0999 **Localization of the rough colony protein (RcpA) of Actinobacillus actinomycetemcomitans by immunofluorescence.** **E.M. HAASE*1, R.J. PALMER JR2, Y. WANG3, C. CHEN3, P.E. KOLENBRANDER2, and F.A. SCANNAPIECO1, 1 University at Buffalo, USA, 2 NIDCR, USA, 3 University of Southern California, USA**

Fresh clinical isolates of Actinobacillus actinomycetemcomitans, a periodontal pathogen associated with aggressive periodontitis, exhibit an adherent rough colony morphology that rapidly converts to a minimally adherent smooth colony morphology. Flp fimbriae are abundantly expressed by cells with the rough phenotype. The rcpA gene is part of the operon responsible for the synthesis, assembly, and expression of these fimbriae. Based on homology studies, RcpA may be a pore through which fimbriae are transported to the cell surface. Objective: To study the cell surface localization of RcpA using monospecific polyclonal antibody. Methods: The specificity of the anti-RcpA polyclonal antibody was verified by probing outer membrane protein (OMP) profiles of both rough and smooth variants 283 and D7, and a RcpA-deficient mutant on Western blot. The reactivity of the antibody to different conformations of RcpA was shown by comparison of OMP samples heated to 100°C and 37°C. Cellular localization was studied by indirect immunofluorescence of rough and smooth variants of strain 283 and visualized by confocal laser microscopy. Results: On Western blot, antibody reacted with RcpA present only in the rough strains, but not the isogenic smooth variants or a RcpA- mutant. Antibody reacted with RcpA presented as a monomer at 100°C, and an aggregate at 37°C. The smooth phenotype cells, as well as the negative control (no primary antibody) showed no reactivity by indirect immunofluorescence. However, the rough phenotype cells exhibited a speckled pattern of variable fluorescence that predominated in regions of adjoining microcolonies. Conclusions: RcpA is variably surface-exposed and appears to be expressed in greatest concentration in regions of microcolony contact. NIDCR grants DE09838 and DE12212, and NIDCR intramural funds supported this work.

1000 **Expression of green fluorescent protein in different serotypes of Actinobacillus actinomycetemcomitans.** **W. TEUGHELS*1, D. VAN STEENBERGHE, J. VANDERLEYDEN1, J. VAN ELDERE1, M. PAUWELS1, K. DIERICKX1, and M. QUIRYNEN1, 1 Catholic University of Leuven, Belgium**

Objectives: Green fluorescent protein (GFP) has become an important tool to investigate dynamic biological processes. The aim of this study was to express GFP in seven serotyped clinical and six serotyped laboratory Actinobacillus actinomycetemcomitans (Aa) strains which were used in epithelial cell adhesion and invasion studies. Methods: Six laboratory (serotypes a, b (2x), c, d, e) and seven clinical Aa strains (serotypes a, b (2x), c, d, e, x) were transformed by electroporation in the presence of a previously developed plasmid pVT1303 (pSU20+LTX/GFPmut2) and a novel plasmid pVT1304 (pPK1+LTX/GFPmut2), both containing a spectinomycin resistance marker. When unsuccessful, electroporation was repeated three times at most. Prior to transformation, all strains were tested for spectinomycin sensitivity. Also electroporation survival rates were determined. Transformed strains were checked on the presence of fluorescence. Contamination was excluded by a commercial enzymatic test. By comparing an instant selective growth method on spectinomycin containing TSBYE agar (TSBYE+S), with and without bacitracin and vancomycin, to a 24 hour non-selective growth method followed by subcultivation on TSBYE+S, long-term maintenance at -80°C was assessed. Results: All strains were spectinomycin sensitive prior to electroporation and able to survive electroporation. Three laboratory strains and two clinical strains were successfully transformed with pVT1303 and exhibited fluorescence. Only one laboratory strain was successfully transformed with pVT1304 but exhibited a brighter fluorescence compared to pVT1303. One laboratory and two clinical strains transformed with pVT1304 obtained spectinomycin resistance but did not exhibit fluorescence. None of the a, c and d serotypes appeared transformable with one of the plasmids. Only the 24 hour non-selective growth followed by subcultivation on TSBYE+S method was effective to regain growth after long-term storage. Conclusions: The results indicate that for Aa the efficiency of transformation and GFP expression is strain/serotype and plasmid specific. Long-term storage of GFP transformed Aa strains seems possible.

1001 **The clonality of Actinobacillus actinomycetemcomitans in localized juvenile periodontitis.** **D. FURGANG*, H. SCHREINER, J. KAPLAN, and D. FINE, UMDNJ New Jersey Dental School, USA**

Objective: Previous literature has implicated one clonal type (serotype b, JP2 leukotoxin promoter sequence) of Actinobacillus actinomycetemcomitans (Aa) as being strongly associated with Localized Juvenile Periodontitis (LJP). Recently our group has developed PCR protocols for the analysis of 5 presumptive virulence factors. These analyses when combined with Arbitrary Primed PCR (AP-PCR) analysis can be used to better define the clonality of Aa. Methods: The DNA from 30 Aa strains (6 reference strains, 4 strains from healthy adults and 20 strains from LJP patients) were isolated and AP-PCR plus analyses for genetic serotyping (serotypes a-e and the new serotype f), cytolethal distending toxin gene analysis (both promoter region and structural genes cdt A, cdt B and cdt C), flp-1 (fimbriae) gene analysis, groEL gene analysis and ltx (leukotoxin promoter region) analysis were performed. Results: All Aa strains fell into known serotypes. There were two major ltx variants found as well as 2 major flp-1 variants. Four cdt promoter region variants were identified. 31 of 33 strains appeared to have intact cdt structural genes. All strains had the intact groEL structural gene. AP-PCR results identified 3 major patterns that were named Types 1, 2 and 3. Twenty-six out of 30 strains (87%) fell into these types. Each type showed a unique pattern of other phenotypes tested. All strains containing the JP2 type ltx promoter region (8 of 8) were isolated from LJP subjects. The three serotype f strains were isolated from LJP subjects. Future research will add to the strains analyzed and determine whether specific clonal types are associated with health. Conclusion: The AP-PCR analyses performed on 33 Aa strains found 3 major clonal types. Further each type was shown to contain differing phenotypic patterns for 5 other presumptive virulence factors tested.

1002 **Directed mutagenesis of the DNA-Adenine-Methyltransferase (DAM) gene in Actinobacillus actinomycetemcomitans (Aa) by conjugation.** **J.E. LIPPMANN*, H. WU, and P.M. FIVES-TAYLOR, University of Vermont, USA**

Actinobacillus actinomycetemcomitans (Aa) is a slow growing, fastidious, Gram-negative, capnophillic bacterium. Of the over 500 different organisms in the oral cavity, Aa is one which has been strongly implicated in juvenile and adult periodontitis. Aa manifests disease by it's ability to produce toxins, adhere to and invade epithelial cells. DNA-Adenine-Methyltransferase (DAM) is generally associated with DNA modification, but it may also act as a global regulator of gene expression in many organisms. By adding methyl groups to various sites along the genome, DAM alters the interactions of regulatory proteins with their target genes. Recently, virulence genes in Salmonella strains have been shown to be modulated by DAM methylation. Objectives: To create a DAM minus strain of Aa SUNY465 for use in evaluating the role of DAM in Aa virulence. Methods: The dam gene was cloned and sequenced. The PCR-amplified gene was disrupted by insertion of an antibiotic cassette into a unique blunt ended restriction site. The disrupted gene was cloned into a conjugative plasmid and transferred from E. coli SM10(lpir) to Aa. Results: The insertional mutation resulted in the loss of DAM methylation of the Aa genome as shown by restriction analysis using Dpn I & Dpn II endonucleases, which cleave DNA at methylated and non-methylated sites, respectively. Confirmation of the genetic mutation was confirmed by colony PCR and Southern blot analysis. Conclusion: The chromosomal gene coding for DNA-Adenine-Methyltransferase of A. actinomycetemcomitans has been cloned, sequenced, and disrupted. This work was supported by NIH-NIDCR grant RO1DE09760.

1003 **Effect on the cell surface hydrophobicity of oral Candida albicans isolates obtained from HIV infected patients following brief and sequential exposure to fluconazole and amphotericin B.** **A.N.B. ELLEPOLA*, Faculty of Dental Sciences, University of Peradeniya, Sri Lanka, and L.P. SAMARANAYAKE, Faculty of Dentistry, University of Hong Kong, Hong Kong**

Candida albicans is considered the most virulent of all Candida species responsible for oral candidoses, which is a common oral manifestation associated with human immunodeficiency virus (HIV) infection. Cell surface hydrophobicity (CSH) of C. albicnas isolates is a pathogenic attribute, which is inextricably involved, in candidal adhesion to both mucosal and inert surfaces. Fluconazole and amphotericin B have been successfully used for treating oral candidosis in HIV infected patients. However, due to the unique environment of oral cavity, the diluent effect of saliva and the cleansing effect of the tongue, it is unlikely that the organisms are exposed to a constant and frequent delivery of the drug during therapy. Objectives, As the concentration of antimycotic agents reach sub-therapeutic levels at dosage intervals in the oral cavity, the impact of fluconazole and amphotericin B on the CSH of oral C. albicans isolates obtained from HIV infected patients, should be of clinical relevance. Methods, Hence the main aim of the study was to measure the CSH using the biphasic aqueous-hydrocarbon assay, of 11 isolates of oral C. albicans following limited (1 hour) and sequential (10 days) exposure to sub-therapeutic concentrations of fluconazole and amphotericin B. Results, The reduction in CSH following exposure to fluconazole and amphotericin B were 6.15% ($p<0.05$ for 18.18% of the isolates) and 22.80% ($p<0.05$ for 54.54% of the isolates), respectively. Conclusions, These findings reveal that such exposure modifies candidal CSH to varying degrees, which appear to be an unrecognized and poorly understood salutary feature of fluconazole and amphotericin B. It further elucidates pharmacodynamic mechanisms by which these drugs may operate in vivo in modulating candidal virulence.

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Withdrawn

1005 **Candida albicans triggers interleukin-8 responses by oral epithelial cells.** **A. DONGARI-BAGTZOGLOU*, H. KASHLEVA, and D. CHOW, Columbia University, USA**

Objective: Oropharyngeal candidiasis is a frequent opportunistic infection associated with an immunocompromised host. Candida albicans (C.a.) is the principal species responsible for this infection. Production of proinflammatory cytokines, such as Interleukin-8 (IL-8), by oral epithelial cells in response to C.a. can be expected to play a major role in the recruitment of professional phagocytes to the infected site. The purpose of this investigation was to determine whether C.a. triggers secretion of IL-8 by oral epithelial cells and investigate mechanisms that trigger such responses. Methods: Oral epithelial cell lines (KB (ATCC), SCC4, SCC15, OKF6/TERT-1 and OKF6/TERT-2 (provided by D. Wong and J. Rheiwald)) and primary gingival epithelial cell cultures were used. Cell cultures were seeded at confluence and were allowed to adhere overnight. Subsequently, stationary phase viable C.a., strains SC5314, ATCC28366 or ATCC32077, were suspended in fresh media and added to human cell cultures at different yeast:oral cell ratios. Supernatants were collected and analyzed for IL-8 content by ELISA. A germination-deficient mutant, otherwise isogenic to strain SC5314, was used to assess the effects of germination on IL-8 responses. In order to ascertain whether direct contact of C.a. with host cells is required to trigger cytokine production, epithelial cells were separated by yeast with cell culture inserts. Results: All cell cultures responded with an increase in IL-8 secretion after 18 hours of coculture with C.a. Loss of fungal viability by fixation abolished cytokine responses. IL-8 responses varied when different C.a. strains were used. Separation using culture inserts or use of the mutant strain substantially impaired cytokine responses. Conclusion:C.a. triggers IL-8 responses by oral epithelial cells in vitro. These responses are contact-dependent, strain-dependent, require yeast viability and are optimal when the yeast germinate into hyphae. Supported by NIDCR RO1 DE13986 and RO3 DE12668.

1006 **Hemolysis assay and hemolysin gene (HLP) expression in switched phenotypes of Candida glabrata.** **G. LUO*, L.P. SAMARANAYAKE, G. TANG, B.P.K. CHEUNG, and J.Y.Y. YAU, The University of Hong Kong, Hong Kong**

Objectives: Phenotypic switching is believed to contribute to the virulence of Candida spp.. Recently it has been shown that phenotypic switching does occur in Candida glabrata (Cg), the second most frequently isolated Candida species. However, the relationship between phenotypic switching and virulence of Cg is unknown. Our recent works showed that production of hemolysis is an important virulence factor of Candida species (Luo et al, J Clin Microbiol,2001). Hence, the aim of this study was to evaluate the differential hemolytic activity and HLP expression in "switched" strains of Cg.

Methods: Switched strains of Cg were obtained by using the method suggested by Lachke et al (Infect Immun, 2000) with minor modifications. The hemolysin production was evaluated using a modification of the plate assay described by Luo et al (vide supra), by spot-inoculating yeast suspension on a sugar enriched sheep blood agar medium.The expression of HLP gene was assessed using a standard RT-PCR procedure, with the housekeeping gene Act1 serving as an internal control (Zhao et al, Microb Pathol,1998).

Results: After 7 days' incubation, four major phenotypes termed white (Wh), light brown (LB), dark brown (DB) and very dark brown (vDB) were obtained. Hemolytic assay showed that phenotypes vDB, DB and LB had significantly higher hemolytic activity than the Wh phenotype. When the levels of HLP transcript in the four switch phenotypes of Cg was assessed by RT-PCR analysis, the levels of transcript were lowest in Wh cells, intermediate in LB cells, and highest in DB and vDB cells, the relative ratios being 1:7:15:17, respectively.

Conclusions: These results indicate that the expression of HLP gene and hemolytic activity of C. glabrata are likely to be associated with the switched phenotypes of the yeast. This is the first report of a distinct correlation between the colony phenotype and a virulent attribute in Cg.

1007 **Assessment of the genetic diversity of Candida isolated from the oral cavity in health and disease.** **M. FRASER*1, A. ROGERS, R. ANDREWS1, and D. ELLIS3, 1 Adelaide University, Australia, 2 Women's and Children's Hospital, Australia**

Oropharyngeal candidiasis is prevalent in up to 95% of HIV-positive individuals. The predominant causative species is Candida albicans.

Objectives: The aim of the study was to assess the genetic diversity of C. albicans isolates obtained from the oral cavity of 101 HIV-positive individuals with or without clinical symptoms and 20 asymptomatic HIV-negative carriers.

Methods: Isolates were cultured on Sabouraud's dextrose agar and up to six well-separated colonies were collected from each sample. Isolates from the HIV-positive individuals included sequential oral rinse samples and swabs of obvious lesions, collected at the Adelaide Dental Hospital over a 2-year period. The asymptomatic carrier samples were from oral rinses. The cell lysates of each colony from a single sample were pooled for analysis. The genetic data was obtained using allozyme electrophoresis, at 15 to 20 independent metabolic enzyme loci.

Results: This study demonstrated the existence of a large degree of genetic variation both within and between patients, with all but 4 of the 287 isolates being genetically distinct. Samples that were comprised of more than one strain or species were obtained from 29.5% of samples from both HIV-positive individuals, during times of infection or treatment, and asymptomatic carriers. Of the single species isolates, 77% were C. albicans, 7% were C. dubliniensis, 0.3% were C. parapsilosis and 15.7% belong to other Candida species. Strain replacement was noted to occur as rapidly as weekly in some patients. There was no obvious correlation between genetic cluster and site of infection, clinical symptoms, antifungal treatment, sex or HIV status.

Conclusion: Oropharyngeal candidiasis is opportunistic and a patient's clinical outcome is primarily reliant on host immune status, not the infecting Candida strain.