Immunogenicity Compared to Wildtype and Perfingolysin Expressing Strains

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Background: In tuberculosis vaccine studies, perfingolysin expressing strains (pfo) of recombinant Mycobacterium bovis (rBCG) have been shown to enhance immunogenicity as compared to wildtype strains whilst pantothenate auxotrophic strains (ΔpanCD) have been shown to be safer and more immunogenic. Our group has recently shown that rBCGΔpanCD expressing HIV-1 Gag is more immunogenic than the wildtype Pasteur strain of BCG in the murine model. In this study, a wild type strain, a ΔpanCDstrain, a pfo strain and a ΔpanCD strain expressing perfringolysin (ΔpanCDpfo) of Danish BCG were used as vectors to express HIV-1 subtype C Gag. Gag specific immune responses induced by a prime with each rBCG-Gag vaccine and boost with modified vaccinia Ankara (MVA) were compared.

Methods: rBCG vaccines were given intraperitoneally to BALB/c mice followed by an intramuscular MVA boost after 28 days. Twelve days after the boost, mice were sacrificed and single cell suspensions of splenocytes were stimulated with HIV peptides. Immunogenicity was assessed using an IFN-γ ELISPOT assay, cytokine bead array and multi-parameter flow cytometry.

Results: All the rBCG-Gag vaccines primed the immune response to a boost with MVA. Cytokine kinetic measurements and flow cytometry indicated a more rapid and robust release of pro-inflammatory cytokines, in response to ex vivo HIV peptides, from the splenocytes of mice vaccinated with rBCGΔpanCD(gag) as compared to rBCGpfo(gag)($p<0.001$- $p<0.05$) and rBCG(gag) ($p<0.01$- $p<0.05$). A rBCGΔpanCD(gag) prime induced increased polyfunctional HIV specific CD4+ and CD8+ T cells as compared to the other strains of BCG. We observed no enhancement of immunogenicity in the rBCGΔpanCDpfo(gag) group as compared to the rBCGΔpanCD(gag) group.

Conclusion: As this study indicated the Danish ΔpanCD vector induced a more robust and rapid Gag-specific immune response than the wild type and perfringolysin expressing BCG Danish vectors, the Danish ΔpanCD vector maybe an attractive option for HIV vaccines.

P12.27

Optimising CN54gp140 Plasmid Delivery by Comparing Intramuscular and Intradermal Vaccination Combinations With and Without Electroporation

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Background: Prime boost vaccination studies employing plasmid DNA have been shown to significantly increase vaccine-induced immune responses with the added advantage of increasing vaccine-induced immune breadth. Furthermore, recent studies have shown that prevailing immune responses to DNA based vaccines can be substantially augmented by the immediate application of short pulses of electric current (electroporation) at the DNA injection site.

Methods: We vaccinated mice with DNA via the intradermal (ID), intramuscular (IM) or combined (ID + IM) routes with and without electroporation (EP), using a Gene Transfer Unit (GTU®) plasmid expressing CN54gp140. Some mice were further boosted with recombinant trimeric CN54gp140. Female BALB/c mice (n=8) were vaccinated every three weeks. Serum and vaginal lavage samples were collected 1 week post each vaccination and tested by ELISA for anti-HIV-gp140 specific IgG and IgA. Spleen derived T cells were evaluated for antigen reactivity by IFN-g ELISpot using 2 peptide pools of 15mers overlapping by 11, spanning the full length of the gp140 molecule.

Results: Antigen-specific T lymphocyte IFN-g cytokine expression and humoral antibody profiles were different after either ID or IM immunization or the combined route vaccinations. Moreover, we found that EP added an additional level of complexity by influencing T and B cell responses both quantitatively and qualitatively. As expected, these vaccinations did not elicit biologically significant levels of mucosal antigen-specific antibody responses.

Conclusion: Multi-route vaccinations can enhance and subtly alter vaccine-induced B and T lymphocyte immunity. All of the vaccine routes benefited from enhancement to the DNA immunization using in vivo EP and the elicited immunity was again influenced by the enhanced transfection efficiency. These strategies are likely to be useful in the future design of vaccine modalities that seek to generate specific immune responses.

P12.28

A gp41 MPER-Specific llama VHH Requires a Hydrophobic CDR3 Determinant for Neutralization but Not for Antigen Recognition

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Background: The membrane proximal external region (MPER) of the HIV-1 glycoprotein gp41 is targeted by broadly neutralizing antibodies such as 2F5, 4E10 and Z13, which recognize antigen and membrane components.

Methods: In this study, we immunized llamas with gp41 proteoliposomes and selected a MPER-specific single chain antibody (VHH), 2H10, whose gp41 epitope overlaps with that of mAb 2F5.

Results: 2H10 binds to the intermediate conformation of gp41 with medium nanomolar affinity. Construction of 2H10 biheads (bi-2H10) increases the binding affinity by a factor of 20. Bi-2H10 neutralizes various sensitive and resistant HIV-1 strains, as well as SHIV strains in a TZM-bl cell assay. We further present structural data from crystallographic and NMR analyses together with mutagenesis data that allowed to map the interaction site on gp41. This revealed that 2H10 has a long CDR3 whose tip exposes a tryptophan residue that is not required for gp41 interaction, but crucial for neutralization.

Conclusion: Our data indicate that 2H10 induced by immunization classifies as a functional MPER antibody as a bihead that requires both antigen recognition and membrane interaction for neutralization.

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P12.29

Transforming Epitope-Specific gp120 Monomer-Based Probes into Immunogens with N-linked Glycan Masking

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Background: HIV-1 gp120 monomer-based probes have been used for the identification of broadly neutralizing antibodies. Such probes could represent starting points in the design of HIV-1 immunogens, though efforts must be made to silence immune responses directed toward non-neutralizing epitopes. One possible approach would be to mask these epitopes by introducing N-linked glycan. A potential complication to such an approach is glycan occupancy: although N-linked glycosylation generally occurs at N-X-T/S sequons, many such sequons are not occupied.

Methods: A computational protocol was developed to identify the putative positions for insertion of N-linked glycan on the gp120 surface. The first step involves the identification of residue positions on the gp120 surface where the insertion of the N-X-T/S sequon is predicted as energetically-tolerable. The second step involves the application of NGlycPred, a Random Forest-based predictor, to predict the glycan occupancy at the inserted sequons.

Results: The glycan occupancy prediction of the protocol is highly correlated to validated N-X-T sequon insertion designs. Multiple sequon insertions to gp120 monomer-based probes were generated based on the protocol.

Conclusion: A computational protocol was implemented to identify putative sites for insertion of N-X-T/S sequons with improved likelihood of glycan occupancy. The protocol is applicable to the design of N-linked glycans for masking non-neutralizing antibody epitopes on gp120-based probes as well as other immunogen candidates.

P12.30

Development of an Imaging Based Virus Aggregation Assay for Vaccine Development

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Background: Vaccination strategies capable of eliciting neutralizing antibody responses to HIV remain elusive despite extensive efforts. Alternative antibody functions offer opportunities for protection without necessarily achieving broad neutralization breadth. Viral immune exclusion through aggregation has been proposed as an alternative protection pathway, but mechanisms for studying this phenomenon at the scale necessary for clinical trials have not been explored.

Methods: Concentrated fluorescent virions of two colors, suspended in hydroxyethylcellulose (HEC) gel, which has been formulated to simulate the diffusion characteristics of cervical mucus, are imaged over time. Mean squared displacement and incidence of colocalized viral particles are determined. The addition of monoclonal antibodies of various specificities and isotypes affects these parameters is explored. Immunoglobulin isolated from HIV-1 positive individuals was examined as well. Correlative scanning electron micrographs of the same preparations were performed to confirm the nature of suspected aggregates.

Results: Multimeric antibodies, rather than monomeric isoforms, selectively hinder the diffusion characteristics of colocalized virions, more so than non-aggregated virions. The incidence of colocalized virions is also increased in a concentration dependent manner. Excessive antibody or virus concentration is seen to obviate aggregate formation. These results are seen in both monoclonal antibody experiments alongside polyclonal patient IgA isolated from breast milk and IgM from serum.

Conclusion: Virus aggregation has been demonstrated as a feasible effector function of HIV-1 specific antibody preparations. This assay platform is amenable to adaptation for medium to high throughput sample screening and low sample size. Monoclonal antibodies as well as patient samples are able to induce similar behaviors. Measurement of viral aggregation as a corollary of vaccine efficacy is deserving of further exploration in a clinical setting.

P12.31

Molecular Epidemiology of the Protective RV144 V2 Loop Epitope

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Background: The immune correlate analysis of the RV144 trial identified antibodies (Abs) targeted at the V1-V2 domain as potentially protective vaccine responses. Reactivity of vaccinee serum Abs with a glycosylated gp70-V1-V2 fusion protein or with certain V2 loop peptides was significantly associated with a lower risk of viral infection. A V2 loop segment approximately comprising amino acid residues 165-178 of gp120 was also identified by pilot vaccinee studies as a key immunogenic region.

Methods: To identify whether the segment identified by pilot studies coincides with the reagents reactive in the case control study, I mapped the locations of the reagents associated with lower risk of viral infection onto the V1-V2 domain structure. With a single segment identified as associated both with immunogenicity in the pilot studies and protection in the case control study, I calculated the Dayhoff evolutionary sequence distance between the the sequences of this segment in the immunogens and in a panel of V2 loop based reagents used in the case control study, each with associated odds ratios (OR) for risk. OR was then plotted against distance.

Results: The same V2 loop segment from positions 165-178 that was specifically most immunogenic in the pilot studies appears to have been associated with protection in the immune correlate analysis. This segment corresponds to the "C" strand of the V1-V2 domain beta-sheet fold, exactly where the broadly neutralizing antibody PG9 binds. Plotting the evolutionary distance between this segment in the RV144 immunogens and synthetic V2 loop based antigens used in the case control study shows that lower risk (lower OR) correlates with greater evolutionary distance.

Conclusion: A V2 loop segment from positions 165-178 of gp120 was highly immunogenic in humans. Abs elicited by the RV144 subtype E immunogen displaying segment may have been protective only if they cross-reacted distantly with subtype B.

P12.32

Novel Strategies for HIV-1 Epitope Delivery Using Foamy Viral Hybrid Proteins and Vectors

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Background: To prevent HIV-1 infection induction of broadly neutralising antibodies (bnAb) like 2F5 and 4E10 reacting with the membrane proximal external region (MPER) of the transmembrane envelope (TM) protein gp41 of HIV-1 may be essential. Since these antibodies appear late after infection, it is thought that they require a prolonged maturation time. Using apathogenic foamy virus (FV) as replicating gene delivery vector may allow antibody maturation through persistent antigen presentation. Based on studies showing an interaction of the MPER with the fusion peptide proximal region (FPPR) in gp41, strategies to graft these regions into backbone proteins of FV were examined and two, comprising the FV TM protein and the accessory Bet protein were tested here.

Methods: The feline foamy virus (FFV) TM protein and Bet hybrid proteins comprising the MPER and FPPR of HIV-1 gp41 were produced and antibody responses were studied in immunized and FFV infected animals by ELISA, Western blot and microarray-based epitope mapping. Also, constructs for stable, high level expression of full length FFV Env were developed.

Results: Mapping sera from animals immunized with the FFV TM protein and infected cats revealed immunogenic epitopes in the FV FPPR and MPER. These were exchanged against their HIV-1 homologues in optimized FV Env. Upon transfection, these constructs allowed production of chimeric virus-like-particles (VLPs) currently used for immunization studies. By immunizing with the Bet fusion proteins containing the gp41 MPER, FPPR or MPER-FPPR, strong responses were induced against gp41 and the 2F5 epitope ELDKWAS, demonstrating the feasibility of this approach. The hybrid sequences are now transferred to infectious FFV clones.

Conclusion: To induce bnAb of the type 2F5 and 4E10, the developed vaccine systems provide two new high safety strategies for HIV epitope delivery by either VLPs or replicating vectors. This work was supported by the Volkswagen Foundation.

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P12.33

Immunogenic Selection of HIV-1 MPER Epitopes for Improved Vaccine Design

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Background: The goal of this study was to design structural mimics of HIV-1 epitopes that have the potential to induce broadly neutralizing antibodies (bnAbs). The structure of the gp41 membrane proximal external region (MPER), targeted by three bnAbs, requires further definition. Experiments were designed to select epitopes with enhanced binding to MPER bnAbs, to identify neutralization-competent MPER structures, and to determine if selected MPER epitopes can broaden the immune response as potential vaccines.

Methods: MPER epitopes were selected by biopanning with phage-displayed peptide libraries against bnAbs 4E10, 2F5 and Z13. Epitopes were screened in competition binding assays where M13-displayed epitopes competed with envelope peptides or infectious HIV-1 for antibody binding. In vivo response to MPER was assessed by M13 immunoprecipitation and neutralization competition assays using HIV-positive plasma, and immunogenicity of select epitopes was assessed in BALB/c mice.

Results: Forty-six unique 4E10 epitopes were identified, representing both MPER homologous and non-homologous sequences. Fourteen epitopes, capable of competing with MPER peptide and HIV-1 for 4E10 binding, bound HIV-positive IgG. Of these, five epitopes absorbed MPER-specific neutralizing activity in HIV-positive patient plasma. Mouse immunization with the selected neutralization-competent MPER epitopes elicited HIV-1 specific cellular and humoral immune responses and boosted the neutralizing activity of a gp145 Env subunit vaccine.

Conclusion: This study demonstrates the diversity of epitope recognition by 4E10 and the unique response to MPER in vivo. The M13-displayed, neutralization-competent structures of the 4E10 epitopes have the potential to elicit and boost HIV-1 neutralizing antibody production in mice. Phage-displayed epitopes can rapidly and inexpensively be selected to provide epitope-specific depth and variation to HIV-1 vaccine designs without requiring modification to major vaccine components.

P12.34

Antigenicity and Immunogenicity of a Novel, Acute HIV-1 Tanzanian Subtype C gp145 Envelope Protein for Clinical Development

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Background: Eliciting broadly reactive neutralizing antibodies remains a challenge in HIV-1 vaccine development, complicated by variations in envelope (Env) subtype and structure, and by the assays used for product down-selection. Since a majority of new HIV-1 infections are subtype C and considering the novel properties of C Envs, a C Env (CO6980v0c22) from an acutely infected Tanzanian was developed as a candidate HIV vaccine.

Methods: The CO6980v0c22 Env sequence was codon optimized and a stable CHO cell line expressing gp145 was established. Purified gp145 was adjuvanted in alum or lipid A-liposomes, injected into New Zealand white rabbits (4/group; 25 ug at weeks 0, 4, and 8), or BALB/c mice (5/group; 10 ug in liposomes at weeks 0, 3, 6, 8). Antibody titers were assessed by ELISA and neutralizing antibodies were measured against pseudoviruses in TZM-bl cells or against infectious molecular clones (IMC) in a PBMC assay.

Results: Secreted gp145 is a novel subunit with the full MPER extended by three lysines. Unlike some gp140 subunits, the 4E10 neutralizing monoclonal antibody (mAb) binds to gp145. IgG1b12 binds weakly, VRC01 binds potently, as does the V2-specific 697D mAb; the gp145 also binds to alpha4beta7 receptor, as demonstrated by flow cytometry. At week 10 post-immunization, rabbit sera showed strong binding antibody titers to several Env antigens, including the clade B V1V2gp70 scaffold protein. While neutralization of the HIV-2 MPER-scaffold pseudovirus was negative, cross-clade neutralization was observed in both rabbits and mice, against Tier 1 subtype B and C pseudoviruses, and against Tier 1 and Tier 2 IMC. Using EGS cross-linking, it appears that the majority of the gp145 multimers are trimeric; this is currently under investigation using electron microscopy techniques.

Conclusion: These data indicate essential immunogenic features of a novel acute C HIV-1 Env that warrants further testing for potential clinical development.

P12.35

The Emptied Bacteriophage T4 Head: A Novel Nanoparticle Vehicle to Simultaneously Deliver HIV-1 Antigens and Genes into Dendritic Cells

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Background: Simultaneous delivery of HIV-1 antigens and genes by a single nanoparticle will greatly aid in the development of effective HIV-1 vaccines. We have created a highly stable, empty bacteriophage T4 capsid shell which can be engineered both inside and out. The empty head can be filled with foreign DNA using a powerful DNA packaging machine. Its surface can be arrayed with proteins as fusions of the outer capsid proteins, Hoc (highly antigenic outer capsid protein) and Soc (small outer capsid protein). The 120 x 86 nm head has a capacity to accommodate 170-kb foreign DNA and 1025 molecules of proteins.

Methods: A "neck" mutant fails to seal the packaged head of phage T4. The packaged viral genome is spontaneously emptied to create empty space inside. The pentameric packaging motor is assembled at the portal vertex. A variety of genes; reporter genes luciferase and GFP, HIV-1 envelope genes gp41, gp145, and gp160, and/or molecular adjuvant gene GMCSF are packaged inside the head. The surface is arrayed with enzymes such as the tetrameric β-galactosidase or trimers of HIV-1 gp145 as fusions of Soc, and dendritic cell targeting ligands such as Dec205 mAb or CD40L as fusions of Hoc.

Results: The emptied phage T4 head efficiently packaged foreign DNA. Packaging is highly promiscuous requiring no specific sequence. Long concatemers, plasmid DNAs, or short PCR-amplified DNAs are packaged efficiently. Multiple genes and multiple copies of each gene are packaged into the same head. The surface is decorated with HIV-1 gp145 trimers and dendritic cell targeting ligands Dec205 mAb or CD40 ligand. The engineered head delivered the massive "payload" into dendritic cells to near 100% efficiency. The delivered genes were abundantly expressed as analyzed by immunoelectron microscopy.

Conclusion: A novel bacteriophage T4 nanoparticle efficiently delivers DNA-inside, protein-outside, prime-boost HIV-1 vaccines to dendritic cells.

P12.36

The C-Terminal Tail of HIV-1 Envelope: A Unique Role for Conserved LLP Arginines in Env Functional Properties

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Background: The C-terminal tail (CTT) of HIV-1 envelope transmembrane protein has recently been the subject of an increasing number of studies to determine its role in the architecture of the HIV virion and in virus replication. Published studies from our lab have previously demonstrated that the lentivirus lytic peptides (LLP) domains contained in the CTT present highly characteristic and conserved physicochemical and structural properties, including a highly preferential incorporation of arginine over lysine at conserved sites.

Methods: We previously reported that nonconservative substitution of selected LLP arginines to glutamate residues in a reference provirus resulted in substantial changes in Env structure and function, reflecting an important role of the LLP arginines in overall Env phenotypes. To further evaluate the role of LLP arginines in Env properties, we have now evaluated the effects of conservative substitutions of selected LLP arginines by lysines in the context of the HIV 89.6 provirus. The various lysine substitution mutants are being characterized for Env incorporation, fusogenicity, infectivity, and antigenicity.

Results: To date, the results of these studies clearly indicate marked changes in Env functional properties as a result of the lysine substitutions for native arginine residues. Thus, these data demonstrate for the first time unique functional properties that are intrinsic to arginine in the LLP domains and that cannot be replaced by the closely related lysine.

Conclusion: Taken together, these observations reveal the critical role of conserved LLP arginine residues in affecting viral envelope phenotypes and further highlight the role of the CTT as a major determinant of overall HIV Env structural and functional properties. As such, their role as determinants of Env antigenicity, immunogenicity and as conserved epitopes is also investigated in experimental immunizations, and will be informative for future vaccine design.

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P12.37

Yeast Surface Display of HIV Env Protein Variants for the Discovery of Novel Vaccine Immunogens

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Background: The discovery of broadly neutralizing antibodies (bnAbs) such as 4E10, pg9 and VRC01 binding to the HIV env spike has electrified the field and shows that, in principle, the human immune system is capable of producing protective antibodies against HIV. All current vaccination strategies have however failed to elicit such bnAbs, leaving room for novel approaches to address this problem.

Protein engineering techniques such as yeast surface display have proven potential for the discovery and affinity maturation of monoclonal antibodies. Antibody libraries are presented on yeast cells and flow cytometric sorting is used to identify antibody variants with improved properties. As antibodies, also HIV env spikes are posttranslationally modified in the secretory pathway and composed of disulphide bond-containing glycoproteins. We here investigated whether HIV spike protein variants can be displayed or secreted from surface of the yeast strain S. cerevisiae.

Methods: A panel of HIV env spike protein-encoding genes was inserted in vectors allowing for the display or secretion of the encoded proteins in S. cerevisiae. The displayed protein variants were analyzed for binding to either bnAbs or pooled HIV immunoglobulin from the serum of infected individuals (HIVIg) using flow cytometry. The secreted protein variants were tested for binding activities on an Octet biosensor.

Results: We found that HIV env spike protein variants can be displayed or secreted from the yeast strain S. cerevisiae. Both displayed and secreted variants showed binding to bnAbs and HIVIg.

Conclusion: This study suggests that yeast surface display is as a viable option for the engineering of HIV env spike protein variants and may become a valuable tool for the discovery of novel vaccine immunogens.

P12.38

Development of Candidate HIV Vaccines Using VSV Vectors to Express Membrane-Anchored MPER Immunogen

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Background: The HIV-neutralizing activity of monoclonal antibodies 2F5, 4E10, and Z13 have shown that the membrane-proximal external region (MPER) of HIV Env is an important vaccine target, but development of an immunogen that elicits similar virus-neutralizing antibody responses against the MPER from a wide range of subtypes has been difficult to achieve. It has been proposed that a MPER immunogen must be membrane-associated to adopt the conformation needed to elicit broadly neutralizing antibodies, and accordingly, we have developed Vesicular Stomatitis Virus(VSV) Vectors that express membrane-anchored MPER epitopes.

Methods: Replication-competent and single-cycle VSV vectors have been developed that express a truncated VSV G protein (G-Stem) in which the natural G ectodomain is replaced with MPER sequence (GStemMPER). The nucleotide sequence of GStemMPER has been optimized by computer based algorithms to ensure genetic stability. Several VSV vector modifications are also introduced for directing immune responses towards MPER rather than G. Those strategies include relocation of GStemMPER and VSV G glycoprotein to achieve maximal MPER expression while minimize the anti-vector immunity, incorporation of different VSV G serotypes and pseudotyping single- cycle VSV vectors with various types of glycoproteins.

Results: Western blot analysis confirmed expression of MPER epitopes. Assessment by FACS showed the cell surface MPER being recognized by 2F5 and 4E10 monoclonal antibodies. Immunoprecipitation study further proved incorporation of MPER into viral particles.

Conclusion: Multiple VSV-GStemMPER vectors are being evaluated in small animal studies to compare their immunogenicity and whether the membrane-anchored form of the MPER immunogen elicits humoral responses with a broad range of HIV neutralizing activity.

P12.39

Vaccination with Dendritic Cells Loaded with HIV-1 Lipopeptides Elicits Broad T Cell Immunity and Control of Viral Load in HIV Infected Patients

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Background: The DALIA trial tested the hypothesis that immunization with HIV peptide loaded Dendritic Cells (DC) may improve HIV immune responses and help to contain viral replication.

Methods: 19 pts with CD4 >500 cells/m3 and HIV RNA <50 cp/ml under HAART received at W0, 4, 8 and 12 ex-vivo generated IFN-α DC loaded with HIV-1 lipopeptides. Analytical treatment interruption (ATI) was conducted from W24. HAART resumption regardless of the reason and CD4 <350 cells/mm3 (or <25%) were considered as end points. HIV-specific immunity was evaluated at baseline, W16, and W48 using: i) ex vivo IFN-γ ELISPOT; ii) intra cellular staining; iii) multiplex analysis. PBMCs were stimulated with HIV peptide pools. Student t-test and Wilcoxon signed-rank tests were used with estimation of the False Discovery Rates (FDR) for controlling test multiplicity.

Results: Vaccine regimen was well tolerated. Following ATI, all pts experienced a viral rebound in 14 days in median (IQR 8-27). Median highest observed VL (peak) was 5 (4.28-5.23) log10 cp/ml. Three patients resumed HAART and eight had CD4 <350 cells/mm3. Median (IQR) SFU/106 PBMC rose from 186 (140-670) at baseline to 761 (470-1154) and 1878 (1102-4443) at W16 and 48, respectively. At the same time points the breadth of the response (nb of peptide pools) increased from 1 (1-3) to 4 (2-5) (P=.009) and 6 (3-7) (P=.008). % of polyfunctional CD4+ (> 2 cytokines among: IFN-γ,TNF-α,IL-2) increased from 0.026% (w-4) to 0.32% (w16) (P=.002). Respective % of CD8+ were 0.26% and 0.35% (P=.005). Production of IL-2, IFN-g, IL-21, IL-13, IL-17 increased significantly at W16 (FDR<.05). An inverse correlation was found between the peak of VL and % of polyfunctional CD4+ (r=-0.63, FDR=.007), production of IL-2 (r=-0.67, FDR=.006), IFN-g (r=-0.58, FDR=.01), IL-21 (r=-0.66, FDR=0.006) and IL-13 (r=-0.78, FDR=.001).

Conclusion: DC vaccination elicited polyfunctional HIV-specific responses associated with a reduced peak viral load following ATI.

P12.40

Evaluation of a Replication-Competent VSV-SIV Vaccine Candidate

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Background: Immunity elicited by live attenuated vaccines confers protection against viral pathogens causing measles, yellow fever, smallpox and others, but this approach is not well suited to HIV vaccine development. Accordingly, to develop a vaccine that incorporates features of a live virus that make it immunogenic without the inherent safety issues associated with attenuated lentiviruses, we are developing replication-competent, recombinant vesicular stomatitis virus (rVSV) vectors for delivery of SIV and HIV vaccines.

Methods: An rVSV vector was constructed in which the natural glycoprotein (G) was replaced with SIVmac239 Env, and an additional transcription unit was introduced to encode SIVmac239 Gag. The chimeric VSV deltaG-SIV virus vaccine was rescued and evaluated in vitro and in vivo.

Results: Infection of susceptible cells with this chimeric rVSV-SIV GagEnv virus produced infectious VSV particles containing functional SIV Env, and lentivirus-like particles containing Gag and Env. Serial passage of rVSV-SIV GagEnv vector in CD4+/CCR5+ cells resulted in improved replicative fitness evident by a 2-log increase in infectious virus titers. This adapted virus retained CD4/CCR5 dependence, infected primary rhesus PBMCs and cells isolated from lymph node and duodenum tissues ex vivo. Primary human monocyte-derived dendritic cells (MDDCs) also were susceptible to infection with the rVSV-SIV GagEnv vector. Indian rhesus macaques were immunized with increasing doses of rVSV-SIV GagEnv intramuscularly (IM) to observe reactions to vaccination with this new vector and to quantify immune responses. At the highest dose of $1x10^8$ PFU, no adverse reactions were observed and serum antibody responses against Gag and Env were elicited, which were boosted by IM immunization with Ad5-SIV Gag and Ad5-SIV Env vectors.

Conclusion: A prototype chimeric VSV-SIV live virus vaccine was developed and evaluated in the NHP model. Chimeric VSV-SIV and VSV-HIV vaccine candidates designed for improved replication and immunogenicity currently are being evaluated in vitro and in vivo.

Topic 12: Vaccine Concepts and Design

P12.41

Preferential Targeting of Conserved Gag Regions After Vaccination with a Heterologous DNA Prime Modified Vaccinia Ankara Boost HIV Vaccine Regime

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Background: Genetic diversity is a major challenge in the design of vaccines against variable viruses, including HIV. Engineering vaccines to induce immune responses that preferentially target conserved antigenic regions could thus contribute to improved HIV vaccines.

Methods: During the Tanzania Mozambique HIV Vaccine trial (TaMoVac 01) phase 2a HIV vaccine trial, HIV negative Tanzanian volunteers received 3x 0.6 or 1mg intradermal injections with a multiclade, multigene DNA vaccine that included 2 plasmids encoding for clade B Gag-p37 (p17 & p24) and a recombinant Gag-p37 (clade B p17 & clade A p24). DNA vaccine recipients were boosted with Modified Vaccinia Ankara (MVA)-CMDR expressing clade A Gag that additionally covered the Gag-p15 region. Vaccine-induced T cell responses were characterized using IFN-gamma ELISpot in 45 participants after stimulation of fresh PBMC with 9 peptide pools subdividing Gag into 9 distinct antigenic regions. Data were analyzed using the Mann-Whitney test and linear regression analysis.

Results: Antigenic regions p17 and p24 included in the DNA prime and the MVA boost were recognized with higher median magnitude than antigenic regions within p15, which was only covered by the MVA boost ($p<0.0001$). Antigenic regions within p24 (clade A&B prime and clade A boost) were better recognized than those within p17 (clade B prime and clade A boost; $p<0.0001$). We then determined the sequence homology between the MVA and the DNA Gag vaccine immunogens for each of the peptide pools. The sequence homology ranged between 91% and 0% (for p15) of total amino acids within a given peptide pool and there was a linear correlation between the sequence homology and the response rate ($p=0.04$, $r2=0.47$).

Conclusion: These preliminary results support the hypothesis that heterologous prime-boost vaccine regimens preferentially induce immune responses targeting regions that are conserved between the priming and boosting vaccines.

P12.42

From HIV Protein Sequences to Viral Fitness Landscapes: A New Paradigm for In Silico Vaccine Design

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Background: An inexpensive prophylactic vaccine offers the best hope to curb the HIV/AIDS epidemic gripping sub-Saharan Africa. Systematic means to guide the design of an effective immunogen for this, and other, infectious diseases are not available. What is required is a method to chart the peaks and valleys of viral fitness as a function of amino acid sequence. An efficacious vaccine would eject the virus from the high fitness peaks, and drive it into the valleys where its compromised fitness impairs its ability to replicate and inflict damage to the host.

Methods: Appealing to spin glass models in statistical physics, we present a novel approach to translate viral sequence databases into landscapes of viral fitness. These inferred models furnish a quantitative description of viral replicative capacity as a function of amino acid sequence. We illustrate this approach in the development of landscapes for the proteins of HIV-1 clade B Gag.

Results: In comparisons to experimental and clinical data, our inferred landscapes demonstrate excellent agreement with: 1) in vitro replicative fitness measurements, 2) clinically observed high-fitness circulating viral strains, 3) documented HLA associated CTL escape mutations, and 4) intra-host temporal adaptation pathways revealed by deep sequencing. These favorable comparisons support our landscapes as reflections of intrinsic viral fitness. We illustrate the value of such descriptions in the computational design of a CTL Gag immunogen.

Conclusion: We present a novel methodology to translate viral sequence data into quantitative landscapes of viral fitness. In an application to HIV-1 Gag, we illustrate excellent agreement of our model predictions with experimental and clinical data, and demonstrate a powerful new approach for HIV immunogen design. We anticipate that this approach may represent a heretofore unprecedented means to synthesize fitness landscapes for diverse pathogens, and may provide the basis for the design of improved prophylactic and therapeutic strategies.

P12.43

In Silico Prediction of the Neutralization Range of Human Anti-HIV Monoclonal Antibodies

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Background: Antigenic variation is a primary obstacle to HIV-1 vaccine development since antibodies (Ab) directed against the viral envelope have widely variable and poorly predictable cross-strain reactivity. The breadth of cross-strain reactivity is usually estimated by in vitro neutralization of a broad panel of HIV-1 viral strains by a query antibody. However, this approach is cumbersome and cannot be scaled up to assess the more than 60,000 circulating HIV-1 viruses.

Methods: To address this issue, we used in silico docking of a flexible peptide, representing the epitope-containing part of a viral gp120, to a static crystallographic conformation of an antigen-combining site of an Ab. This procedure was applied to predict whether neutralization would occur between the pair. To train the prototype method we used a panel of 59 V3 sequence diverse pseudoviruses (psVs) controlled for masking effects. All psVs had an associated experimentally derived IC50 value for neutralization by anti-V3 monoclonal Abs 2219 and 447-52D.

Results: We optimized the method for each of the two Abs by determining an optimal docking model (optimal boundaries of a docking peptide and an optimal Ab crystallographic conformation) giving the largest area under the prediction ROC curve (AUC) on the training set of 59 psVs. The prediction accuracy for the optimized method was then estimated: the AUC was equal to 0.96 (95% CI (0.91; 1)) for 2219, and to 0.88 (95% CI (0.79; 0.97)) for 447-52D.

Conclusion: The method accurately predicts the neutralization of any HIV-1 strain by mAbs 2219 or 447-52D based solely on neutralization assay independent energetics and 3D structural parameters. The neutralization range of these anti-V3 mAbs can therefore be precisely determined in silico. Furthermore, given the fact that mAbs 2219 and 447-52D have completely different binding modes, we anticipate that our approach is extensible to other antibody-viral complexes with known structure.

P12.44

Comparison of Cellular and Humoral Immune Responses Induced by Primary, Consensus or Mosaic HIV-1 Env DNA Vaccines

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Background: The genomic variability of HIV viruses has impeded the development of globally relevant HIV vaccines with ability to induce strong and broad cellular and humoral immune responses. Many strategies, such as codon/RNA optimization, addition of highly efficient leader sequences, use of consensus or mosaic antigens, and electroporation (EP) have all been applied to enhance the breadth and magnitude of immune responses induced by DNA vaccines.

Methods: Highly optimized clade B and C consensus envelope DNA vaccines (pEY2E1-B and pEY3E1-C) were developed. Their immunogenicity was compared to the immune responses induced by an optimized mosaic gp160 envelope vaccine (pMosEnv) or optimized primary clade B and C envelope vaccines (pPK61-14 and p96ZM651gp140-CD5). The immunogenicity of these constructs was studied in different murine models. All vaccines were delivered using electroporation. Antibody responses were determined by ELISA and cellular responses by IFN-γ ELISPOT.

Results: Antibody analysis supported that the consensus immunogens induced stronger clade-specific antibody response than the primary immunogens, while the mosaic antigen was a poor inducer of antibody responses. Compared to the primary vaccine constructs, both consensus and mosaic constructs were more potent at driving diverse cellular immune responses. The strongest cross-reactive immune responses against various consensus peptides libraries were induced by pEY2E1-B and pEY3E-C. The consensus immunogens were up to three times more potent at driving subtype-specific responses that recognized the different cross-clade immunogens. pMosEnv exhibited robust cellular responses when a PTE peptide set was used.

Conclusion: We conclude that the highly optimized consensus immunogens may fold into a relatively native conformation and exhibit improved focus on antibody and T cell conserved regions, while the mosaic immunogen provides important T cell responses but does not preserve the native structure of envelope to allow for similar antibody responses in their current forms. Further exploration of these immunogens will be interesting.

Topic 12: Vaccine Concepts and Design

P12.45

Engineered Microneedle Arrays for Transcutaneous HIV Vaccine Delivery

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Background: Needle-free delivery has the potential to enhance vaccine efficacy and safety, as well as facilitate cost-effective global vaccine distribution and storage. To this end, microneedle arrays provide the ability for pain-free, safe, and convenient materials delivery through disruption of the outer layers of the skin to access potent immune-competent cell populations residing within the epidermal/dermal tissues.

Methods: We have employed this strategy to design a series of vaccine-delivery strategies based on the rapid delivery of dried vaccine-containing surface-coatings from biodegradable microneedles upon gentle application to the skin. We have demonstrated the utility of this approach for the rapid and convenient delivery of plasmid DNA as well as recombinant adenoviral vectors expressing model HIV antigens.

Results: When used to deliver a plasmid expressing SIV-Gag together with poly(I:C) in mice, these engineered microneedles elicited stronger cellular and humoral immunity than traditional naked DNA injections and were comparable in potency to DNA administered with in vivo electroporation. Furthermore, microneedle delivery of a luciferase-expressing plasmid in explanted skin from Rhesus macaques showed significantly enhanced expression (~100-fold greater) relative to injected dose-matched controls. In parallel, we designed coatings to encapsulate replication-deficient adenoviral vectors, and dried microneedle-formulated Ad vaccines were observed to maintain biological activity at room temperature for in excess of 8 weeks, while control formulations rapidly lost activity within 7 days. Microneedle-delivery of adenoviral vectors expressing SIV-Gag was observed to produce cellular and humoral immune responses comparable to traditional intramuscular/intradermal injection. Thus, this microneedle-based delivery strategy has the potential to provide enhanced vaccine stability while maintaining efficacy equivalent to traditional approaches.

Conclusion: Together, these results demonstrate the promise of microneedles for effective and safe vaccine delivery while showcasing the additional potential for greater stability in storage and more potent immunogenicity.

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P12.46

A New Strategy for Repeated Application of Adenovirus Based Vectors: Proof-of-Concept in Rhesus Macaques Challenged with SIVmac239

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Background: It is well recognized that highly active antiretroviral therapy (HAART) can control HIV/AIDS and prolong patient's life. However, HAART is associated with drug toxicity, drug resistant, and patient's affordability. Therefore, developing an effective therapeutic vaccine that could induce HIV-specific immune responses may provide a solution to reduce the need of antiretroviral therapy. Adenovirus based vaccines have been extensively evaluated as a vaccine vehicle for HIV/AIDS and a variety infectious diseases, however, it has been a major concern that anti-Ad5 neutralizing antibodies in general population or after the first dose of immunization can hinder the its practical application. The idea of using less prevalent adenovirus serotypes lack of long-term safety record including oncogeneicity. Moreover, any adenovirus serotype will inevitablely induce neutralizing antibodies after one single use which render the strategy of using more serotypes infeasible.

Methods: In this study, we explored a novel "one-size-fits-all" strategy, namely AVIP (Adenoviral Vector Infected PBMC), to circumvent the attenuated efficacy of Ad-based vaccines due to anti-Ad immunity.

Results: We demonstrated that this AVIP strategy can elicit SIV-specific responses in both Ad5-seropositive and Ad5-seronegative macaques. Interestingly, comparable SIV-specific but weaker Ad5-specific responses were elicited in Ad5-seronegative monkeys received AVIP immunization as compared to direct injection of Ad5-SIV vaccines. Then a cohort of Ad5-seropositive SIVmac239-infected monkeys received AVIP immunization with Ad5 vectors expressing SIVmac239 Gag, Pol and Env with HAART. After therapeutic immunization, SIV-specific response was induced in vaccinated monkeys whereas a slower responses were observed in non-vaccinated monkeys. The functional immunological profiles were also being detected, and we are also monitoring whether this immunological benefit would afford a virological benefit.

Conclusion: Our goal is to further develop the AVIP strategy as a simple but practically effective method for repeated delivery of Ad5-based vaccines in humans for HIV and other diseases.

P12.47

Oral Delivery of Transgenic Plant-Derived HIV-1 p24 Antigen in Low Doses Shows a Superior Priming Effect in Mice Compared to Higher Doses

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Background: The gut associated lymphoid tissue (GALT) includes around two thirds of the total lymphoid system. CD4+ T-cells in the GALT are a main target for HIV during primary infection. Thus, immunization targetting GALT is likely to be of importance for an effective vaccine strategy. Transgenic plants expressing HIV antigens can reach GALT conveniently. This system allows multiple boosts, has simple logistics (no cold chain, no injections) and large production capacity.

Methods: Three groups of mice were given extract from plant lines expressing HIV-1 p24 at (A) low level (20 ng/feeding); (B) high level (460 ng/feeding); (C) control (wild type, 0 ng). No adjuvant was included. The extracts were administered by gastric tube day 0, 14 and 28. On day 55 all mice were given an intramuscular (i.m.) boost with 10 micrograms of purified p24 antigen. Immune responses were determined by measurement of p24-antibodies in serum by ELISA.

Results: The mice immunized by the low dose plant line (A) showed a higher systemic immune response after i.m. boost compared to the high dose group (B). The w.t. controls (C) had undetectable p24-responses. The responses in group A were 3 to 10 times higher (ELISA OD values) than in group B. Pre-boost antibody responses were at background levels in all groups. Preliminary analyses indicate a predomninant Th1-type response (antigen-specific IgG2a higher than IgG1).

Conclusion: Simple and inexpensive means of vaccination are important in order to reach large numbers of people with effective vaccine regimens. The HIV-1 p24 low dose transgenic plant extracts given orally showed a superior priming effect in mice compared to the p24 high dose extracts. This could be an immunization method and route worth exploring further.

P12.48

Antigenic Mimicry of Mammalian Oligomannose by a Naturally Occurring Bacterial Oligosaccharide and Its Implications for HIV Vaccine Design

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Background: Oligomannose sugars on gp120 are of significance to HIV vaccine design because of the description of the broadly neutralizing mAb 2G12 and, more recently, of the potent and broadly neutralizing 'PGT' series of mAbs, which bind with high affinity to clusters of oligomannose moieties on gp120. Attempts to elicit oligomannose-specific antibodies have focused mainly on immunizing with antigenic clusters of synthetic oligomannose or natural oligomannose. These strategies have had limited success, suggesting that alternative approaches are needed. Here, we present the surface lipo-oligosaccharide (LOS) of Rhizobium radiobacter Rv3 that antigenically mimics the 2G12 epitope, as one potential new avenue of exploration.

Methods: The chemical structure of the Rv3 OS was determined by NMR and its antigenic similarity to the 2G12 epitope determined by ELISA and X-ray crystallography. Immunogenicity of the Rv3 OS and its ability to elicit antibodies of 2G12-like specificity was assessed by immunizing mice with heat-killed Rv3 bacteria.

Results: The detailed chemical analysis of the Rv3 OS revealed that its carbohydrate backbone consists of a unique tetramannose backbone that is analogous to the D1 arm of mammalian oligomannose. 2G12 bound with at least similar affinity to purified Rv3 OS as reported for oligomannose. The 2.4 Å-structure of the 2G12:Rv3 OS complex shows that 2G12 contacts all four mannosyl moieties in the Rv3 backbone that mimic the D1 arm, with the majority of contacts occurring with the terminal mannose disaccharide. Antibodies elicited by immunizing with heat-killed bacteria bound a synthetic tetramannose epitope and monomeric gp120.

Conclusion: Although the elicited antibodies failed to exhibit the desired neutralizing activity, our data suggest that presentation of an antigenic analog of mammalian oligomannose in a bacterial context presents a novel avenue for pursuing immunogens to elicit oligomannose-specific HIV neutralizing antibodies. The development of Rv3-based bioconjugates using human carbohydrate vaccine carriers is ongoing.

Topic 12: Vaccine Concepts and Design

P12.49

Skin Tattooing as an Effective Tool for Delivering DNA and Protein Vaccine Immunogens

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Background: Skin is an excellent site for vaccine administration due to the abundance of antigen presenting cells and easy access. When DNA or protein is delivered into the skin, uptake and expression of immunogens can lead to a rapid and robust induction of immune responses.Skin tattooing has been suggested as a useful tool delivering vaccines intradermally. We have chosen to use the skin tattooing technique to deliver both DNA and protein immunogens that are focusing the immune response on the highly immunogenic V3 region of HIV-1 gp120.

Methods: A GFP-containing plasmid was used first to optimize the protocol for skin tattooing on BALB/c mice. Subsequently, BALB/c mice were immunized using a DNA prime/Protein boost regimen. DNA skin tattooing was used first as the prime to deliver a gp120-encoding DNA plasmid intradermally at weeks 0, 2, and 4. The animals were then given a fusion protein, V3-bearing cholera toxin subunit B (V3-CTB), via skin tattooing (Group1; n=5) or intramuscular injection (Group2; n=5) at weeks 10 and 14. Two weeks after the final immunization, the sera were collected and analyzed.

Results: GFP expressed in skin cells was visualized by confocal microscopy showing that skin tattooing can effectively deliver DNA into these cells. ELISA assays using sera drawn after the final immunization confirmed that tattoo-delivered DNA followed by V3-CTBelicited V3 binding antibodies, and these antibodies have potent neutralizing activities in an HIV pseudovirus neutralization assay.

Conclusion: Immunization by skin tattooing is an easy, effective, and economical way to administer both DNA and protein vaccines. Interestingly, the needle perforations may serve as a potent and natural adjuvant for the immunization, making skin tattooing a promising vaccine delivery technique.

P12.50

Different Biological Activity of CD154-SIVgp41 Fusion Protein Vaccine Component in Naïve or Pre-immune Individuals

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Background: Trimeric gp41 might be an important target for neutralizing antibodies; however, the poor immunogenicity of this region may be due to its lack of exposure on native virus. CD154 (CD40L) is a trimeric glycoprotein found on activated CD4 T-cells that binds to CD40 on APCs and leads to B-cell activation and differentiation to plasma cells. We designed a novel immunogen with the potential for inducing neutralizing antibodies to gp41 and for stimulating activity on APC and B-cells.

Methods: A flexible (FL) and helical (HL) linkers were used to join CD154 and SIV gp41, which are trimeric glycoproteins with opposing polarities. Recombinant vaccinia viruses (VVs) were engineered to express CD154FLgp41 and CD154HLgp41 glycoproteins. PBMCs from SIV naïve and immune macaques were exposed to wild type VV (VVwt) or to recombinant VVs expressing SIV Gag, gp160, and Nef (VVgen), or SIV Gag and Nef, and CD154-gp41 fusion proteins. Cytokine production and cell activation were analyzed by Luminex and flow cytometry, respectively.

Results: Both linkers allowed proper protein folding and CD154 biological activity. Compared to VVwt, VVgen, and VVCD154FLgp41, PBMCs from naïve macaques exposed to VVCD154HLgp41 expressed higher levels of IL-6, IL-1Rα, IL-1β, RANTES, GRO-α, TNF-α, IL-8, IP-10 and IL-10. In contrast, PBMCs from SIV-immune macaques expressed more IL-6, MIP-1α, MIP-1β, INF-α, IL-1β, and MCP-1. Interestingly, lymphocytes from SIV-immune macaques were activated by VVCD154HLgp41 and VVCD154HLgp41 more than cells exposed to SIVgen.

Conclusion: Fusion proteins of CD154 and gp41 with either HL or FL assembled into trimers and activated immune cells. However, there was a differential cytokine expression for the fusion protein containing the HL, including chemokines that inhibit HIV entry. This increased biological activity may indicate that the helical linker allows for a more functional folding of the CD154 moiety. The CD154HLgp41 fusion protein will be tested in NHP as a novel vaccine component.

P12.51

Co-immunization with HIV Env DNA and Protein Elicit Long-Lasting Strong Cellular and Humoral Immune Responses

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Background: We have previously reported that potent, long-lasting HIV-1 Env-specific cell-mediated immune responses could be elicited in rhesus macaques and mice using plasmids encoding env DNA as the immunogen. Subsequent experiments showed that combination of DNA and protein in the form of inactivated virus particles provided significant protection from infection and high viremia. We examine a vaccine platform combining DNA and recombinant Env protein co-immunization at the same time to generate both strong cellular and humoral immune responses.

Methods: Mice or macaques were immunized with HIV env gp120 DNA vaccine and/or purified gp120 protein from clade B or clade C isolates. Mice were immunized twice at 4 weeks interval with DNA only, protein only formulated in EM005 adjuvant, or DNA&protein/EM005. Macaques were immunized twice at 4 weeks interval with DNA only, DNA&protein, DNA&protein/EM005.

Results: DNA&protein co-immunization enhances the Ab responses compared with DNA or protein only in mice. DNA&protein co-immunization generated similar levels of cellular immune responses compared to mice immunized with DNA only but those levels were significantly higher than those obtained in mice immunized with protein only. The establishment of a mouse model that gives similar results with the macaque model enhances our ability to test many variations and optimize the vaccine. Importantly, in macaques this strategy elicited higher binding and neutralizing Ab responses than DNA only and the neutralizing Abs showed broad activity. The presence of the EM005 adjuvant further enhanced the Ab responses. These responses were correlated with the up-regulated activation of dendritic cells by EM005. The longevity of the Ab response was superior.

Conclusion: The strategy of DNA and protein co-immunization has potential for development as a prophylactic HIV-1 vaccine. Our challenge studies show that DNA and protein co-immunized animals developing long-lasting Ab titers were protected from infection.

P12.52

Viral Vector Delivery of Env Trimer Immunogens

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Background: Our objective is to develop viral vaccine vectors that will elicit neutralizing antibodies that are specific for the functional attachment protein on the HIV particle. To achieve this goal, we are developing vectors that express membrane-anchored Env trimers that closely mimic authentic functional glycoprotein spikes.

Methods: We are using vesicular stomatitis virus (VSV) as a vector platform for delivery of Env immunogens as transmembrane glycoproteins. We have investigated a variety of vector designs and Env modifications to identify combinations that balance the practical requirement for vector genetic stability with factors influencing antibody responses including immunogen abundance, efficient post-translational processing, and presentation of antigenic determinants representative of a functional trimeric spike.

Results: Substituting domains in Env with analogous regions from VSV G, we have developed a number of immunogens that are efficiently expressed and incorporated in the infected cell plasma membrane, and in most cases, progeny virus particles. Antigenicity was evaluated using a panel of monoclonal antibodies specific for various Env epitopes.

Conclusion: We identified modified Env immunogens that contain determinants for most classes of known broadly neutralizing monoclonal antibodies including those with specificity for the CD4 binding site (b12, PGV04), V3 and carbohydrate (PGT126), the MPER (2F5 and 4E10), the glycan shield (2G12), and structures formed by V1/V2 and carbohydrate (PG9, PG16, PGT145). Results from ongoing immunogenicity studies with vectors encoding SIV or HIV Env immunogens (subtypes A, B, or C) indicate that the modified trimers elicit antibody responses in small animals and nonhuman primates, and that some live vectors induce mucosal antibodies. Study sera are being analyzed for virus neutralization activity and fine specificity.

Topic 12: Vaccine Concepts and Design

P12.53

Optimizing Expression of Functional HIV Envelopes in rVSV-ΔG Vaccine Vectors

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Background: Our objective is to develop replicating recombinant vesicular stomatitis virus (rVSV) vectored HIV vaccine candidates that deliver membrane-bound trimeric HIV Env in a functional conformation.

Methods: Using a combination of nucleotide sequence optimization and protein domain swapping, we have generated a panel of novel gene inserts for VSV vectors that encode chimeric HIV-1 and VSV glycoprotein immunogens (EnvG). A stable VERO cell line engineered to express human CD4 and CCR5 was used to rescue rVSV vectors in which the G gene was replaced with coding sequence for several different EnvG proteins.

Results: Analysis of cells transfected with plasmid DNA expressing EnvG constructs revealed abundant cell surface expression of chimeric glycoproteins. The expressed proteins retained CD4-dependent membrane fusion activity, which is one of the main characteristics of functional HIV Env. The chimeric EnvG in which the signal peptide (SP), transmembrane (TM) and cytoplasmic tail (CT) domains of HIV Env were replaced with functionally related domains of VSV G were expressed efficiently and supported vector propagation to high titer specifically in CD4+CCR5+ cells. Flow cytometric analysis demonstrated that cell-surface expressed EnvG chimeras were recognized by a spectrum of HIV-1 specific broadly neutralizing monoclonal antibodies, including those that bind preferentially to the trimeric spike. Western blot analysis on purified viruses indicated that EnvG glycoproteins that contained the VSV G TM and CT were incorporated efficiently into VSV particles. Interestingly, an EnvG in which the Env MPER domain was replaced with membrane-proximal sequence from G was more effectively processed and incorporated into virus particles.

Conclusion: Chimeric EnvG glycoproteins expressed efficiently from plasmid DNA and rVSV vectors in membrane-bound, fusion-competent conformation and displayed relevant HIV-1 broadly neutralizing antibody epitopes. Small animal studies are underway to assess Env-specific humoral immune responses elicited by rVSV vectors encoding EnvG immunogens.

P12.54

Rational Modification of an HIV-1 gp120 Results in Enhanced Neutralization Breadth When Used as a DNA Prime

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Background: The identification of phenotypic features of the HIV-1 envelope glycoprotein that correlate with neutralization breadth is an important goal of HIV vaccine research. Recently we compared the immunogenic potential of two gp120s differing in their ability to utilize CD4; B33 (highly macrophage topic) and LN40 (non-macrophage tropic). Using a DNA prime protein boost regimen in New Zealand White Rabbits, LN40-primed sera displayed enhanced breadth compared to the B33-primed group, with differences in immunogenicity between groups modulated by specific residues within and flanking the V3 loop and the CD4bs. To better understand the role of these residues in eliciting breadth, we introduced reciprocal mutations between LN40 and B33 at these critical positions.

Methods: Three groups of four rabbits were primed with one of three chimeric LN40/B33 gp120 DNAs, followed by a polyvalent protein boost. Time course and endpoint titers were determined via ELISA. Neutralization breadth was analyzed by Monogram against a panel of sixteen viruses using a Phenosense neutralization assay. Anti-gp120 serum specificities were determined using a set of overlapping peptides spanning the entire gp120 via ELISA.

Results: We found that sera primed with a B33 chimera containing specific LN40 residues within the V3 loop and the CD4 binding loop displayed enhanced neutralization breadth against a cross-clade panel of Tier 1 and 2 viruses compared to the B33-primed group. Interestingly, a second B33 chimera containing two additional LN40 substitutions (Stu-Bsu R373/N386) within C3/V4 primed the broadest response, being broader than even the LN40-primed group. Additionally, peptide ELISAs showed differences in reactivity between priming groups which were most pronounced for the C3/V4 region, suggesting an important role for these regions in modulating serum antibody responses against gp120.

Conclusion: While the role of R373/N386 is still under investigation, these results represent potentially promising steps towards the rational, targeted design of a better gp120 immunogen

P12.55

Functional Properties of an Ensemble of Candidate Germline-Encoded Precursors of the Anti-MPER Antibody 4E10

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Background: We have previously engineered computationally-designed 'epitope-scaffold' constructs for the broadly neutralizing, MPER-specific antibody 4E10, consisting of the epitope grafted as a structural unit onto non-HIV scaffold proteins for optimal presentation during immunization. 4E10 epitope-scaffolds display dissociation constants for mature 4E10 ranging down to picomolar values and can elicit epitope-specific responses during immunization. Sera from immunized animals failed to potently neutralize HIV, at least partially due to differences between human and non-human germline repertoires. Successful use of epitope-scaffolds as vaccine immunogens will require optimizing interactions with both the mature antibody target and appropriate precursors, while preserving or generating neutralization potency during maturation.

Methods: Since a unique germline-encoded precursor sequence for 4E10 cannot be unambiguously assigned, we have generated an ensemble of the twelve likeliest candidates in order to study their functional and recognition properties. Interaction parameters between mature and candidate germline-encoded precursor (CGP) antibodies for engineered epitope-scaffolds, peptides, membrane components and HIV proteins were determined in surface plasmon resonance analyses and three-dimensional structures of free and ligand-complexed forms were determined by x-ray crystallography. Neutralization potencies were determined and polyspecificity and autoreactivity were analyzed with large-scale peptide arrays.

Results: Unlike other anti-HIV CGPs, which display negligible affinities for HIV-related ligands, 4E10 CGPs display affinities for epitope-scaffolds which, while 1,000- to 100,000-fold weaker, still reach into the nanomolar range. Structural studies show remarkable conservation of recognition mechanisms while functional studies show retention of anti-HIV activity. Polyspecificity of both mature and CGP forms is very limited, but the significant autoreactivity of mature 4E10 appears directed to specific targets.

Conclusion: The functional gap between mature 4E10 and its CGPs is narrower than in other HIV-related systems. Strategies based on our results can be proposed to generate mature- and CGP-specific epitope-scaffolds for use in prime-boost vaccinations to target specific CGPs with desirable functional properties while potentially avoiding autoreactivity.

P12.56

Native Envelope-Based Immunogens Derived from Critical Timepoints in the Development of Breadth Elicit Rapid Neutralizing Antibodies in Rabbits

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Background: HIV-1 evolves rapidly within the host, resulting in the development of diverse variants called a viral "quasispecies" population. A major goal of vaccine efforts is the design of Envelope (Env)-based immunogens effective at eliciting broadly neutralizing antibodies. We hypothesize that B cells become programmed to develop broad NAbs by exposure to Envs presented by the viral quasispecies variants. We propose that similar programming could be achieved by a vaccine concept exposing the host to such Env quasispecies variants isolated from an individual who developed broad NAbs over time.

Methods: Full-length functional env genes were cloned longitudinally from elite neutralizer CI10014 by single genome amplification, and a combination of in silico sequence analysis and in vitro neutralization was used to select vaccine candidates. Four immunization strategies were tested in rabbits: (1) sequential env evolution as it occurred in CI10014, with multiple clones per timepoint (Sequential); (2) sequential vaccine approach using only one clone per timepoint (Simplified Sequential); (3) an approach uniquely focused on env clones derived from timepoints where env evolution drove the development of breadth (Jump into Breadth); and (4) single env variant (Clonal). The gp160-DNA and gp140-trimer immunogens were co-administered.

Results: NAbs were detected at six weeks, after only two immunizations and increased after additional immunizations. The Jump into Breadth strategy elicited significantly higher NAbs than the Clonal and Sequential strategies. Modest heterologous neutralization was obtained against Tier 1 clade A and B viruses.

Conclusion: Exposure to env immunogens derived from timepoints preceding and contemporaneous with the appearance of neutralization breadth elicited higher NAbs than exposure to a single variant or a longitudinal collection of Envs. This study explores the use of multiple native, related HIV-1 Envs as immunogens and emphasizes the critical importance of understanding the development of breadth in an elite neutralizer subject.

Topic 12: Vaccine Concepts and Design

P12.57

Structural Definition of a Novel CD4-Induced Epitope That Is Targeted by a Single-Headed Immunoglobulin to Effect Broad and Potent HIV Meutralization

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Background: HIV-1 enters cells by sequentially binding the CD4 receptor and a coreceptor, either CCR5 or CXCR4. Functional constraints result in a high degree of conservation of the receptor-binding sites making them potential targets for intervention. The coreceptor-binding site on HIV-1 envelope gp120 glycoprotein is protected from the humoral immune system by conformational masking and steric occlusion. The site becomes available after a conformational change in gp120 following CD4 engagement, but at that point in the entry process, the proximity of the viral and cellular membranes makes the site inaccessible to bulky antibody molecules. Thus, in spite of being highly conserved, this region has not been considered a viable vaccine target.

Methods: Single domain antibody vHH120.4 was isolated from llama immunized with gp120 covalently linked to a CD4-mimetic peptide. Full-length versions of this antibody (IgG2B and IgG3) were created and tested for neutralization. The structure of vHH120.4 bound to gp120 from the HIV-1 YU2 strain was determined at 2.1Å resolution.

Results: Both IgG2B and IgG3 versions of the vHH120.4 potently neutralized over 95% of a panel of circulating Tier 2 HIV-1 isolates. Structural analyses of vHH120.4 bound to gp120 revealed a novel CD4i epitope that involves antibody interactions with region on gp120 encompassing the bridging sheet and the base of the V3 loop, the β19 strand, the CD4-binding loop, and the glycan at Asn 386. This epitope overlaps the classically defined CD4-induced epitopes recognized by antibodies 17b and 48d, but is shifted towards the site of CD4 attachment.

Conclusion: The discovery of a neutralizing CD4-induced epitope indicates that not all CD4-induced sites are masked from neutralization. Whether human antibodies can also utilize the newly defined vHH120.4 epitope for effective neutralization remains to be determined; HIV-1 envelope probes designed to specifically select antibodies targeting this epitope are now being developed.

P12.58

Purification of Chemically Fixed HIV-1 Spikes for Oriented Display on Nanoparticles

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Background: HIV-1 neutralizing antibodies (Abs) bind to the envelope glycoprotein (Env) spike, which functions as a trimer of gp120-gp41 heterodimers that is anchored in the viral membrane. However, Env trimers are low in copy number and coexist with irrelevant forms of Env and its byproducts, which typically elicit non-neutralizing Abs.

Methods: Here, we have attempted to generate immunogens by fixing trimeric spikes of HIV-1JR-FL using a defined chemical crosslinker, purifying the crosslinked spikes from virions, and immunodepleting them of irrelevant Env contaminants using non-neutralizing Abs.

Results: Purified, crosslinked spikes were bound by a panel of neutralizing Abs, but not by non-neutralizing Abs, and are virtually devoid of non-trimeric Env. However, at least one neutralizing epitope on gp120, the crown of V3, appears to be occluded by crosslinking. An immunization was performed using the purified, crosslinked Env trimers as a boosting agent following a DNA prime using full-length env, either as soluble protein or captured onto small proteoliposome nanoparticles (PLNs). The binding titers of the Ab response to crosslinked Env spikes were quite weak, possibly reflecting an overall weaker immune response. Boosting animals with trimer-PLNs however elicited a qualitatively different neutralizing Ab response than uncrosslinked Env on virions, with sporadic activity against neutralization-resistant HIV-1 isolate JR-CSF, reduced neutralization of sensitive Tier 1 isolates, and reduced antibody responses against host protein.

Conclusion: Immunizing animals using purified, crosslinked Env spikes captured on PLNs elicited a qualitatively different and broader neutralizing Ab response than uncrosslinked, heterogeneous Env. We conclude that with further changes to the crosslinking and immunization strategy crosslinked and purified Env spikes hold promise as a vaccine candidate.

P12.59 LB

DNA Vaccines that Express the MPER of HIV-1 gp41 Elicit Different Antibodies Depending Upon Their Transmembrane and Cytoplasmic Domains

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Background:

The HIV-1 gp41 MPER contains the epitopes for 4 broadly neutralizing (bNt) antibodies (Abs), making it a target for vaccine design. We developed a panel of DNA-vaccine candidates that link the MPER, or larger portions of gp41's external domain, to two different transmembrane (TM) and cytoplasmic (CT) domains. We report on the ability of DNA vaccines expressing these constructs to elicit MPER-specific Nt Abs in rabbits.

Methods: DNA vaccines encoding various gp41 ectodomain fragments, and the TM and CT of either the platelet-derived growth factor receptor (PGDFR-TM), or the TM and truncated CT of gp41 (TM1), were used to immunize rabbits; immune sera were tested for reactivity against the MPER displayed on the cell surface and for pseudovirus neutralization.

Results: Immunizations with plasmids expressing larger fragments of the gp41 ectodomain tethered to the PDGFR-TM elicited lower-titer Abs against the MPER, as compared to the MPER tethered to the PDGFR-TM, which elicited MPER-specific Abs that included those targeting the 2F5 epitope Affinity purification of these Abs on 2F5-epitope peptide resulted in Abs that bound MPER expressed on cells, but neutralized both pseudoviruses bearing HIV envelope and those bearing AMLV envelope. Immunization with DNA vaccines encoding the MPER fused to the gp41 TM1, elicited low-titre Abs that cross-reacted weakly with the MPER and strongly with regions in the CT. All immunization failed to produce Abs that cross-react with the 4E10 epitope.

Conclusion: While the initial immunization studies reported here demonstrate that MPER reactivity is elicited by DNA immunization, the MPER-TM1 construct appears to elicit Abs against epitopes in its 27-aa CT domain. Current work is focused on replacing the gp41 TM with an engineered TM that will optimally expose the epitopes for the bNt MAbs, particularly 4E10.

P12.60 LB

Determination of Structural Constraint within the HIV Proteome through Analysis of Amino Acid Microenvironments

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Background: The design of an effective T cell based vaccine relies on determining the most highly constrained regions of the HIV proteome.

Methods: Using publicly available crystal structures from the Protein Databank (PDB), we performed a systematic analysis of the local microenvironment of every amino acid within the HIV Proteome for which structural data was available (65.8%). Structural constraint parameters included involvement in protein secondary structure, relative solvent accessibility, and involvement of amino acid side chains in intermolecular interactions (Van der Waals bonds, hydrogen bonding, salt bridges, disulfide bridges, pi-pi bonds, and pi-cation bonds). Calculations of these constraints were carried out using validated methods of protein structure analysis and distance geometry, with weighted values cumulatively summed to provide a constraint score for every amino acid.

Results: Amino acids with a higher constraint score were observed to strongly correlate with low values of entropy within viral sequences from every clade of HIV. Analysis of constraint score variation across the HIV proteome reveals that the p24 capsid protein to be the most highly interconnected and constrained. Evaluation of amino acids within known HLA-restricted epitopes further elucidated a preference of controller alleles for buried and interconnected amino acids, while progressor alleles predominantly targeted exposed and non-connected amino acids. Thermodynamic stability analysis further demonstrated a strong correlation with amino acid constraint and change in predicted Gibbs' Free Energy.

Conclusion: Our analyses reveal that evaluation of local amino acid microenvironments represents a novel method for the determination of constraint within the HIV proteome, setting the stage for more robust targeting of these constrained regions and enhanced immunogen design.

Topic 12: Vaccine Concepts and Design

P12.61 LB

Design And Development of a New Lentiviral Based Anti-HIV Therapeutic Vaccine

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Background: Theravectys develops a new generation of prophylactic and therapeutic vaccines using optimized lentiviral vectors. It's most advanced product, a therapeutic anti-HIV vaccinal treatment, will enter clinical Phase I/II within a few weeks. This vaccination will allow seropositive patients to gain an immunological status identical to the so-called "Functional Cured" patients who develop an efficient immunological response capable of controlling the infection without therapy

Methods: Vaccine candidates are integrative and self-inactivated live-recombinant lentiviral vectors. They encode an HIV antigen, under the regulation of a patented promoter that is preferentially induced in APC (generating of a strong, specific and long lasting T-cell immune response), and showing a basal level expression in all cells (allowing their elimination by the settled immune response).

Furthermore, Theravectys developed a vaccination regimen based on iterative immunizations with lentivectors encoding the same HIV transgene, relying on different VSV-G serotypes for pseudotyping without generating cross-neutralizing antibodies. These candidates were classified as "Live recombinant vectored vaccines" (EMA, 2011)

Results: Theravectys set up an innovative manufacturing process combining high production yields, impurity profiles compatible with direct injections into humans and high immunogenicity.

Pilot and GMP batches have been manufactured and GLP preclinical studies (amongst which biodistribution, shedding and toxicity) performed, that showed the restricted diffusion of the vaccine candidates after injection and their fast disappearance within few weeks, correlated with an absence of macroscopic and microscopic toxicity

Conclusion: These data allowed the settlement of an anti-HIV therapeutic Phase I/II clinical trial that should receive the authorization of the French regulatory agency before the end of July.

This trial will be held in France and Belgium and plans the enrollment of 36 HIV-1 infected patients. Theravectys' anti-HIV vaccine treatment will be assessed at three doses and safety, tolerability and immunogenicity compared to a placebo group. Results are expected by September 2014 with intermediary analyses in September 2013

P12.62 LB

CN54gp140: Product Characteristics, Preclinical and Clinical Use - Recombinant Glycoprotein for HIV Immunization

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Background: The usefulness of HIV envelope proteins for vaccine design is widely accepted since the RV144 HIV-1 prime-boost vaccine trial. It is assumed that a trimeric structure close to the natural form of the HIV envelope is preferable.

Methods: We have expressed the soluble form of the HIV envelope of the C/B' strain 97/CN/54 in CHO cells. The production process consisted of a large-scale fed-batch fermentation, an antibody-based affinity chromatography plus additional purifying steps. CN54gp140 was extensively characterized for purity and identity. All glycosylation sites were characterized by mass spectrometry. Immunogenicity and safety were evaluated in mice, rabbits, mini-pigs, sheep as well as non-human primates. The antigen was formulated for clinical phase I studies in Tris buffer (MUCOVAC I, HIVIS07) in HEC gel (MUCOVAC I) as well as chemically conjugated to hsp70 (MUVAPRED).

Results: The vaccine antigen candidate CN54gp140 proved to be of high purity and long-term stability. The immune response was strongest with i.m. application whereas the mucosal routes (i.vag., i.n.) were less immunogenic. Safety was demonstrated in animal models as well as in the clinical phase I studies MUCOVAC I, MUVAPRED and HIVIS07.

Conclusion: CN54gp140 is a highly immunogenic trimeric envelope protein which can be manufactured in sufficient quality and quantity for clinical application. It proved to be immunogenic in several animal models. Finally, it is well tolerated in several formulations and combinations in humans.

P12.63 LB

Pre-Clinical Development Of BCG.HIVA(CAT) Strain, An Antibiotic-Free Selection Strain For HIV-TB Pediatric Vaccine

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Background: Our starting platform was based on a heterologous BCG prime and MVA boost regimen delivering a common immunogen called HIVA. In this study, we have i) developed a BCG.HIVA^{CAT} strain containing an antibiotic free selection system (Cobra); ii) evaluated the specific HIV-1 immune responses induced after newborn BALB/c mice immunization with BCG.HIVA^{CAT} prime and MVA.HIVA.85A boost; iii) evaluated the specific-TB immune responses induced after newborn BALB/c mice immunization with BCG.HIVA^{CAT} prime and MVA.HIVA.85A boost and iv) evaluated the influence of age on specific HIV-1 immune responses using the same vaccination schedule.

Methods: 7-days-old newborn and 7-weeks-old adult mice were either left unvaccinated or vaccinated subcutaneously with 10^5 cfu of BCG.HIVA^{CAT} or BCGwt, and 16 weeks later were boosted intramuscularly with 10^6 pfu MVA.HIVA.85A. The mice were sacrificed 2 weeks later. The HIV-1 and TB-specific cellular immune responses were analyzed in spleen cells by intracellular cytokine staining and IFN-γ ELISPOT.

Results: The frequencies of TB-specific CD8$^+$ T-cells producing IFN-γ (P11 stimulation), and spleen cells producing IFN-γ (P11, P15 and PPD stimulation), were higher in BCG.HIVA^{CAT} or BCGwt primed and MVA.HIVA.85A boosted mice compared with mice vaccinated with MVA.HIVA.85A alone (i.e. 231, 108 and 24 sfu/10^6 PPD stimulated splenocytes respectively). The specific HIV-1 immune responses (P18I10 stimulation) were lower in BCG.HIVA^{CAT} or BCGwt primed and MVA.HIVA.85A boosted mice compared with mice vaccinated with MVA.HIVA.85A alone (i.e. 270, 276 and 412 sfu/10^6 P18I10 stimulated splenocytes respectively). When adult and newborn mice were immunized using the same vaccination schedule, the HIV-1-specific immune responses in adult mice were higher than in newborn mice (0.45% vs 0.2% CD8$^+$ T-cells producing IFN γ).

Conclusion: In conclusion we demonstrated the immunogenicity of BCG.HIVA^{CAT} and MVA.HIVA.85A in newborn mice but additional experiments should be performed in newborn mice testing different routes and doses that might provide different levels of immunogenicity.

P12.64 LB

Novel Computational Methods for Predicting Epitopes of Potent and Broadly Neutralizing HIV-1 Antibodies

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Background: Recent efforts in HIV-1 vaccine design have focused on immunogens that evoke potent neutralizing antibody responses to a broad spectrum of viruses circulating worldwide. However, the development of effective vaccines will depend on the identification and characterization of the neutralizing antibody epitopes. Consequently, we developed bioinformatics methods to predict epitopes using corresponding genotypes and phenotypes generated using a highly sensitive and reproducible neutralization assay.

Methods: Using 264 clonal envelope (gp120) sequences from a panel of multiclade HIV-1 viruses with matching neutralization titers to an array of neutralizing monoclonal antibodies (b12, PG9,16, PGT121 - 128, PGT130 - 131, PGT135 - 137, PGT141 - 145, and PGV04), we correlated IC_{50} titers with envelope mutations, and used this information to predict antibody epitopes. Structural patches were generated as amino acid groupings based on solvent-accessibility, diameter, atomic depth, and interaction networks within 3D envelope models. These patches were then evaluated as possible antibody targets by applying a boosted algorithm comprised of machine learning and statistical models. We identified residues with statistically significant correlation with IC_{50} titers as sites that impact neutralization sensitivity. Residues frequently occurring within the significant patches were mapped onto envelope structures as potential antibody binding sites.

Results: Predicted epitopes were identified based on strong correlations with neutralization response to each antibody. Residues highly associated with the IC_{50} titers and patch clusters predicting neutralization response to these antibodies were located within V1/V2, and V3. The predicted response by the algorithm was highly concordant (>80%) with the neutralization sensitivity of all antibodies.

Conclusion: We developed and applied computational methods to rapidly survey protein structures and identify epitope regions associated with neutralization response. This data mining algorithm can help identify immunological hotspots, and provide rapid and accurate insight into regions that are targeted by potent and broad neutralization responses. Studies are ongoing to confirm these novel epitopes.

Topic 12: Vaccine Concepts and Design

P12.65 LB

Liposomal Formulation of Gp41 Derivate with Adjuvant MPLA: Vaccine Design, Immunogenicity in Animals and Safety in Humans

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Background: Gp41 of the HIV envelope, especially the MPER, contains highly conserved epitopes recognized by neutralizing monoclonal antibodies such as D5, 2F5 and 4E10. The correct presentation of the antigens is considered to be key for eliciting neutralizing immune response. The lipid bilayer of liposomes can mimic the virus surface and therefore provides the appropriate presentation of this membrane protein. In addition, liposomes can integrate the adjuvant monophosphoryl lipid A (MPLA).

Methods: The gp41 derivate FPA2 was mutated to increase its solubility and modify its structure for exposure of neutralizing epitopes. The protein was expressed in E. coli. The purified protein was solubilized with detergent. Liposomes were prepared containing antigen FPA2 and MPLA, a Toll-like receptor 4 agonist, using a continuous ethanol crossflow injection technology. Immunogenicity and safety was determined in rabbits and macaques. The liposomes formulated in HEC gel were applied i.n. followed by two intramuscular boosts in healthy female volunteers in a phase I study of safety and immunogenicity.

Results: Liposomes containing FPA2 and MPLA manufactured under GMP conditions were uniform and stable at 2-8°C. Immunogenicity was demonstrated in rabbits and monkey after mucosal (i.vag., i.n.) prime, followed by intramuscular boosts. Immune response was induced systemically and in mucosal surfaces. Preliminary data from the clinical phase 1 study in healthy volunteers demonstrated good safety with three nasal applications of 200 µg of the formulated protein in HEC gel and of two i.m. boosts.

Conclusion: The liposomal formulation of the gp41 subunit of HIV envelope together with MPLA as adjuvant proved to be well tolerated in animals and human. Liposome-formulated FPA2 was able to induce binding antibodies measured by ELISA and neutralizing activity in both blood and vaginal secretions in animals. Immunogenicity testing in humans is ongoing.

P12.66 LB

Novel AIDS Vaccine Approach using Epithelial Stem Cells as Mucosal Antigen-Presenting Cells

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Background: Because HIV transmission occurs predominantly across mucosal surfaces, the ideal vaccine strategy to prevent infection would be to target HIV at mucosal entry sites of transmission. We investigated a novel vaccine approach, which aim is to elicit long-term immunity against HIV infection at the entry site of the virus. This strategy relies on the expression of viral proteins from epithelial stem cells at the basal layer of the epithelium and using a promoter that is specific for terminally differentiated epithelial

Methods: The involucrin promoter, which is exclusively expressed in terminally differentiated epithelial cells, was chosen and used as a tool. We generated a GFP-tagged replication competent SIVdeltaNef and a GFP-tagged replication deficient SIVdeltaVifdeltaNef constructs under the transcriptional control of the involucrin promoter (pINV). Viral stocks used to deliver these constructs to basal epithelial cells were obtained by their co-transfection with a plasmid encoding for VSV-G envelope proteins used as pseudotyping envelope protein of significantly broadened host cell range.

Results: When administered intradermally to mice, we found that GFP-reporter gene under the transcriptional control of the involucrin promoter was expressed in the upper layers of the epidermis. Although transduced cells were very low in number, high and sustained anti-GFP antibody production was observed in vivo. After production of high concentrations of infectious viral particles, we demonstrated the integrity of our constructs (regions encoding for GAG, POL and GFP) in the VSV-G pseudotyped viral particles to be used for inoculation in nonhuman primate model for AIDS.

Conclusion: After integration of pINV-driven constructs, basal layer cells will divide and differentiate thus triggering SIV antigens expression as well as both direct and cross priming. Long-term antigen expression in upper layers of the epithelium may occur even after multiple cycles of epithelia renewal, thus eliciting a long-term immunity against HIV/SIV infection at the site

P12.67 LB

Simple, Scalable And Robust Purification Of Two HIV-1 Subtype C gp120 Monomer Subunit Antigens For Phase II Clinical Trial In Republic Of South Africa

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Background: Development of an effective vaccine against HIV-1 is challenging due to various viral evolutionary mechanisms to evade human immune system. The partial efficacy of the recent RV144 vaccine efficacy trial in Thailand provides hope for improvements of vaccine regimens for higher efficacy. A Phase IIb proof-of-concept clinical trial in the Republic of South Africa (RSA) is planned to confirm and extend the results of the RV144 trial with the vaccine strategy of poxvirus vector prime plus envelope protein boost.

Methods: We selected two HIV subtype C gp120 vaccine antigens, TV1.C gp120 and 1086.C gp120, formulated with Novartis proprietary adjuvant, MF59 as protein boosts of the clinical trial.

Results: To produce TV1.C gp120 and 1086.C gp120 monomers, we generated CHO stable cell lines for both gp120, which consistently expressed gp120 subunits with high yield. Simple, scalable and robust antigen purification processes were developed to generate both gp120 proteins. The ion-exchange based purification strategy enabled the separation of gp120 monomer from dimer and produced gp120 monomer with high purity and homogeneity.

Conclusion: Purified gp120 monomers were stable, either alone or in combination, and when formulated with adjuvant MF59. Finally, the early evaluations showed that both gp120 monomers were immunogenic and able to elicit high neutralizing antibody titer.

P12.68 LB

2G12/PGT-Binding Yeast Glycoprotein Gp38 Elicits Mannose-Specific HIV-1 Env Cross-Reactive Antibodies

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Background: The increasing numbers of broad neutralizing antibodies (bNAbs) that target carbohydrates of HIV envelops highlight the importance of designing immunogens to elicit such types of bNAbs for an effective HIV vaccine.

Methods: PGT bNAbs-cross reactive proteins were detected by Western blots and identified by nano-LC-MS/MS. Rabbit antisera were raised with single PGT bNAbs-reactive yeast glycoprotein, and tested by ELISA, Western blots and glycan microarray. HIV-1 pseudoviruses were generated in 293T cells, and neutralization assay was performed using TZM-bl cells.

Results: Using the newly identified, glycan-specific PGT bNAbs to search for their binders from a triple mutant(TM) strain of Saccharomyces cerevisiae, we found that the PGT bNAbs not only bind to the previously identified 2G12-reactive glycoproteins but also recognize several unknown proteins in TM yeast. One of them was identified as a short version of Gp38 with N-terminus truncation. Based on immunization of rabbits with various formulations and strategies, we found that a high titer of HIV-1 Env cross-reactive antibodies was induced when using a promiscuous T-cell epitope peptide conjugated Gp38 in a formulation with a Toll-like receptor 2 agonist and aluminum salts. The Gp38-elicited antibodies could bind to a broad range of monomeric gp120s from HIV and SIV. Moreover, the antibodies could also efficiently neutralize HIV-1 pseudoviruses when the viruses were produced in the presence of a mannosidase inhibitor kifunensine, which enriches high-mannose Man9GlcNAc2 N-linked glycans. Glycan microarray analysis showed that these antibodies bind to the synthetic Manα1,2-Manα1,2-Man containing oligosaccharides

Conclusion: These data suggest that yeast glycoprotein Gp38, as well as its truncated form, is an efficient binder to the glycan-specific HIV bNAbs, and that Gp38 is able to induce a strong glycan-specific HIV-reactive antibody response when incorporated with appropriate adjuvants. These results encourage us to further explore the strategies to induce 2G12/PGT-like antibodies using Gp38 as well as its truncated form.

Topic 12: Vaccine Concepts and Design

P12.69 LB

Targeting HIV Gag p24 To DICR On Dendritic Cells Induces T Cell And Potent And Long-Lasting Antibody Responses In Non-Human Primates

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Background: Targeting Dendritic Cells (DCs) with anti-DC receptor antibody-antigen fusion proteins is a novel approach in vaccine development for inducing potent humoral and cellular immune responses.

Methods: We engineered an anti-human DCIR recombinant antibody cross-reacting with the cynomolgus macaque receptor fused via the heavy chain C-terminus to HIV-1 Gagp24 protein (anti-DCIR.Gagp24). HIV patient PBMC cultures were incubated with anti-DCIR and control hIgG4.Gagp24 fusion proteins. After 10 days, the total T cells were challenged with HIV Gagp24 peptide pools, and then antigen-specific cytokine production was detected using intracellular staining. Macaques were also immunized i.d. 3 times with anti-DCIR.Gagp24 or control hIgG4. Gagp24 with or without polyI:C. Gagp24-specific IgG titers from serum were measured by ELISA and the magnitude of the antigen-specific IFNγ responses was assessed by ELISPOT.

Results: In vitro, low doses of anti-DCIR Gagp24 prototype vaccine, but not the control hIgG4.Gagp24, expand of Gagp24-specific T cells. These in vitro-expanded antigen-specific T cells were multifunctional, simultaneously producing multiple cytokines (IFNγ, TNFα and MIP-1β). In vivo, in non-adjuvanted naïve animals, serum anti-Gagp24 antibodies were detectable 2 weeks after priming and titers were substantially increased after the 1st and the 2nd boost with anti-DCIR.Gagp24 and were durable, while in the control hIgG4.Gagp24 group the responses were minimal. Poly I:C increased the magnitude of the responses in the anti-DCIR.Gagp24 and hIgG4.Gagp24 groups to a similar level in both groups. T cell responses induced by anti-DCIR Gagp24 could be enhanced after priming with a recombinant modified vaccinia virus Ankara (MVA) encoding HIV Gag/Pol/Nef. Boosting with anti-DCIR.Gagp24 plus poly I:C generated high titers of anti-Gagp24 antibody titers and strongly enhanced IFNγ-producing T cells following priming with MVA HIV Gag/Pol/Nef.

Conclusion: Our results demonstrate that heterologous prime-boost immunization with vectors and DC-targeting protein-based vaccines is a promising vaccination approach to optimize humoral and cellular immunity for therapeutic and preventive applications against AIDS.

P12.70 LB

Virus-Like Particles Highly Expressing DC-SIGN Concentrate Trimeric HIV-Envelope Proteins With Noncovalently Linked Immunoreactive Gp120 And Gp41

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Background: We have hypothesized that membrane-bound macromolecular HIV-envelope proteins (mHIV-env) as a subunit, with gp120 and gp41 in their innate conformation, can be used as an effective immunogen to elicit broadly neutralizing antibodies (bnAb) against sexually or perinatally transmitted HIV-1. To test this hypothesis we have synthesized infection-free mHIV-env from the HIV-1 transmitted in humans (Vyas et al, Biologicals 2012;40:15-20). The process has been simplified for making large amounts of mHIV-env coupled with DC-SIGN expressing virus-like lipoparticles (VLP) as a candidate immunogen.

Methods: Virus isolates of plasma-derived HIV-1 (PHIV) from infected blood donors while negative for anti-HIV (earliest acute infection) were selected for expansion in an optimized cell substrate (OCS) prepared from lymphocytes of four donors. Virions in the culture supernatants were inactivated by extracting membrane cholesterol with 200mM cyclodextrin for 4 hours to maximally expel p24, RT, and viral RNA from the permeabilized virions. The residual host/viral DNA/RNA in the inactivated virion shells were hydrolyzed with protease-free Benzonase without loss of gp120. The mHIV-env was coupled with VLP highly expressing DC-SIGN (Integral Molecular, Philadelphia, PA).

Results: The inactivated virions apparently disintegrated into amorphous mHIV-env proteins when reacted with Benzonase. While the mHIV-env passed through membranes with 1,000kD cut off, it was retained by 30, 100, or 300kD cut off membranes as determined by gp120 EIA. The mHIV-env was coupled to DC-SIGN highly expressed on VLP. Analyses of DC-SIGN bound mHIV-env showed conformation-dependent immune reactivity with anti-gp120(b12). Remarkably, the gp41 noncovalently bound to gp120 was quantitatively detected by human monoclonal anti-gp41. The simplified method for preparing mHIV-env yielded trimeric heterodimers with an estimated molecular mass of 500kD, which was concentrated 10-100X with VLP expressing DC-SIGN.

Conclusion: The VLP with mHIV-env bound to DC-SIGN at 10-100X more concentration than the 7-14 spikes per native virions provide a putative immunogen capable of inducing broadly neutralizing antibodies.

P13.01

Pediatric HIV Infection Due to Maternal Transmission: A Solvable Problem in a Peri-urban Setting in Bamako, Mali

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Background: In 2005, GAIA Vaccine Foundation established a mother-to-child transmission prevention (MTCTP) program (Chez Rosalie) in the community-based clinic of Sikoro, a peri-urban low-resource setting in Bamako, Mali. HIV testing of the pediatric population by PCR was recently implemented. Here we report the status of children born to HIV-seropositive mothers followed at the clinic.

Methods: The MTCTP program at Chez Rosalie was one of the first such programs to be established. Standard finger-stick approaches to testing children for HIV at 18 months resulted in very few children tested, as women frequently did not return to the clinic with their children at this time. In 2010, PCR-testing of newborns was integrated into mothers' postpartum follow-up appointments, during which artificial milk and antiretrovirals (ARV) were also distributed as part of PMTCT. As a result of this change, the test has now been performed for 69 children of the 202 HIV-seropositive mothers enrolled in the Chez Rosalie PMTCT program in Sikoro.

Results: 62 children (90%) were HIV-negative by PCR, and seven children (10%) were HIV-positive. Of the seven HIV-positive children, only one was born to a mother followed at the clinic. This mother was diagnosed late in her pregnancy and did not strictly adhere to MTCTP, including exclusive formula feeding her child (the national policy at the time). The other six HIV-positive children were born either at home or in another clinic where MTCTP was not available.

Conclusion: PCR testing of newborns increased the number of children screened for HIV infection in this MTCTP program. Both treating mothers with ARVs prior to delivery and providing newborns with formula (or exclusive breastfeeding while on ARV) reduced prevalence of pediatric HIV infection by close to 98%. Based on our results, the introduction of MTCTP at the community level is one of the most successful non-vaccine HIV prevention interventions.

P13.02

Assessment of T Cell Immune Responses Towards HIV-1 Epitopes Designed by Reverse Immunogenetic Approach: Proof-of-Concept in HIV+ Cameroonian Children

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Background: We tested whether 23 HIV-1 epitopes designed using a bioinformatics approach were recognized by HIV-infected children living in ethnically diverse Cameroon, which harbors a high HIV strain variety.

Methods: Areas containing both promiscuous HLA-class I and II epitopes covering more than 90% of the HLA haplotypes present in the African population were identified within the most HIV-1 conserved sequence of each protein. Twenty-three peptides have been designed targeting gag (10), nef (6), tat (4), vpr (2) and vpu (1) proteins.

We enrolled 33 children vertically infected with HIV (age range: 3 months – 12 years), naïve for antiretroviral therapy, with CD4% >15% or CD4 count >350 cells/ml.

T cell recognition of single peptides was assessed by IFN-γ ELISpot. Pooled peptides (gag, nef, regulatory proteins) were used to assess helper (CD40L expression) and cytotoxic (CD107a expression on cell surface) functions, and proliferative capacity (CFSE dilution) by flow cytometry.

Results: The majority (76%) of children recognized at least one peptide or peptide pool in at least one test, but none of the functional assays alone would have identified all responders.

Half (48%) of the peptides were recognized by at least one child in ELISpot assay, nef peptides being the most frequently targeted (by 67% of the responders). Three previously undescribed epitopes were identified, while 2 epitopes commonly considered immunodominant were not recognized.

In some children the same peptides were able to elicit different functions, while in other children diverse functions were induced by different peptides. The breadth of the epitope recognition and the number of different functions elicited were directly correlated and independent from the length of infection (age).

Conclusion: These data provide a proof-of-concept for the rational design of T cell immunogens by reverse immunogenetic approach and support the parallel use of different functional assays for epitope mapping.

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