THE FOURTH ESWI INFLUENZA CONFERENCE

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POSTER PRESENTATIONS SCIENCE POLICY INTERFACE

ORAL PRESENTATIONS

MONDAY 12TH SEPTEMBER 2011

SPA 1: CLINICAL IMPACT AND DIAGNOSTIC APPROACHES

A102O

Pulmonary pathology of pandemic influenza A/H1N1 virus (2009) infected ferrets upon longitudinal evaluation by computed tomography

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We investigated the development of pulmonary lesions in ferrets following infection with the 2009 pandemic A/H1N1 influenza virus by means of computed tomography (CT) and compared the scans with gross pathology, histopathology and immunohistochemistry. Ground-glass opacities observed by CT-scanning in all infected lungs corresponded to areas of alveolar oedema at necropsy. These areas were most pronounced on day 3 and gradually decreased from day 4 to day 7 post-infection. This pilot study shows that non-invasive imaging procedure allows quantification and characterization of influenza induced pulmonary lesions in living animals under biosafety level 3 conditions and can thus be used in preclinical pharmaceutical efficacy studies.

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SPA 1: CLINICAL IMPACT AND DIAGNOSTIC APPROACHES

A103O

Seasonal influenza and acute myocardial infarction: a self-controlled case series study using UK primary care data

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Introduction

Previous studies suggest associations between some infections such as respiratory and urinary tract infections and acute vascular events such as myocardial infarction (MI). It is not clear whether influenza has a specific triggering effect. This is important from a public health perspective as influenza is the only viral respiratory infection for which effective prophylaxis and treatment is available. Here we investigate the hypothesis that influenza infection can act to trigger acute MI using the self-controlled case series method in primary care data.

Materials & methods

The General Practice Research Database (GPRD) contains computerized anonymised longitudinal medical records on over 5 million patients in the UK. Records were obtained for a cohort of patients aged over 18 years who had experienced an incident myocardial infarction between January 1st 1999 and December 31st 2008. A period of at least 6 months was required between registration with a GPRD practice and occurrence of MI. Exposure was defined as GP attendance with an influenza-like illness or with an acute systemic respiratory tract infection. Participants required records of both an MI and a respiratory illness for inclusion in primary analysis.

We used the self-controlled case series method to undertake within-person comparisons of the incidence of MI occurring in the period following GP attendance with a respiratory infection compared to baseline time periods. Derived from the cohort method, self-controlled case series has the major advantage of eliminating the effect of fixed confounders. We considered that the probability of a respiratory illness being caused by influenza would vary according to 1) the codes used to classify respiratory illness, 2) influenza vaccination status and 3) community levels of influenza virus circulation according to surveillance data. We stratified analyses on these variables to explore whether there was a specific triggering effect of influenza on MI or whether any effect was general to respiratory tract infection.

Results

From a cohort of 38,274 patients with a record of first incident MI, 16,258 had also consulted their GP for a respiratory infection during the study period. For these, median age at MI was 72 years and 58.9% occurred in males. Mean duration of follow up was 7.9 years. There were 39,029 episodes of respiratory illness (mean = 2.4 episodes per person), of which 2,252 episodes were coded as influenza (mean = 1.10 episodes per person).

Rates of MI were substantially higher in days following a respiratory tract infection – incidence ratio (IR) 3.07 (2.59-3.63) for 1-3 days – with the effect tapering over time: IRR 2.51 (2.13-2.95) for 4-7 days; IR 2.18 (1.91-2.49) for 8-14 days; IR 1.59 (1.42-1.77) for 15-28 days. There was no statistically significant difference between episodes coded as influenza compared to those coded as general systemic respiratory infection – IR 3.40 (2.34-4.43) versus IR 2.40 (2.19-2.63) for a single risk period of 1-14 days. Similarly, unvaccinated episodes (thought to be more likely to represent influenza infection) were not associated with significantly higher incidence ratios than vaccinated episodes – IR 2.55 (2.29-2.85) compared to IR 2.13(1.82-2.48). Finally, no difference was seen in incidence ratios between time periods when community levels of circulating influenza were at baseline (IR 2.44 (2.19-2.71)) compared to normal or high seasonal activity (IR 2.07 (1.61-2.66)).

Conclusions

Acute respiratory infections, including influenza, are associated with a transient increased risk of MI. However there is no evidence that this effect is greater for influenza than for other respiratory viruses that cause systemic respiratory illness.

MONDAY 12TH SEPTEMBER 2011

SPB 1: VIRUS HOST INTERACTION / PATHOGENESIS / TRANSMISSION (1)

B101O

Pandemic H1N1/09 in pigs: a reservoir host and model for human disease.

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The emergence of human pandemic A(H1N1)pdm09 virus involved the reassortment of a diverse influenza gene cassette derived from classical (H1) and Eurasian (N1) swine, avian and human viruses. It is genetically and immunologically distinct from other H1N1 viruses and it was initially speculated that antigenic cross-reaction with any recently circulating influenza virus would be insignificant. The disease has been relatively mild and uncomplicated in the majority of the human population, although a number (~0.5%) of fatalities have occurred usually in individuals with intercurrent disease.

We have established that young pigs are susceptible to infection with A(H1N1)pdm09 and will readily transmit the virus to in-contact susceptible animals*. Infection of pigs with A(H1N1)pdm09 in the field has also been reported in >25 countries establishing it as a new global swine pathogen. The current series of experiments explores the virulence variation between isolates and the ability of prior exposure to endemic Eurasian avian-like swine influenza (avH1N1) to provide a level of cross-protection, in addition to providing correlates to human infection and disease.

Methods

Pathogenesis of A(H1N1)pdm09 strains: A/California/07/09 and A/England/195/09, in 6 and 12 week old pigs respectively was carried out using ante- and post-mortem analyses at dpi 2, 3, 4/5, 7 and later (dpi 10/14).

The prior immune protection experimental design utilised four A(H1N1)pdm09 (Eng195) infected 'seeder' pigs (IN@$5.5x10^6$ EID_{50}) at 12 weeks-of-age to which either naïve or avH1N1 prior exposed animals (PE, A/swine/England/453/09 at 6 weeks post-infection, HI antibody negative) were placed in contact during three infections windows: dpi 2-4, 4-6 or 8 onwards. A second round of contact transmission was established between the primary contacts and either naïve or PE animals after each infection window. All animals were monitored daily for clinical signs, virus shedding and serology.

Results

The pathogenesis of the two virus isolates (Cal07 v Eng195) was substantially different. Cal07 induced mild to moderate clinical disease whilst that in Eng195 infected animals remained mild. More viral RNA was shed from the nasal cavity during Eng195 infection, particularly between days 2-5. However, the total virus load in the respiratory tract was greater for Cal07 ($P<0.03$). This difference may be attributed to a mutation at the receptor binding site of Cal07 D225G (H3 numbering) also noted in more severe human cases.

Successful transmission of Eng/195 from the naïve 'seeder' pigs to naïve in-contacts was achieved during the dpi 2-4 (6/6) and 4-6 windows (6/6), but not from the dpi 8 window (0/5). Second round transmission from the first infection window contacts to further naïve animals was also successful. The 'seeder' and in-contact pigs developed limited clinical signs of infection and had similar level of nasal viral shedding (3.5-4.0 log_{10} REU). All animals sero-converted from dpi/dpc 14 (HI titres 40-160).

In contrast, when PE in-contact animals were used there was only weak transmission (<2.0 log_{10} REU) at the first (6/6) and second (5/6) windows and none from the third (0/5). No specific clinical signs of infection were observed in the PE pigs post contact with 'seeder' animals. No transmission to either naïve or PE animals was observed in the second round of transmission. Antibody responses are currently being dissected.

Conclusions

The virulence of A(H1N1)pdm09 infection in humans varies according to underlying health, immunological state and virus variants. These variables also occur in the swine population and the pig can be used as a satisfactory model for human infection. It also appears that immunity to endemic swine (av-like) H1N1 should provide a substantial level of protection in the pig population against infection and transmission of A(H1N1)pdm09, field data is currently being analysed for comparison with human data.

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SPB 1: VIRUS HOST INTERACTION / PATHOGENESIS / TRANSMISSION (1)

B102O

The effect of avian species on the transmissibility of highly pathogenic avian influenza (HPAI) H5N1 virus: turkeys, chickens and ducks

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The emergence of a variant of highly pathogenic avian influenza (HPAI) H5N1 virus that has shown a marked increase in its virulence for wild waterfowl, previously shown to be resistant, has raised further questions on the transmission mechanisms of these viruses. Groups of ten 3-week-old Pekin ducks and turkeys were each infected with $4\log_{10}$ EID_{50} of A/turkey/Turkey/1/2005 H5N1 HPAI clade 2.2 virus and were placed immediately in contact with ten uninfected, age- and species-matched birds within the same enclosure or in two adjacent enclosures separated by 10cm and 50cm respectively. Cloacal and oropharyngeal swabs were collected and the presence of virus was determined by matrix gene real-time RT-PCR. Blood was sampled weekly via the brachial vein from surviving birds and haemagglutination inhibition assays were performed. Mortality was observed in all groups of directly infected and in-contact ducks (range 10-90%), but only in directly infected turkeys and those housed within the same enclosure (100%). Although infection and transmission was successful within the same enclosure for both turkeys and ducks, transmission between pens (10cm and 50cm groups) was only detected with ducks with 100% infection observed in both groups. All ducks seroconverted while no seroconversion was observed in those turkeys that survived infection. These results further highlight the increased susceptibility of ducks to this unusual group of viruses and a potential difference in the mode of transmission between *Anseriformes* and *Galliformes*.

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SPB 1: VIRUS HOST INTERACTION / PATHOGENESIS / TRANSMISSION (1)

B103O

Avian Influenza Virus Hemagglutinins H2, H4 and H8 support a highly pathogenic phenotype in chicken

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Introduction

Highly pathogenic avian influenza viruses (HPAIV) evolve from low-pathogenic precursors specifying the hemagglutinin (HA) serotypes H5 or H7 by acquisition of a polybasic HA cleavage site. Since the reason for that HA serotype restriction has remained unclear, we aimed to distinguish between compatibility of a polybasic cleavage site to the HA protein or unique predisposition for insertion mutations.

Methods

Using reverse genetics, we introduced a polybasic cleavage site into the HA of several low-pathogenic avian strains with serotypes H1, H2, H3, H4, H6, H8, H10 or H15 and rescued HA reassortants after co-transfection with the seven remaining gene segments from either a low-pathogenic H9N2 or high-pathogenic H5N1 strain. To investigate the virulence of the HA reassortants, we subjected them to oculonasal infection in chicken. For assessment of pathogenicity according to the OIE criteria, we then determined the intravenous pathogenicity index (IVPI). The most virulent reassortants were studied in contact transmission experiments.

Results

Oculonasal inoculation with those reassortants resulted in varying pathogenicity in chicken. Recombinants containing the engineered H2, H4 or H8 in the HPAIV background were lethal for all animals and exhibited IVPI of 2.79, 2.37 or 2.85, displaying values equivalent to conventional H5 or H7 HPAIV. Furthermore, all three reassortants were shed via oral and cloacal routes at lower titers compared with the homologous H5N1 HPAIV and were transmitted to some contact animals. Once infected, those contact animals succumbed to death whereas no surviving chickens developed any clinical symptoms, displayed viral antigen in several organs or sero-converted.

Conclusion

Taken together, with a polybasic HA cleavage site present, nonH5/H7 viruses can exhibit a highly pathogenic phenotype yet requiring further adaptation for establishment in the field. Therefore, the well-known restriction of authentic HPAIV to the serotypes H5 and H7 is likely due to their unique predisposition for acquisition of a polybasic HA cleavage site.

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YOUNG SCIENTIST

SPB 1: VIRUS HOST INTERACTION / PATHOGENESIS / TRANSMISSION (1)

B104O

Pathogenesis of influenza A/H5N1 virus infection in ferrets differs betweenintranasal and intratracheal routes of inoculation

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Introduction

Most patients infected with highly pathogenic avian influenza A/H5N1 virus suffer from severe pneumonia resulting in acute respiratory distress syndrome, with extra-respiratory disease as an uncommon complication. Intranasal inoculation of ferrets with influenza A/H5N1 virus causes lesions in both the respiratory tract and extra-respiratory organs, primarily brain. However, the route of spread to extra-respiratory organs and the relative contribution of extra-respiratory disease to pathogenicity are largely unknown.

Materials and methods

Ferrets (n=8) were inoculated intranasally with influenza virus A/Indonesia/5/2005 (H5N1; 5x10^6 TCID50). At seven dpi or earlier when ferrets became moribund, ferrets were killed and necropsies were performed according to standard procedures. Samples of multiple organs, including lungs and brains were collected to determine virus titers and for histological evaluation. After fixation in formalin, tissues sections were stained with HE for histological evaluation or with an immunoperoxidase method using a monoclonal antibody directed against the nucleoprotein of the influenza A virus for detection of virus-infected cells.

Results

From day 1 dpi onwards, all ferrets developed severe clinical signs including lethargy, anorexia and neurological signs. Upon histological examination, only 3 of 8 ferrets had a mild or moderate broncho-interstitial pneumonia. In contrast, all 8 ferrets had moderate or severe CNS lesions, characterized by meningo-encephalitis, choroiditis and ependymitis, and centred on tissues adjoining the cerebrospinal fluid.

Conclusions

These findings indicate that influenza A/H5N1 virus spread directly from nasal cavity to brain, and that CNS lesions contributed more than pulmonary lesions to the pathogenicity of influenza A/H5N1 virus infection in ferrets. In comparison, intratracheal inoculation of ferrets with the same virus reproducibly caused severe broncho-interstitial pneumonia. The implications are that the method of virus inoculation needs to be considered carefully when designing ferret experiments as a model for influenza A/H5N1 in humans.

MONDAY 12TH SEPTEMBER 2011

SPA 2: VACCINES: CURRENT AND NOVEL APPROACHES

A201O

Plant-made influenza virus-like particles: an effective way of producing next generation vaccines

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Today, the globalization of trade and volume of international travel provides more opportunities for infectious diseases to spread rapidly and globally. As seen with the outbreak of the 2009 A/H1N1 influenza, within 2 days of the first WHO confirmed cases from Mexico, the virus was reported in 5 additional countries. Due to difficulties in producing the vaccine, initial doses became available only after 26 weeks in the US and supplies for the protection of all US citizens would have taken almost a year to produce. This increasing risk is posing new challenges to public health agencies around the globe, stressing the need for effective vaccines sourced from highly flexible manufacturing facilities that can be mobilized rapidly and cost-effectively. The greatest potential to meet this challenge lies in the use of recombinant DNA technologies. Medicago is paving the way for such a promising alternative approach combining the speed of transient expression technology with the efficacy of VLPs as antigen presentation scaffolds. The technology has been developed so that it has a minimum lag-time, can be established with limited resources and can initiate vaccine production within three weeks of the identification of the genetic sequence from a pandemic strain. The influenza VLP vaccines developed by Medicago are made by the expression of the wild-type sequence coding for the viral hemagglutinin surface protein that will bud out from the plant plasma membrane and forms enveloped VLPs resembling influenza viruses. The plant-made VLPs have been extensively characterized namely with regards to lipids, glycans and protein impurities. The safety and immunogenicity of one pandemic and one seasonal influenza vaccines have been evaluated in healthy adults. Both vaccines were found safe and well tolerated and induced a robust immune response. No allergic responses were noticed. This talk will present the data from the phase II clinical trial for a H5 VLP made for the A/Indonesia/5/05 H5N1 influenza strain and from the phase I clinical trial for a H1 VLP made for the A/California/7/09 H1N1 influenza strain with particular attention to humoral and cell-mediated immune responses induced by the plant-made VLPs.

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SPA 2: VACCINES: CURRENT AND NOVEL APPROACHES

A202O

Development of a human cell-based Virus-Like Particle (VLP) Vaccine for Pandemic Influenza

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Traditional egg-based Influenza vaccines have limitations, particularly for use in a pandemic setting. Highly Pathogenic Avian Influenza (HPAI) viruses, such as the H5N1 strains that continue to circulate and infect people in Asia and the Middle East are too virulent to grow in eggs, and may require handling in a biohazard containment facility. Lentigen has developed a recombinant Virus-Like Particle (VLP) vaccine for H5N1 Influenza that is produced from human cells in continuous culture. HEK 293 cells were genetically modified with a Lentiviral vector that expresses Influenza A/Vietnam/1203/04 (H5N1) Matrix (M1), Hemagglutinin (HA), and Neuraminidase (NA) proteins. This cell line was engineered to produce and secrete the VLP vaccine into the culture medium for at least 3 months for continuous harvest of vaccine. The supernatants were harvested daily, concentrated and purified by several orthogonal chromatographic methods. The vaccine was analyzed to confirm the presence of HA, M1 and NA proteins. HA and M1 were shown to be present by Western Blot. HA was also analyzed by hemagglutination and SRID, while NA was detected by a neuraminidase activity assay. VLP production was confirmed by particle analysis and electron microscopy. Finally, *in vivo* immunogenicity of the vaccine was evaluated in mice. Groups of 12 BALB/c mice were vaccinated intramuscularly with the VLP vaccine at 0.2ug, 0.7ug or 2.3 ug doses on days 0 and 28. Sera from days 0, 28, 42 were tested for anti-HA IgG and hemagglutination inhibition (HAI) activity. Serum anti-HA IgG end-point dilutions reached 1/100 on day 28 and 1/10,000 on day 42 after vaccination. The geometric mean HAI end-point dilutions were greater than 1/40 on day 28 and greater than 1/200 on day 42. These results are consistent with a highly immunogenic immune response being generated by the H5N1 VLPs. The animals were then challenged with live H5N1 virus to determine the protective nature of the immune response. While the control animals died, all vaccinated animals survived and had no significant weight loss. Therefore, the vaccine generated a protective immune response in these vaccinated mice. These results demonstrate the feasibility of using Lentiviral vector-transduced human cells for continuous production of highly immunogenic influenza VLP vaccine. Current development work focuses on process development for manufacturing scale-up and confirmation of the vaccine immunogenicity in ferrets in preparation for IND submission and initiation of phase I clinical trials in humans.
The program was supported by PATH.

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SPA 2: VACCINES: CURRENT AND NOVEL APPROACHES

A203O

High-yield influenza vaccine production due to trypsin-mediated inhibition of interferon signaling

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For cell culture-based influenza vaccine production using adherent Madin-Darby canine kidney (MDCK) cells, trypsin is added to the medium to enable multi-cycle virus replication by the proteolytic activation of viral hemagglutinin. In this study, we were able to demonstrate that trypsin also provides an efficient inhibition of cellular pathogen defense mechanisms. In particular, a trypsin concentration of 5 BAEE U/mL (4.5 µg/ml porcine trypsin), which is typically used in vaccine manufacturing, strongly reduced interferon (IFN) signaling. This was probably due to the degradation of secreted IFN. At the same time, this concentration supported fast virus spreading and high virus yields. By contrast, infections without trypsin resulted in higher IFN signaling and correlated with enhanced apoptosis induction of host cells, which significantly reduced virus yields. However, suppression of IFN signaling alone by overexpression of antagonists only partially rescued virus titers in the absence of trypsin. To achieve high virus titers, fast virus spreading was additionally needed to outrun the cellular defense and apoptosis induction. Using 5 BAEE U/ml trypsin even allowed efficient replication of the attenuated delNS1 virus in IFN-competent MDCK cells.

In summary, trypsin provides optimal conditions for high yield vaccine production in MDCK cells by keeping antiviral defense as well as apoptosis induction at a low level and concurrently enabling viruses to replicate before this cellular response becomes fully activated.

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YOUNG SCIENTIST

SPA 2: VACCINES: CURRENT AND NOVEL APPROACHES

A204O

Improved haemagglutinin antigen content of pandemic H1N1v candidate vaccine viruses with chimeric haemagglutinin molecules

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Background

Wild type (wt) pandemic A(H1N1) influenza viruses grow exceedingly poorly in eggs in line with wt seasonal A(H1N1) viruses. The H1N1 candidate vaccine virus (CVV) NIBRG-121 was generated in response to the 2009 pandemic at NIBSC using reverse genetics and comprises the haemagglutinin (HA) and neuraminidase (NA) genes from A/California/7/2009 on a PR8 backbone. Early characterisation of NIBRG-121 demonstrated a much higher titre in eggs than wt strains; however, the virus did not grow as well as seasonal H1N1 CVV. Due to the potential high demand for a pandemic vaccine, a virus that provides for a maximum number of doses per manufacturing batch is highly desirable.

Materials and Methods

Based upon results obtained in our laboratory in a previous study on improving CVV for H5N1 vaccine manufacture, we created two new viruses from NIBRG-121. In these viruses, the six internal genes derived from PR8, the NA gene derived from A/California/7/2009, whilst the HA gene was a chimera with sequences deriving from both PR8 and A/California/7/2009 HAs. Thus, virus NIBRG-118 has an HA in which the 3' non-coding region, signal peptide and ectodomain derive from A/California/7/2009 whilst the transmembrane domain, cytoplasmic tail and 5' non-coding region derive from PR8. Virus NIBRG-119 has an HA with the ectodomain of A/California/7/2009 and all other domains (3' and 5' non-coding regions, signal peptide, transmembrane domain and cytoplasmic tail) from PR8. The new viruses were compared to NIBRG-121 in a number of assays to assess virus growth and antigen yield. Growth characteristics were assessed by determination of haemagglutination titres and EID_{50} as well as growth kinetics in eggs. Purified virus concentrates made from these bulks were analysed for yield of virus protein and HA antigen. Antigenicity of the viruses was assessed using an HI assay.

Results

EID_{50} results, HA data and growth curves showed that the two new viruses had significantly improved growth in eggs over NIBRG-121. Total protein yield was significantly increased for the two new viruses and importantly, they gave a better overall yield of HA antigen in eggs.

Conclusions

Two viruses with chimeric HA genes containing the HA ectodomain of A/California/7/2009 and the other regions of the HA gene from either PR8 or A/California/7/2009 had significantly improved growth and yield characteristics in eggs and are therefore promising candidate vaccine viruses for use in production of pandemic H1N1 vaccine. The virus in which only the ectodomain derived from A/California/7/2009 and all other regions of the HA gene were from PR8 was most improved. Antigenically, the modified viruses remained A/California/7/2009-like. This is the second time we have demonstrated that a virus with a chimeric HA is superior as a CVV (previously we improved an H5N1 CVV for which the underlying mechanisms appear to be different). Ultimately, if the introduction of PR8 regions into HAs of various subtypes were to be shown to reproducibly improve HA antigen yields, a new type of CVV for seasonal and pandemic influenza vaccines could be introduced for routine use. This approach could generate high yielding strains more rapidly than optimising classical CVVs by repeated passaging of the initial high growth reassortants.

MONDAY 12TH SEPTEMBER 2011

SPA 2: VACCINES: CURRENT AND NOVEL APPROACHES

A204P

Vaccine R&D Development Success Rates Quantified; Considerations for Vaccine Investors

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Aim

Immunization strategies are single-handedly considered to have *"prevented more premature deaths, permanent disability, and suffering, in all regions in the world, than any other medical discovery or invention* [Andre.Vaccine.2001;19:2206]". Vaccines are the most cost-effective strategy with the potential to prevent - or even cure - external pathogen infections, chronic infections, allergic conditions, auto-immune diseases and cancer [Davis.Vaccine.2010;28:1353].

Nevertheless, human prophylactic vaccines for communicable diseases are not attractive for the biopharmaceutical industry. There is a zero-tolerance attitude towards adverse events for vaccines administered to healthy individuals and risk groups. Moreover, diseases largely affecting developing countries do not promise a return-on-investment. Agenda setting by altruistic foundations have helped counter this latter development.

The aim of this research is to generate risk profiles for human vaccines currently in development. Forecasting statistics - including, but not limited to; length and risks of development - are important for investors seeking strategic financial advice. The poster presents an empirical review, evaluating the vaccine pipeline on development timelines and phase transition rates. When combined the two factors produce the risk profile. Data from an earlier publication showing the risk profile for vaccines in development during 1983-1994 [Struck. NBiotech.1996;14:591] is compared to data from 1998-2009.

Method

Our methodology and assumptions are based on Struck [Struck.NBiotech.1996;14:591] and Dimasi [Dimasi.NClinPharmTher.2010:1]. Data is collected on five value chain phases due to data availability, observing human vaccines in development during 1998-2009[1]. An active research strategy is chosen to develop the vaccine dataset consulting various sources including; commercial database (Medtrack), governmental sources, company sources, official press statements and scientific publications. Using the commercial database as a starting point, a total of 902 products in development from 313 companies are included in the dataset[2].

Result

The filtered dataset contains 619 unique human products from 195 individual firms covering 55 therapeutic areas. The risk profile for vaccines in development during 1998-2009 only partially behaved as predicted; the average timeline has lengthened by 0.56 years as forecasted by Dimasi [Dimasi.NClinPharmTher.2010:1], yet the cumulative success rate is substantially lower at an estimated probability of 0.09.

Data stratification by disease area showed significant differences (Table).

	Struck Data [Struck.NBiotech.1996;14:591]	Own Data	Stratification; Influenza
Average Duration Development (Years)	10,00	10,56	7,84
Market Entry Probability (Probability)	0,22	0,09	0,16

Discussion/Conclusion

Risk profiles are important descriptive tools, with information that is necessary for investment decisions and project continuation. In general; vaccine projects in development during 1998-2009 have a longer timeline with a lower probability of success when compared to vaccine development in 1983-1994.

As a final point; the industry is reluctant in reporting vaccine candidates in pre-clinical phase, or with a discontinued status. We feel the industry can benefit from reviewing the vaccine life-cycle, especially when considering the importance and potentials of this technology [Sahoo.BI.2008].

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[1] The five development phases included in the analysis; Preclinical, Clinical Phase I, II and III, Registration and discontinued projects are included.

[2] Data was collected on the 12th of May 2010. Medtrack is comparable with Pharmaprojects, the latter being mostly used in publications

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SPA 2: VACCINES: CURRENT AND NOVEL APPROACHES

A208P

Entrepreneurship in Vaccine Development

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Aim

A major challenge in every science & technology driven industry is the continuous development of novel products, concepts and designs based on new scientific achievements. However, the biopharmaceutical industry is facing a *productivity gap*; the proportion invested in the value chain compared to the number of products approved is unbalanced [Carney.DDT.2005;10(15):1011]. Developing a product from discovery to market entry is a complex, risky, lengthy and costly process, that requires generating new knowledge for commercial and societal exploitation.

The aim of this research is to explore the roles and strategic interactions between scientists, entrepreneurs, and companies in advancing the health and life sciences, with special focus on prophylactic vaccine development for viral infectious diseases (Influenza). First the theoretical background on entrepreneurial styles by Miner [Miner.JBusVent.1992;7:103] is placed in context during vaccine product development (Table 1). Second, the market dynamics of vaccines compared to pharmaceutical products is evaluated based on the cost of investment and statistical chance for completing each step in the development process (Table 2, summary of graph).

Method

Literature Research

Result

Table 1

Development Phases	Description	Average phase length / Year	Who is involved; Project Level?	Who is involved; Organizational Level?	Funding Source **	Type of Entrepreneur at Project and Organizational Level	Institutional level
Lead discovery, Selection and Optimization	Research and Development; *in silico*, *in vitro* and *in vivo*	NA	Scientists, PhD, Professor	Academia, Governmental, Private Biotech Laboratory or In-house	IP Fund, PreSeed Fund, Venture Capital	Craftsman Inventor-entrepreneur Oppertunistic-entrepreneur	TTO, governmental institution
PreClinical Phase	sub-phases *in vitro* and *in vivo* for preliminary quality, toxicity, pharmaco-dynamic and dosage profiles	1.5	Scientists, PhD, Professor	Academia, Governmental, Private Biotech Laboratory or In-house	Proof-of-Concept Fund, Seed Fund, Venture Capital	Craftsman Inventor-entrepreneur Oppertunistic-entrepreneur	TTO, governmental institution

Clinical Development	Introducing the drug in (healthy) human volunteers and patients to establish safety, efficacy and dosage profiles. Three phases; I (healthy volunteers), II (small group of target individuals) and III (larger group of target individuals).	Clinical Phase I 2.5	Physician, Scientist, CRA	Bioventure, CRO, In-House	Valoriza-tion Grant, Venture Capital	Inventor-entrepreneur Oppertunistic-entrepreneur	Pharmaceuti-cal Industry
		Clinical Phase II 2.8	Physician, Scientist, CRA	Bioventure, CRO, Hospital, In-House	Biotech Firm, Venture Capital	Opportunistic-entrepreneur	
		Clinical Phase III 2.6	Physician, Scientist, CRA	CRO, Hospital, In-House	Biotech Firm, Venture Capital	Large established firms	
						Corporate entrepreneurs (Intrapreneurs)	
Approval Phase	The application for market approval.	2	Marketing and Legal	Pharmaceutical Industry	Pharma-ceutical Industry		
Marketing Life-Cycle	The process which allows the product to be distributed to the target population.	NA *	Marketing and Legal	Pharmaceutical Industry	Pharma-ceutical Industry		
Post-Market-ing Approval	After market entry, the product is watched closely for any rare side-effects.	NA *	Physician, Scientist, Marketing and Legal	CRO, Hospital, In-House	Pharma-ceutical Industry		

CRA Clinical Research Associate

CRO Contract Research Organization, outsourced for completing (parts-of) clinical trials

TTO Technology Transfer Office

NA Not Available

* For as long as the vaccine product remains in the market
** Applies to the Netherlands. This column also includes credits and special tax breaks.

Table 1

Product	Cumulative Cost	Cumulative Success Rate
Vaccine	US $ 0,5 Billion [Andre.DevBio.2002;110:25]	0,09 [OwnData]
Biopharmaceutical	US $ 1,2 Billion [Dimasi.JHEcon.2003;22(2):151]	0,19 [Dimasi.NClinPharmTher.2010:1]

Conclusion

This research is a context study of science-based venturing, specifying the distinctive roles of the researcher, the entrepreneur, large firms, and their effective interactions to result in successful vaccine development and commercialization. The ultimate goal in life science-based venturing is the development of knowledge, technology and products whilst abiding to international ethic, safety, and efficacy standards. With an annual compound growth rate of 23% since 2004 [DMBI.2010:HC00004-001], this therapeutic intervention will remain an essential part in preserving our health in the future.

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SPA 2: VACCINES: CURRENT AND NOVEL APPROACHES

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Influenza Vaccines; Learning from Failure

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Aim

Developing a vaccine product targeting a viral infectious disease is an unmet medical need within an international political setting. With current modes of transportation, diseases are not geographically isolated and the spread is difficult to predict (for example: recent H1N1 outbreaks). Governments, medical facilities and industries worldwide are affected and have to respond with appropriate strategies.

Influenza causes both seasonal epidemics and pandemics. During a seasonal outbreak 5-20% of the population can be infected and a substantial amount stays at home for an average of 3 to 5 days. In economic terms, the annual burden in the US alone is US $ 87 Billion [Keech.PharmceEcon.2008;11(26):911]. In some cases certain severe and lethal manifestations can develop, particularly in high-risk groups. During a pandemic, individuals without increased risk may also develop serious complications from the infection [Lagacé-Wiens. CritCareMed.2010;38(4):supp].

Developing influenza vaccines is an internationally coordinated event involving the World Health Organization, academic institutions, biotechnology companies and governments. Once a target compound has been identified; it enters a linear development path governed by national and international legislation to establish efficacy and safety, eventually resulting in a commercial product. This takes around US $ 500 Million and ten years [Struck.NBiotech.1996;14:591] [Andre.DevBio.2002;110:25]. Nevertheless, only 9% of the candidates evolve into a successful vaccine [OwnData] and the remaining projects are terminated due to any number of reasons [Kola.NRevDD.2004;3:711].

The aim of this research is to take a snap-shot of influenza vaccine industry pipeline and evaluate the reasons behind unsuccessful products.

Method

1. Development pathway for influenza vaccines, and involved actors, was constructed through desk research
2. Commercial database Medtrack © in combination with internet sources, were consulted to identify;
    a. Information on all companies developing Influenza vaccines
    b. All influenza vaccine products
        i. In any stage of development, and any region of the world
        ii. In development between Jan 1997 and April 2011
    c. Discontinued vaccine programs during any stage of development
3. Academic and commercial experts were approached to identify reasons behind discontinuation

Result

From the 359 influenza vaccines, 32 were identified as discontinued during any stage of product development for any number of reasons (Table; Preliminary Results).

Development Phase	Quantity	Reasons for Discontinuation
Research/ Pre-Clinical	6	· Product no longer appears on company website or in annual report · Suspended program to pursue other products in pipeline
Clinical Phase I	5	· Product no longer appears on company website or in annual report · Product manifested mild side-effects in a few participants
Clinical Phase II	4	· Product no longer appears on company website or in annual reports · Upon sponsor company acquisition, product no longer appears on company website or annual reports
Clinical Phase II/III	1	· Discontinued trial after discussion of preliminary results with Data and Safety Monitoring Board
Clinical Phase III	1	· Product no longer appears on company website or in annual reports
Market	12	· Product no longer appears on company website or in annual reports · Stopped development due to disappointing sales · Regulatory or taxation issues · Revoked from market due to lethal side affects · Product development partnerships terminated
Unknown	3	· Product no longer appears on company website · Partnership terminated

Discussion/Conclusion

- Global vaccine pipeline contains 24% programs targeting influenza
- Each influenza vaccine project is developed under a unique set of circumstances
- The preliminary results are not indicative for a common factor to clarify project discontinuation
- Therefore we ask ourselves; Can failure be predicted?
- Moreover we claim that failures should be shared more openly, so that we can learn for future influenza vaccine development

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YOUNG SCIENTIST

SPB 2: VIRUS-HOST INTERACTION / PATHOGENESIS / TRANSMISSION (2)

B201O

The influenza virus protein pb1-f2 inhibits the induction of type 1 interferon at the level of the mavs adaptor protein

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PB1-F2 is a 90 amino acid protein that is expressed from the +1 open reading frame in the PB1 gene of some influenza A viruses and has been shown to contribute to viral pathogenicity. Notably, a serine at position 66 (66S) in PB1-F2 is known to increase virulence compared to an isogenic virus with an asparagine (66N) at this position.

Recently, we have found that an influenza virus expressing PB1-F2 N66S suppresses interferon (IFN)-stimulated genes in mice. To confirm and characterize this phenomenon, we employed several *in vitro* assays. Overexpression of A/Puerto Rico/8/1934 (PR8) PB1-F2 in 293T cells decreased RIG-I mediated activation of an IFN-β reporter and secretion of IFN as determined by bioassay. Of note, PB1-F2 N66S showed enhanced IFN antagonism activity compared to PB1-F2 wildtype which was also observed in the context of viral infection with a PR8 PB1-F2 N66S virus that expresses a functional NS1. In addition, PB1-F2 N66S decreased the induction of IFN when expressed from a PR8 virus with an NS1 dsRNA/TRIM25 binding mutation compared to PB1-F2 wildtype. To understand the relationship between NS1 and PB1-F2 regarding IFN antagonism, we investigated the induction of IFN when NS1 and PB1-F2 were co-expressed in an *in vitro* transfection system. In this assay we found that PB1-F2 N66S further reduced IFN induction in the presence of NS1. By inducing the IFN-β reporter at different levels in the signaling cascade, we found that PB1-F2 affects IFN production at the level of the mitochondrial antiviral signaling protein (MAVS). Furthermore, immunofluorescence studies revealed that PB1-F2 co-localizes with MAVS. In summary, we have characterized the anti-interferon function of PB1-F2 and we suggest that this activity contributes to the enhanced pathogenicity seen with PB1-F2 N66S- expressing influenza viruses.

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YOUNG SCIENTIST

SPB 2: VIRUS-HOST INTERACTION / PATHOGENESIS / TRANSMISSION (2)

B202O

Why is HPAI H5N1 virus not transmissible via aerosol? An extensive mutational and phenotypic analysis of mutant and reassortant H5N1 viruses

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Introduction

Since 1997, over 550 laboratory-confirmed cases of highly pathogenic avian influenza (HPAI) H5N1 virus infections in humans have been reported, with a case fatality rate of ~60%. Although sustained human-to-human transmission of HPAI H5N1 virus has not occurred, a key question is if this might happen in the future. For efficient replication and transmission in humans, avian viruses need to adapt to their new host via mutation or reassortment. We hypothesized that at least three important adaptive changes may facilitate transmission of H5N1 virus via aerosols in mammals: 1) better attachment to and infection of cells in the upper respiratory tract (URT), 2) increased replication in the URT, and 3) efficient release of virus particles.

Methods

We introduced several known adaptation mutations and exchanged several gene segments in an attempt to adapt HPAI H5N1 virus for efficient replication and possibly transmission in mammals. We used reverse genetics techniques with prototype clade 2.1 strain A/Indonesia/5/05, *in-vitro* test systems, a ferret animal model, and deep sequencing techniques to investigate molecular changes affecting virus replication and transmission.

Results

We first generated 27 receptor binding site mutants of the hemagglutinin (HA) gene and tested the preference for α2,3-linked sialic acids (SA) and α2,6-linked SA, the avian and human virus receptors respectively. HPAI H5N1 virus A/Indonesia/5/05 (Indo/5/05) harbouring N182K or Q222L/G224S mutations in HA demonstrated a similar attachment pattern to the URT and lower respiratory tract (LRT) of ferrets and humans as human influenza viruses (Chutinimitkul et al., J. Virol., 2010). However, virus shedding from the URT of inoculated ferrets was reduced, and these viruses did not transmit in a ferret transmission model.

To overcome the reduced replication capacity of Indo/5/05 with α2,6-SA preference, we introduced the known adaptive mutations E627K or D701N in PB2 or exchanged the entire PB2 gene by PB2 of the human A/NL/26/07 (H1N1) virus. Both mutations increased virus replication in the trachea of inoculated ferrets and virus titers detected in the nasal turbinates (NT) were very high, up to 10E7 TCID50. Nevertheless, levels of virus shedding from the URT remained low. Consequently, we hypothesized that virus particles are produced in large quantities in the URT, but are subsequently not released efficiently from the cells. Therefore, we further manipulated A/Indo/5/05-222L/224S/627K, by replacing the neuraminidase (NA) gene by NA of sH1N1 (NL/26/07), pH1N1 (NL/602/09), HPAI H7N7 (A/NL/219/03) or A/Indo/5/05 with an elongated stalk domain. Introduction of the NA of sH1N1 resulted in increased virus replication *in vitro*. However, upon evaluation in ferrets, none of the viruses tested displayed a marked difference in replication.

Since our rational approach to manipulate Indo/5/05 did not result in the desired replication and transmission properties in ferrets, we next attempted H5N1 virus adaption to mammals by serially passaging wt Indo/5/05 and A/Indo/5/05-222L/224S/627K in ferrets 10 times. The genetic composition of these viruses was determined by deep sequencing technology using a Roche 454 platform. Preliminary data showed that the introduced mutations in HA (Q222L/G224S) and PB2 (E627K) were still present in viruses from passage 10, and that multiple amino acid substitutions had occurred in all 8 segments of these viruses. An extensive evaluation of the phenotypic effect of these changes *in vitro* and *in vivo* on replication and transmission is currently in progress, and the results will be presented.

Conclusion

Our results may provide new insights in the requirements for adaptation of avian influenza viruses for efficient replication and transmission in mammals.

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YOUNG SCIENTIST

SPB 2: VIRUS-HOST INTERACTION / PATHOGENESIS / TRANSMISSION (2)

B203O

Demographic and climatologic characteristics of human avian influenza (A/H5N1)

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Introduction

Human avian influenza (A/H5N1) has threatened the world with its high mortality rates and potential to cause a deadly pandemic. Better understanding of the demographic and climatologic characteristics of H5N1 will help to inform global prevention and control efforts.

Methods

H5N1 case data were retrieved from the WHO's Global Alert and Response (GAR) system (1997-2011). Case demographics and climatologic characteristics of study years were described.

Results

1. ***Geographic distribution:*** Human A/H5N1 cases have been highly concentrated in two geographic areas in the world: Southeast Asia (Indonesia, Vietnam, Thailand, South China/Hong Kong, Cambodia, Bangladesh, Laos, Myanmar and Pakistan) and the Middle East (Egypt, Turkey, Azerbaijan, Iraq, and Djibouti). Cases in Southeast Asia and the Middle East accounted for 67.1% and 29.2% of global cases, respectively. Countries with the highest case counts were Indonesia (n=176), Egypt (n=143) and Vietnam (n=119).
2. ***Age distribution:*** The highest proportion of cases was in the age group 0-9 years (28.6%). The majority of cases (75%) were aged less than 30 years and 90% were less than 40 years, showing a highly skewed lognormal distribution.
3. ***Gender distribution:*** Cases were approximately evenly distributed across gender in most age groups, except in age group 0-9 years (54.2% cases were male) and age group 20-29 years (64.4% cases female).
4. ***Mortality distribution:*** Overall, the case-fatality rate was 58.3%, but it varied geographically. Among those countries with more than 10 cases, Cambodia had the highest case-fatality rate (86.7%), followed by Indonesia (82.4%), Thailand (68%), Vietnam (49.6%), China (46.7%), Turkey (33.3%), and Egypt (32.9%).
5. ***Interannual distribution:*** Total case counts varied across years, with higher numbers recorded in 1997, 2006 and 2009, all of which were El Niño years when equatorial surface sea water transited to a warmer status. Incidence was lower in La Niña years.
6. ***Seasonal distribution:*** The seasonality of avian influenza presents different patterns in the three highest-rate countries. In Egypt, a distinct annual peak is observed in March after dewpoint temperature reaches its annual valley. In Vietnam, the main peaks arise in January and March, corresponding to the coldest and least sunlit periods of the year. A small peak also shows in July, the warmest month in the rainy season. In Indonesia, seasonality of avian flu is less clear, but major peaks still coincide with the months of least sunlight, most rainfall or warmest temperatures.

Discussion & Conclusion

Reasons for the high H5N1 incidence in Southeast Asia and the Middle East are unclear, but could be attributed to unsanitary conditions in local poultry markets, populations' hygiene practices, bird reservoir/migration, and monsoon climate in these tropical-subtropical regions. The fact that most H5N1 cases and deaths were children and young adults suggests greater susceptibility and more exposure among these age groups. The reason why higher case counts were seen in boys under 10 years and women 20-29 is unclear, but could be due to greater exposure (eg. boys are more likely to play with birds and many young farmers are women). These data also represent case counts only and population denominator data are needed for further comparison of incidence rates. Higher mortality rates in some countries may reflect insufficiency in awareness, prompt treatment, and health infrastructure. It is noteworthy that human avian influenza shares very similar seasonal patterns with seasonal flu and appears to be associated with dewpoint extremes, less solar radiation, and excessive precipitation. The effects of global warming and El Niño on human avian flu warrants further investigation. Our findings have implications for H5N1 forecasting and targeting of intervention strategies.

No conflict of interest

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SPB 2: VIRUS-HOST INTERACTION / PATHOGENESIS / TRANSMISSION (2)

B204O

The virulence of 1997 H5N1 influenza viruses in the mouse model is increased by correcting a defect in their NS1 proteins

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The NS1 protein of human influenza A viruses binds the 30-kDa subunit of the cleavage and polyadenylation specificity factor (CPSF30), a protein required for 3'end processing of cellular pre-mRNAs, thereby inhibiting production of interferon-beta (IFN-beta) mRNA and presumably other antiviral mRNAs. The NS1 proteins of pathogenic 1997 H5N1 viruses contain the CPSF30-binding site, but lack the consensus amino acids at positions 103 and 106, F and M, respectively, that are required for the stabilization of CPSF30 binding, resulting in non-optimal CPSF30 binding in infected cells. Nonetheless, these viruses are lethal for chickens and humans in nature and in laboratory experiments are lethal for mice and ferrets. Here we demonstrate that strengthening CPSF30 binding, by changing positions 103 and 106 in the 1997 H5N1 NS1 protein to the consensus amino acids, results in a remarkable 300-fold increase in the lethality of the virus in mice. Unexpectedly this increase in virulence does not lead to increased lung pathology, but rather is characterized by faster systemic spread of the virus from the lung, particularly to the brain, where increased replication and severe pathology occur. This increased spread is associated with increased cytokine and chemokine levels in extrapulmonary tissues. We conclude that strengthening CPSF30 binding by the NS1 protein of 1997 H5N1 viruses enhances virulence in mice by increasing the systemic spread of the virus from the lungs, particularly to the brain.

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YOUNG SCIENTIST

SPB 2: VIRUS-HOST INTERACTION / PATHOGENESIS / TRANSMISSION (2)

B205O

Human-like PB2 627K influenza virus polymerase activity is regulated by importin-a1 and -a7

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Influenza A viruses may cross species barriers and transmit to humans with the potential to cause pandemics. Interplay of human- (PB2 627K) and avian-like (PB2 627E) influenza polymerase complexes with unknown host factors have been postulated to play a key role in interspecies transmission. Here, we have identified human importin-α isoforms (α1 and α7) as positive regulators of human- but not avian-like polymerase activity. Importin-α3 generally restricted polymerase complexes independently of their origin. Human-like polymerase activity correlated with efficient recruitment of α1 and α7 to viral ribonucleoprotein complexes (vRNPs) without affecting subcellular localization. We also observed that human-like influenza virus growth was impaired in α1 and α7 downregulated human lung cells. Mice lacking α7 were less susceptible to human- but not avian-like influenza virus infection. Thus, α1 and α7 are positive regulators of human-like polymerase activity and pathogenicity without affecting nuclear transport.

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SPA 3: PANDEMIC AND EPIDEMICS

A301O

The pandemic is not over yet – influenza surveillance in Danish intensive care units during the 2009/10 and 2010/11 influenza seasons

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The Danish influenza surveillance was intensified during the pandemic season of 2009/10 by the monitoring of the burden on intensive care units (ICUs) in order to inform policy makers on the severity of disease and to timely detect shortages in ICU bed capacity. An improved ICU surveillance system was put in place with a short notice, when an increased number of ICU patients with influenza A and B was reported from the United Kingdom in December 2010.

The surveillance system consisted of active reporting by all Danish ICUs of aggregate and case-based data from week 46-2009 to week 11-2010 and from week 49-2010 to week 14-2011. During the 2009/10 season the case definition was a patient in a Danish ICU with laboratory confirmed Influenza A (H1N1)v infection or with influenza-like-illness after close contact with a person with laboratory confirmed infection. In the 2010/11 season the case definition was a patient in a Danish ICU with a laboratory confirmed infection with Influenza A or B. The number of newly admitted patients was reported weekly. The percentage of beds occupied by influenza patients was assessed on Monday morning 8 am. The number of influenza patients present in Danish ICUs at that time was divided by the total number of patients present in the ICUs. The case-based data included demographic information, as well as information on underlying illness and treatment with extracorporeal membrane oxygenation (ECMO).

The total number of ICU patients with Influenza A (H1N1)v reported in the 2009/10 season was 96. The total number of influenza cases in the 2010/11 season was 156, 115 with Influenza A and 41 with Influenza B. The maximum percentage of beds used for influenza patients in the 2009/10 season was 4.5% and as high as 10.2% in the 2010/11 season.

Case-based data for the 2009/10 season was available for 53 patients and was compared with data from the 115 patients with Influenza A in the 2010/11 season. The mean age was lower in the 2009/10 season with 44 years (range: 3-80 years) than the mean age of 52 years (range: 1 week-83 years) in the 2010/11 season (Student t-test: $p<0.001$). The distribution among men and women was similar (male:female ratio of 1:0.7 and 1:0.8). Eleven of 52 patients (21.2%) in the 2009/10 season had no underlying illness. During the 2010/11 season 15 of 87 influenza A patients (17.2%), for whom information on underlying condition was available, were reported to have no underlying illness. Six of the 53 influenza A patients (11.3%) of the 2009/10 season were treated with ECMO compared to 11 of 115 influenza A patients (9.6%) in the 2010/11 season.

In conclusion, the total number of influenza patients was higher in 2010/11 than during the season of 2009/10. The percentage of ICU beds occupied with influenza patients was, at its peak, two times higher in 2010/11 compared with 2009/10, showing that the pandemic strain was still causing severe disease. Furthermore, 26% of the influenza patients in Danish ICUs in 2010/11 were caused by influenza B, which contributed to the increased pressure on the ICUs. Finally, the age of Influenza A patients in ICUs in 2010/11 was higher compared to the 2009/10 season.

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YOUNG SCIENTIST

SPA 3: PANDEMIC AND EPIDEMICS

A302O

Age-specific mortality and years of life lost associated with the A/H1N1 influenza pandemic in Mexico, 2009-2010

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Aims

More than two years after the emergence and rapid global spread of a novel influenza A/H1N1 virus in March 2009, the mortality impact of the 2009 pandemic remains controversial, in part due to delays in vital statistics reports traditionally used to measure influenza-related mortality. Accurate estimates of the morbidity and mortality impact of the pandemic are essential to guide intervention strategies and identify priority groups for vaccination and treatment. Using detailed vital statistics, we estimate age-specific mortality rates and the years of life lost associated with the A/H1N1 pandemic in Mexico from April 2009 to April 2010 and compare the health burden of the pandemic with that of past influenza seasons.

Methods

We analyzed monthly age- and cause-specific Mexican death records from January 2000 to April 2010 to estimate seasonal excess mortality from pneumonia and influenza (P&I), respiratory diseases, respiratory and cardiac diseases, and all causes, using seasonal regression approaches supplemented with influenza virus activity data. We integrated age-specific life expectancy estimates with excess deaths to calculate years of life lost in pandemic and epidemic seasons. We compared the age-distribution of pandemic-related excess mortality with that of laboratory-confirmed A/H1N1 deaths and influenza-like-illness (ILI) deaths reported in 2009 to a large Mexican private health system, covering 40% of the population.

Results

We identified 4 consecutive waves of excess mortality in Mexico during spring, summer, fall, and winter, 2009-2010. Overall, we attribute 4.5 excess P&I deaths per 100,000 (95% CI 3.4 – 5.6 per 100,000) and 26.0 excess all-cause deaths per 100,000 (95% CI 13.2 - 38.8 per 100,000) to the A/H1N1 pandemic virus in Mexico, corresponding to 650,000 years of life lost (95% CI 510,000-790,000). These estimates were 1.2 to 4.8-fold higher than for an average influenza season in the inter-pandemic period from 2000-2008, and were comparable to the impact of the severe 2003-04 influenza epidemic in Mexico. Individuals 5-59 years of age were disproportionately affected by the A/H1N1 pandemic virus, compared to seasonal epidemics, especially during the first three waves of the pandemic in 2009. By contrast, seniors over 60 years were essentially spared throughout 2009 but experienced a recrudescence of excess mortality in winter 2010, similar in magnitude to that of previous influenza epidemics. A substantially larger proportion of excess deaths were coded as P&I during the pandemic compared to epidemic seasons (26% vs. 8%, P<0.0001). Age patterns in laboratory-confirmed pandemic A/H1N1 deaths and ILI deaths mirrored those in excess P&I deaths among individuals under 60 years of age. However, laboratory surveillance substantially underestimated pandemic–related mortality in older individuals.

Conclusion

We report a substantial mortality and years of life lost burden associated with the A/H1N1 influenza pandemic in Mexico, during 4 consecutive waves from April 2009 to April 2010—each wave differing in intensity and age patterns. Younger populations experienced unusually high mortality rates relative to seasonal influenza, while individuals over age 60 were partially spared, especially during the early months of the pandemic. The Mexican experience suggests that despite high influenza testing rates in all age groups in 2009 (~30%) when influenza was the predominant respiratory virus in circulation, identification of influenza-related illness was particularly difficult in senior patients. Comparison with mortality estimates from other countries suggests that Mexico experienced a particularly high pandemic mortality burden in 2009-2010. Analysis of detailed vital statistics from other countries is required to confirm the reported geographical variation in A/H1N1 pandemic burden globally.

Abbreviations: P&I: Pneumonia and Influenza; ILI: Influenza-Like-Illnesses

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YOUNG SCIENTIST

SPA 3: PANDEMIC AND EPIDEMICS

A303O

Influenza in the Tropics: High Burden and Defined Seasonality in a Nicaraguan Pediatric Cohort

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Introduction

Despite the fact that annual influenza epidemics have been documented in both temperate and tropical countries, the epidemiologic features of influenza in tropical countries have received little attention to date. Of particular importance is the collection of long-term data from well-defined populations in developing countries.

Methods

To examine the incidence, epidemiologic features, and transmission of influenza virus infection in children two to fourteen years of age in Managua, Nicaragua, we established a prospective cohort study of influenza. The Nicaraguan Influenza Cohort Study was conducted between June 2007 and December 2010, with yearly participation of ~3,800 children. Children were enrolled prospectively, and data was systematically recorded at all medical visits. Parents were encouraged to bring their child to the study health center at the first sign of illness, and adherence was high, with 94% of children presenting during the first 72 hours after symptom onset. All medical care was provided free-of-charge through the study. A random sample of 25% of all children presenting with influenza-like illness (ILI) were tested for influenza viruses by RT-PCR; viral isolation was performed on all RT-PCR-positive samples. Full-length sequencing of virus was performed through the Influenza Genome Sequencing Project. Weekly influenza incidence in the cohort was estimated by applying the percentage of samples positive for influenza in the calendar week to the total number of children who presented with ILI, divided by the person-time for that week.

Results

The incidence of influenza in the cohort during the first three years of the study was 20.0 influenza cases per 100 person-years. Yearly incidence varied from 15.5 cases per 100 person-years to 27.5 cases per 100 person-years. Both influenza A and B were observed over the study period, with dominance of influenza A H3N2 in 2007, H1N1 and influenza B in 2008, pandemic H1N1 2009 in 2009, and H3N2 and influenza B in 2010. We observed a defined seasonality of influenza in all years, with peak activity occurring between May and August. The first case of pandemic influenza was detected in the cohort on June 1, 2009; this was also the first case detected in Nicaragua. Although pandemic influenza began during the normal seasonal influenza period, the peak influenza activity shifted and occurred later than seasonal influenza in other years.

Conclusions

This study documents that Nicaraguan children experience a substantial burden of influenza and that Nicaragua, a tropical developing country, experiences a defined seasonality of influenza. Additionally, transmission dynamics of pandemic influenza differed from those observed for seasonal influenza. This study has provided critically needed data on the epidemiology and transmission of influenza in Central America.

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SPA 3: PANDEMIC AND EPIDEMICS

A304O

Incidence of Influenza in Healthy Adults and Healthcare Workers: A Systematic Review and Meta-Analysis

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Background
Working in healthcare is often considered a risk factor for influenza; however, this risk has not been quantified.

Purpose
To systematically review evidence describing the annual incidence of influenza among healthy adults and healthcare workers.

Data Sources
OVID MEDLINE (1950 to 2010), EMBASE (1947 to 2010) and reference lists of identified articles.

Study Selection
Observational studies or randomized trials reporting influenza infection rates for healthy adult subjects and healthcare workers were included. Influenza infection was defined as a four-fold rise in antibody titer, or positive viral culture or polymerase chain reaction.

Data Extraction
Data on year of publication, influenza seasons under study, circulating influenza subtypes, study design, population, vaccination status, diagnostic methods, number of subjects studied and number infected were collected

Data Synthesis
From 24,707 citations, 29 studies covering 97 influenza seasons with 58,245 study participants were included. Pooled influenza incidence rates (IR) (95% confidence intervals (CI)) per 100 healthcare workers per season and corresponding incidence rate ratios (IRR) (95% CI) as compared to healthy adults were as follows. All infections: IR 18.7 (95% CI, 15.8 to 22.1), IRR 3.4 (95% CI, 1.2 to 5.7) in unvaccinated healthcare workers; IR 6.5 (95% CI, 4.6 to 9.1), IRR 5.4 (95% CI, 2.8 to 8.0) in vaccinated healthcare workers. Symptomatic infections: IR 7.5 (95% CI, 4.9 to 11.7), IRR 1.5 (95% CI, 0.4 to 2.5) in unvaccinated healthcare workers, IR 4.8 (95% CI, 3.2 to 7.2), IRR 1.6 (95% CI, 0.5 to 2.7) in vaccinated healthcare workers.

Limitations
Limitations applicable to indirect meta-analyses are relevant to our results.

Conclusion
Compared to adults working in non-healthcare settings, healthcare workers are at significantly higher risk of influenza.

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SPA 3: PANDEMIC AND EPIDEMICS

A305O

Case-based reported mortality associated with influenza A(H1N1) 2009 virus infection in the Netherlands: pandemic versus first post-pandemic season.

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Introduction

In contrast to seasonal influenza epidemics, were the majority of deaths occur amongst elderly, a considerable part of the 2009 pandemic influenza related deaths concerned relatively young people. In the Netherlands, all deaths associated with laboratory-confirmed influenza A(H1N1) 2009 virus infection had to be notified, both during the pandemic season (2009-2010) and the first post-pandemic season (2010-2011). To assess whether and to what extent pandemic mortality patterns were reverting back to seasonal patterns, a retrospective analyses of all notified fatal cases associated with laboratory-confirmed influenza A(H1N1) 2009 virus infection was performed.

Material & methods

The notification database, including detailed information about the clinical characteristics of all notified deaths, was used to perform a comprehensive analysis of all deceased patients with a laboratory-confirmed influenza A(H1N1) 2009 virus infection. Characteristics of the fatalities with respect to age and underlying medical conditions were analyzed, comparing the pandemic and the first post-pandemic season.

Results

A total of 65 fatalities with a laboratory-confirmed influenza A(H1N1) 2009 virus infection were notified in the pandemic season and 38 in the post-pandemic season. During the pandemic season, the population mortality rates peaked in persons aged 0-15 and 55-64 years. In the post-pandemic season, peaks in mortality were seen in persons aged 0-15 and 75-84 years. During the post-pandemic season, the height of first peak was lower compared to that during the pandemic season. Underlying immunological disorders were more common in the pandemic season compared to the post-pandemic season (p=0.02), and cardiovascular disorders and other underlying disorders were more common in the post-pandemic season (p=0.005 and p=0.01, respectively).

Conclusions

The mortality pattern in the post-pandemic season still resembled the pandemic season with a peak in relatively young age groups, but concurrently a clear shift toward seasonal patterns was seen, with a peak in mortality in the elderly, i.e. $\geq$75 years of age.

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YOUNG SCIENTIST

SPB 3: VIRUS STRUCTURE & REPLICATION

B302O

Interaction of the influenza A virus polymerase subunit PB1 with the host nuclear import factor RanBP5

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Introduction

The influenza A virus RNA polymerase is a heterotrimer, and transcribes and replicates the viral genome in the cell nucleus. Newly synthesised RNA polymerase subunits must therefore be imported into the nucleus during an infection. While various models have been proposed for this process, the consensus is that the PB1 and PA subunits form a dimer in the cytoplasm and are transported into the nucleus by the β-importin RanBP5, with the PB2 subunit imported separately to complete the trimeric complex. In this study, we characterised the interaction of PB1 with RanBP5 further and assessed its importance for viral growth.

Material & methods

To detect protein association, tagged proteins were transiently expressed in cultured cells, purified, and detected along with co-purifying proteins. Transiently expressed proteins were also assessed for stability (by pulse-chase), polymerase activity (by primer extension of RNA products), and finally for nuclear accumulation (by immunofluorescence). The conservation of RanBP5-binding residues was determined from sequence analysis, and their contribution to viral fitness assessed by characterising mutant viruses.

Results

The N-terminal region of PB1 mediates its binding to RanBP5, and basic residues in a bipartite nuclear localisation signal (NLS) are required for RanBP5 binding. Mutating these basic residues to alanines does not reduce the stability of PB1 or prevent it forming a dimer with PA, but does reduce RanBP5 binding. RanBP5-binding mutations decrease, though do not entirely prevent, the nuclear accumulation of PB1. Polymerase function was not substantially affected by mutations in either part of the NLS, though mutating both parts together caused a slight change in the ratio of positive and negative-sense RNA products. Finally, RanBP5-binding residues are highly conserved. They do not appear to undergo host adaptation, and PB1 from avian influenza viruses can efficiently bind human RanBP5. Mutations affecting RanBP5 binding are incompatible with or severely attenuate viral growth.

Conclusions

We have identified the RanBP5-binding site of PB1, showing that RanBP5 binds a previously identified NLS. As expected, RanBP5-binding mutations reduce the nuclear accumulation of PB1, showing a direct role for RanBP5 in the nuclear import of PB1. However, the precise function of RanBP5 appears to be complex: nuclear accumulation of PB1 requires other factors, notably PA, and even RanBP5-binding mutants retain a degree of nuclear accumulation. Despite this, the RanBP5-binding site is highly conserved. The interaction with RanBP5 does not appear to be a determinant of host tropism, and mutation of the binding site is poorly tolerated, suggesting that RanBP5 may play additional important roles in the viral lifecycle, potentially as a cytoplasmic chaperone for an exposed basic region of PB1.

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SPB 3: VIRUS STRUCTURE & REPLICATION

B303O

A supramolecular assembly formed by influenza A virus genomic RNA segments

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The genome of influenza A viruses consists of eight viral RNAs (vRNAs) that form viral ribonucleoproteins (vRNPs). Growing evidence supports the existence of a packaging mechanism allowing selective incorporation of the vRNAs into the viral particles. Notably, analysis of defective interfering RNAs and reverse genetics experiments identified sequences, most often located within 100 nucleotides at each end of the coding regions, that are required for the selective packaging of each vRNA. However, the mechanism ensuring selective packaging of one copy of each vRNA into the viral particles remains largely unknown.

We recorded the tomograms of two large fields of view in which 10 and 12 human H3N2 influenza A viral particles displayed individual vRNPs, and we were able to obtain 3D-reconstructions of four particles. In these reconstructions, 7 vRNPs surround a central one and form a common platform at the budding tip of the virions. The thickness of the platform varies between vRNAs: it is about 18-20 nm in most cases, but ranges from 5 to 30 nm. The compaction ratio of all vRNPs within virions was found to be similar and equal to about 31.5 nucleotides per nm. Thus, the platform from which the individual vRNPs emerge is thick enough to contain all but one known packaging signals.

We next analyzed the interactions between the vRNAs *in vitro* by native gel electrophoresis, in this absence of viral and cellular proteins. We detected eight intermolecular interactions that link all vRNA segments in a single network of intermolecular interactions. Using mutagenesis and oligonucleotide mapping, we showed that the regions involved in the three prominent interactions correspond to previously identified packaging regions, even when they could be narrowed down to a few nucleotides. In particular, we showed that mutation of two codons in vRNA 7 that have been shown by P. Digard and co-workers to affect influenza A/PR/8/34 virus growth, virion assembly and genome packaging, decreases the interaction between vRNAs 7 and 6. Classical reverse genetics and competition experiments showed that these mutations strongly affect incorporation of vRNA 7 in human H3N2 influenza viral particles. These results strongly suggest that at least some of the intermolecular vRNA interactions we detected *in vitro* are relevant to vRNA packaging.

Focusing on vRNA 7 and its interaction with vRNA 6, we set up an *in vitro* assay allowing to study the interaction between these vRNAs in the presence of nucleoprotein (NP). This experiment indicated that NP does not prevent interaction between these vRNAs and that it does not affect its specificity. Finally, we analyzed the reactivity of the 5' region of vRNA 7 in intact viral particles and in individual vRNPs to dimethyl sulfate (DMS), a chemical probe that crosses the viral membrane. In intact viruses, all but two A and C nucleotides in the 5' region of vRNA 7 were protected from DMS modification by base-pairing or proteins. Upon disruption of the interactions between vRNPs, the reactivity of these two nucleotides was not affected but 15 additional nucleotides, including some involved in the interaction with vRNA 6 *in vitro* and known packaging signals, became reactive. Thus, in viral particles, at least some packaging signals are involved in interactions between vRNP.

Collectively, our findings support a model in which the eight genomic RNA segments are selected and packaged as an organized supramolecular complex held together by direct base-pairing of the packaging signals. By using their length to discriminate between vRNPs and making the assumption that base-pairing must preferentially occur between adjacent vRNAs, we show that only a small number of vRNP dispositions within the virions are compatible with our data.

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SPA 4: ANTIVIRALS AND RESISTANCE

A401O

Hemagglutinin activating host cell proteases provide promising drug targets for influenza treatment

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Background

The influenza virus hemagglutinin (HA) is synthesized as precursor protein HA0 and has to be cleaved by a host cell protease into the subunits HA1 and HA2 to gain its fusion capacity. Cleavage of HA is crucial for infectivity and spread of the virus and, therefore, relevant proteases should be considered as potential drug targets. However, to date protease inhibitors were not applied for influenza treatment, most likely due to limited knowledge about relevant proteases until recently.

Most influenza viruses, including human viruses, contain a single arginine at the cleavage site and require activation by trypsin-like enzymes. We identified the transmembrane proteases HAT (human airway trypsin-like protease) and TMPRSS2 (transmembrane protease serine S1 member 2) known to be present in the human airway epithelium as proteases that cleave HA with a monobasic cleavage site. HAT is expressed as an enzymatically active protease at the surface of cells and cleaves newly synthesized HA as well as HA of incoming virus, whereas TMPRSS2 activates newly synthesized HA within the cell during its transport to the plasma membrane and is not capable of cleaving HA at the stage of virus entry.

Aims and results

Here, we investigated the potential of substrate-analogue peptide mimetic protease inhibitors to block influenza virus propagation due to inhibition of HA cleavage by TMPRSS2 and HAT. Treatment with inhibitors prevented proteolytic maturation and spread of influenza viruses in stable HAT- and TMPRSS2-expressing cells, with HAT being easily accessible to exogenous inhibitors, whereas inhibition of TMPRSS2 required cellular uptake of the compounds. The most potent inhibitors were tested in human airway epithelial cells and were found to reduce titers of different seasonal and pandemic influenza A viruses and influenza B viruses up to 1.000-10.000fold at non-cytotoxic concentrations. Interestingly, some inhibitors markedly suppressed virus propagation even when added to infected cells at 24 h post infection. At present, the efficacy of the protease inhibitors compared to or in combination with current influenza antivirals is under investigation

Conclusion

Our studies demonstrate that inhibition of HA cleavage provides an effective approach to suppress influenza virus propagation and specific peptide mimetic protease inhibitors could lead to novel and potent drugs for the preventative and therapeutic treatment of influenza infections. In doing so HAT and TMPRSS2 represent promising targets for drug development.

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SPA 4: ANTIVIRALS AND RESISTANCE

A402O

Influenza virus and intracellular redox state: characterization of redox-sensitive molecular targets for innovative antiviral strategies

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Several viruses, including influenza virus, induce an imbalance of intracellular redox state towards pro-oxidant conditions. The oxidized state can be caused by different mechanisms among which depletion of the main intracellular antioxidant glutathione (GSH), and/or increased production of Reactive Oxygen Species (ROS). Different studies have suggested that these alterations contribute to influenza virus replication and to the pathogenesis of virus-induced disease, but the molecular mechanisms underlying the redox imbalance and its role in activating redox-dependent intracellular signaling, remain to be clarified.

On this basis, our study was aimed at identifying the source of intracellular ROS produced during influenza A virus infection of human pulmonary cells (NCI-H292), and at characterizing the molecular pathways activated by virus-induced intracellular redox imbalance and their role in viral replication.

We found that three to four hours post infection (p.i.), influenza virus induced a significant increase of intracellular levels of ROS, that, five hours p.i., returned to those measured in uninfected cells. In parallel with the return of ROS to basal levels we found a significant decrease in intracellular GSH content, probably due to a GSH consumption in scavenging ROS. Accordingly, the addition of the radical scavenger TROLOX, a cell permeable water soluble analogue of vitamine E, prevented intracellular GSH depletion.
The most potent source of ROS is the recently discovered family of NADPH oxidase enzymes (NOX), which consist of seven isoforms functionally expressed in a variety of cells. Recently NOX2 isoform has been demonstrated to be responsible for the lung inflammation caused by influenza A virus. Then, to investigate the role of these enzymes in influenza virus-induced oxidative stress, cells were infected and treated with diphenyleneiodonium (DPI), an inhibitor commonly used for inhibiting NOX activity. DPI treatment inhibited ROS over-production and prevented intracellular GSH depletion, indicating a significant contribution of NOX to virus-induced oxidative stress. The screening of mRNA expression of NOX isoforms in infected cells revealed an increase of NOX4 isoform, suggesting the involvement of this enzyme in influenza virus-mediated ROS production.
It is known that p38 MAPK pathway is regulated by levels of ROS that actively work as intracellular signaling mediators. Influenza virus is known to activate this pathway for controlling viral replication and induction of inflammatory response. However, the possible correlation between the virus-induced intracellular redox alterations and p38 MAPK activation, remain to be elucidated. To this scope the effect of DPI on p38 MAPK phosphorylation in infected cells was evaluated. The treatment with DPI inhibited p38 MAPK activation, indicating that virus-induced NOX activity is responsible for the activation of this kinase. Recently, our group has demonstrated the involvement of p38 MAPK in the regulation of viral ribonucleoprotein (vRNP) translocation from the nucleus to the cytosol. Then, we studied vRNP traffic in the presence of DPI. In this condition, we found that NP was retained in the nucleus suggesting a potential role of NOX activity in the regulation of vRNP traffic. In addition, viral titer released from cells infected with different influenza virus strains (human and avian), was strongly reduced by DPI treatment.

Overall these data suggest that redox-sensitive cell pathways exploited by the virus are promising target for effective anti-influenza strategies. In particular, modulation of NOX activity may represent a new therapeutic approach for controlling replication of different influenza virus strains.

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SPA 4: ANTIVIRALS AND RESISTANCE

A403O

Generation of (multi)drug-resistant influenza A/H1N1 (2009) viruses by reassortment

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Introduction

In Germany, the monitoring for antiviral resistance of influenza viruses gathered from community respiratory specimens showed a prevalence of 100% oseltamivir resistant A/H1N1 viruses during the 2008/2009 influenza season due to the neuraminidase substitution H275Y. All viruses tested remained susceptible to zanamivir and M2 ion channel inhibitors. Resistance analyses of A/H1N1 (2009) viruses circulating in the community revealed oseltamivir resistance in only three of 2066 cases. All resistant viruses acquired the neuraminidase mutation H275Y in absence of any therapeutic selective pressure in immunocompetent and otherwise healthy patients. On the other hand, the mutation S31N that conferred resistance to M2 ion channel inhibitors was detected in 1381 A/H1N1 (2009) viruses. Reassortment between these two influenza virus strains of subtype A/H1N1 could lead to new viruses that are resistant to oseltamivir and M2 ion channel inhibitors. Aim of the study was to determine the probability of emergence of such multi(drug) resistant influenza viruses and the further characterisation of reassortants evolved by *in vitro* coinfection.

Methods

The reassortment between A/H1N1 viruses related to A/Brisbane/59/2007 (Brisbane-like viruses) and A/H1N1 (2009) California-like viruses similar to the strain A/California/7/2009 was accomplished by *in vitro* coinfections of MDCK cells with different ratios. After plaque-purification the progeny viruses were further propagated in MDCK cells. Investigations of their gene segment compositions were carried out by real-time RT-PCR and cycle sequencing for HA and NA genes and pyrosequencing-based analysis of PB2, PB1, PA, NP, M and NS gene segments. The reassortants obtained were further analysed regarding their resistance and enzymatic properties as well as their *in vitro* growth capability.

Results

Sequencing analyses of 114 progeny viruses revealed 75% reassortant viruses and 25% parental viruses of A/H1N1 Brisbane-like origin. Interestingly, although A/H1N1 (2009) viruses had a three-quarter proportion in the second coinfection, complete California-like genome compositions were not detected. Altogether, 45 different out of 254 possible genome constellations occurred in our study. Some of them appeared more than once. Multiresistant reassortants, characterized by Brisbane-like NA, carrying the H275Y substitution and California-like M-gene, including the S31N mutation could be identified for 31 times. Only five reassortant viruses contained the HA and the M-gene segment from A/H1N1 (2009) and the NA from the Brisbane-like virus. One virus harboured the California-like HA and the Brisbane-like NA and M segment. Analysis of the enzymatic properties of these reassortants and the parental viruses showed 2.5-fold higher values of the Michaelis-Menten constant for the resistant NA (Brisbane-like origin) compared to the sensitive NA originated from Brisbane- and California-like viruses.

Multiresistant reassortants with segment compositions of California-like HA/M genes and Brisbane-like NA as well as Brisbane-like HA/NA genes and California-like M gene segments were further analysed. Viruses with HA/NA from the same parental virus grew with impaired growth kinetics and reached the plateau two times faster compared to HA/NA misbalanced viruses. However, both virus variants grew with similar titres (PFU/ml).

Conclusion

A/Brisbane/59/2007-like viruses are replaced currently by A/H1N1 (2009) viruses. However, they might reappear after the human population acquires immunity to the new California-like strain. The data of our study indicate the possibility of the emergence of A/H1N1 viruses resistant to oseltamivir and M2 ion channel inhibitors due to reassortment events. Although, these multidrug resistant viruses seem to possess a decreased viral fitness, the emergence of oseltamivir resistant A/H1N1 viruses in 2007/2008 showed the ability of influenza viruses to overcome such reduced fitness properties. Therefore, close monitoring for antiviral resistance is essential.

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YOUNG SCIENTIST

SPB 4: ECOLOGY AND EPIDEMIOLOGY OF ANIMAL INFLUENZA

B401O

Spatial Dynamics of Human-origin H1 Influenza A Virus in North American Swine

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Introduction

The emergence and rapid global spread of the swine-origin H1N1/09 pandemic influenza A virus in humans underscores the importance of swine populations as reservoirs for genetically diverse influenza viruses with the potential to infect humans. However, despite their significance for animal and human health, relatively little is known about the phylogeography of swine influenza viruses in the United States.

Materials and methods

This study utilizes an expansive data set of hemagglutinin (HA1) sequences (n = 1520) from swine influenza viruses collected in North America during the period 2003 – 2010. With these data we investigate the spatial dissemination of a novel influenza virus of the H1 subtype that was introduced into the North American swine population via two separate human-to-swine transmission events around 2003.

Results

Bayesian phylogeographic analysis reveals that the spatial dissemination of this influenza virus in the US swine population follows long-distance swine movements from the Southern US to the Midwest, a corn-rich commercial center that imports millions of swine annually. Hence, multiple genetically diverse influenza viruses are introduced and co-circulate in the Midwest, providing the opportunity for genomic reassortment.

Conclusions

Overall, the Midwest serves primarily as an ecological sink for swine influenza in the US, with sources of virus genetic diversity instead located in the Southeast (mainly North Carolina) and South-central (mainly Oklahoma) regions. Understanding the importance of long-distance pig transportation in the evolution and spatial dissemination of the influenza virus in swine may inform future strategies for the surveillance and control of influenza, and perhaps other swine pathogens.

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SPB 4: ECOLOGY AND EPIDEMIOLOGY OF ANIMAL INFLUENZA

B402O

Neutralizing antibodies against pandemic h1n1, seasonal h1n1 and avian-like h1n1 swine influenza virus in swine contacts and swine, Western-Europe

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Serological studies for swine influenza viruses (SIVs) in humans with occupational exposure to pigs have only been reported from the Americas, but not from Europe. Human infections with SIV have been rare and mostly reported in contacts with swine, as serological studies suggest that swine workers are at increased risk of zoonotic infection with SIV. In this study, we analyzed neutralizing antibodies against pandemic H1N1 2009 influenza virus, a 2007/2008 seasonal H1N1 virus and an avian-like, enzootic H1N1 SIV in 211 swine contacts in Luxembourg compared to a matched general population. In addition, neutralizing antibodies to SIV and H1N1 2009 in 203 swine were analyzed.
The results showed that swine workers have more often and higher titers of antibodies against pandemic flu (17.5%) and SIV (27%) than the control population. Age was a risk factor for SIV but not for pandemic H1N1. Pigs showed a strong positive antibody response to avian like H1N1 and a weaker to H1N1 2009 but not to European swine H3N2 or H1N2. Antibodies against SIV and pandemic correlated with each other in pig workers (R = 0.7) (but not in the general population) and to a lesser extend in pigs (R = 0.45) but not with the 2007/2008 seasonal H1N1. Sequential infections with variants of human seasonal H1N1 viruses or contact with swine H1N1 influenza viruses seems to increase the risk of serological cross-reaction with the pandemic H1N1.

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SPB 4: ECOLOGY AND EPIDEMIOLOGY OF ANIMAL INFLUENZA

B403O

Multiple introductions of h5n1 influenza viruses into lao people's democratic republic

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Lao PDR has experienced several outbreaks of HPAI H5N1 since the first introduction in early 2004. Several clades of H5N1 viruses have been isolated including clade 1, 2.3.2, and 2.3.4. Systematic surveillance of domestic poultry has been intermittent in Lao PDR, but has detected serum antibodies to subtypes H5 and H9 in ducks, and isolated H5N1 from apparently healthy ducks in 2006.

The aim of this study was to survey influenza A viruses in domestic poultry (ducks and chickens) in Live-bird markets, village backyard poultry, and poultry farms in Lao PDR in areas identified as carrying an increased risk of H5N1 presence as indicated by previous outbreaks and trade routes.

Cloacal, tracheal, environmental swab samples, as well as sera samples were collected during March 2010 and screened for the presence of influenza A RNA by rRT-PCR or influenza-A-specific antibodies by ELISA (IDEXX Flock-Chek MultiS Screen).

2.3% of swabs were found to be positive for influenza A RNA (duck 2.4%, chicken 1.9%, environmental 0.7%). Sequencing and phylogenetic analysis (MEGA 5.02, clustalW alignment and Neighbor-Joining trees) identified three distinct groups of H5N1 viruses: two subgroups of clade 2.3.4 and one group of clade 2.3.2. None of the surveillance sequences were closely related to previously reported H5N1 viruses in Lao PDR, but were instead closely related to recent isolates from China, Vietnam, Mongolia, Russia and Eastern Europe. No LPAI viruses were identified in swabs during March 2010.

One of the two subgroups of clade 2.3.4 viruses identified was closely related to A/chicken/Lao/LH1/2010, which was isolated during a chicken farm outbreak of HPAI H5N1 in Vientiane Capital that occurred approximately 1 month after surveillance sampling. These viruses were also closely related to A/chicken/Vietnam/NCVD-394/2010-like viruses, representing a subgroup within clade 2.3.4 that was recognized in 2010 in Vietnam.

The remaining clade 2.3.4 viruses were closely related to A/Guizhou/1/2009, and A/chicken/Vientam/NCVD-404/2010, representing the second recently recognized Vietnamese subgroup within clade 2.3.4.

Clade 2.3.2 viruses were detected in one locality and were closely related to isolates from Mongolia, Russia, and Eastern Europe. All of the Lao PDR viruses were reassortants. Two were clade 2.3.2 viruses with an NP of clade 2.3.4 origin, while a third was a 2.3.2 virus with PB2, NP, and NA genes most similar to clade 2.3.4 viruses. The HA segment of these three reassortants were identical suggesting that the re-assortment event may have occurred recently.

The prevalence of influenza A-specific antibodies in surveillance sera was 13% (duck 14%, chicken 8%). Sera were further tested by hemagglutination inhibition assay for specific antibodies to H3, H4, H5 (clade 2.3.2 and 2.3.4), H6, and H9 (lineage G1 and Y280). Evidence for the circulation of H4, H5, H6, and H9 viruses was found, with H9-specific antibodies detected most frequently.

This study has identified highly pathogenic H5N1 viruses in swabs of domestic ducks, including two distinct groups of clade 2.3.4 viruses, as well as clade 2.3.2 viruses that were interclade reassortants. Furthermore, sera analysis indicates the presence of H4, H6, and H9 viruses in addition to H5 viruses in Lao PDR. Phylogenetic analysis indicates that the viruses detected were the result of several recent introductions into Lao PDR rather than the spread of endemic viruses. Further risk-assessment and identification of introduction routes of highly pathogenic H5N1 viruses into Lao PDR are ongoing.

No conflict of interest

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YOUNG SCIENTIST

SPB 4: ECOLOGY AND EPIDEMIOLOGY OF ANIMAL INFLUENZA

B403O

Molecular epidemiology of Highly Pathogenic Avian Influenza in Bangladesh

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Bangladesh has one of the highest numbers of reported cases of Highly Pathogenic Avian Influenza (HPAI-H5N1) in the world from 2007 through 2011. HPAI-H5N1 is thought to be endemic in the country. However, little is known about the genetic diversity and the evolution of the circulating viruses in Bangladesh. Analysis of genetic and spatio-temporal information can explore additional dimension of epidemics than the analysis of genetic or of space-time information alone. In the current study we analyzed DNA sequences of virus collected during successive epidemic waves in Bangladesh.

We analyzed the hemagglutinin gene of 30 chicken and one human isolates from 2007 through 2009 in Bangladesh. For each Bangladeshi isolate, we determined the polybasic amino acid sequence motif of the cleavage site and amino acid substitution pattern. Phylogenetic history was reconstructed using Neighbor-Joining and Bayesian methods that take into account temporal and spatial origin of the samples. In addition, we used Mantel correlation tests to analyze the relation between genetic relatedness and spatial and temporal distances.

The polybasic amino acid sequence motif of cleavage site in hemagglutinin (HA) protein was identical to that of Qinghai lineage except two of the isolates from the year 2010. Phylogeography revealed that virus circulating in Bangladesh from 2007 through 2010 belongs to clade 2.2. The result suggests that HPAI-H5N1 in Bangladesh results from a single introduction and that the disease is now possibly endemic. We detect several significant amino acid substitutions in the receptor binding sites. Some of these substitutions are predicted markers for human receptor specificity. The most significant finding of exploratory spatial and temporal analysis is that identical virus caused independent outbreaks over a distance of 270 km and within 14 days of each other. This might indicate long distance spread through vectors such as migratory birds and vehicles and challenges the effectiveness of the10 km radius movement restriction zone. The Mantel correlation test confirmed significant correlation between genetic distances and temporal distances between the viruses, but not between genetic and geographical distances. We found two different clusters within the Bangladeshi isolates in Bayesian phylogenetic analysis. Viruses from 2009 and 2010 formed different cluster than 2007 and 2008, genetically more diverse which indicates possible subclade formation with the progression of time.

The study indicates possible endemicity of the HPAI-H5N1 virus in Bangladesh. Furthermore, the formation of a subclade capable of transmission to humans cannot be ruled out. Hence, we need to intensify surveillance and review the current control programme.

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SPA 5: HOW TO EVALUATE VACCINE EFFECTIVENESS AND EFFICACY?

A501O

I-MOVE, a European network to monitor influenza vaccine effectiveness in the EU: Results from the multi-centre case control studies, seasons 2008-9, 2009-10, 2010-11

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Introduction

The Influenza Monitoring Vaccine Effectiveness in Europe (I-MOVE) network was established in 2007 to have rapid estimates of seasonal and pandemic influenza vaccine effectiveness (VE) and to monitor VE along the seasons. Multi-centre case control studies were conducted in 2008-9 (pilot season), 2009-10 (pandemic season) and 2010-11, with 5, 7 and 8 European countries participating respectively. The studies were carried out in the framework of the existing sentinel influenza surveillance.

Methods

Participating sentinel practitioners interviewed and swabbed patients with influenza-like illness (ILI) (only those aged> 64 or >59 years in the pilot season) during the influenza seasons. We included in the analysis patients adhering to the EU ILI case definition, and swabbed <8 days after onset of symptoms. Vaccination coverage was compared between ILI patients RT-PCR or culture positive for any type of influenza and those testing negative. A valid vaccination corresponded to one dose received > 14 days before ILI symptom onset.
Using a one stage model, we computed pooled vaccine effectiveness (VE) as 1 minus the odds ratio with study site as fixed effect. Using logistic regression we adjusted VE for all potential confounding factors (age, sex, week of onset, chronic diseases and related hospitalisations, smoking history, prior influenza vaccinations, number of practitioner visits in previous year (2009-10 and 2010-11 only)). We conducted a multiple multivariable imputation using chained equations to estimate missing values.

Results

(Preliminary results only (up to week 10 2011) are included in the 2010-11 analysis.)
We included 327 (> 64 y), 2902 (all ages) and 3886 (all ages) ILI patients, in 2008-9, 2009-10 and 2010-11 respectively. Of these, 138 (42%), 918 (32%) and 1891 (47%) were positive for any influenza. Vaccination coverage among ILI cases included in the study was 62% (in 2008-9; elderly only), 7% (pandemic season) and 7% (in 2010-11).The adjusted VE against all influenza was 53% (95% CI 6;78), 72% (95% CI 46;85; A(H1N1)2009 only) and 46% (95% CI 23;62) respectively.
In 2009/10 the adjusted VE against A(H1N1)2009 was 84.8% (95% CI 31;97) in the <15 y; 72% (95% CI 33;88) in the 15-59 age group and not computable above 60 years. In 2010/11 it was 48% (95% CI 21;66) overall and 73% (95% CI -5;93), 21% (95% CI -41;55) and 70% (95% CI 33;87) for the 0-14, 15-59 and ≥60 age groups respectively. The preliminary results of 2009/10 and 2010/11 were already available in February.

Conclusions

The I-MOVE multicentre case control study provided rapid and stratified pooled influenza VE estimates during the seasons and pandemic. The sample size per study site has increased throughout the seasons. VE of the seasonal 2010/11 vaccine against A(H1N1)2009 seems to be lower than the pandemic VE.

I-MOVE is a well established network based on good collaboration between EU countries. This network shows how to monitor vaccine effectiveness across Europe. A similar model could be used for monitoring the effectiveness of other vaccines.

A502O

Exploring uncertainty in the potential benefits of childhood seasonal influenza vaccination

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Introduction

In recent years the expansion of seasonal influenza vaccination programs to healthy children has gained increased attention, with an ongoing debate over the role childhood vaccination can play in controlling the spread of influenza in the community. A major aim of this analysis was to explore some important aspects of seasonal variability and data uncertainty which may affect the benefits of childhood influenza vaccination.

Methods

Using an age-stratified Susceptible Exposed Infectious Recovered (SEIR) model we examined the potential benefits of seasonal influenza vaccination of children aged 6 months to 17 years in Australia.

Results

At lower reproduction numbers, we found that high uptake of a well matched vaccine has the potential to substantially reduce the spread of influenza in the population. Under some less favourable conditions a significant number of deaths may still be prevented. Most of the mortality reduction from widespread vaccination of children was estimated to come from the indirect protection conferred to other age groups, with the number of deaths prevented in the elderly outweighing the direct impact in children.

Conclusions

Several factors were highly influential in determining the potential benefits from vaccination. These included seasonal variation in the vaccine match and the infectivity of the influenza epidemic, as well as uncertainty as to the magnitude of influenza mortality. Estimating the likely benefits of a change in vaccine policy is important in determining if it represents value for money. To our knowledge, this is the first study to explore childhood seasonal influenza vaccination in Australia using a dynamic transmission model.

Acknowledgements

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A503O

Long-term trends in influenza-related mortality and benefits of routine vaccination programs, France, 1975-2008

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Introduction

Despite well-established routine seasonal influenza vaccination programs targeted at seniors in high-income countries, influenza remains a public health issue, with approximately 90% of influenza-related deaths occurring among seniors over 65 years. The mortality benefits of influenza vaccination among seniors remain debated, in part due to the absence of randomized control trials focused on this age group and the difficulties in measuring influenza-related mortality in observational studies.

Here we estimate long-term trends in influenza-related excess mortality during 1975-2008 in France in relation with changes in influenza vaccine coverage and circulating strains.

Material and methods

We compiled weekly mortality rates from pneumonia and influenza, respiratory, circulatory, other causes, and total mortality among seniors over 65 years for 33 inter-pandemic seasons in France, 1975 to 2008. We stratified the data by 7 different age classes and applied Serfling cyclical regression approach to each death outcome and age class. Seasonal excesses mortality rates were estimated as the mortality in excess of a seasonal baseline occurring during influenza epidemic weeks. We obtained data on influenza vaccine coverage, weekly viral activity, and influenza dominant subtypes during the study period.

To evaluate the mortality benefits of vaccination, we regressed seasonal influenza-related excess mortality rates against seasonal vaccination coverage. We also tested differences in the distribution of excess mortality rates in periods with low and high vaccination coverage. All analyses were stratified by age and accounted for year-to-year variation in influenza dominant subtype.

Results

Excess all-cause mortality due to influenza represented 6% on average of the number of all-causes deaths observed during winter months among seniors, and did not exceed 12% in the severe 1985/86 season. The average seasonal excess mortality rate attributable to influenza was estimated at 19.5 per 100,000 for pneumonia and influenza, 35.3 for respiratory causes, 32.9 for circulatory causes, 35.2 for other causes and 102.2 for all-causes.

The A/H3N2 influenza subtype was dominant in 14 of the 33 seasons studied, while A/H1N1 was dominant in 3, B in 2, and mixed circulation was reported in 14 seasons. Influenza-related excess mortality was on average ten-fold (range 7-13) higher in A/H3N2-dominant seasons than in other seasons ($p<.0001$) and a larger proportion of excess deaths was found in younger individuals in A/H3N2 seasons ($p<.0001$ for all death outcomes).

Excess mortality rates were highly correlated among death outcomes years ($0.83 < r < 0.88$, $p<.0001$).

Detailed age-specific annual influenza vaccination rates were available since 1981 among seniors over 75 years. Regression analysis for the period 1982-2008 suggested that vaccination did not reduce excess mortality rates from any cause in this age group (P>0.14).

Among seniors 65-74 years, we found no difference in excess mortality rates years between the low vaccination period 1981-1988 (vaccination coverage < 20%) and the high vaccination period 1999-2008 (vaccination coverage > 60%) in these age groups (Mann-Whitney test, P>0.23). Similarly there was no temporal trend in excess mortality rates from any cause between 1982 and 2008 in these age groups (P>0.07).

Conclusions

Overall, patterns in influenza-related excess mortality rates in France were similar to those reported in other high income countries, with a higher burden in A/H3N2-dominant seasons than seasons dominated by other subtypes. In line with previous population studies in the US and Italy, there was no significant decline in excess mortality among seniors as influenza vaccine coverage increased from 14% in 1979 to 67% in 2008. While we cannot rule out a relatively low mortality decline among seniors that may be undetectable by trend analysis, or the effect of confounding factors, our study and others suggest a lack of effectiveness of routine seasonal influenza vaccination programs in this immunologically-frail population.

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SPA 5: HOW TO EVALUATE VACCINE EFFECTIVENESS AND EFFICACY?

A504O

Cross-protective Efficacy of a Seasonal Trivalent Influenza Vaccine against 2009 Pandemic Influenza A (H1N1) Virus Observed in a Large, Randomized Placebo-controlled Trial

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Introduction

During the 2009 H1N1 pandemic, numerous observational studies of trivalent influenza vaccine (TIV) effectiveness and analyses of stored sera from TIV trials yielded conflicting results, suggesting the effect of seasonal TIV against pandemic H1N1 to be protective, ineffective or even detrimental. A large, randomized, placebo-controlled efficacy study of a seasonal TIV conducted in 2008 and 2009 was able to assess the possibility of cross-protection against the 2009 pandemic H1N1 virus.

Material & Methods

This phase IV, randomized, observer-blind, placebo-controlled study enrolled healthy adults aged 18 to less than 65 years over two consecutive influenza seasons (2008 and 2009) across 23 sites in Australia and New Zealand. Participants were randomized in a two-to-one ratio to receive a single injection of 0.5 mL TIV or placebo. The primary objective was to estimate the efficacy of a TIV (Fluvax®, CSL Limited, Parkville, Victoria, Australia) against laboratory-confirmed influenza.

Nasal or throat specimens were collected from subjects reporting an influenza-like illness (ILI) from day 14 after vaccination until 30 November of the respective study year, the time typically marking the end of influenza circulation in the southern hemisphere. Laboratory-confirmed influenza was determined by viral culture and/or real-time reverse transcription polymerase chain reaction (rRT-PCR).

Results

A total of 15,044 participants were randomized (7,544 in 2008, 7,500 in 2009). For the two influenza seasons combined, vaccine efficacy against any influenza infection was 42% (95% CI 30%, 52%) whereas efficacy against vaccine-matched strains was 60% (95% CI 44%, 72%).

The incidence of laboratory-confirmed influenza due to 2009 pandemic H1N1 was identified among 94 of 2459 (3.8%) placebo recipients and 115 of 4875 (2.4%) vaccine recipients. The efficacy of the 2009 TIV against 2009 pandemic H1N1 was highest in older compared with younger participants. Among those 55 to 65 years of age, vaccine efficacy was 65.8% (95% CI, 4.6%, 87.7%) whereas in those 18 to 39 years of age efficacy against 2009 H1N1 was 38.1% (95% CI, 16.1%, 54.4%). Estimated vaccine efficacy among those 40 to 54 years of age was 21.6% but confidence intervals were wide (95% CI, -56.3%, 60.7%). These differences in vaccine efficacy between age groups were not statistically significant (Logistic regression P=0.4162). However, the incidence rates were significantly different between the age groups (Logistic regression $p<0.0001$) reaching 4.7% among the 18-39 year old placebo recipients, nearly double the 2.4% rate in the 40-55 year and the 55-65 year age groups.

Discussion & Conclusions

This is the first large randomized controlled study to demonstrate cross-protection afforded by a seasonal TIV against laboratory-confirmed 2009 pandemic H1N1 in humans.

Speculating on the potential mechanisms for the cross protection against outbreak virus, stimulation of cross-reactive antibodies is one likely explanation. Cross-reactive antibodies may be vaccine strain-specific or from boosting of antibodies to pre-existing influenza strains.

There are several potential reasons why the results of this interventional study differed from those arising from epidemiologic studies of vaccine effectiveness, such as differences in endpoints, study design, characteristics of populations vaccinated and geographic location, and possibly, not all TIVs are indeed the same.

As the monovalent H1N1 vaccine was not available during the first wave of the 2009 influenza pandemic, this study suggests that any use of seasonal vaccine most likely had a positive impact on mitigating the peak incidence of pandemic H1N1 infections.

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YOUNG SCIENTIST

SPB 5: GENETIC AND ANTIGENIC EVOLUTION

B501O

35 years of antigenic evolution of influenza A/H3N2 virus is dictated by 7 amino acid positions flanking the hemagglutin receptor binding site

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Introduction

Influenza A virus epidemics affect approximately 5-15% of the worlds' population and are responsible for an estimated 0.25 – 0.5 million deaths annually. Vaccination is the primary method to reduce the public health impact in risk groups. Antibodies against the hemagglutinin (HA) protein can provide protective immunity against disease and HA is therefore the main component of influenza vaccines. However, antigenic drift—the accumulation of mutations as a result of population immunity—warrants the need for frequent updates of the vaccine. The hemagglutination inhibition (HI) assay is routinely used to detect antigenic differences among circulating influenza viruses. Since the introduction of the A/H3N2 subtype in humans in 1968 at least 11 "antigenic clusters" of viruses have emerged, each of which was subsequently replaced by antigenically distinct viruses [D.J.Smith et al., Science, 305, 371 (2004)].

Aim

We wished to study the molecular basis of the antigenic evolution of influenza A/H3N2 virus from 1968 to 2003.

Methods

For each of the 11 identified antigenic clusters we selected representative strains with an HA sequence resembling the consensus amino acid sequence of the cluster. The HA genes were cloned and recombinant viruses were produced. Thirty viruses with a chimeric HA gene were generated for a first coarse mapping of the antigenic determinants. The minimal amino acid substitutions involved in each cluster transition were identified by producing ~130 recombinant viruses with specific mutations introduced in HA using site-directed mutagenesis methods. All viruses were tested by HI assays with ferret antisera, and results were analyzed using antigenic cartography methods.

Results

HI assays using viruses with chimeric HAs revealed that amino acids 109-301 of the HA1 globular head determined the antigenic properties. Site-directed mutagenesis studies revealed that introduction of only one to three amino acid changes were sufficient to cause the antigenic differences between clusters. HA positions 145, 155, 156, 158, 159, 189 and 193 were exclusively responsible for these antigenic changes. At least 8 out of 10 cluster transitions were predominantly caused by a single substitution. With the exception of position 159 all positions were involved in a cluster transition more than once. Six of seven positions align to form an antigenic ridge on the membrane distal periphery of the HA receptor binding site. By introducing only 5 key amino acid substitutions in a 1968 virus we changed the virus antigenically to a 2003-like virus. Moreover, inoculation of ferrets with a wildtype 1968 virus or a mutant virus with the single substitution responsible for the first cluster transition revealed that a single substitution at a key antigenic site can substantially skew the polyclonal antibody response to A/H3N2 virus.

Conclusion and discussion

Only seven key positions in HA were predominantly responsible for the antigenic evolution of A/H3N2 subtype viruses over a 35-year period. The resampling of amino acid changes at key positions that caused major antigenic change suggests that these positions are under stronger selection pressure than others, and may indicate a potential role in future antigenic change. The small number of positions and limited amino acid usage involved in past antigenic evolution suggests that the potential number of escape variants may be much smaller than previously anticipated and, as a consequence, the antigenic evolution of influenza A/H3N2 virus becomes more predictable.

B502O

Discordant antigenic drift of neuraminidase and hemagglutinin in recent h1n1 and h3n2 influenza viruses

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Introduction

Seasonal influenza virus epidemics are driven by antigenic changes in viral surface glycoproteins, which evade pre-existing humoral immunity. Antigenic drift is a feature of not only the hemagglutinin (HA) protein, but also of neuraminidase (NA). Vaccine strain selection depends on epidemiology information as well as HA gene sequence phylogeny and serologic analysis of candidate strains with the hemagglutination inhibition (HI) assay, with changes visualized by genetic and antigenic cartography. Antigenic variation of NA also contributes to immune escape but the cumbersome nature of the standard colorimetric neuraminidase inhibition (NI) assay has precluded analysis of NA drift to support strain selection.

Aims

To compare the antigenic and genetic evolution of the N1, N2, H1, and H3 components of H1N1 and H3N2 vaccine formulations during the past 15 years.

Methods

NI titers measured by a miniaturized thiobarbituric acid method and HI titers were analyzed by multidimensional scaling (MDS) algorithms to generate antigenic maps based on ferret antisera. NI results were confirmed using human sera from a clinical vaccine study that were also analyzed in the NI assay. Aligned nucleotide sequences of NA and HA1 were used to construct phylogenetic trees. Amino acid sequence alignments for HA1 and NA were used to calculate amino acid distance matrices to create genetic maps with MDS algorithms.

Results

There was a general agreement between the phylogenetic trees and genetic maps produced for the H1 and N1 genes of H1N1 viruses and the H3 and N2 genes of H3N2 viruses, with evolutionary rates slightly higher for HA as compared to NA.
Comparing the antigenic and genetic data for NA, we found that large numbers of amino changes in NA on some occasions did not result in significant antigenic drift while in other instances few amino acid substitutions in NA resulted in significant antigenic drift. For instance, NA of TX/91, NC/99, and SI/06 H1N1 strains were virtually identical in antigenic maps but very distinct in genetic maps, while NA of WY/03 and NY/04 H3N2 strains were genetically very similar but antigenically quite distinct. These data indicate that genetic sequences of NA are poor predictors for antigenic properties of influenza A viruses.
When we compared the antigenic data for NA and HA, we observed that antigenic drift of HA is often asynchronous with drift of NA. For instance the antigenic distance between HA of the WU/95 and SY/97 H3N2 vaccine strains is very well known, but the antigenic distance between NA of these strains was negligible. On the other hand, relatively minor antigenic drift such as observed between HA of SI/06 and BR/07 H1N1 strains was accompanied by substantial antigenic drift in NA. Thus, the antigenic drift of HA and NA of influenza H1N1 and H3N2 viruses was clearly discordant in the 15-year period.
NI data generated with human sera from a clinical vaccine study revealed that the assessment of NA drift using human antisera generally reflected the antigenic phenotypes described above.

Discussion & conclusion

Our data provide evidence of discontinuous antigenic drift of NA that is discordant with the antigenic drift of HA across several contemporary H1N1 and H3N2 strains. The finding that substantial antigenic drift of NA can be observed when genetic change in NA is minimal, highlights the need to evaluate antigenic structure and not just sequence changes when characterizing circulating or emerging virus strains. Rigorous analysis that allows selection of vaccine candidates with antigenically-matched NA, could improve vaccine efficacy and may be of particular benefit when the HA of the vaccine strain is poorly matched to the circulating seasonal or pandemic strain.

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SPB 5: GENETIC AND ANTIGENIC EVOLUTION

B503O

Estimating Reassortment Rates and Epistasis in Influenza viruses from European Swine

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Background

Swine have often been considered as a mixing vessel for different influenza strains; swine may become infected with viruses with avian-like or human-like sialic acid binding receptors, but before 2009 swine influenza had not been observed to give rise to a pandemic. However, the co-circulation in pigs of a North American H3N2 strain, itself a triple reassortant, with Eurasian swine H1N1, presumably in swine, led to the production of a novel reassortant virus which caused the 2009 human pandemic.

Aims

The aims of this work are to characterise the reassortants present in European swine; estimate the rate of reassortment of subtypes in swine; and derive a measure of epistasis between different segment combinations.

Data

To gain a better understanding of the diversity and evolution of the pandemic (H1N1) 2009 precursor strains we undertook complete genome sequencing of archived European swine isolates using an Illumina platform. From over 600 archived isolates available, an initial selection of approximately 100 was made to include one isolate per subtype per country per year, prioritising H1N1 sequences from 1990 onwards. In this study we focus on subtypes H1N1-H3N2 and analyse the new sequences together with a sample of 370 complete genome sequences from avian, swine and human hosts in the NCBI database.

Methods

The evolutionary histories of the viral segments in the swine 'flu lineages were investigated using time resolved phylogenetic trees and rates of evolution were calculated in different lineages. We identified a clade of H1N1, H1N2 and H3N2 mostly European Eurasian swine viruses which were monophyletic in PB2, PB1 and PA, and examined the reassortments between the HA and NA segments with respect to this backbone. We assigned trait labels to the PB2 sequences based on the clades of their corresponding HA and NA segments, and then inferred the transition rates between these traits over the set of time resolved PB2 trees generated in BEAST in order to provide an estimate of the reassortment rates between different pairs of lineages. Additionally, since a distribution of likely trees and rate models was generated, the statistical significance of each rate was also calculated. To gain a measure of epistasis between the HA and NA segments, the observed distribution of HA and NA pairings was compared to null models in which the HA and NA traits were evolved independently down the PB2 trees, utilising the rates inferred previously and 1000 evolutions per tree.

Results

Our analysis of evolutionary rates between avian, swine and human clades shows that in all segments the rates for the Triple Reassortant swine and Eurasian Swine clades have been higher than either the Avian or Human Seasonal clades, implying that the viruses were adapting to their new swine hosts (from avian and human for Triple, or just avian in the Eurasian clade). Furthermore we detected high rates of reassortment between HA H1-Human Seasonal to HA H1-Eurasian Swine in our European clade, as much as 0.5 per year (i.e. a reassortment every 2 years), but a somewhat lower rate of 0.2 per year for N1-Eurasian Swine to N2-Human Seasonal. We also found that the HA and NA in H1N1 Eurasian Swine was a particularly favoured combination in comparison to the null models.

Conclusions

In conclusion we find that HA and NA segments from co-circulating lineages have undergone rapid reassortment onto an internal segment backbone in recent European swine influenza viruses, and in consequence we should expect further reassortments between currently circulating swine strains and the pandemic strain.

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SPB 5: GENETIC AND ANTIGENIC EVOLUTION

B504O

Neutralizing antibody epitopes on the hemagglutinin of recent H3N2 viruses

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Introduction

H3N2 influenza viruses have now circulated in the human population for 43 years, accumulating sequence changes in the HA and NA that are believed to be partly due to selection for escape from antibodies. Other selection pressures for change may be to optimize receptor recognition or increase transmissibility, but it is likely that most non-antigenic changes are a consequence of random drift and have no selective advantage. Phylogenetic trees of H3 HA show a main trunk of evolution with short side branches, and examination of the trunk mutations, that persist and accumulate, has led to identification of antigenically significant mutations that overlap the five antigenic sites mapped on to the H3 HA of the earliest H3N2 isolates.

Methods

To obtain experimental evidence for important antigenic sites on modern H3 HAs, we have used human and mouse monoclonal antibodies (mAbs) to select escape mutants of an A/Wisconsin/67/2005-like virus and to partially map epitopes by mutagenesis of amino acids in antigenic sites A and B. To provide more accurate mapping of epitopes, we have determined the crystal structure of H3 HA of A/California/7/2004.

Results

As expected, the overall fold of the 2004 HA is very similar to that of the 1968 H3 structure, but the 20% amino acid sequence variation in HA1 since then has resulted in considerable rearrangement of side chains and their interactions. The epitopes of the mAbs we have in hand map to antigenic sites A and B.

To establish the significance of these mAbs, we have tested sera of people vaccinated in recent years against antigenic site mutants of HA. We find that most individuals show immunodominance of antigenic site B over 4 years of influenza vaccinations, while a minority have dominance of site A.

Conclusions

The overall fold of A/California/7/2004 H3 HA is identical to that previously determined of X-31 (1968) HA, but the new side chain dispositions alter the surface of HA1. Antigenic site B is dominant in people vaccinated in recent years. A better understanding of immunodominance may allow prediction of future antigenic drift.

TUESDAY 13TH SEPTEMBER 2011

SPB 5: GENETIC AND ANTIGENIC EVOLUTION

B505O

Comparative aspects of infectious salmon anaemia virus, a fish orthomyxovirus, and influenza viruses

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Infectious salmon anaemia (ISA) is an orthomyxovirus infection of sea-farmed Atlantic salmon (Salmo salar) inducing a systemic and lethal condition characterised by circulatory failure including ascites, severe anaemia and variable haemorrhages and necrosis in several organs. The disease course is prolonged with low daily mortality (0.05-0.1 %) typically only in a few cages, but cumulative mortality may become very high. The pathology and tissue tropism are poorly described, but infection of endothelial cells and leucocytes have been demonstrated. The disease has been diagnosed in Canada, Scotland, Shetland Islands, the Faroe Islands, USA and Chile.

As in influenza viruses, the ISAV genome consists of eight negative sense, single-stranded RNA segments, encoding at least 10 proteins. It has been shown to undergo segment reassortment and recombination, with links to virulence. Compared to influenza viruses, the organisation of ISAV genes and gene products are unique. The virus' two major surface glycproteins are the haemagglutinin-esterase (HE), responsible for receptor binding and -release, and the fusion protein (F), responsible for the fusion of viral and cellular membranes. Highly pathogenic ISAV strains are characterised by a differential deletion in the HE stalk region, accompanied by a leucine$_{266}$, or a short sequence insertion (recombination), in F next to the putative cleavage site R$_{267}$. Present knowledge strongly suggests that the precursors of all pathogenic ISAV strains are low pathogenic so-called HPR0 genotypes carrying a full-length HE gene and a glutamine$_{266}$ in F. In recent years HPR0's have been detected with increased frequency in apparently healthy wild and farmed Atlantic salmon. They are almost exclusively found in gill tissue and identified by RT-PCR. All attempts to propagate this virus in cell culture have been unsuccessful. Not associated with classical clinical and pathological changes consistent with ISA, the HPR0's have occasionally been associated with pathology in gills (proliferative gill inflammation).

The single amino acid substitution or recombination event in ISAV F close to R267 may be analogous to that in HPAIV subtypes H5 and H7, where pathogenicity is acquired through the mutational change of cleavage specificity following species crossover from waterfowl to domesticated poultry. As in influenza A virus, a functional balance between the HE receptor-binding and -destroying activities may also be required for ISAV replicative fitness and virulence. A differential ability of ISAV particles to elute from Atlantic salmon erythrocytes compared to that from other salmonid species has been documented, and possibly linked to virulence. The forces driving the deletions in the HE stalk region could be analogues to the phenomenon described for influenza A virus, were varying lengths in the NA stalk region has been associated with host range. In ISAV, this may represent viral adaptation to Atlantic salmon from an unknown reservoir leading to disease in densely populated fish farms. Also, the importance of internal genes in virulence is well documented for influenza viruses. Although only one HPR0 genome has been sequenced, potentially unique sites in HPR0 internal genes have been identified.

An ongoing three year project aims at elucidating many of the mysteries surrounding the HPR0 genotype, and the comparative aspect to influenza viruses will be important. HPR0 genomes will be compared to that of virulent strains to increase our knowledge regarding ISAV evolution and the potential presence of additional virulence factors. Functional properties associated with the HPR0 genotype will also be investigated. Pyrosequencing on samples from ISAV diseased fish is ongoing, and preliminary results from this work will be discussed.

TUESDAY 13TH SEPTEMBER 2011

SPA 6: VACCINE SAFETY

A601O

Possible outcomes of reassortment in vivo between wild type and live attenuated influenza vaccine strains

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Introduction

Reassortment of influenza viruses in nature has been well documented. Genetic reassortment plays a key role in emergence of new influenza A strains, including pandemic viruses. Permissive host can be simultaneously coinfected with multiple influenza viruses. During genetic reassortment gene segments are exchanged between parental viruses that may lead to some enhancement of virulence of reassortant progeny. At present, vaccination with live attenuated cold–adapted (ca) reassortant vaccine (LAIV) is used as an effective public health measure for influenza prophylaxis. However, there are concerns about a potential of simultaneous infection of human host with ca and wild type (wt) influenza viruses which might produce progeny that contain novel, more virulent genotypes.

Aim

To investigate potential consequences of reassortment of wt with LAIV strains *in vivo*.

Methods

Viruses: A/California/07/2009 (pH1N1); A/Sydney/5/97 (H3N2); PR8; PR8–based reassortants for inactivated vaccine subtype H5N1 (NIBRG–23, VN1203); A/Leningrad/134/17/57 (H2N2), *ca* master donor virus (MDV) for Russian LAIV; MDV–based vaccine strains. *Animals*: female albino guinea pigs (300 to 350 g) were coinfected intranasally with a mixture of two or three viruses without anaesthesia. Reassortants from nasal washes were isolated by limited dilution. Genome composition of reassortants was monitored by RFLP analysis. Capacity of viruses to grow at optimum, low (*ca* phenotype) and elevated temperatures (*ts* phenotype) was determined by titration in chicken eggs and expressed as EID_{50}. *Macroscopy of chicken embryos*: a maximum macroscopic lessions were scored as follows: 0 – no visible changes; 1 – mild; 2 – moderate; 3 – strong; 4 – severe.

Results

Genome composition of guinea pig–derived reassortants. A limited number of true reassortants were isolated. The majority of isolated clones were identical to the ca parents. Genome composition analysis of reassortants of PR8–based H5N1 viruses with MDV showed that all isolates were triple reassortants which had inherited PB2 and NA genes from MDV, PA from PR8 and HA from H5N1. The genome formula of reassortants of H1N1 2009 virus with ca MDV or MDV–based vaccine strains varied. *Ca/ts phenotype*. Reassuringly, a large majority of reassortants generated *in vivo* had the phenotype typical of the MDV. *Macroscopical observation*. Infection of chicken embryos with a H1N1 2009 virus caused significant pathological changes (score = 4). The macroscopy of chicken embryos infected with H5N1–PR8 reassortants demonstrated that they were also severely affected (scores = 3–4). The most frequently observed macroscopic lesions included focal head and skin hemorrhages, head edema, delayed embryo development, weight loss, kinked neck, and skin loss. Most dominant gross lesions were observed in embryo head while body was less affected. In contrast, the mock (PBS), MDV, and LAIVs infected chicken embryos didn't show any visible macroscopic lesions (score = 0). Macroscopy scores in groups of reassortants didn't dramatically differ from the control (mock). Visualization results revealed that all reassortants when inoculated in eggs did not induce substantial macroscopic lesions and were not more virulent than wt parents (score = 0–1).

Conclusion

Ca and/or *ts* reassortants were generated as a result of the coinfection of *wt* viruses with *ca* strains *in vivo*. Reassortment of *wt* virus with LAIV strain in guinea pigs have resulted in progeny virus which reduced macroscopic lesions of chicken embryos. None of reassortants were more virulent than *wt* parents, or revealed a significantly higher macroscopy lesions than control. Macroscopy differences can be used as additional marker of attenuation. Our results suggest that genetic reassortment between *wt* and vaccine strains is unlikely to lead to virulent reassortant progeny. These findings provide additional support of LAIV safety data.

This research was supported by PATH.

TUESDAY 13TH SEPTEMBER 2011

SPA 6: VACCINE SAFETY

A602O

Scientific investigations into febrile reactions observed in the paediatric population following vaccination with a 2010 Southern Hemisphere Trivalent Influenza Vaccine

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Aims

During the 2010 Southern Hemisphere (SH) season, an increased incidence of febrile reactions was reported in the paediatric population following vaccination with the trivalent influenza vaccine (TIV). These adverse events appeared to be more prevalent with the CSL Limited TIV, and a series of scientific investigations were initiated to determine the root cause. The primary objectives were: 1) investigate the immunogenicity/reactogenicity of the CSL 2010 TIV as compared to previous season and comparator vaccines; (2) Identify vaccine components that may have contributed to the adverse events; (3) identify surrogate parameters that can be used to prepare future TIVs which are safe and effective in the paediatric population.

Methods

CSL Limited has implemented a detailed investigational plan to elucidate the mechanisms underlying these adverse events, including *in vitro* cytokine/chemokine assays following stimulation of adult and paediatric whole blood, as well as mammalian cell lines and primary cells (Center for Biologics Evaluation and Research; CBER), profiling of molecular signatures using microarrays, biochemical tests for neuraminidase activity and content (Center for Disease Control and Prevention; CDC), and *in vivo* studies in rabbits, ferrets, new born rats and rhesus non-human primates (NHPs). Various TIVs (approved commercial vaccines as well as re-engineered) and their individual monovalent pool harvest components were examined in these assays and animal models. The full array of cytokines and chemokines screened by Luminex arrays were IL-1α, IL-1β, IL-1RA, IL-6, IL-8, IL-9, IL-10, IL-17, IP-10, MCP-1, MCP-3, MDC, MIP-1α, MIP-1β, RANTES, GRO, TNF-α, IFN-α, IFN-γ, G-CSF, Eotaxin, PGE_2 and Activin-A.

Results

Studies using myelomonocytic cell lines conducted by CBER confirmed the findings of the rabbit pyrogenicity studies, that the CSL 2010 TIV and corresponding individual strains do not contain bacterial-derived pyrogens. Assays using 21 adult blood donors have been completed and assays using paediatric blood have been initiated. Statistical analysis of the cytokine/chemokine data from the adult whole blood assays indicates that the CSL 2010 TIV is generally, more stimulatory than previous season TIVs. Analysis of the individual viral components implicates a combination effect of the specific viral strains used in the 2010 SH TIV formulation. NHPs (n=4 per vaccine cohort) immunized with adult doses of various CSL TIV formulations or competitor 2010 TIVs indicated no statistically significant change from baseline in body temperature (T=0, 4, 8, 24, 72h) or complete blood cell count parameters (T=72h). Genomics microarray analyses have been performed and data is being analysed. Preliminary results indicate that CSL TIV formulations generally induce more potent gene signatures than the competitor vaccine and that replacement of the H1N1 pandemic A/California/7/09 strain in the 2010 TIV with another H1N1 strain alters the gene signatures. However, the monovalent A/California/7/09 pandemic vaccine, containing 15mg HA, induced weaker gene signatures. This possibly implicates a combination effect of the three viral components in the CSL TIV or relates to the total antigen content administered. Serum haemagglutinin inhibition (HAI) and microneutralization (MN) titres (T= 28d) and cytokine profiling (T= 0, 4, 8, 24h, 7, 14, 21d) have been examined. Analysis of neuraminidase (NA) activity indicates that the H1N1 A/California/07/2009 contains substantially higher NA activity than previous H1N1 strains and other H3N2 and B strains.

Discussion/conclusion

Although the scientific investigations are still ongoing, the emerging hypothesis is that there are one or more viral-derived components within the CSL 2010 TIV that may contribute to the unusually high proportion of fever and other febrile reactions in children during the 2010 SH season. Identification of the causal components may result in the identification of surrogate parameters that can assist in the formulation of future TIVs to minimise the incidence of febrile reactions in the paediatric population.

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SPA 6: VACCINE SAFETY

A603O

Safety comparison of MF59-adjuvanted and non-adjuvanted seasonal influenza vaccines in 107,661 elderly subjects in Italy

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Introduction

Influenza infection is a major cause of illness, morbidity and mortality throughout the world with the elderly in particular being at high risk for morbidity and mortality. Due to immunosenescence non-adjuvanted seasonal influenza vaccines are only 40-60% efficacious in the elderly population, so adjuvanted influenza vaccines have been developed to amplify the immunogenicity and enhance effectiveness of influenza vaccination. The MF59-adjuvanted Influenza vaccine, Fluad® (Novartis Vaccines), has been shown to be more effective than the equivalent non-adjuvanted vaccine in adults older than 65 years of age. We report here on the first large-scale, prospective post-licensure evaluation of the safety of the MF59-adjuvanted vaccine in the elderly.

Methods

We performed this evaluation in the context of an observational, non-interventional, prospective cohort effectiveness and safety study (LIVE) in the Italian region of Lombardy during the 2006-7, 2007-8 and 2008-9 influenza seasons. The Lombardy region has highly developed computerized clinical information systems. Adults older than 65 years of age were invited to participate in the study. The choice of which influenza vaccination was appropriate to give to each individual—MF59-adjuvanted trivalent influenza vaccine (Fluad®) or non-adjuvanted trivalent influenza vaccine (Agrippal®) with identical antigen composition appropriate for each season—was decided by the individual vaccine provider and not assigned by study protocol. Potential adverse events of special interest (AESI) were pre-defined based on US FDA, European ECDC and WHO serious safety outcome lists, and were identified from hospital utilization databases and then reviewed and validated against recognized case definitions to identify confirmed cases of hospitalization for AESI. The rates of such cases in both biologically plausible and a six month time windows following vaccination were compared.

Results

Overall, during the three study years there were 170,988 vaccine doses (88,449 adjuvanted, 82,539 non-adjuvanted) administered to a total of 107,661 study participants. Despite the large sample sizes for the two vaccine groups, cases of hospitalization for any of the AESIs were rare in both groups. A total of 460 hospitalizations in 401 subjects for conditions coded as possible AESIs within six months of any study vaccine, but only 56 within the predefined biologically plausible time window, were identified.

After validation, there was no elevated risk for any outcome, rates being similar in both the non-adjuvanted and the MF59-adjuvanted vaccine groups. The most frequent AESI in a biologically plausible time window with "definite, probable or possible" linkage to any vaccination was convulsions, for which incidence rates per 10,000 person-month were 0.73 (95% CI: 0.27, 1.58) and 0.45 (0.12, 1.16) for non-adjuvanted and MF59-adjuvanted vaccines, respectively. Applying the same criteria, a single case of Bell's Palsy, two cases each of idiopathic thrombocytopenia (ITP) and vasculitis were reported in the adjuvanted vaccine group, and one case of ITP in the non-adjuvanted group (all giving incidence rates ≤ 0.23/10,000 pm). There were no cases of Guillain-Barré, anaphylaxis or encephalitis.

Conclusion

Data from this large-scale prospective cohort evaluation provide further evidence that MF59-adjuvanted influenza vaccination is safe in the elderly. The safety of the MF59-adjuvanted influenza vaccine, and its demonstrated superior immunogenicity and effectiveness over non-adjuvanted influenza vaccine in the elderly, support its routine use in this population.

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SPA 6: VACCINE SAFETY

A604O

Annual vaccination against influenza hampers the development of infection-induced heterosubtypic immunity

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Introduction

Annual vaccination of young healthy children against seasonal influenza has been implemented in the national vaccination programs of a number of countries. However, we recently demonstrated that vaccination against seasonal influenza prevented the induction of heterosubtypic immunity against influenza A/H5N1 otherwise induced by infection with seasonal influenza A viruses in both mice and ferrets (1-3). The absence of heterosubtypic immunity correlated with the absence of a virus-specific cross-reactive CD8+ T cell response. These findings might be important for the development of vaccination strategies against future pandemic viruses (4). However, the impact of vaccination on the development of the virus-specific CD8+ T cell immunity in children is unknown. In this study, influenza A virus-specific cellular and humoral immune responses were compared between unvaccinated and vaccinated children.

Materials and methods

Peripheral blood mononuclear cells (PBMC) and plasma samples were collected from cystic fibrosis (CF) patients vaccinated annually (n=14) and unvaccinated healthy children undergoing correctional surgery (n=27). PBMC of these children were tested for the presence of virus-specific T cells by intracellular IFN-γ staining and plasma samples for the presence of virus-specific antibodies against various influenza A virus strains and multiple other antigens used in the national immunization program.

Results

No differences in virus-specific CD4+ T cell and antibody responses were observed between groups, while an age-dependent increase of the virus-specific CD8+ T cell response was observed in unvaccinated children that was absent in vaccinated children. The age-dependent increase in the virus-specific CD8+ T cell response in unvaccinated children correlates with the age-dependent increase in the seroprevalence of antibodies against influenza viruses in children demonstrated in a large sero-epidemiological study recently(5).

Conclusions

Our results obtained *in vivo* in animal models and ***in vitro*** with PBMC from children indicate that annual influenza vaccination is effective against seasonal influenza, but hampers the development of virus-specific CD8+ T cell responses. These findings highlight the importance of the development of broadly-protective vaccines and indicate that when annual influenza vaccination of young children is implemented, vaccination of this age group against pandemic influenza should have a priority.

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TUESDAY 13TH SEPTEMBER 2011

YOUNG SCIENTIST

SPB 6: MATHEMATIC MODELLING

B601O

Understanding the dynamics of intracellular virus replication -how does influenza control its RNA and protein synthesis?

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Introduction

Influenza viruses have developed sophisticated strategies to direct cellular resources toward virus replication. To most efficiently utilize these resources while escaping detection by the cell, the virus has to precisely regulate the synthesis of its components in a temporal and quantitative fashion. In recent years, numerous studies have revealed various mechanisms by which viral proteins can achieve this regulation. However, a lot of regulatory interactions are still controversially discussed and little is known about their relative importance and interplay in the course of an infection. Here, we propose to study the regulatory effects of the influenza virus nucleoprotein (NP) and matrix protein (M1) by placing them into a coherent mathematical framework. Such a model provides the unique opportunity to gain a quantitative understanding of how these proteins shape viral replication dynamics.

Method

We developed a structured mechanistic description of influenza A virus growth in a mammalian cell. It accounts for the complete intracellular life cycle of the virus including attachment, endocytosis and uncoating, the synthesis of viral RNA and viral proteins, and the assembly of progeny virions. The model is used to investigate the regulation of influenza virus replication during two crucial stages of the infection cycle: the switch from viral mRNA (vmRNA) transcription to genome (vRNA) replication and the initiation of vRNA export from the nucleus.

Results

With respect to the switch from transcription to replication it was proposed that NP either directly interacts with viral polymerases altering their activity or acts through stabilizing complementary RNA (cRNA). By simulating these two alternative hypotheses, we show that each of them yields a specific pattern of cRNA accumulation. This facilitates the design of new experiments to asses the individual contribution of both mechanisms to regulation. Our model also suggests that a negative feedback via M1 plays a major role to avoid overproduction of viral RNA. Moreover, validation against a variety of published data sets allows estimating kinetic constants for the above mentioned processes. With these results, we find that budding from the cell membrane is a potential bottleneck for progeny virus production.

Conclusion

Ultimately, the presented approach shows that a comprehensive understanding of the influenza virus life cycle with respect to its regulation and dynamics benefits from detailed mathematical modeling. If validated thoroughly, a structured model can support the development of antiviral drugs and the optimization of influenza vaccine production in mammalian cells.

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SPB 6: MATHEMATIC MODELLING

B602O

Estimation of household and community transmission parameters of seasonal and pandemic influenza in a cohort of households in Hong Kong

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Background

Thirty percent of influenza transmission occurs in households. Therefore, elucidating household transmission dynamics is critical to understand how influenza spreads during an epidemic and to inform potential countermeasures. The household secondary attack proportion (SAP) describes the expected proportion of household contacts which result in infection by an index case, while the community probability of infection (CPI) describes the probability an individual becomes infected outside of households. The literature contains few estimates of the SAP and CPI from empirical data, not permitting direct comparisons of these two parameters between pandemic and seasonal influenza.

Methods

Serum specimens were collected from all members of 117 Hong Kong families in April 2009 before the 2009 H1N1 pandemic and again in August-October 2009. Serological assays were used to identify pandemic A/H1N1 and seasonal A/H1N1 and A/H3N2 infections using hemagglutination inhibition assays and pandemic A/H1N1 using viral neutralization assays during the follow-up period. Statistical modeling approach to household final size distribution was employed to estimate the CPI and SAP for seasonal and pandemic viruses for children and adults separately.

Results

Overall, the estimated CPI for pandemic A/H1N1 was higher in children 0.15 (95% confidence interval, CI: 0.10, 0.21) than adults 0.05 (95% CI: 0.03, 0.11; children vs. adults $p>0.05$) in adults, compared to 0.09 (95% CI: 0.05, 0.14) and 0.05 (95% CI: 0.02, 0.09; children vs. adults $p=0.09$) for seasonal A/H3N2 in children and adults, respectively. The SAP for pandemic A/H1N1 was higher in children (0.23; 95% CI: 0.08, 0.39) than adults (0.06; 95% CI: 0.00, 0.14; children vs. adults $p<0.001$), whereas the SAP for seasonal A/H3N2 was similar in children (0.06; 95% CI: 0.00, 0.21) and adults (0.09; 95% CI: 0.01, 0.19; children vs. adults $p>0.05$). However, the estimated SAP for children in seasonal A/H3N2 increased substantially once individuals with higher baseline antibody titers were excluded resulting in no statistically significant differences in age-specific transmissibility between pandemic A/H1N1 and seasonal A/H3N2.

Conclusions

Our results suggest that pandemic and seasonal influenza A viruses had similar transmissibility in a cohort of initially uninfected households. While unadjusted CPI and SAP estimates were lower for seasonal influenza than for pandemic influenza, once we restricted our analysis to a subgroup of individuals that had low immunity to seasonal influenza, the SAP and CPI were quite similar between seasonal A/H3N2 and pandemic viruses.

TUESDAY 13TH SEPTEMBER 2011

YOUNG SCIENTIST

SPB 6: MATHEMATIC MODELLING

B603O

Latitudinal variation in the seasonality of influenza A and B in China

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Aims

Geographical variations in the seasonality of influenza have been well described globally but remain poorly understood. Recent experimental and epidemiological studies suggest that seasonal variation in specific humidity may affect influenza transmission in inter-pandemic and pandemic periods. Travelling waves of influenza epidemics driven by latitude have been described in tropical areas such as Brazil, while by contrast influenza transmission may be driven by large population centers in more temperate areas. Here we characterize the seasonality patterns of influenza in 30 Chinese provinces spanning the latitudes of +20°N to +46°N and investigate the impact of geographic, climatic, and population factors on influenza seasonality.

Material and Methods

We analyzed weekly laboratory-confirmed reports of influenza positive specimens and percent positive in 30 Chinese provinces from January 2003 to March 2009. Wavelet analyses and Fourier decomposition were used to characterize influenza seasonal patterns by province and virus type (influenza A and B). Seasonal patterns were tested against geographical coordinates, temperature, humidity, precipitation, population size and density, population movements, and socio-economic factors.

Results

Influenza A virus displayed strong winter seasonality in temperate Chinese provinces above 33°N (range of peak timing, December-February), while seasonality of this virus was more diverse in subtropical provinces at lower latitudes (range of peak timing, December-August). By contrast, influenza B virus had winter seasonality in most of China (range of peak timing, December-March), except for the southernmost Hainan province where viral activity peaked in late July. Both influenza A and B displayed latitudinal gradients in seasonal amplitude, duration, and timing of epidemics, with Southern provinces experiencing weaker seasonal amplitude, longer epidemic duration, and later timing of viral activity (correlation between seasonal amplitude and latitude: rho=0.84, $P<0.001$ for influenza B and rho=0.63, $P=0.001$ for influenza A). Interestingly, latitudinal gradients were most pronounced for influenza B, which became increasingly dominant among influenza samples at lower latitudes ($P<0.001$). Latitude was the only factor associated with influenza seasonal patterns in China, and the association remained after adjustment for sample size. Results were robust to the use of influenza positive counts or percent positive.

Conclusion

Our data highlight latitudinal gradients in the seasonal patterns of inter-pandemic influenza across China, reminiscent of the travelling waves of influenza previously reported for Brazil. In contrast to previous studies in Europe and the US, we did not find a clear association between timing of influenza activity and population or environmental factors. To our knowledge, this is the first study to report intriguing differences in the seasonal drivers and prevalence of influenza A and B on a large scale, with influenza B displaying winter seasonality across a broader range of latitudes than influenza A. Further studies are warranted to identify the drivers of influenza seasonality beyond latitude and assess whether differences in evolutionary dynamics, persistence, or migration patterns, could explain the observed seasonality differences between influenza types in China.

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SPB 6: MATHEMATIC MODELLING

B604O

Methods for inferring pandemic transmission patterns within and between locations using Bayesian phylogenetics

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Introduction

The 2009 H1N1 pandemic was first detected in North America in early April 2009, from where it spread and persisted in many regions of the world throughout the course of the pandemic. In this work we investigate the use of Bayesian phylogenetic methods to estimate the number of independent introductions into a specific location, whether viral lineages persist in the same location from one pandemic wave to the next, and how the origin of viral outbreaks changes over the course of the pandemic.

Materials and methods

We applied Bayesian time resolved phylogenetic methods to samples of 300-500 unique whole genome influenza sequences consisting of between one and ten sequences per region (country, state or city) per month. Using isolate location and pandemic wave as viral traits (e.g. USA-1st wave, Canada-2nd wave) in a continuous time Markov process integrated over time-structured phylogenetic trees, the rates of transmission from one location and time period to the next were estimated, and probable source and sink regions identified. In particular we investigate the origins of pandemic influenza imports into and within North America to assess its relative importance to the global spread of influenza versus local transmission between sub-regions within this continent and identify possible transmission routes (e.g. via air travel). A recently described robust counting algorithm was used to infer the number of transitions between different locations along each branch in the tree; enabling an estimation of transmission times into a particular location from any other location to be obtained, whilst incorporating a measure of phylogenetic uncertainty arising from the data.

Results

Initial results show the global pattern of pandemic transmission as a network with strong links between the USA and Europe, as is to expected from air traffic patterns. However, we find less support for the persistence of the same viral lineages between the early and late waves in several locations and that 2nd wave infections were the result of new imports in many cases. Further analyses using all available North American genome sequence data will be carried out to investigate the times of transmission between locations and identify the strongest transmission routes within North America. Additionally, the transmission patterns revealed by the Markov jumps method will help to identify where there have been several introductions of the virus into a single location. This is important when estimating epidemiological parameters from sequence data, as it is assumed that the data represent a single epidemic.

Conclusions

We employ the Markov jumps method to reconstruct the spread of pandemic influenza and demonstrate its useful implications for understanding persistence of viral lineages within local regions and their global transmission patterns.

No conflict of interest

TUESDAY 13TH SEPTEMBER 2011

SPB 6: MATHEMATIC MODELLING

B605O

Controlling for antigenic differences simplifies the interpretation of human serology data

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Introduction

Antigenic change of influenza A and B viruses can be quantified by the antibody titers of sera raised in humans, or other species, through infections and vaccinations. Serum samples can be assayed against different virus strains to assess their cross-reactivity. Typically, several sera are raised in experimental animals, each against a different virus strain. The antigenic evolution of influenza can then be visualised as an antigenic map by transforming these antibody titers using antigenic cartography, but this procedure is only appropriate for first infection sera. Much work has focused on human serological data, but these data have been considered difficult to interpret because of variations in infection history and uncertainty in expected response. However, human sera are more relevant when considering the effects of vaccination or infection in humans. Here we introduce a new method for the visualisation and interpretation of human serology data.

Aim

To investigate whether controlling for the antigenic difference between test viruses resolves irregularities and difficulties in the interpretation of human serology data.

Materials and Methods

Serum samples were collected during an adult vaccine trial conducted in 2002 (vaccine strain - A/H3N2/Panama/2001/1999, n=92). Pre- and post-vaccination sera were measured using the haemagglutination inhibition assay against 28 influenza A/H3N2 viruses isolated between 1993 and 2004. Ferret sera were titrated against the same 28 viruses, and the results used to create a two-dimensional antigenic map using antigenic cartography. The human serum titers against each virus were projected upwards from the location of the virus in the antigenic map. The values between these points were interpolated to create a surface, termed an antibody landscape. Individuals were grouped into four clusters based on their pre-vaccination titres, and antibody landscapes were compared across groups.

Results

Human serum titers were found to be similar to each other when the test antigens were close together in the antigenic map, which justifies the use of interpolation when constructing the antibody landscapes. As expected, the average pre-vaccination landscape was high against older strains (indicating strong immunity), but low against more recent strains (indicating less immunity) which would not have circulated at the time of sample collection. Vaccination increased antibody titers and so the post-vaccination landscape was higher. There was a large degree of variability between the pre-vaccination landscapes of individuals, probably due to their diverse infection histories. However, the post-vaccination landscapes were similar in shape, being much broader than the antibody landscapes seen with single infection ferrets. The increase in titer was higher when the pre-vaccination titer was low, which acted to smooth out an individual's post-vaccination landscape. It was also noted that no statistically significant differences were found in the response between male and female, and those aged above or below 60 years.

Conclusions

The antibody landscapes approach is appropriate for visualising and analysing human serology data. Controlling for antigenic variation substantially simplifies the interpretation of serology, and allows detailed interpretation of both individual- and population-level features of antibody-mediated immunity and the antibody response to vaccination.

WEDNESDAY 14TH SEPTEMBER 2011

SPA 7: LATE BREAKERS

A701O

Live cell fluorescence imaging of influenza A virus RNA polymerase and ribonucleoproteins using replication-competent virus encoding tagged PB2 polymerase subunit

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Introduction

State-of-the-art live cell microscopy provides unprecedented insights into events occuring in the living cell. However, application of these approaches to influenza virus proteins and ribnucleoproteins (RNPs) is currently limited by the lack of a versatile method enabling their visualization in single infected cells. This is due to the difficulty of encoding fluorescent fusion proteins within the viral genome. Here, we report the production of the first unimpaired recombinant influenza virus which allows expression of individually fluorescent PB2 polymerase subunits in infected cells. The virus was used to probe intracellular dynamics of viral polymerase and to study the trafficking of vRNPs during the course of an infection.

Material & methods

Recombinant A/WSN/33/H1N1 influenza virus encoding tagged PB2 subunit was produced by reverse genetics and characterized by conventional molecular biological techniques (RT-PCR, sequencing etc) and by plaque assay on MDCK cells; activity of the RNPs containing tagged PB2 was determined by CAT-ELISA assay. Confocal microscopy techniques (time lapse imaging, fluorescence recovery after photobleaching, single particle tracking) and fluorescence correlation spectroscopy (FCS) were used to image and characterize the dynamics of PB2 in live HEK293T and Vero cells infected with the recombinant or the wt A/WSN/33/H1N1 virus.

Results

The produced recombinant virus allowed expression of GFP labelled PB2 polymerase subunits in infected cells. The virus was not attenuated and the reconstituted RNPs preserved transcription/replication activity. The signal of the fluorescent tag was specific and mostly co-localized with PB2 detected by immunostaining. In infected cells, the nuclear export of the fluorescent PB2 was dependent on CRM1 pathway which is used by influenza vRNPs; in the cytoplasm, the fluorescent PB2 co-localized with vRNPs (stained with a specific antibody); these observations indicated that the fluorescent PB2 is incorporated into vRNPs. The intranuclear dynamics of viral RNA polymerase was probed by FCS in the nuclei of the infected cells. It was observed that the mobility of viral polymerase was significantly restricted in the infection context, as compared to a transient expression system, indicating interactions with slowly moving partners. Furthermore, vRNP trafficking was characterized using the recombinant virus. Following nuclear export and a transient pericentriolar accumulation, viral RNPs appeared in the cytoplasm as particles which showed slow (>0.25 µm/s) non-directional and intermittent rapid (~1 µm/s) directional movements. Some of the directional movements overlapped with microtubules, however, upon disruption of the microtubule network, rapid directional movements still sporadically occurred, indicating involvement of mechanisms other than microtubules for vRNP transport.

Conclusions

Our data establish the potential of the recombinant viruses for visualization of viral proteins during a quasi wild-type infection. This new virus, or adaptations of it, will be of use in elucidating many aspects of influenza virus host cell interactions as well as in screening for new anti-viral compounds.

WEDNESDAY 14TH SEPTEMBER 2011

SPA 7: LATE BREAKERS

A702O

Influenza virus contamination of common household surfaces and its role in household transmission, Bangkok, Thailand

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Background

The relative contribution of surface contamination in household transmission of influenza viruses is unclear. A complex interaction of environmental (e.g. humidity) and host factors may influence influenza transmission. We conducted a study of influenza virus surface contamination in households with an influenza infected child to measure the prevalence of contamination, the effect of hand washing, the association with household humidity and effect on transmission.

Methods

As part of a prospective randomized trial to assess the effect of non-pharmaceutical interventions on household transmission of influenza, we enrolled a subset of households with children who had laboratory-confirmed influenza virus in Bangkok, Thailand. These households had been randomly assigned to hand washing (HW) or control arms. Enrollment occurred over two influenza seasons: 19 June-13 August, 2009 and 25 June-12 November, 2010. The HW arm received hand washing education and liquid soap. We swabbed six common household surfaces and fingertips of index patients and symptomatic household members on day 3 after enrollment in 2009 and day 1, 3 and 7 after enrollment in 2010. Specimens were tested by real-time reverse transcriptase polymerase chain reaction and positives were cultured for live virus. A handheld psychrometer was used to measure dew point to evaluate absolute humidity of enrolled households.

Results

We enrolled 191 households (90 in 2009 and 101 in 2010). Overall, 24 (13%) of surfaces and 38 (20%) of fingers swabbed were positive for influenza viruses. One finger swab grew live virus. There was no significant difference between HW and control arm in the median age of the index case (6.3 years in HW vs. 6.5 years in control arm, p=0.72) or presence of secondary influenza infection in the household on day 3(37% in HW vs. 29% in control arm, p=0.26). Reported hand washing frequency per day was significantly higher in HW arm (3.6 vs. 2.8 times/day in control arm, p=0.006).

In 101 households enrolled in 2010, 26 (26%), 23 (23%) and 3 (3%) fingers of the index children were positive for influenza on days 1, 3 and 7, respectively. The prevalence of influenza virus on fingers on any day was similar in the HW (44%) and control arm (41%) (Chi-square p=0.77). On days 1, 3 and 7, 9 (9%), 8 (8%) and 4 (4%) households had contamination of at least one surface; no specimens grew live virus. Across all 3 days, the prevalence of surface contamination in the HW (18%) was not significantly different from the control arm (20%) (Fishers p=1.00). In a joint analysis of households enrolled in 2009 and 2010, the prevalence of influenza virus on the fingers of the index children on day 3 was similar in the HW (22%) and control arm (18%) (Chi-square=0.45). The HW households had a significantly lower prevalence of surface contamination on day 3 (7/95, 7%) than control households (17/96, 18%); prevalence risk difference (PRD) 10 % (p=0.03). The secondary influenza attack rate in the HW (52%) exceeded that in the control arms (37%) but the difference was not statistically significant. Households with a dew point below average had significantly higher prevalence of surface contamination (18/94, 19%) than those with a dew point above average (6/97, 6%); PRD 13% (p=0.007).

Conclusion

Influenza virus particles can persist on fingertips of infected children and surfaces in their households up to at least a week. Only one fingertip specimen was able to grow in culture. Low absolute humidity was associated with an increase in influenza surface contamination. Hand washing reduced surface contamination without reducing secondary influenza in the household. Influenza transmission through contact with contaminated surfaces may not be an important route of household influenza transmission in

WEDNESDAY 14TH SEPTEMBER 2011

YOUNG SCIENTIST

SPB 7: IMMUNOLOGY

B701O

Structure of a broadly neutralizing antibody derived from sorted single human B cells that occludes the receptor-binding pocket of H1N1 hemagglutinin

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Introduction

Influenza vaccines elicit neutralizing antibodies targeting the principal protective antigen, hemagglutinin (HA). Current vaccines confer limited protection against antigenic variants, because antibodies to variable epitopes dominate the immune response. Rare antibodies with broad specificity do occur in response to vaccination or to infection and have been isolated from phage-displayed libraries in the past. A novel method to isolate antibodies to HA from the B-cell repertoire of a vaccinated individual allowed us to isolate broadly neutralizing antibodies. We expect that structural studies of this antibody and other antibodies recovered using this method will provide valuable information to guide design of immunogens for use as more effective influenza vaccines.

Materials & methods

Immunoglobulin genes from B cells of a subject immunized with killed influenza vaccine Fluzone® 2007-2008 were cloned and sequenced as previously reported (1), and used to identify an antibody (Ab0082) that neutralizes all tested H1N1 strains, including the 2009 pandemic strain A/California/04/2009. This antibody was expressed as an Fab and bound to HA of A/Solomon Islands/03/2006. Size exclusion chromatography and X-ray crystallography were used to characterize the antibody:antigen complex and to determine its structure.

Results

Ab0082 forms the expected 3:1 complex with HA of A/Solomon Island/03/2006. The crystal structure of this complex reveals the molecular basis for this antibody's apparent breadth. The antibody, contacting exclusively the head domain of HA, extends its long CDR-H3 loop into the binding pocket for sialic acid, the cellular receptor. The CDR-H3 loop of Ab0082 mimics the chemical structure of sialic acid.

Conclusions

A broadly neutralizing antibody (Ab0082) derived from a human B cell occludes the receptor-binding pocket of H1N1 HA. We suggest that targeting the receptor-binding pocket is an under-appreciated means to evoke an immune response resistant to antigenic variation; that Ab0082 is representative of desirable class of antibody; and that Ab0082 is a potential target for structure-based immunogen design.

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WEDNESDAY 14TH SEPTEMBER 2011

YOUNG SCIENTIST

SPB 7: IMMUNOLOGY

B702O

Cross-protective immunity to influenza A(H1N1) 2009 virus induced by seasonal A(H3N2) virus is mediated by virus specific T cells

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Introduction

Influenza A viruses (IAV) are a major cause of respiratory tract infections and cause excess morbidity and mortality every year. Recently, H1N1 viruses caused the first pandemic of the 21st century, which started in Mexico in March 2009. The 2009 pandemic was considered mild and caused a limited number of fatal cases. In the absence of antibodies to the pandemic strain, heterosubtypic immunity induced by infection with influenza A viruses of unrelated subtypes may have afforded protection.

In the present study we investigated whether infection of mice with an influenza A virus of the H3N2 subtype, could afford protection against infection with a pandemic 2009 A(H1N1) virus (pH1N1). To determine which components of the immune system contribute to protective immunity, we adoptively transferred, B cells, sera, CD8+ and CD4+ T cells, CD8+ T cells or CD4+ T cells isolated from A/H3N2 virus infected donor mice to naïve recipients and challenged them with pH1N1 virus.

Materials and Methods

C57BL/6 J mice were infected intranasally with influenza virus A/Hong Kong/2/68 (A/H3N2) or were mock-infected with PBS. Four weeks later, mice were infected with 2009 pH1N1 influenza virus A/Netherlands/602/09. Lung virus titers were determined four and 7 days post challenge infection. The frequency of virus specific CD8+ T cells in spleens and lungs were assessed 7 days post infection.

For adoptive transfer experiments splenocytes were collected four weeks after infection with A/H3N2 virus or mock-infection and used for the isolation of B cells, T cells, CD4+ T cells and CD8+ T cells using commercially available lymphocyte isolation kits.

Four hours after adoptive transfer of serum, B cells, T cells, CD4+ or CD8+ T cells, recipient mice were infected were infected pH1N1 virus. Seven days post challenge infection, lung virus titers, histopathological changes in the lung and T cell responses were assessed.

Results

Mice primed by infection with an A/H3N2 virus were protected from pH1N1 challenge infection and displayed less body weight compared to un-primed, mock-infected animals and had significantly reduced lung virus titers 7 days post infection. Antibodies induced by infection with A/H3N2 virus did not cross-react with pH1N1 virus in HI and VN assays.

To determine which subpopulation of lymphocytes provided protection, B cells, total T cells, CD4+ T cells or CD8+ T cells from A/H3N2-primed and un-primed mice were transferred to naïve recipient mice, which were challenged with pH1N1 virus four hours later. The transfer of post-infection T cells afforded protection against the challenge infection. Loss of bodyweight was prevented and virus replication and hisotpathological changes were undetectable 7 days post infection. The adoptive transfer of post infection CD8+ T cells only provided partial protection. These mice displayed reduced weight loss and histopathological changes although lung virus remained high.

Discussion

Mice that experience an infection with a seasonal A/H3N2 influenza virus developed heterosubtypic immunity against 2009 pandemic H1N1 virus. Virus-specific CD8+ T lymphocytes in concert with virus-specific CD4+ T cells afforded robust protection against challenge infection with pH1N1 virus. Thus the induction of virus-specific cell mediated immunity may be a feasible venue for the development of a universal influenza A vaccine.

WEDNESDAY 14TH SEPTEMBER 2011

SPB 7: IMMUNOLOGY

B704O

Characterization of cd4-cd8- double negative T cells in different lung compartments after influenza infection

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Introduction

Regulatory T cells (Treg) have been shown to be involved in regulating immune responses in auto-immune disease, transplant rejection and tumor immunity. CD4-CD8- double negative (DN) T cells are a subset of Treg cells that develop along unique pathways and that suppress T-cell responses in an antigen specific manner, such as during antibacterial immunity to *Francisella tularensis*.

Aim

As the importance of DN T cells in influenza is currently unknown, the aim of this study was to characterize the phenotype, location, origin and role of these DN T cells during Influenza infection

Materials en methods

C57Bl/6 mice (8w) were infected intranasally with $10^{\wedge 5}$ $TCID_{50}$ H_3N_2 influenza virus X-31. Analysis was done at at various days post infection (dpi). Lungs were snap-frozen in OCT for histology. Lung tissue, mediastinal lymphnode (MLN) and broncho-alveolar lavage fluid (BALf) were analysed by multiparameter flow cytometry.

Results

The number of DN T cells was significantly higher in infected mice at 8dpi and these enhanced numbers were maintained at 32dpi in lung, MLN and BALf. DN T cells were not found in the iBALT (inducible Bronchus Associated Lymphoid Tissue) structures that are typically formed 17 days after infuenza infection and are important for local IgA synthesis.

Two subsets of DN T cells can be described: αβ TCR^+ and γδ TCR^+ DN T cells. Upon activation, conventional T cells can internalize CD4 and CD8, also leading to a DN phenotype. This is not the case for the γδ TCR^+ DN T cells. In lung and MLN, 50% of the αβ TCR^+ DN T cells were revertants from classical CD8 T cells, as 90% of these had intracelullar CD8 chains. In contrast, none of the αβ TCR^+ DN T cells accessible by BALf were revertants, making it likely that antigen presentation by epithelial cells was insufficient to generate DN T cells. In the lung, DN T cells were MHC I restricted, $CD28^-$, $CD27^+$, $CD127^+$ and were able to produce IL-17 and IFN-γ after *in vitro* stimulation.

Conclusion

DN T cells are a T cell subset that is often neglected while evaluating immune responses against influenza virus infection, and could represent a subset of effector or Treg population that was activated in a unique manner. Specific DN-T cell depletion and adoptive transfer studies are underway to further understand the potential of these cells in immunopathogenesis of influenza and application to vaccine design.

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TUESDAY 13TH OF SEPTEMBER 2011

SPI 5: WILL THERE BE ANOTHER FLU PANDEMIC? SHOULD WE BE PREPARED?

I101O

Did pandemic preparedness activities aid the response to influenza A/H1N1 pandemic in 2009? A study in seven randomly selected countries from the WHO European Region.

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Introduction
Although the 2009-10 influenza A(H1N1) pandemic was of low severity compared with those of the 20th century, this was the first chance for Member States to implement a real-life pandemic response, drawing on plans made and planning activities undertaken in the preceding few years, notably from 2004 onwards. The aim of the project was to review the extent to which those plans and planning activities proved useful; and to identify areas of pandemic planning that require further strengthening.

Methods
We randomly selected seven Member States within the WHO European Region to participate in a comprehensive qualitative study to evaluate the pandemic planning activities (PPA) undertaken before March 2009, in relation to the subsequent pandemic response mounted from May 2009 onwards. WHO expert teams visited each country and interviewed stakeholders from health and civil response ministries, national public health authorities, regional authorities, family doctors and hospital physicians using a 'whole of system' approach from frontline services to central government.

Results
Using content analysis, we identified six consistent major themes, which were essential elements of successful PPA: communication; coordination; capacity; adaptability/flexibility; leadership; and mutual support. PPA had generally been successful, with multi-sectoral involvement, political support and dedicated funding emerging as important success factors. However, in future PPA, greater emphasis still needs to be placed on these areas, as well as improving planning for: communications (with the public and health professional end-users); vaccine procurement and logistics; flexibility of response; use of diagnostic tests; and real-time surveillance.

Conclusions
PPA were successfully undertaken in the WHO European Region prior to the 2009 pandemic. These activities proved to be effective and were generally appropriate for the response made in 2009. Nevertheless, consistent themes also emerged regarding specific areas of ''under-planning" common to most countries surveyed. These should now be rectified in the post-pandemic recovery phase.

POSTER PRESENTATIONS

SPA 1: CLINICAL IMPACT & DIAGNOSTIC APPROACHES

A101O

Burden of influenza-related hospitalisations and emergency room visits in children under 15 years of age in Spain

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Introduction

Data on the burden of laboratory-confirmed influenza in children and secondary transmission within household members of children with influenza are lacking in many countries in Europe. Such data will complement development of appropriate national vaccination policies for children. The purpose of this study was to quantify burden of laboratory-confirmed influenza for hospitalisations and emergency room (ER) visits among children.

Methods

In a single hospital in Spain during 2008–2009, all children aged <15 years hospitalised or attending the ER with acute respiratory tract infection (ARI) and/or isolated fever (oral temperature ≥37.5°C) and whose parents consented to their participation were tested for influenza by culture, RT-PCR and a rapid diagnostic test kit. Study data including clinical features, severity, complications, risk factors and socioeconomic impact of influenza in children were collected at enrollment and by telephone interview of the parent/guardian 21–30 days later. Data were stratified into the following age groups: <6 months, 6–23 months, 24–59 months and ≥5 years. Influenza-positive subjects were defined as those with at least one laboratory test positive for influenza.

Results

500 out of 12,169 children hospitalised or presenting at ER with fever and/or ARI received parental consent to participate and were enrolled in the study, of whom 477 were included in the according-to-protocol cohort (median age 41.0 [range 1–179] months; 54.3% male). Only three children had been vaccinated for the current influenza season (2008–9). Of the 477 children presenting with ARI/isolated fever, 32.3% (95% CI: 28.1–36.7) were influenza-positive (median age 55.0 [1–179] months for influenza-positive subjects and 32.0 [1–174] months for influenza-negative subjects). Of the influenza-positive children, 52.3% and 47.7% had influenza A and B, respectively. Positive agreement for the detection of influenza was 45.7%, 51.5%, and 98% for the rapid test/RT-PCR, rapid test/culture and RT-PCR/culture comparisons, respectively. Negative agreement for the same comparisons was 96.1%, 93.1%, 88.6%, respectively. Overall, 6.1% of children had pre-existing medical conditions, with the most frequent being chronic pneumopathy (2.1%). 12.8% experienced complications, mainly acute otitis media (AOM; 8.2%). 85.1% of influenza-positive children had sudden onset ARI/isolated fever vs 76.5% of influenza-negative. Influenza-positive children also presented with more sore throats (14.9% vs 7.1%), muscle pain (12.3% vs 4.6%) and fever (89.0% vs 66.3%), compared with influenza-negative children. Children with influenza B were significantly older than children with influenza A (mean age 77.5 vs 48.3 months, respectively), and presented more often with sudden onset ARI/fever (91.8% vs 78.8%). Influenza B was associated with increased sore throat (21.9% vs 8.8%), headache (19.2% vs 3.8%) and arthralgia (6.8% vs 0%), and decreased AOM (2.7% vs 16.3%), compared with influenza A. Influenza A was associated with more complications overall (20% vs 2.7%). School absenteeism was higher amongst influenza-positive, compared with influenza-negative children (82.8% vs 59.4%, respectively). Similarly, more caregivers of influenza-positive children missed days off work (39.7% vs 22.6%). 87.5% of influenza-positive children were aged <5 years and attended day-care, compared with 66.5% for influenza-negative. All children were prescribed ARI/fever-related medications during their hospitalisation or ER visit, with antipyretics prescribed most frequently (85.7% influenza-positive vs 63.8% influenza-negative).

Conclusions

This study showed that influenza has a considerable impact on children aged <15 years. Unusually, disease due to influenza B was more severe than disease due to influenza A. Additionally, influenza B was as prevalent as influenza A in children with acute respiratory infections. AOM was the most frequent complication following influenza infection in children. All available effective preventive measures for childhood influenza should be considered.

SPA 1: CLINICAL IMPACT & DIAGNOSTIC APPROACHES

A102P

Clinical symptoms of pandemic influenza H1N1 2009 and effects of neuraminidase inhibitors for university students

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Introduction

In April 2009 the United States and Mexico first confirmed an outbreak of influenza, which was caused by the pandemic H1N1 2009 influenza virus. The virus spread all over the world. In June, the World Health Organization announced that the status of the influenza epidemic was at alert Phase 6: a pandemic of influenza. Influenza spread rapidly throughout Japan. At Kagawa University, the Health Center engaged in consultation and surveillance of influenza in the students and staff. It was determined that patients diagnosed with pandemic influenza should phone the Health Center and report their attribution, date of onset, laboratory test results, symptoms, and prescription. We report the Clinical symptoms of pandemic influenza H1N1 2009 and effects of neuraminidase inhibitors for university students.

Methods

From 12 August 2009 to 30 March 2010, 644 undergraduate and 27 graduate students (368 male, 134 female, average age ± SD = 20.4 ± 2.0) were diagnosed as pandemic influenza H1N1 2009 by flu test kit in Kagawa University. Statistical analysis: Student's t test and ANOVA were performed. The level of significance was $P<0.05$.

Results

Results of flu test kits revealed that 11.3% (644/5,713) undergraduate and 3.3% (27/817) graduate students at the university were positive for the virus. For the pandemic influenza cases, 78.7% of patients had a fever greater than 38°C; 82.4% had a cough; 67.1% had general fatigue; 59.0% had a sore throat; 58.6% had rhinorrhea; and 58.3% had a headache. The duration until defervescence was 2.0 ± 1.3 days in the oseltamivir-treated patients (N = 339) and 2.1 ± 1.0 days in the zanamivir-treated patients (N = 142). The duration of fever was 2.6 ± 1.5 days in the patients treated with oseltamivir on the date of onset and 2.3 ± 1.0 days in the patients treated with zanamivir on the date of onset. The duration of fever was 3.1 ± 1.1 days in the patients treated with oseltamivir on the first day after onset and 3.1 ± 1.0 days in the patients treated with zanamivir on the first day after onset. The duration of fever was 3.6 ± 1.2 days in the patients treated with oseltamivir on the second day after onset and 3.8 ± 1.1 days in the patients treated with zanamivir on the second day after onset.

Conclusions

It was supported the later intake of the neuraminidase inhibitor, the longer the duration of fever. Duration from date of first administration until defervescence was more than five days in 4.5% oseltamivir-treated patients. A duration more than five days was not observed in the zanamivir-treated patients.

SPA 1: CLINICAL IMPACT & DIAGNOSTIC APPROACHES

A103P

Virological diagnosis of flu in the French army: new perspectives

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Introduction

The analytical performance and practicability in routine use of the Clart® Pneumovir (Genomica, Spain) and Xpert® Flu (Cepheid, USA) tests were compared with those of real-time RT-PCR for the virological diagnosis of influenza A infection in the laboratories of the French army.

Patients and methods

The evaluation was carried out during the 2009/2010 flu season, on rhinopharyngeal samples from soldiers presenting acute respiratory tract infection. The first 100 patients were included, with a male/female ratio of 3 and a mean age of 24 years. For each sample, we carried out: (1) real-time RT-PCR for the detection of influenza A (M, H and N genes). This technique is the gold standard; (2) simultaneous detection of 17 respiratory viruses, including influenza A, by PCR amplification coupled with DNA microarray detection (Clart® Pneumovir test) and (3) a totally automated multiplex RT-PCR, with single-use cartridges, distinguishing between the A/H3N2 and seasonal A/H1N1 and A/H1N1 2009 viruses (Xpert® Flu test).

Results

Analytical performance: RT-PCR detected 53 cases of influenza A infection (52 H1N1 2009 and 1 seasonal H1N1). The sensitivity of the Clart® Pneumovir test with respect to RT-PCR was 49% for influenza A, whereas that of Xpert® Flu was 92.5%. This lower sensitivity was observed for low viral loads. For discordant tests, the mean threshold values (CT) for RT-PCR were significantly higher for negative Xpert® Flu tests than for negative Clart® Pneumovir tests (28.6 vs 24.3, Wilcoxon W= 88, p = 0.03).

Practicability: For Xpert® Flu, cartridge preparation took 5 minutes and the reaction took 75 minutes. For RT-PCR, the manipulations took 20 minutes and the automated analysis took 1 hour 50 minutes. For Clart® Pneumovir, the test took a long time (at least 6 hours) and required a large number of manipulations: extraction of nucleic acid, preparation of the PCR mixture, preparation of washing buffers and conjugate, washing of the microarrays and the hybridisation reaction. Sophisticated machinery was also required: thermocycler, thermomixer, microarray reader.

Reagent costs: The costs were €60 for Clart® Pneumovir, €45 for Xpert® Flu and €23 for real-time RT-PCR (automated extraction and mixture of 4 viruses: generic influenza A, H3N2, seasonal H1N1 and H1N1 2009).

Conclusions

RT-PCR remains the technique of choice for the diagnosis of influenza A, because it combines high sensitivity and specificity with moderate reagent costs. Nevertheless, it requires trained personnel, it takes time and it is not very adaptable to conditions in the field, particularly outside the opening hours of the laboratory. The Xpert® Flu test overcomes these constraints through its ease of use and, despite a slightly lower sensitivity and high reagent costs, may represent a future solution, particularly during external military operations. The Clart® Pneumovir test is not a viable alternative to RT-PCR for the diagnosis of flu, due to its low sensitivity and the high costs of the reagents required (€60 per test).

SPA 1: CLINICAL IMPACT & DIAGNOSTIC APPROACHES

A104P

Diagnosis of acute respiratory virus infections in a military community: the contribution of PCR coupled with DNA microarray detection.

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Introduction

We compared the performance of the Clart ® Pneumovir (Genomica, Spain) test with that of RT-PCR for in the diagnosis of influenza and rhinovirus infections in young adults. This test, which is based on PCR amplification coupled with DNA microarray detection, allows the simultaneous detection of 17 respiratory viruses.

Patients and methods

This evaluation was carried out during the 2009/2010 flu season in three military units in South West France. Nasopharyngeal swabs from subjects with acute respiratory infection were used for: (1) real-time RT-PCR detection of influenza A virus (the M, H and N genes), influenza B virus and rhinovirus; (2) the simultaneous detection of 17 respiratory viruses with the Clart ® Pneumovir test.

Results

The study began at the start of August 2009 and ended with inclusion of the 100th patient in the last week of 2009. The sex ratio (m/f) was 3 and the mean age of the patients was 24 years. RT-PCR identified 53 cases of influenza A infection (52 of H1N1 2009 and a 1 of seasonal H1N1), 1 case of influenza B, 32 cases of rhinovirus and two cases of enterovirus infection. The sensitivity of the test Clart ® Pneumovir with respect to RT-PCR was 49% for influenza A and 72% for rhinovirus. In isolated infections, fever (> 38°C) occurred in 71% of subjects with flu and 58% of subjects with rhinovirus infection (chi^2 = 0.32, p = 0.57, NS) and was not a useful criterion for diagnosis. Influenza-rhinovirus co-infections are not uncommon (n = 14) and the viral load for influenza (mean threshold value = 22.8 CT) was not significantly lower than that in single infections (21.6 CT, Wilcoxon W: 317, p value = 0.33). In our population, the flu epidemic was not preceded by an outbreak of rhinovirus infections as described elsewhere.
The other viruses identified were: parainfluenza 1 (4 cases), parainfluenza 2 (1 case), RSV (A and B; 1 case each), adenovirus (1 case) and bocavirus (1 case).

Conclusions

The Clart Pneumovir ® test is not a valid alternative to RT-PCR for the diagnosis of influenza and rhinovirus infections, because of its low sensitivity and the high cost of reagents (€60 per test).

SPA 1: CLINICAL IMPACT & DIAGNOSTIC APPROACHES

A105P

Statistical estimates of hospital admissions attributable to seasonal and pandemic influenza for Canada

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Introduction

The number of admissions to hospital for which influenza is laboratory confirmed is considered to be a substantial underestimate of the true number of admissions due to an influenza infection. During the 2009 pandemic, priority was given to full case ascertainment and laboratory confirmation of all patients admitted to hospital with an H1N1/2009 infection in Canada, though the ascertainment rate remains uncertain.

Material & methods

The discharge abstracts of persons admitted with any respiratory condition mentioned were extracted from the Canadian Discharge Abstract Database (DAD), maintained by the Canadian Institute of Health Information, for April 2003 to March 2010. The DAD covers approximately 80% of hospital separations in Canada. All diagnostic fields were searched to identify patients with the influenza virus identified (ICD-10 code J10, and J09 during the pandemic period), as a proxy for laboratory confirmation. Stratified, weekly admissions were modelled as a function of viral activity, seasonality, and trend using Poisson regression models similar to previously published estimates of the influenza burden in Canada. The ICD-10 category of J12.1 was used as a proxy variable for the level of respiratory syncytial virus (RSV) activity and the weekly number of J05 (croup) admissions in infants and children under the age of 3 years and without any mention of influenza of were used as a proxy variable for parainfluenza-1 (PIV-1), as it has been shown to be important to include these two viruses when modelling respiratory admissions for children. Where sufficient statistical power was available, separate multipliers (influenza-attributed/influenza confirmed admissions) were estimated for each season and for the fall and spring wave.

Results

Results are preliminary. The number of hospital admissions attributable to influenza varied considerably from year to year. For seasonal influenza, 1 out of 10 admissions attributable to influenza were coded to J10 (influenza virus identified) as the diagnostic condition most responsible for the admission and the influenza virus was indentified in 1 out of every 6.4 admissions attributable to influenza. During the 2009 pandemic period, the ascertainment rate improved substantially with corresponding figures of 1 out of 2.4 and 1 out 1.6 admissions (95% CI: 1.5-1.7) respectively. During the pandemic period only 17% of admissions attributed to the pandemic did not have any mention of influenza. Compared to previous H1N1 seasons, the influenza-attributed hospitalization rate for persons under the age of 65 years was approximately 6 times higher during the 2009 H1N1 pandemic. Around age 65 the rate ratio decreased sharply. Prior to the pandemic period, the J11 code (influenza, without virus identification) was infrequently used, while during the pandemic, the J11 code captured most of the admissions without pneumonia that were attributable to influenza.

Conclusions

Case ascertainment was much improved during the pandemic period. Most of the discharge abstracts for admissions attributable to H1N1/2009 but without any mention of influenza had a diagnosis of pneumonia. Rate ratios comparing H1N1/2009 to previous H1N1 seasons are consistent with some protection associated with the unusually severe H1N1 strain that circulated in Canada in 1951.

SPA 1: CLINICAL IMPACT & DIAGNOSTIC APPROACHES

A106P

Prevalence of influenza C virus in hospitalized and outbreak settings

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Background

The prevalence of influenza C in hospitalized patients and suspected respiratory outbreaks of viral etiology in Canada is not known with any degree of certainty.

Aim

We have developed a RT-PCR with an analytical sensitivity of between 7 to 10 genome copies per reaction which will be applied to detect this virus in a selected sample of convenience. These samples have previously been extensively tested to rule out other viral respiratory agents through a combination of in-house and commercially available molecular assays. The sample panel will comprise of at least 500 unique patient samples selected during the 2010 to 2011 respiratory season from the following categories, (a) children less than 10 years of age who are either hospitalized or seen in the Emergency Department (b) respiratory outbreaks of suspected viral etiology.

Results & Conclusions

Results and data interpretation from the pilot project will be completed during July and August 2011 and will be available for presentation at this meeting.

SPA 1: CLINICAL IMPACT & DIAGNOSTIC APPROACHES

A107P

The influenza caused by new virus A/H1N1/CA/2009 in patients in the general medical practice.

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Early diagnosis of the influenza caused by new virus A/H1N1/CA/2009 (INA) is necessary for the correct treatment of patient.

The purpose

to study the special features of INA in patients in the general medical practice.

The material and the methods

The analysis of 44 dispensary documents of patients was carried out, who turned themselves into the polyclinic during the epidemic lift of morbidity INA from October to December 2009 because of acute respiratory virus infection (ARVI). All patients were inspected by polymerase chain reaction (PCR) to the presence RNA of virus INA, seasonal influenza (ISA) and influenza B (IB) in the nasopharyngeal mucous. The patients were investigated by the clinical analysis of the blood, the analysis of urine, the chest roentgenography. According to the result PCR there were the patients with INA (21 people) and ARVI (23 people). The frequency of the subjective and objective signs of disease, acute diseases of recent 12 months and chronic diseases was evaluated.

The results

The patients INA (1) turned into the polyclinic during 1-9 days of disease, and ARVI (2) - 1-16 days. The duration of disease was from 6 to 21 days (1) and from 6 to 27 days (2). In resent 12 months the acute respiratory diseases were in 76,2% people INA and 26,1% people ARVI (OR=6,01; 95%CI 1,35-28,5; p<0,01). The leading subjective symptoms in sick INA were cough (90%), headache (80%), weakness (60%), pain in muscles (50%), and in sick ARVI - weakness (80%), headache (70%), sore throat (60%) and cough (50%). The temperature of the body higher than 37°C in sick INA was noted in 100% of cases, and ARVI - 87% of cases, and above 38°C - 28,6% (1) and 21,5% (2). Compared with ARVI in sick INA more frequent were noted the obstruction of nose (47,2% and 17,4%, p<0,001), hyperemia of throat (95,2% and 87,0%), a change in respiratory noise (52,4% and 30,4%, p<0,001), dry wheezes (14,3% and 0,0%). Complications with INA were revealed in 28,6% (1) and with ARVI – in 13,0% (2) (OR=2,68, 95% CI 2,12-3,40; p<0,001). In the analysis of the blood for INA in comparison with ARVI there was significantly more often the acceleration SE (61,1% and 31,6%), neutropenia (44,4% and 21,1%), lymphocytosis (44,4% and 31,6%), monocytosis (33,3% and 10,5%), leucopenia (16,7% and 5,3%) and thrombocytopenia (16,7% and 10,5%). Microproteinuria was revealed in 54,5% of INA and 40,0% of ARVI cases.
Conclusion INA has characteristic clinical features and changes in the analysis of the blood, which have diagnostic and prognostic significance.

SPA 1: CLINICAL IMPACT & DIAGNOSTIC APPROACHES

A108P

Clinical Features and Duration of Virus Shedding of Children and Adults with Influenza A/H1N1 (2009) Infection in Austria

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Background

Only a limited number of studies are available comparing clinical and virological features (like the duration of RNA detectability and the duration of shedding infectious virus particles) of children with confirmed Influenza A/H1N1 (2009) infection to those of infected adults.

Method

Retrospective analysis of medical charts (patient's characteristic, symptoms, comorbidities, duration of hospital stay, complications, and therapy) and virological data (cycle threshold value of the initially performed diagnostic PCR) from 375 patients (146 children ≤ 18 years and 229 adults) with confirmed influenza A/H1N1 (2009) virus infection. In addition: determination of the duration of RNA detectability as well as the duration of shedding of infectious virus particles in 161 patients (25 children, 136 adults) receiving antiviral therapy.

Results

The median age of the patients was 25.5 years. Children had significantly higher body temperatures ($p < 0.001$) than adults and suffered more frequently of rhinitis ($p < 0.001$), abdominal pain (p 0.047) and emesis ($p < 0.001$). In contrast, adults complained more often about arthralgia/myalgia ($p < 0.001$) and chephalea (p 0.012). In our group of patients underlying conditions were more common in adults ($p < 0.001$), but in children pandemic influenza A/H1N1 (2009) infection were significantly more often associated with complications like otitis media or fever convulsions (p 0.011). Children were significantly longer hospitalized than adults (p 0.048).

Symptoms were significantly shorter in patients receiving antiviral therapy compared to the untreated group (1.94 versus 4.20 days; p 0.001). When antiviral treatment was started within 24h of symptom onset, patients had a 2.8 days shorter duration of symptoms than non-treated patients with no significant differences between paediatric patients and adults.

The analysis of the duration of viral RNA detectability in patients receiving antiviral therapy within 48 hours after symptom onset revealed that almost 80% of patients had a negative PCR result at day 3 after initiation therapy. Nevertheless, in some patients RNA remained detectable for up to 10 days. Although infants had significantly higher viral loads than adults (p 0.010) at the initial diagnostic PCR, no difference in the duration of RNA detectability between children and adults was found. As far as the analysis of the duration of shedding of infectious virus particles is concerned, 95.8% of the patients under treatment had negative results within the first 2 days with no difference in the duration of shedding of infectious virus between children and adults.

Conclusions

Although both, children and adults, with an influenza A/H1N1(2009) infection had symptoms characteristic for influenza like illness, our data demonstrate, that children can also show a different clinical picture than adults (abdominal pain, emesis and rhinitis). In addition we found, that paediatric patients were significantly longer hospitalized. Our data confirm previous findings reporting that children are more affected from an influenza A/H1N1(2009) infection than adults.
Concerning the duration of RNA detectability and shedding of infectious virus particles, we showed, that in patient receiving antiviral treatment within 48 hours after symptom onset RNA can be detected in some patients for up to 10 days, whereas the majority of patients under therapy do not shed infectious virus particles for longer than two days. This finding may be of relevance for the clinical handling of patients.

YOUNG SCIENTIST

SPA 1: CLINICAL IMPACT & DIAGNOSTIC APPROACHES

A109P

Signs and Symptoms Predicting Influenza in Children: A Matched Case-Control Analysis of Prospectively Collected Clinical Data

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Introduction

Distinguishing between influenza and other viral respiratory infections on clinical grounds alone poses a major challenge to clinicians, and the clinical diagnosis of influenza is particularly difficult in young children. Identification of signs and symptoms that predict influenza infection would be of great importance especially in settings where adequate diagnostic methods are not easily available. Early recognition of influenza would enable the optimal use of influenza-specific antiviral drugs and help avoid unnecessary use of antibiotics. Although the vast majority of pediatric patients who seek medical care during any influenza outbreak are outpatients, no previous study has assessed the predictive value of various signs and symptoms in different age groups of such children.

Materials and Methods The clinical data for this case-control analysis were derived from a prospective cohort study of children's respiratory infections conducted during two consecutive winter seasons of 2000-2002 in Turku, Finland. Before the start of each respiratory season, children <13 years of age were recruited into the follow-up cohorts without the use of any exclusion criteria. The parents of the children were asked to bring their child to a specific study clinic every time the child had fever or signs of respiratory infection. During each visit, a nasal swab was obtained for viral detection, and a study physician examined the child and recorded his/her signs and symptoms on a structured form.

The cases consisted of all 353 children with culture-confirmed influenza from whom adequate information about the signs and symptoms was available. These cases were individually matched by age, sex, and timing of the visit with control children who tested negative for influenza. Subjective symptoms (headache, myalgia, and sore throat) were analyzed only in children aged ≥3 years. Initially, a univariate analysis was performed to identify potential predictors of influenza. To determine the signs and symptoms that independently predicted influenza, all symptoms with P<0.1 in the univariate analysis were included in multivariate conditional logistic regression analyses.

Results

In the univariate analysis, fever was the strongest predictor of influenza, with the highest odds ratio (58.46; 95% CI, 19.36-176.53) observed for fever ≥40.0°C. Other signs and symptoms with P<0.1 in the univariate analysis were impaired general condition, gastrointestinal symptoms, pharyngitis, cough, headache and myalgia. However, when all these signs and symptoms where included in the multivariate model, fever remained the only symptom that independently predicted influenza. The predictive capability of fever increased with incremental elevations in child's temperature. In the multivariate analysis of all children, the odds ratios for influenza in children with temperatures of 38.0-38.9°C, 39.0-39.9°C, and ≥40.0ºC were 13.55 (6.90-26.63), 25.93 (12.20-55.14), and 50.10 (16.25-154.45), respectively.

The highest positive likelihood ratio (LR; 6.00) was observed for fever ≥40.0ºC, while a temperature of <38.0ºC was a strong negative predictor of influenza (LR 0.16). Other signs and symptoms that significantly increased the likelihood of influenza were impaired general condition (LR 3.50); gastrointestinal symptoms (LR 2.07); pharyngitis (LR 1.36), and in children ≥3 years of age headache (LR 2.60) and myalgia (LR 2.43).
Conclusions Besides fever, few other clinically useful signs or symptoms to predict influenza infection were identified in this carefully matched case-control study performed among unselected outpatient children. The optimal use of influenza-specific antiviral drugs in children may require the use of rapid influenza tests.

YOUNG SCIENTIST

SPA 1: CLINICAL IMPACT & DIAGNOSTIC APPROACHES

A110P

Effect of exercise on mortality risk associated with influenza: evidence from an elderly cohort in Hong Kong

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The benefits of physical exercise on reduced risks of respiratory infections have been reported in previous studies. However, few studies have examined the effects of exercise on influenza associated mortality risks. In this study we assessed such effects by using the data of an elderly cohort in Hong Kong. This cohort included 66,820 community dwelling subjects aged 65 years or over (22,679 men and 44,141 women), who voluntarily enrolled in 18 Elderly Health Centres (EHC) managed by the Department of Health in Hong Kong during 1998 to 2001. The death registration records during the follow-up period till December 2009 were linked to the baseline data by their unique Hong Kong identity numbers. According to the self-reported frequency and duration of regular exercise at the time of their first visits to EHC, the subjects were divided into three groups: sedentary, moderate and frequent exercise. Moderate exercise was defined as not doing exercise everyday or for less than 30 minutes each time and frequent exercise as doing exercise for more than 30 minutes everyday. Cox proportional hazard models with time-dependent interaction terms of influenza virus activity and exercise were adopted to estimate the effect modification by exercise. Here influenza virus activity is defined as weekly proportions of specimens positive for influenza. Several confounders were adjusted for in the model as time-independent covariates (age, gender, smoking history, drinking habits, co-morbidity score, education and housing) and time-dependent covariates (co-circulation of respiratory syncytial viruses, environmental factors and concentrations of air pollutants). In both gender combined group, the interaction between exercise habit and influenza was significant (p=0.046) in the mortality with underlying cause of cardiovascular and respiratory diseases. The excess risks (ER) of all natural mortality associated with every 10% increase in influenza virus activity was 5.7% (95% confidence interval, 0.3%, 11.5%), 3.8% (0%, 7.6%) and 1.0% (-2.2%, 4.4%) for sedentary, moderate and frequent exercise groups, respectively. Compared to the sedentary group, frequent exercise was found to associate with a lower ER of all natural cause and cardiovascular/respiratory mortality attributable to influenza for men and women separately, but the results were not consistent for moderate exercise between all natural cause and cardiovascular/respiratory mortality. In conclusion, exercise was associated with reduced mortality risk attributable to influenza in the elders, but further investigation on beneficial effects of exercise with different frequencies and durations is warranted.

SPA 1: CLINICAL IMPACT & DIAGNOSTIC APPROACHES

A111P

Clinical Characteristics & Treatment Effectiveness in Possible Human Cases of H5N1

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Aims

To describe suspected human cases of avian influenza, and to compare them with cases having laboratory confirmation of influenza A (H5N1) infection.

Methods

he Avian Flu Registry collects epidemiological, clinical, and treatment information on laboratory confirmed and suspected human cases of influenza A (H5N1). The registry classifies non-laboratory confirmed cases according to the level of evidence that they are true avian influenza cases. A possible case has an influenza-like illness and is epidemiologically linked by time, place, and exposure to a likely or confirmed human and/or avian H5N1 case, but lacks laboratory confirmation of influenza A H5n1 infection (not tested or negative result). A likely case meets the same requirements as a possible case, except that likely cases have either an indeterminate viral test result or have died of an unexplained respiratory illness before tests could be performed. This analysis is derived only from countries that reported both cases with laboratory-confirmation of H5N1 ("confirmed") and unconfirmed cases. For the purposes of this analysis likely and possible cases are grouped together as "unconfirmed" cases.

Results

Data are available for 256 confirmed cases and 215 unconfirmed cases from eight countries: Azerbaijan, China, Indonesia, Nigeria, Pakistan, Thailand, Turkey, and Vietnam. Forty-eight percent of confirmed cases and 55% of unconfirmed cases are male. The mortality rate for confirmed cases (70%) is substantially higher than that of unconfirmed cases (17%), although nearly similar proportions of each group were treated with oseltamivir (55% of confirmed and 61% of unconfirmed cases). Both confirmed and unconfirmed cases presented for medical care fairly quickly after symptom onset, with median days to presentation for medical care of 1 and 2 days respectively. However, the median time from symptom onset to oseltamivir treatment was longer for confirmed cases (7 days vs. 3). Both groups showed a statistically significant survival benefit from treatment with oseltamivir, although the benefit was substantially greater for confirmed cases than unconfirmed. The survival rate for confirmed cases increased from 19% among cases who did not receive any antiviral therapy to 40% among those who were treated with at least one dose of oseltamivir ($p = 0.0003$), whereas for unconfirmed cases, the survival rate increased from 76% among those without antiviral treatment to 87% among those treated with oseltamivir ($p=0.04$). For confirmed cases, the greatest survival benefit from oseltamivir was seen in cases who initiated treatment within 2 days after symptom onset (RR=5.73, 95% CL 2.69-12.22), and the treatment benefit continued in cases initiating oseltamivir up to 6-8 days after symptom onset (RR=1.82, 95%CL 1.07-3.09). But for unconfirmed cases, there does not appear to be an additional survival benefit associated with early treatment with oseltamivir.

The clinical characteristics at presentation for medical care differed in many ways for confirmed and unconfirmed cases. Tachypnea, diarrhea, and fever were reported more frequently in laboratory confirmed cases. Unconfirmed cases reported rhinorrhea, excessive sputum production, and sore throat more frequently. The case fatality rate by symptom was significantly higher in laboratory confirmed cases for all symptoms. Discussion/Conclusions The unconfirmed cases of avian influenza likely represent a mix of true avian influenza cases and cases of other illnesses. Unconfirmed cases were more likely to present with symptoms of an upper respiratory tract illness as compared with laboratory confirmed cases. Unconfirmed cases may have received antiviral treatment more quickly since they generally presented for medical treatment after human cases of avian influenza had been confirmed in their region. Both confirmed and unconfirmed cases showed statistically significant survival benefit from oseltamivir treatment, with greater benefit accruing to the confirmed case group.

SPA 1: CLINICAL IMPACT & DIAGNOSTIC APPROACHES

A112P

Evaluation of a fluorescence-based point-of-care test for detection of influenza viruses circulating recently in Germany

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Background

During recent years, amplification of reverse transcribed viral RNA by the polymerase chain reaction (RT-PCR) in a real-time format is used by an increasing number of laboratories for timely and sensitive detection of influenza viruses. However, there is an urgent need for rapid and accurate detection of influenza viruses. A quick test result allows for prompt administration of appropriate antiviral therapy, assists in isolation of patients in hospitals and emergency centers to reduce nosocomial spread of infection, and can identify local epidemics of influenza in a timely manner. POC tests are rapid tests that can be used for bedside diagnosis or for diagnosis of patients in emergency units and the physicians' practice. These rapid tests are convenient to perform and can be completed in approximately 15 min. Currently available POC tests have been described to be less sensitive for the detection of pandemic A/H1N1 (2009) viruses (17%-70%) compared to seasonal viruses. Here we present a new fluorescence-based POC test for detection of A/H1N1 (2009) viruses as well as recently circulating A/H3N2 and influenza B viruses.

Methods

Patient specimens tested positive by real-time PCR were selected for comparative analysis according to their PCR threshold (CT) values. The majority of samples were obtained from the nationwide community based influenza sentinel. The Quidel Influenza A+B Fluorescent Immunoassay (FIA) was used in this study; this instrument employs a lateral-flow immunofluorescence technique to detect influenza virus nucleoprotein. Using this test allows for the differential detection of influenza A and influenza B antigens. After a sample extraction step using 260 µl of the specimen, an aliquot was pipetted onto the test cassette and incubated for 15 min. The cassette was then inserted into the FIA Analyzer. Each strip was scanned within one minute and the fluorescent signal was analyzed automatically, using method-specific algorithms, to yield a positive or negative result.

Results

In total, 160 patient specimens were categorised into four different groups. Group A represented CT values of 20-24, group B values of 25-30, group C values of 31-34 and group D values of ≥35, respectively. About 40 samples belonged to each of the four groups. The sensitivity for detection of A/H1N1 (2009) was on average 80.2% with 100% for group A and B, 93% for group C and 28% for group D, respectively. Influenza A/H3N2 viruses were detected with an average sensitivity of 79.8% showing the highest values with 100% for groups A and B, 65% for group C, and 59% for group D, respectively. Influenza B viruses were characterised and gave the somewhat lower sensitivity rates of 67.5% for type B viruses of the Victoria-lineage and 62.5% for viruses of the Yamagata-lineage. As found for type A viruses, a sensitivity of 100% was determined for type B viruses belonging to groups A and B. However, the sensitivity decreased significantly with CT values higher than 30. The positive predictive values were generally 100%, and the negative predictive values varied between 69.6% (B-Yam), 75.5% (B-Vic), 80.7% (H3N2) and 86.9% (H1N1 (2009)).

Conclusion

The study revealed a high sensitivity for the fluorescence-based POC test. A good performance could be shown especially for A/H1N1 (2009) viruses characterised by a detection rate of 80.2% compared to real-time PCR. About 90% of the H1N1 (2009)-positive specimens belonged to group A-C. When only CT values of group A-C viruses were analysed, the sensitivity for detection of H1N1 (2009) viruses was 96.6%. The majority of A/H3N2 and type B viruses represented also groups A-C. In summary, the fluorescence test can detect the overall majority (85-90%) of actually circulating influenza virus with a very high sensitivity.

SPA 1: CLINICAL IMPACT & DIAGNOSTIC APPROACHES

A113P

IgM and IgG antibody responses to novel H1N1 virus during the first wave of 2009 pandemic in US

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Background

Novel swine origin H1N1 virus (pH1N1) emerged in the spring of 2009 and quickly spread worldwide. The possibility of detecting a pH1N1 specific IgM response to aid in serodiagnosis was investigated. Using ELISA, we measured pH1N1-specific IgM and IgG antibody responses in confirmed cases and unexposed controls.

Material and Methods

Recombinant HA (rHA) from pH1N1 (A/Texas/05/09, a CA/07/09-like virus), seasonal H1N1 (A/Brisbane/59/07), H3N2 (A/Brisbane/10/07), H5N1 (A/Vietnam/1203/04), and H13N9 (A/shorebird/Delaware/68/04) viruses were expressed in Sf9 cells. Purified rHA was confirmed as trimeric by size exclusion chromatography and used as antigen in an indirect ELISA. One-hundred-thirty normal human sera collected in 2008 (donors aged 3-79 years) and 63 convalescent sera from serologically confirmed pH1N1 influenza infection cases (aged 6 months-80 years) were used as negative and positive samples, respectively, to establish the cut-off value for IgM and IgG seropositivity. The sensitivity and specificity of the ELISA were evaluated for two populations of pH1N1 cases. Thirteen children ≤5 years old whose convalescent sera had HI titers ≤10 to a panel of seasonal H1N1 viruses (Brisbane/59/07 or New Caledonia/20/99) were considered to be primary infections. Forty individuals, all >15 years old, were considered non-primary infections. IgM and IgG concentrations were measured by quantitative ELISA using a standard curve. A positive control was included on each plate. The ratio of the concentration of the test serum to the control serum was determined (T/P) and used to evaluate sensitivity and specificity as well as fold-rise in acute and convalescent serum samples.

Results and discussion

Specific IgM antibodies were detected in 62% (8/13) of primary infection cases at a corresponding specificity of 95% (18/19) for ages 3-5 years. For non-primary infections, only 10% (4/40) of individuals achieved an IgM concentration greater than the cut-off value. The corresponding specificity was 90% (77/86). Specific IgG antibodies were detected in 100% (13/13) of primary infection cases with a corresponding specificity of 100% (19/19) for ages 3-5 years. For non-primary infections, 95% (38/40) achieved an IgG concentration greater than the cut-off value. The corresponding specificity is 77% (66/86).

Influenza serodiagnosis is typically dependent upon the collection of appropriately timed acute and convalescent serum samples in order to demonstrate a rising antibody titer to the suspect virus. We evaluated the fold-rise in IgG concentrations for 32 paired serum samples from non-primary infection cases with acute sera collected within seven days of ILI onset and convalescent sera at least 10 days later. 91% of cases had a ≥3-fold rise in IgG concentration to pH1N1, but 47% also had a ≥3-fold rise to seasonal H1N1 (Brisbane/59/07). To evaluate cross reactivity to other rHAs, six paired sera were tested by IgG ELISA using seasonal H1N1, H3N2, H5N1, and H13N9 rHAs and the fold-rise to each rHA determined. Infection with pH1N1 boosted the IgG response 2-fold or higher 79% (19/24) of the time, though the fold-rise was always highest with the pH1N1 rHA.

Conclusions

Specific IgM antibody responses were detected in convalescent sera from 62% of pH1N1 cases presumed to have experienced a primary influenza infection while specific IgG antibody responses were detected in all these cases. Specific IgM antibody was not detected in 90% of individuals presumed to have had prior influenza virus infection while 95% of these individuals had a specific IgG antibody response. With paired sera, 91% of non-primary influenza infection cases had a ≥3-fold rise in pH1N1 HA specific antibody concentration. The IgG antibody response to pH1N1 was cross-reactive with rHAs from H3, H5, and H13 subtypes suggesting that infections with subtypes other than pH1N1 could result in false positives by ELISA.

SPA 1: CLINICAL IMPACT & DIAGNOSTIC APPROACHES

A114P

Characterization of the performance of a novel multiplexed molecular assay for the detection of Influenza A+B on multiple extraction and real-time amplification platforms.

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Introduction

Respiratory infections are the most common acute infection in both adults and children. A significant causative agent of the most severe respiratory infections is the Influenza virus. Influenza A and B (Flu A/B) spread in regular epidemics resulting in the deaths of more than 250,000 people worldwide annually. We have recently developed an assay based upon novel, room temperature stable, lyophilized reagents for use in multiplexed RT-PCR for the detection and differentiation of Flu A/B. These reagents could be especially useful in low resource settings where flexible shipping and storage conditions can be important. This assay utilizes a simplified workflow that significantly reduces the number of steps required for testing, with thermocycling times of about one hour. An evaluation of these reagents was performed on automated and manual extraction systems and multiple real-time amplification platforms to compare the performance characteristics of the assay with a variety of systems commonly found in hospital laboratories.

Methods

RNA from either clinical specimens or spiked matrix samples was extracted using either automated or manual procedures. 5 µL of the sample was added to 15µL of the reconstituted master mix. Real-time PCR on the samples was performed using the ABI® 7500 FastDx, Cepheid® SmartCycler or Stratagene Mx3000p. Each sample also contained an integrated process control that controls for the extraction and amplification of the sample.

Results

The limit of detection (LoD) for 6 Influenza strains (3 Flu A and 3 Flu B), including the pandemic H1N1 strains, was determined to be between 0.9 TCID50/mL to 28 TCID50/mL depending upon the strain evaluated and the PCR platform utilized. Analysis of a panel of 50 microorganisms at high titers was found to have no cross-reactivity in the assay. Evaluation of the assay with 5 different transport media found that there were no significant differences when tested at 3x LoD in assay performance. Inclusivity of the assay was analyzed with 20 Flu A strains and 13 Flu B strains and the assay was capable of detecting each strain tested. In addition, 18 different Flu A strains found in animals were also able to be detected with this assay. Evaluation of the assay with leftover, blinded clinical specimens found Flu A in 62 of 66 Flu A culture positive samples and 15 of 111 Flu A culture negative samples. The Flu A results discrepant with culture were confirmed using a commercially available, FDA cleared PCR test for a corrected sensitivity and specificity of 100%. The assay detected Flu B in 24 of 27 Flu B culture positive samples and 1 of 150 Flu B culture negative samples. The Flu B PCR culture discrepant results were confirmed using a commercially available, FDA cleared PCR test and sequencing for a corrected sensitivity and specificity of 100%.

Conclusion

Results from these studies using multiple extraction and amplification platforms indicate that this novel real-time assay is sensitive and specific for the detection of Flu A/B and capable of being utilized with a variety of laboratory equipment.

SPA 1: CLINICAL IMPACT & DIAGNOSTIC APPROACHES

A115P

Monitoring of morbidity and mortality due to severe influenza illness in Scotland.

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Introduction

During the 2010/11 influenza season, the first indication that influenza was beginning to circulate within the UK was a sudden increase in the number of hospitalised severely ill influenza cases requiring intensive treatment. In light of this, Health Protection Scotland (HPS) implemented monitoring of severe influenza illness cases requiring intensive care treatment and deaths due to confirmed influenza illness to inform public health planning and management of NHS intensive care pressures.

The aim of this surveillance was to provide more detailed information on the clinical and virological characteristics of individuals developing severe influenza illness, and in particular to identify whether there were any specific risk factors or features which predisposed an individual to a fatal outcome. In addition, it was hoped that this information could provide an indication of the effectiveness of public health interventions such as influenza immunisation and antiviral treatment. The key findings of the interim analysis of the information collected through this surveillance system are summarised below.

Method

Information on individuals admitted to hospital with severe acute respiratory illness due to influenza (SARI) requiring intensive care management, or who died due to confirmed influenza were reported by NHS boards in Scotland to HPS during the 2010/11 Influenza season. NHS boards within Scotland were asked to submit a form detailing information on patient demographics, clinical presentation, virology results, antiviral treatment, immunisation eligibility and history, risk factors, secondary complications and outcome (including cause of death) for individuals who met the above criteria.

Results

A total of 177 individuals (114 SARI cases and 63 deaths) were reported to HPS during December 2010 to March 2011 and whom met the criteria. The majority of SARI cases and deaths reported (where virology results were available or known) were due to Influenza A H1N1 2009 (87%). Influenza B was detected in a much smaller proportion of SARI cases and deaths (7%). There was no significant difference in the proportion of cases due to influenza A and B in the SARI cases compared to the deaths.

The median age of the SARI cases was 45 years compared to 55 years in those who died, with Influenza B illness generally associated with older age. Both severe influenza illness and death were associated with the presence of a number of risk factors, with chronic respiratory illness the most common condition recorded (34%). Chronic heart disease and obesity were more commonly stated as a risk factor in those individuals who died. The mortality rate associated with Influenza B was higher (58%) than Influenza A H1N1 (2009) (42%) but this was not statistically significant.

Conclusion

The high proportion of SARI and deaths due to Influenza A H1N1 2009 (87%) is very different to that seen in the community where influenza B positivity rates were very similar to Influenza A H1N1 (2009) (47 and 51%, respectively). This finding suggests that influenza A H1N1 (2009) may be associated with more severe influenza illness and death.

Severe influenza morbidity and mortality is associated with the presence of a number of chronic health problems. There is some evidence that chronic heart disease and obesity may contribute to an increased risk of death, and also that having influenza B may be associated with an increased risk of death. These findings are being investigated further.

Information gained through the severe influenza morbidity and mortality surveillance undertaken in Scotland was extremely useful in informing local and national influenza public health policy during the 2010/11 influenza season, and in managing NHS intensive care pressures.

SPA 1: CLINICAL IMPACT & DIAGNOSTIC APPROACHES

A116P

Presenting symptoms of influenza b patients: a prospective study in sentinel practices in france and turkey in 2010/11 season

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Introduction

Symptoms of influenza like illnesses (ILI) attributable to influenza are well described but strain specific information is limited. As part of the IBGP (Influenza B in General Practice) study, in which we aim to describe clinical and socio-economical facets of influenza B, we here describe our first findings in France and Turkey. The analysis of symptoms presentation is important to determine if the diagnosis can be established clinically with reasonable confidence.

Methods

The study was conducted between 27/12/10 and 17/04/11 in sentinel networks in France and Turkey. General Practitioners of the networks were invited to participate and 154 in France and 145 in Turkey agreed. Recruitment was based on ILI patients investigated virologically. Information was collected at the time of presentation (day 0) and recorded on routine investigation form. Laboratories received swabs by post and examined it by PCR. The GP was informed promptly when a B virus was detected and a suitable influenza B negative person was identified as a matched Control (broad age group, time of diagnosis...). Case/Control patients were phoned and where agreeable, were consented for the study and followed-up on Days 7(±2) and 28(±5). Differences between Case and Control cohorts were examined using StataMP11.

Results

All influenza B detected during the study period in broad age groups in both networks is given in table 1, together with cases/controls recruited with fully completed investigation forms, and also the proportion of vaccinated against flu with the current seasonal vaccine. Altogether we studied 356 Case/Control pairs. Cases recruited represented approximately 44% in France and 50% in Turkey of all influenza B detections. In France 70% of cases were aged <14 years compared with 19% in Turkey where recruitment was maximal in the age group 15-59 years. Vaccination rates in the two countries were similar in both cases and controls in children and adults (all <5%) but differed in persons over 60 years (approximately 50% in France and 8% in Turkey).

Table 1: Cases and controls recruited by country and age group: proportions vaccinated against influenza

	AGE 0-14			AGE 15-59			AGE 60+			ALL AGES*		
		Recruits			Recruits			Recruits			Recruits	
	Total B detections	Cases (vac%)	Controls (vac%)	Total B detections	Cases (vac%)	Controls (vac%)	Total B detections	Cases (vac%)	Controls (vac%)	Total B detections	Cases	Controls
France	319	141 (2.8%)	99 (1.4%)	107	44 (2.2%)	44 (2.2%)	31	16 (56.2%)	13 (42.9%)	406	201	156
Turkey	72	30 (3.3%)	29 (3.4%)	155	111 (2.7%)	113 (4.4%)	43	14 (7.1%)	13 (7.6%)	307	155	155

*Some samples did not have age identified

The influenza epidemic periods were similar in both countries extending over the first 10 weeks of the year and peaking in week 4. Recruitment of cases was synchronous with total cases identification in each country but in France the recruitment of controls lagged behind cases. Fever and cough were the commonest symptoms in Cases both children and adults. Significant differences (5% level) between cases and controls were found in France for myalgia and headache in children and for the frequency of cough in adults. In Turkey the only difference was in the proportion of adults with fever.

Discussion and conclusion

This study has demonstrated effective recruitment of influenza B cases in the two countries, where the epidemic occurred at the same time. However, age distribution of cases largely differed of children recruitment in France, which is possibly due to disparities in healthcare access. Vaccination rates in older persons were higher in France where free vaccination is strongly promoted, differently from Turkey: paying vaccine and slight public campaign.

These results demonstrate it is possible to collect detailed clinical and socio-economical data on influenza B across Europe. The IBGP project will be probably extended to other European countries during the 2011/12 winter.

SPA 1: CLINICAL IMPACT & DIAGNOSTIC APPROACHES

A117P

Rapid, high performance influenza A+B fluorescent immunoassay and analyzer

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Introduction

Quidel Corporation has developed a new generation of rapid tests based on a detection technology that yields significantly enhanced assay performance and builds upon the advantages of lateral flow assay design by incorporating fluorescent immunoassay technology with an easy-to-use fluorescent immunoassay Analyzer (FIA Analyzer). The Analyzer is a small "stand-alone" instrument that incorporates microchip technology with purpose-designed software to scan the test strip, analyze the data and report a final, differentiating, qualitative result for influenza A and B. A formal clinical study was conducted in North America during the winter of 2011 to validate the instrument and determine the clinical performance of Quidel's Influenza A+B Fluorescent Immunoassay (FIA).

Materials & Methods

After obtaining informed consent, patients presenting with influenza-like illness (ILI) were enrolled into the clinical study. Depending upon the patient's age and the policy of the participating clinical trial sites, nasal swabs (NS), nasopharyngeal swabs (NPS), or nasopharyngeal aspirates (NPA) were collected for testing. When testing either swab type, one swab was tested directly in the Influenza A+B FIA and a second was inserted into VTM and an aliquot was then cultured in shell vials containing MDCK cells for later characterization with DFA typing reagents (Millipore or Diagnostic Hybrids). Another aliquot of the VTM was tested directly in the Influenza A+B FIA. For the NPA, one aliquot was tested directly in the FIA, the remaining NPA was suspended in VTM and an aliquot tested in the FIA and another placed in cell culture. To test a specimen the swab or NPA aliquot is suspended in approx. 260 µl of extraction reagent and incubated for one minute. About 120 µl of the extracted sample is then dispensed into a cassette. The Analyzer scans the test strip through a window in the test cassette and prints out a result at 15 minutes after charging the cassette with the test sample.

Results

Two thousand one hundred thirteen (2113) patients were consented and provided specimens for this study. The FIA results compared to culture for each sample type, obtained without prior suspension of in VTM, are shown in the table below:

Sample type	Number of Specimens	Sensitivity for Influenza A	Specificity for Influenza A	Sensitivity for Influenza B	Specificity for Influenza B
NS	658	85	94	82	93
NPS	726	94	93	91	95
NPA	650	91	94	87	93

The FIA results compared to culture for each sample type, obtained after suspension in VTM, are shown in the table below:

Sample type	Number of Specimens	Sensitivity for Influenza A	Specificity for Influenza A	Sensitivity for Influenza B	Specificity for Influenza B
NS	751	81	98	83	96
NPS	720 or 721	92	97	84	97
NPA	642	97	97	84	96

Conclusion

The Quidel Influenza A+B FIA and FIA Analyzer give rapid, high performance results within approximately 15 minutes of sample collection. The test results are interpreted automatically by the Analyzer, thus avoiding the need for subjective interpretation or test lines commonly associated with lateral flow devices. The clinical sensitivity and specificity for influenza A ranged from 81 to 97% and 93% to 98%, respectively, depending upon sample type and processing. For influenza B, clinical sensitivity and specificity ranged from 82% to 91% and 93% to 97%, respectively. This clinical study validated this test system and clearly demonstrate the test system's ease-of-use, speed to result, and high clinical performance .

Conflict of interest

Employee: John Tamerius and Richard Egan are employees of Quidel Corporation.;

SPA 1: CLINICAL IMPACT & DIAGNOSTIC APPROACHES

A118P

Simultaneous Influenza and RSV Rapid Testing in Infants and Children with Influenza-Like Illness (Charité Influenza-Like Disease = ChILD Cohort)

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Aims

Current guidelines recommend antiviral treatment of pediatric influenza infection below the age of two, ideally within the first 48 hours after symptom onset. Early influenza diagnosis and differentiation from other relevant respiratory pathogens such as respiratory syncytial virus (RSV) are crucial to target infection control measures as well as treatment with neuraminidase inhibitors during their time of maximum efficacy.

Methods

All patients presenting to the Charité pediatric emergency rooms during weekly surveillance days fulfilling the case definition for ILI, as well as all inpatients under the age of 2 fulfilling the same case definition, were screened prospectively (Charité Influenza-Like Disease = ChILD Cohort). Nasopharyngeal swabs/aspirates taken from ChILD cohort patients underwent immediate and simultaneous rapid testing on-site with QuickVue RSV10 and Influenza A&B dipstick immunoassays from the same sample, as well as PCR testing for influenza and RSV at the adjacent Robert Koch Institute.

Results of QuickVue simultaneous rapid testing were tabulated against PCR as gold standard to determine specificity, sensitivity, positive and negative predictive values for influenza and RSV, respectively.

Results

During the peak of the local 2010/11 influenza season from Jan 26th – April 31st 2011, a total number of 395 consecutive ChILD cohort subjects 0-16 years of age (mean age 2.7 yrs, median age 1.3yrs, SD 3.5yrs) were screened prospectively, yielding sensitivities, specificities, positive (PPV) and negative predictive values (NPV) as displayed below:

	all (n=395)		**<=12 mo (n=164)**		**>12 mo (n=231)**	
	Influenza	**RSV**	**Influenza**	**RSV**	**Influenza**	**RSV**
Sensitivity (%)	62.7	67.8	62.7	67.8	62.7	67.8
Specificity (%)	98.0	98.5	98.0	98.5	98.0	98.5
PPV (%)	91.4	88.9	91.4	88.9	91.4	88.9
NPV (%)	88.3	94.6	88.3	94.6	88.3	94.6

Sensitivity and NPV were higher in infants < 1year of age when compared to children >1 year of age. Differentiation between influenza A and B in rapid testing was confirmed by PCR in all cases.

Conclusions

Influenza and RSV point-of care diagnostics in pediatric acute care using simultaneous rapid testing with QuickVue Influenza A&B and RSV10 dipstick immunoassays deliver highly specific and useful information within 10 minutes of taking one nasopharyngeal aspirate or swab. Infants, who also carry the highest risk of influenza morbidity and mortality, showed the highest sensitivity with rapid testing - likely due to elevated viral loads in this age group. While negative test results do not rule out infection, positive test results have important implications directing timely antiviral treatment and infection control measures in the busy emergency room or tertiary inpatient hospital setting.

SPA 1: CLINICAL IMPACT & DIAGNOSTIC APPROACHES

A120P

An outbreak of Influenza A (H1N1) 2009 in a Neonatal Intensive Care Unit

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Introduction

Outbreaks of influenza A (H1N1) 2009 have rarely been reported in NICUs. Annual immunization of all health care workers (HCW) against seasonal influenza is recommended but compliance rate to vaccination is low and exposure to infected staff as the source of nosocomial outbreaks has been described. Influenza is usually regarded as a mild illness in the newborn period. Nevertheless, increased morbidity and fatal cases have also been described. We report an outbreak of influenza A (H1N1) 2009 in a tertiary level NICU that resulted in considerable morbidity.

Materials and methods

The outbreak occurred in a tertiary level neonatal unit in the winter of 2011 during the community peak of the seasonal influenza A (H1N1) 2009. A total of 22 neonates (13 boys) were hospitalized at that time in the unit, of which 11 (50%) were born preterm. Median age was 45 days; 11 (50%) infants were <30 days of age. Median gestational age was 33.8 weeks (range 24-40 weeks) and median corrected gestational age (CGA) 40.1 weeks (range 28 to 68 w). Median birth weight was 2213 g (range 590 to 3750 g) and median weight when the outbreak started was 3072 g (range 1220 to 5590 g).

Influenza A (H1N1) 2009 was detected in nasopharyngeal aspirates by indirect immunofluorescence assay (IFA) and PCR. Oseltamivir was administered for prophylaxis and treatment and dosing was based on CGA and modified by weight. Term by CGA neonates received treatment with 3mg/Kg/dose twice daily for 5 days or prophylaxis with 3mg/Kg once daily for 10 days. Preterm by CGA neonates received prophylaxis with 1mg/Kg/dose of oseltamivir twice daily for 10 days. All infants were closely monitored for the manifestation of symptoms compatible with influenza and for potential clinical adverse effects of antiviral treatment. Laboratory studies were performed on days 0, 5 and 10 of treatment.

Results

A total of 3 neonates (all born preterm) developed influenza A (H1N1) 2009 and received oseltamivir treatment. Two of them developed clinical and radiological evidence of pneumonitis requiring respiratory support while the third had a mild uncomplicated illness. All the rest exposed neonates received oseltamivir prophylaxis. No significant adverse clinical or laboratory effects were noted during treatment. Strict infection control measures were applied in the unit successfully and no additional cases of influenza were noted. Within the five days prior to the manifestation of the outbreak among neonates, 5/33 staff nurses developed laboratory confirmed influenza A (H1N1) 2009 while another 5 had Influenza-like illness (ILI). NICU's personnel immunization rates against influenza A (H1N1) 2009 were reviewed and only 15% of the nursing staff had been vaccinated.

Conclusions

1) Nosocomial influenza can cause considerable morbidity in newborns since 2/3 patients in this report developed moderately severe illness.

2) As shown in this report, unvaccinated staff members who contract influenza can introduce the disease to this vulnerable population with serious impact. Therefore, in addition to infection control measures, the implementation of HCW immunization is of outmost importance.

3) In this observational study, oseltamivir treatment was well-tolerated even among premature infants. Antiviral treatment was effective since patients with influenza had complete recovery and none of those who received prophylaxis developed the disease. These findings need to be confirmed by further studies including larger numbers of patients.

SPA 1: CLINICAL IMPACT & DIAGNOSTIC APPROACHES

A121P

Rapid diagnostic testing enhances diagnostic accuracy for infection due to rsv and influenza in infants and children less than two years of age

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Introduction

Fever and cough have been shown to be fairly accurate clinical predictors of influenza in older children and adults; however they are not accurate predictors in infants and young children. Our objective was to evaluate the clinical performance of rapid diagnostic testing for influenza A and B and RSV in infants and young children presenting with fever and respiratory symptoms during the winter months.

Materials & methods

A prospective evaluation of infants and children <2 years of age was conducted during January-March 2011 at the Dell Children's Medical Center Emergency Department (ED) in Austin, Texas, USA. Eligibility criteria included fever either documented (≥ 38ºC) or subjective and one or more respiratory symptoms (cough, congestion or coryza). All subjects provided a nasopharyngeal aspirate (NPA) specimen for evaluation with the QuickVue Influenza A+B test and the QuickVue RSV-10 rapid tests. Both rapid CLIA-waived tests were run at the point-of-care and results given to the treating physician to assist with patient care. The remainder of the specimen was placed into an equal amount of viral transport medium and stored at 2-8ºC. Laboratory testing conducted within 24 hours of specimen collection included direct specimen testing with DFA and cell culture using R-Mix Too shell vials followed by confirmatory DFA testing.

Results

A total of 596 infants and children < 2 years of age were enrolled and provided specimens for rapid and confirmatory testing. Direct specimen testing and/or cell culture was positive for one or more viruses (RSV, influenza A and/or influenza B) in 304 of the 596 samples (51%). The QuickVue Influenza A+B test demonstrated the following clinical performance for influenza A: sensitivity 91.9% (57/62), specificity 98.5% (526/534), PPV 87.7% (57/65) and NPV 99.1% (526/531). For influenza B the following was obtained: sensitivity 86.0% (37/43), specificity 98.2% (543/553), PPV 78.8% (37/47) and NPV 98.9% (543/549). The QuickVue RSV-10 test demonstrated the following clinical performance for detection of RSV: sensitivity 93.6% (190/203), specificity 96.7% (380/393), PPV 93.6% (190/203) and NPV 96.7% (380/393).

Conclusions

Considering the numerous viral pathogens encountered by infants and young children during the first year of life, rapid diagnostic testing utilizing the QuickVue Influenza A+B and the RSV-10 tests provided very accurate diagnostic information for treating physicians to use for further decision-making regarding testing and treatment in this cohort of infants and young children.

SPA 1: CLINICAL IMPACT & DIAGNOSTIC APPROACHES

A122P

Detection and characterization of influenza C viruses by real-time RT-PCR

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Background

Influenza C viruses usually cause a mild febrile upper respiratory tract infection in children and in young adults, but can also cause lower respiratory tract infections such as bronchitis or pneumonia. Seroepidemiological studies revealed a wide distribution of influenza C viruses worldwide, but little is known about the epidemiology and clinical impact of influenza C virus infections in the general population, including the US population, due to the lack of surveillance and difficulty of isolating and culturing these viruses. Development of more sensitive and rapid diagnostic methods will allow for better detection of influenza C viruses and lead to improved understanding of the epidemiological behavior and clinic impact of influenza C virus infections.

Material and Methods

A universal influenza C (InfC) real-time RT-PCR (rRT-PCR) assay was designed for detection of influenza C viruses. Oligonucleotide primers and probes were designed from a conserved region of the matrix (M) gene based on 108 M gene sequences, including 58 sequences of recent (1990-2000) viruses. The sequences were obtained from Genbank database of National Centers for Biological Information (NCBI), NIH and the Global Initiative on Sharing Avian Influenza Data (GISAID). No more than one nucleotide mismatch within selected primer or probe sequences was found among 108 M gene sequences.

rRT-PCR reaction parameters of InfC assay were optimized using the QuantiTect SYBR Green RT-PCR Kit (QIAGEN, USA) and the Invitrogen SuperScript™III Platinum® One-Step quantitative RT-PCR Kits (Life Technologies) on the Stratagene Mx3005P and the Applied Biosystems™ (AB) 7500 Fast Dx Real-Time PCR systems.

Result and Conclusion

SYBR Green I based real-time PCR, performed using forward and reverse primers, demonstrated amplification of a single amplicon with a sharply defined melting temperature of 79.6°C. Non-specific amplification was not observed in any reactions. Thermal gradient analysis of InfC probe hydrolysis (Taqman) assay reactions indicated comparable performance at annealing temperatures ranging from 50ºC-62.5C. Reaction annealing temperature was set to 55ºC, which is 5°C below the maximum optimal annealing temperature (60°C) to accommodate potential nucleotide mismatch in the primer/probe regions due to virus variability or evolution. Reaction efficiency and RSq of the InfC assay was determined to be 98.3% - 100% and 0.998 by testing ten-fold serial dilution series of viral RNA of C/Yamagata/11/1981 and C/Kansas/2/1979. The assay showed robust reactivity over a dynamic range of 5 orders of magnitude of viral RNA with a co-efficiency of variation (CV) of 2.13% at lowest concentration detected. Cross reactivity was not observed with tested cultured human influenza A/H1N1pdm09, A/H3N2 and B viruses. Fourteen (0.7%) InfC positive clinical specimens were detected among 2129 specimens collected in Kenya in 2008, and one InfC positive specimen was detected among 66 US specimens collected from September 2010 through February 2011. All positive specimens were confirmed by genetic sequence analysis. Sequences of selected primers and probe were 100% identical to corresponding regions of all eight M-genes sequenced from these InfC positive specimens received from Kenya.

The InfC rRT-PCR assay described here is a highly sensitive and specific assay that is capable to detect historical and currently circulating influenza C viruses

SPA 1: CLINICAL IMPACT & DIAGNOSTIC APPROACHES

A123P

A comparison of 3 clinical triage tools for pandemic influenza: CAT, CURB-65 and PMEWS

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Introduction

Clinical triage tools assessing individual patient's risk of severe outcomes have an important role in pandemic situations where secondary care capacity is limited. CURB-65 is a well known marker of severe respiratory illness. Other proposed tools include the Pandemic Medical Early Warning Score (PMEWS) which includes physiological and social factors, and the Community Assessment Triage criteria (CAT). CAT includes clinical assessments that can be carried out in primary care like severe respiratory distress, respiratory exhaustion, oxygen saturation <92 %, severe clinical dehydration and altered consciousness.

Aim

To compare the clinical validity and utility of CAT, CURB-65 and PMEWS as predictive markers for severe outcomes in pandemic influenza

Methods

A retrospective analysis of the predictive ability of CAT, CURB-65 and PMEWS was carried out in a cohort of patients with PCR-confirmed pandemic (swine) A/H1N1 influenza using hospital surveillance data from the Clinical Information Network (FLU-CIN), UK. The 3 tools were compared on their ability to predict admission to high dependency (level 2) or intensive care (level 3) or mortality using area under ROC curve (AUROC) comparison. In addition, the sensitivity, specificity, positive predictive value and negative predictive value were calculated for each of the tools using various score thresholds. Subgroup analyses were carried out by age and in the case of children CAT and PMEWS were compared to CURB scores.

Results

CAT was a better predictor for severe outcomes (AUROC 0.76) than either CURB-65 (AUROC 0.64) or PMEWS (AUROC 0.66). A subgroup analysis by age showed that CAT was the best predictor for severe outcomes for both adults (CAT AUROC 0.76; CURB-65 AUROC 0.69; PMEWS AUROC 0.67) and children (CAT AUROC 0.76; CURB AUROC 0.53; PMEWS AUROC 0.69). A cut-off CAT score ≥3 provided the best clinical utility in terms of predicting severe outcomes (AUROC 0.65; 95% CI: 0.59-0.70).

Conclusion

CAT scores are a potentially useful triage tool to assess severe illness in patients and may provide better clinical utility in a pandemic situation than CURB-65 or PMEWS.

SPA 1: CLINICAL IMPACT & DIAGNOSTIC APPROACHES

A124P

Do current definitions of influenza-like illness accurately represent experiences for adults and children diagnosed with the 2009 H1N1 influenza virus?

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Background

The CDC defines an influenza-like illness (ILI) as one with a fever and cough or sore throat while PHAC defines ILI as illness with fever and cough and sore throat, arthralgia, myalgia, or prostration. During a pandemic, accurate definitions are necessary to aid practitioners in determining when to recommend both antivirals and non-pharmaceutical infection prevention interventions.

Methods

We recruited healthcare workers in Halifax, Quebec City, Hamilton, and Vancouver as well as healthcare workers, other working adults, and their household members in Toronto for a prospective cohort study between May 28, 2009 and February 28, 2010. Participants completed baseline questionnaires and weekly and illness diaries between enrolment and March 31, 2010 to determine the epidemiology of the 2009 A/H1N1 influenza pandemic.

Results

Questionnaires were completed for 1109 HCW, 561 other adults, and 217 children. 92 (9%) of the 1030 nasal swabs (from 678 individuals) were positive for influenza (90/92 were A/H1N1) and 90/92 occurred during the first (May 17-Aug 8) or second (Oct 4-Dec 12) wave of the pandemic. Swabs from children were more likely to test positive (50/240 or 21%) than those submitted from adult participants (42/790 or 5.6%; $p<0.001$). Only 2 participants met the CDC but not the PHAC definition. The sensitivity of the ILI definitions was 28% for the entire study period (95% CI: 18,39) as compared to 29% (20,39) during the defined influenza waves, and was 35% (21,52) for adults and 22% (13,36) for children. The positive predictive value (PPV), the proportion of people who test positive if meeting the ILI definition, was higher for children (25%; 14,40) than adults (6%; 3,10) and was higher during the influenza waves (19% (13,27) than during the entire study period (9%; 6-13). No other combination of symptoms more accurately predicted influenza than the CDC ILI definition.

Conclusion

Although the PPV of the ILI definitions was relatively low during the 2009 influenza pandemic, it was higher during the influenza season and was better for children than adults. During the 2009 pandemic, more than 65% of persons with influenza did not meet the ILI definition.

SPA 1: CLINICAL IMPACT & DIAGNOSTIC APPROACHES

A125P

Assessing accuracy of rapid influenza diagnostic tests

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Introduction

Precise diagnosis of influenza virus infection requires a laboratory with trained staff and adequate supplies and equipment. In resource-constrained settings, such laboratories are not always accessible and test results are unlikely available timely enough to assist clinicians' decision for treatment. To timely detect influenza outbreaks and to commence influenza-specific antiviral treatment in such circumstances, point-of-care rapid influenza diagnostic tests (RIDTs) are considered to be useful. An increasing number of RIDTs have become available in the market leading to a need to assess RIDTs' accuracy.

Objective

The objective of this review was to assess existing evidence for the accuracy of RIDTs compared with RT-PCR of culture in diagnosing influenza either type A or type B.

Material & Methods

We conducted electronic searching using a predefined search strategy. The electronic databases included the following: PubMed, CABI, clinicaltrials.gov, J-STAGE, and Cochrane Library. The authors also searched websites of pharmaceutical industries. A three-stage approach was used to review the title, abstract and full text against inclusion criteria. Data extraction was undertaken using a pre-defined, piloted template. Their methodological qualities were assessed using modified QUIDAS criteria. Results were synthesised using a narrative approach combined with summary receiver-operating curve and forest plotting. On tests with ten or more included studies, sub-categorised analyses were carried out according to virus antigenic type and subtype, methodological qualities of included studies, and sources of samples.

Results

A total of 891 citations were identified, among which 600 were excluded after reviewing the title, 126 following the abstract review, and 76 at the full-text stage. Eighty-seven citations were eligible for final inclusion. The summary receiver-operating curve showed that RIDTs were highly specific tests with varied sensitivity regardless of test types. Studies on four commercially available test types were processed to sub-categorised analyses, yet they indicated that none of the following factors could alone generally explain varied sensitivity: virus type and subtype, methodological qualities, and sources of samples.

Conclusions

This study indicated that patient characteristics and the amount of virus in samples might correlate with sensitivity or that factors affecting sensitivity might differ across test types. The existing evidence, however, is limited in data with regard to patient characteristics and the amount of virus in samples. Further analyses with sufficient data are required to determine factors associated with accuracy of RIDTs.

SPA 1: CLINICAL IMPACT & DIAGNOSTIC APPROACHES

A126P

Does the length of refrigerated specimen storage affect influenza testing results by RT-PCR? An analysis of surveillance specimens in Kenya, 2008-2011

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Introduction and Aims

Real time reverse transcription-polymerase chain reaction (rt RT-PCR) is increasingly used for routine influenza surveillance. Extended storage time in the refrigerator may decrease the viability of samples and therefore affect testing outcomes. Currently the World Health Organization recommends that samples be stored at 4ºC up to 48 hours for viral isolation, but does not provide formal recommendations for samples tested by rt RT-PCR. In Kenya, sentinel surveillance sites are far from the testing laboratory; consequently, specimens are often refrigerated for longer than 48 hours. We conducted retrospective secondary data analysis to determine whether the duration of time of influenza sample storage time affected influenza positivity and Cycle Threshold (CT) values.

Methods

Since October 2006, the Kenya Ministry of Health and the US Centers for Disease Control and Prevention have conducted outpatient and inpatient influenza surveillance at 11 sentinel surveillance sites across the country. Nasopharyngeal and oropharyngeal samples are collected from patients with influenza-like-illness and severe acute respiratory illness. Specimens are stored at 2-8ºC and transported in cool boxes to the National Influenza Center in Nairobi, where they are tested for influenza by rt RT-PCR.

We used SAS version 9.1 (SAS Institute Inc, Cary, North Carolina, USA) to analyze data on length of storage time, influenza test results and CT values from surveillance samples collected from April 11, 2008 --January 3, 2011. For our first analysis, storage days were grouped into three categories, with 0-3 days from the time collected as the reference group. For the second analysis, we looked at storage days individually, using 0 days as the reference. We used multivariate logistic regression to determine the relationship between storage days and positivity. In our model we included patient age, days since illness onset, and surveillance site. Only samples that were in refrigeration for duration of 0≤7days were included in analyses.

Results

Of 17,494 samples collected during the study period, 9,720 had storage data available and were included in the analysis. Of the 9,720 samples, 1113 (11.5%) were positive for influenza, of which 902 (9.3%) were Influenza A, 251 (2.6%) were Influenza B, and 40 (0.4%) were positive for both A and B. 5241 (53.9%) were from male patients. The mean age of patients was 3.4 years, and the majority of samples (63.0%) were from patients <2 years old.

In the multivariate analysis, the influenza positivity of samples stored for 0-3 days was similar to that of samples stored for 4-5 days (11.9% vs. 11.7%, p=0.48). However, the influenza positivity of samples stored for 0-3 days was significantly higher than that of samples stored 6-7 days (11.9% vs. 7.6% p=0.02). For the analysis of influenza positivity by individual storage days using multivariate logistic regression, there was not a statistically significant association between the duration of storage and influenza positivity.

Mean CT values were lower among samples stored for 0-3 days and 4-5 days compared to samples stored for 6-7 days (28.0 vs. 27.6 vs. 30.2, p=0.04). There was not a statistically significant difference in mean CT-values by individual storage days (p=0.22).

Conclusion

We found that influenza positivity by rt RT-PCR was not affected by the duration of specimen storage at 2-8ºC up to 5 days. However, after 5 days of storage, influenza positivity decreased slightly and CT values among influenza-positive specimens increased slightly. In light of these findings, we recommend that samples can remain in storage at 2-8ºC for up to 5 days. This additional storage time may be especially useful in rural areas, where frequent transport of samples to central laboratories can be challenging.

SPA 1: CLINICAL IMPACT & DIAGNOSTIC APPROACHES

A127P

Course of influenza in adult patients hospitalized during 2010 - 2011 epidemic season in Latvia

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Introduction

The aim of the study was to identify main influenza viruses in 2010-2011 epidemic season and course of influenza in hospitalized patient.

Material & methods

It was retrospective analysis of patient's medical documentation in 2010-2011 epidemic influenza season from archive of Infectology Center of Latvia. Obtained data were processed with Epi Info and MS Excel.

Results

Retrospective study analyzed 350 adult patients with medical cards with laboratory confirmed influenza diagnosis. In influenza epidemic outbreak during December and January disease was caused mainly by influenza A virus, while in February and March, influenza B virus. From tissue culture isolates, most of them were A/California/07/09 type influenza virus, but assessing the type B virus, most were B/Brisbane/60/08. Patients were mostly people under 30 years of age. About a third of patients admitted to hospital were from risk groups, mainly with chronic heart disease, pregnancy, immunodeficiency and age of the patients over 65 years, most patients had more than one risk factor. Belonging to a health-risk groups is attributed to a higher risk of complications and longer hospitalizations. On average, patients were hospitalized for third-fourth day after the onset of the disease. Through the interview about the epidemiological history, it was found that about half of the patients were able to produce a contact with an infected person, while the other half of contact with infected people did not know. From the study included only 2 were vaccinated against influenza. A more serious was type A influenza, because it had a higher risk of developing complications, more frequently required hospitalization in intensive care unit, as well as a whole had a longer hospital treatment, compared with more ongoing type B influenza. Prior to the hospitalization of specific antiviral therapy had received only nine patients, all cases were used oseltamivir. 343 of hospitalized patients received specific antiviral therapy, therapy was started on third-fourth day after the onset of the disease. 331 cases were administered oseltamivir (of which 4 cases were used intravenous administration), 11 cases, oseltamivir and zanamivir combination, in one case, zanamivir. 5% of influenza patients with ARDS needed hospitalization in intensive care unit, for part of them invasive ventilation was used and specific antiviral therapy was administred through parenteral route. In 2010 - 2011 season, in the Infectology Center of Latvia 5 patients had died, all of them were with one or more risk factors of disease, there were developed bilateral hemorrhagic pneumonia, and antiviral therapy for these patients was started late, which also may have contributed to more severe course of disease.

Conclusions

The overall conclusion is that the 2010 - 2011 influenza epidemic season in the first half disease was caused by a pandemic H1N1 influenza virus, so it could be regarded as pandemic influenza A/H1N1 follow, but in the seasons second half disease was more caused by type B influenza virus, and H1N1 was found in fewer and fewer. It is important to keep track of what is the current influenza season, leading the virus to be able to effectively prescribe treatment, and patients heal more quickly and would require shorter hospitalization which would cost less.

YOUNG SCIENTIST

SPB1 / SPB2 - VIRUS HOST INTERACTION/PATHOGENESIS/TRANSMISSION

B101P

The associations between the transmission and mortality of pandemic influenza and environmental variables

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Background

The 2009 pandemic influenza has caused over 19,633 deaths globally. Incidence declined after massive vaccination and numerous unreported infections in populations but new regional outbreaks have continuously been reported with dramatic temporal and geographic variations.

In 1981, Hope-Simpson proposed that influenza seasonality may be connected to seasonal variations of solar radiation. More recently, low humidity has been found to strongly modulate the survival and transmission of influenza virus. In 2009, Zhou further revealed and quantified the associations between influenza seasonality and dewpoint/temperature, sunlight exposure and precipitation (http://www.itmm.gov.cn/rdqx/rdqxen/ch/reader/view_abstract.aspx?file_no=20090101&flag=1). Nevertheless, little is known about potential correlations between climatic factors and influenza pandemics, which occur less frequently.

Method

Analytical statistics were conducted to examine the associations between the incidence/mortality of the 2009 pandemic and environmental variables for 3 general scenarios: the winter of temperate/frigid regions, the summer of tropical areas, and during seasonal transitions, with several examples of each across all continents and climate zones.

Results

The outbreak and spread of the pandemic, including incidence and death rate, were found strongly and significantly ($P = 0.0001 \sim 0.05 <$) associated with extremes of dewpoint (deviations from annual means), less sunlight exposure (due to either fewer sunshine hours and/or lengthy indoor stay during precipitation or extreme weathers) and successive rainfall. Specifically, in the winter of temperate/frigid regions, the incidence and death rate of the pandemic were highly associated with low dewpoint, with peaks of death rate arising 3-4 days and 11-13 days after the dewpoint minimized. These lags are close to the average period between symptom onset and death and similar to those found in seasonal mortality studies. In the summer of tropical areas, higher incidence and death rate of the pandemic were related to hot temperatures with peaks of death rate being closer to the hottest days. During seasonal transitions, pandemic activity was strongly correlated to the strength and frequency of dewpoint deviations from normal levels. The associations found were consistent in different populations, time scales, and climate zones. In all scenarios, there was evidence of reduced sunshine and extreme dewpoint/temperature in the days or weeks preceding elevated influenza activity. This may explain some waves and irregular patterns in day-to-day and week-to-week incidence and death rate of the pandemic.

Discussion & Conclusion

Our study confirms that climatic factors influence the outbreaks and mortality of the pandemic, and that there are likely to be more than one critical factor. Some influences may act on a short-term basis and others operate over a longer period. A complete influenza transmission model may have to include environmental variables besides virological factors.

Our findings may have implications for complementary strategies to contain pandemics besides vaccination. Close monitoring of weather variations and patterns that can trigger/aggravate underlying medical conditions during pandemics is warranted to better plan for healthcare load and intensive care burden. As a portion of patients are vulnerable to the variations of atmospheric pressure corresponding to temperature extremes, constant pressure for intensive care settings is worthy of consideration. The association with solar radiation suggests the possibility of application of UV germicidal equipment in healthcare industry, or supplementation with vitamin D that is usually produced by UV-B. Sanitary air conditioning with higher dewpoint (more humid) settings in frigid seasons and wearing of respirators to keep the respiratory tract warm and humid may help ameliorate pandemics.

The pathological and epidemiological mechanisms through which environmental factors influence pandemic activity deserve further studies. A plausible explanation is that synoptic variables can simultaneously affect virus, transmission media and hosts, including the survival and activity of the virus, bio-aerosols, herd immunity, body's metabolism, underlying medical conditions, host contact rate, and human behaviors.

No conflict of interest

SPB1 / SPB2 - VIRUS HOST INTERACTION/PATHOGENESIS/TRANSMISSION

B102P

Pathological changes in mice lungs during experimental infection with natural and mice-adopted strains of h1n1 (2009) pandemic influenza virus.

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Materials and methods

Animals. We used mice (BALB/c lineage) model for adaptation and Pathogenicity investigation.
Virus. We used A/Russia/01/2009 strain, isolated from lethal human case.
Adaptation. On the first passage mice were inoculated intranassaly with A/Russia/01/2009. For all other passages lungs homogenate was used. Total number of 8 passages was made. The adopted strain was named A/Russia/01/2009-mice. Full-length sequences of both strains are available in GeneBank.
Morphology. Hemotoxilin and eosin stained lung tissue samples were used.

Introduction

A/H1N1 (2009) pandemic has started an outbreak in pigs in Mexico in March 2009. The virus genome was an unique combination of avian, pigs and human originating influenza viruses genes. The new virus was very contagious but overall lethal rate was low. After the pandemic end A/H1N1 pdm started circulating in human population. In this case it seems very important to evaluate adaptation and pathogenicity increasing possibilities for this virus.

Results

Using a BALB/c mouse model, we compared the virulence of generated mouse-adapted variants with that of the original strain. First of all we determined MLD_{50} for A/Russia/01/09-mice. Six-week old female BALB/c mice were inoculated intranasally with 50 ml of successive tenfold dilutions of the mouse-adapted virus suspended in PBS, and observed for 20 days for lethality. Control mice were inoculated with PBS. The MLD_{50} value was (1,775 ± 0,2) lg $TCID_{50}$/ml, calculated based on the Reed and Muench method.
After determination of MLD_{50} value we conducted experiments to investigate the pathogenicity of the virus A/Russia/01/09-mice (H1N1). We infected two groups (n=10) of 6-week BALB/c mice with 0.5 and 10 MLD_{50}. We weighed animals in a day during 15 days post infection (dpi). After 6–7 dpi, in BALB/c mice infected with 0.5 MLD_{50} we observed clinical signs, including decreased activity, huddling, hunched posture, and ruffled fur as well as 11 ± 7% weight loss. Two mice died on day 14 post infection. In the second group we observed clinical signs on 3–4 dpi. The decrease in weight was 12 ± 4% at that time. The first mice deaths was registered on the 6 dpi. The weight loss was fast and reached 35%. At the same time the wild strain A/Russia/01/2009 at the same doses (in $TCID_{50}$) did not influence BALB/c mice. However, when we increased the infection doses till $10^4 - 10^6$ $TCID_{50}$ per mouse in 2–3 days we observed clinical signs of a disease. Morphological investigation had shown that A/Russia/01/2009-mice initiates inflammation and small-focal pneumonia on the first d.p.i. Total pneumonia was observed on the 3 d.p.i. In the respiratory compartment of lungs dis- and atelectasis, intraalveolar blood infusions, microcirculation disruption were observed. The original strain A/Russia/01/2009 did not cause mortality in mice. On the 3-6 d.p.i inflammation process causes bronchiospasm. On the 6 d.p.i. inflamotory-cellular infiltration in bronchial tubes and purulent bronchitis can be observed. Small-focal pneumonia was located mostly in periobronchial compartment. On the 10 d.p.i. intraalveolar blood infusions, and microcirculation disruptions could be observed. Blood-vessel compartment in both groups showed the signs of dystonia, blood-vessel walls alteration and inside-vessel thrombosis. To understand the molecular basis for increased virulence acquired by the virus A/Russia/01/2009 during mouse adaptation, the complete genomes of the wild-type and mice-adapted counterparts were sequenced. Sequence analysis revealed that a total of 7 amino acid substitutions affecting HA, NA, PB1 and PB2 proteins were involved in adaptation of 2009 pandemic strain to mice. So H1N1(2009) pdm has a great adaptation potencial in terms of in-mamal pathogenicity. That can cause emerging of highly pathogenic strains during its circulation in human population.

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SPB1 / SPB2 - VIRUS HOST INTERACTION/PATHOGENESIS/TRANSMISSION

B103P

Only Two Residues Are Responsible for the Dramatic Difference in Receptor Binding between Swine and New Pandemic H1 Hemagglutinin

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In view of its critical role in influenza A virus (IAV) tropism and pathogenesis we evaluated the receptor binding properties of hemagglutinin (HA) proteins of the closely related swine and new pandemic human IAVs. We generated recombinant soluble trimeric H1 ectodomains of several IAVs and analyzed their sialic acid binding properties using fetuin-binding and glycan array analysis. The results show that closely related swine and new pandemic H1 proteins differ dramatically in their ability to bind these receptors. While new pandemic H1 protein exhibited hardly any binding, swine H1 bound efficiently to a number of α2-6-linked sialyl glycans. The responsible amino acids were identified by analyzing chimeric H1 proteins and by performing systematic site-directed mutagenesis of swine and new pandemic human H1 proteins. The difference was found to map to residues at position 200 and 227. While substitution of either residue significantly affected the binding phenotype, substitution of both was found to act synergistically and reverse the phenotype almost completely. Modeling of the T200A and E227A substitutions into the crystal structure of the new pandemic human H1 protein revealed the loss of potential hydrogen bond formation with Q191, which is part of the 190-loop of the receptor binding site, and with the penultimate galactose, respectively. Thus, a residue not belonging to the receptor binding site may affect the interaction of HA with its receptor. Interestingly, while alanine at position 200 is found in most new pandemic human viruses, the residue at position 227 in these viruses is invariably a glutamic acid.

SPB1 / SPB2 - VIRUS HOST INTERACTION/PATHOGENESIS/TRANSMISSION

B104P

Pattern of Human Leukocyte Antigen Allele Co-expression Influences Immunodominance of Anti-influenza Cytotoxic T Lymphocytes Responses

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Introduction

A number of factors have been proposed to contribute to immunodominance (ImDc) of cytotoxic T lymphocyte in their response to influenza A (flu). The exact mechanisms leading to ImDc are not clear. Investigating factors leading to ImDc in humans or normal mice is not easy because of multiple MHC class I allele expression. We hypothesized that co-expression of different allele combinations will determine whether one observes ImDc.

Materials & Method

To investigate our hypothesis, we generated 'humanized' transgenic (Tg) mice which are deficient for endogenous mouse MHC class I molecules and express only one human MHC class I allele at a time. To assess whether co-expression of multiple, as opposed to expression of single, MHC class I alleles influences the pattern of anti-flu CTL epitope recognition and ImDc, novel doubly HLA transgenic mice were established on a H2-K-/-/D-/- background and characterized. These include Tg HLA-A2/B7, Tg HLA-A2/B27, and Tg HLA-B7/B27.

Results

In agreement with our hypothesis in flu-infected, doubly Tg HLA-A2/B7 or HLA-A2/B27 mice, IFN-γ ELISpot assays with A2/M1.58-66 and B7/NP418-426 or B27/NP383-391 flu epitopes showed strong recognition of both peptides for both alleles. In contrast, in flu-infected doubly Tg HLA-B7/B27 mice a significantly reduced level of B27/NP383-restricted CTL response was detected while there was virtually no change in the response level of B7/NP418-restricted CTL. These results demonstrate a form of ImDc. Subsequent studies suggested co-expression of HLA-B7 with HLA-B27 may lead to a partial deletion of Vß8.1+ B27/NP383-restricted CD8+ T cells in these doubly Tg B7/B27 mice.

Conclusion

These results indicate that the pattern of allele co-expression influences the CTL response following flu infection.

SPB1 / SPB2 - VIRUS HOST INTERACTION/PATHOGENESIS/TRANSMISSION

B106P

Crk adaptor protein expression is required for efficient replication of avian influenza A viruses and controls JNK mediated apoptotic responses.

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Introduction

The non structural protein 1 (A/NS1) is an important virulence factor of influenza A viruses (IAV) by its interference with the innate immune response of the host cells e.g. by interaction with host cell proteins. For the interaction with cellular proteins, the A/NS1 harbors e.g. several src homology domain (SH)-binding motifs which are required for interaction with proteins, such as the p85beta subunit of PI3-kinase. A SH3-binding motif (aa 212-217 [PPLPPK]) within A/NS1 has been shown to be essential for binding to the cellular adaptor proteins Crk/CrkL. Both regulate diverse pathways in the cell including activation of the MAP kinase JNK, which was previously shown by us to mediate antiviral responses.

Material and methods

To elucidate Crk/CrkL functions in IAV infected cells we used a siRNA approach for knock-down of Crk/CrkL expression. For monitoring IAV replication, standard plaque titrations were conducted. Protein interaction studies were carried out by co-immunoprecipitation assay and Western blot analyses. Furthermore Western blot studies were used to evaluate the activation state of cellular signaling pathways and the onset of cell death. Finally FACS based PI stainings and Nicoletti assays were performed for the analysis of cell death, as well.

Results

By studying functions of the Crk/CrkL adaptor proteins in IAV infected cells, we could demonstrate that only those IAV that encode an A/NS1 protein harboring the functional SH3-binding motif PPLPPK are attenuated upon downregulation of CrkI/II or CrkL, but not of CrkII alone. The PPLPPK site-harboring candidate strains could be discriminated from other strains by a pronounced viral activation of the JNK-ATF2 signaling module that was even further boosted upon knock-down of CrkI/II. Interestingly, this enhanced JNK activation did not alter type-I IFN-expression, but rather resulted in increased levels of virus-induced Caspase-9 cleavage and cell death.

Our current studies in particular focus on the mechanistical role of the different Crk proteins in the IAV induced activation of the JNK signaling pathway. Our obtained data so far substantiate the existence of non redundant functions of the different Crk adaptor proteins in IAV induced JNK signaling.

By investigating the direct impact of the different Crk adaptor proteins on the complex formation of JNK and its activators MKK4 and MKK7, we try to substantially elucidate the, to date unknown, cellular mechanism for the IAV induced activation of JNK signaling.

Discussion

Taken together, our results so far imply that binding-capacity of A/NS1 to Crk/CrkL has evolved in virus strains that over-induce the antiviral acting JNK-ATF2 signaling-module and helps to suppress the detrimental apoptosis promoting action of this pathway. Furthermore, with regard to the exposure of pandemic outbreaks, we revealed a new piece of the puzzle to understand zoonosis descended from avian origin.

SPB1 / SPB2 - VIRUS HOST INTERACTION/PATHOGENESIS/TRANSMISSION

B107P

"To see what is in front of one's nose needs a constant struggle." Treating the host response to severe influenza

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Influenza scientists study influenza viruses and develop vaccines and antiviral agents, yet they have left unanswered three critical questions. First, what are the mechanisms responsible for influenza-related acute lung injury (ALI), multi-organ failure and death? Second, what is the significance of underlying low-grade inflammation that seems characteristic of most patients with severe influenza, regardless of age? Third, what explains the different mortality rates of children and young adults in the 1918 and more recent influenza pandemics?
Non-influenza scientists who study ALI, sepsis, inflammation, cardiovascular diseases, trauma, burn injury and energy metabolism have helped to answer all three questions. First, the host response to severe ALI due to many causes, including inactivated H5N1 influenza virus, proceeds along common cell signaling pathways. Second, most individuals with underlying risk conditions - cardiopulmonary and renal diseases, diabetes, obesity, asthma and pregnancy – have altered levels of many cytokines; compared with normal individuals, their "innate immune rheostats" seem to be set at different and more precarious levels. Third, children have lower mortality rates than young adults in influenza pandemics for the same reasons they have lower ALI mortality due to many other conditions: measles, sickle cell chest syndrome, cerebral malaria, severe trauma and burn injury. Importantly, the dysregulated host response to severe influenza includes numerous molecular targets that are positively affected by anti-inflammatory and immunomodulatory agents such as statins, PPAR agonists (fibrates and glitazones) and AMPK agonists (metformin) (Table). Fibrates, glitazones and AMPK agonists reduce mortality in influenza virus-infected mice by 40-50%, and do so without increasing virus replication. Statins given to patients hospitalized with laboratory-confirmed influenza reduce hospital mortality by 41%.

Cell signaling targets and the effects of immunomodulatory treatment

- Up regulate HO-1 and decrease TLR signaling by PAMPs and DAMPs
- Down regulate NF-kappaB and pro-inflammatory cytokines (e.g., TNFa, IL-1, IL-6)
- Up regulate anti-inflammatory cytokines (IL-10, TGFb)
- Up regulate pro-resolution factors (lipoxin A4, resolvin E1)
- Down regulate HMGB1and late mediators of inflammation
- Up regulate adipokines (adiponectin) that decrease inflammation
- Up regulate eNOS, down regulate iNOS, restore iNOS/eNOS balance and stabilize cardiovascular function
- Decrease formation of reactive oxygen species and oxidative stress
- Decrease tissue factor and its associated pro-thrombotic state
- Attenuate the C5a-C5aR-related increase in vascular endothelial permeability
- Stabilize the actin cytoskeleton in endothelial cells and adherens junctions, thereby increasing pulmonary barrier integrity and decreasing vascular leak
- Attenuate acute disease-associated pulmonary hypertension
- Regulate the balance between Th17 and Treg cells
- Differentially modify caspase activation and apoptosis in epithelial and endothelial cells, macrophages, neutrophils and lymphocytes in the lung and other organs
- Up regulate PGC-1a, improve mitochondrial function and restore mitochondrial biogenesis

Despite the best efforts of in?uenza scientists, health officials and companies, the global response to the H1N1 pandemic fell far short of what was needed: > 90% of the world's people did not have timely access to affordable vaccines and antiviral agents. Instead, they had to depend on 19th Century public health "technologies" to see them through. In the 21st Century, they should have had something better. We urgently need research to determine whether these immunomodulatory agents could be used to manage patients with severe influenza who are at risk of multi-organ failure and death. These agents are currently produced as inexpensive generics in developing countries. In any country with a basic health care system, they would be available on the first pandemic day, and the cost of treating each patient would be less than €1.00. Knowing this, everyone attending this ESWI conference should ask, "Why isn't this research going forward?"

SPB1 / SPB2 - VIRUS HOST INTERACTION/PATHOGENESIS/TRANSMISSION

B109P

Correlation of influenza infection with glycan array

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Introduction

The past 6 years has seen the introduction of glycan arrays containing large numbers of sialic acid (Sia) containing compounds and these arrays have been used to demonstrate the relative binding affinity of influenza viruses to different glycans. Though informative, there has been little correlation of the results of these arrays with actual influenza virus infection. We therefore used influenza viruses that have been arrayed and infected a subset of chinese hamster ovary cells (CHO) cells which have had mass spectrometric analysis performed and determined the validity of these arrays to predict actual infection.

Materials and Methods

CHO Pro-5 cells were infected with seasonal H1N1 and H3N2 viruses, H9N2 and H5N1 avian viruses and H1N1pdm viruses. After 24 hours cells were fixed and stained for influenza nucleoprotein followed by image analysis by Aperio software. Lec11 cells that had increased fucosylation and Lec1.3 cells that lack N-linked sialylation were also used.

Results

CHO Pro-5 cells, which only contain Sia alpha 2-3 linkages were readily infected by WSN/33, H5N1 viruses and G1/H9N2 viruses. Only low rates of infection were seen with H1N1 seasonal virus and there was no significant infection with H3N2, Y280/H9N2 or H1N1pdm viruses. Infection was increased when there was increased fucosylation present in the Lec11 cells and only H5N1 viruses infected Lec1.3 cells. These infection results correlated with findings from arrays produced by the Consortium for Functional Glycomics (CFG) and Center for Disease Control and Prevention (CDC).

Conclusion

Our findings support the validity of using glycan arrays to investigate the differential tropism of influenza viruses and receptor preference.

SPB1 / SPB2 - VIRUS HOST INTERACTION/PATHOGENESIS/TRANSMISSION

B112P

Regulation of interferon gene expression by influenza a and b viruses in human dendritic cells - rapid antiviral activation in influenza b virus infection

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One of the major cellular responses to virus infection is the synthesis of interferons (IFNs). In the present study we have analyzed the ability of influenza B virus to induce IFN responses in comparison to influenza A virus. Both influenza A and B viruses were unable to induce a significant IFN production in human monocyte-derived dendritic cells (moDCs). However, both viruses triggered the expression of IFN-alpha, IFN-beta, IFN-lambda1 and IFN-lambda2 genes: influenza B induced a fast IFN gene expression that was drastically turned off at later phases of infection, whereas influenza A enhanced IFN gene expression more slowly. The early induction of IFN genes was mediated by IRF3 phosphorylation that was evident already at 1h after infection with influenza B virus but it was not seen until 2h after influenza A virus infection. NS1 protein is known to inhibit the IFN responses by interfering with the activation of IRF3. However, transfected NS1A and NS1B inhibited the activation of IFN-lambda1 promoter equally well in a promoter-reporter luciferace assay. For analyzing the effects of the early viral replication on the IRF3-mediated IFN responses we UV-irradiated the viruses and studied IFN induction in cells infected with killed viruses. Inactivated influenza B viruses, but not those of influenza A, were capable of induce the IFN responses in virus-transduced DCs. As there seems to be some early events unique to the influenza B virus infection that triggers the IRF3-mediated IFN responses, we produced the full-length ssRNA segments of influenza A and B viruses in vitro. All the viral ssRNA segments, whether they originated from influenza A or B viruses, induced IRF3 activation and IFN gene expression equally well. Next we isolated vRNP particles from the viruses and transfected these complexes into HEK293 cells. Interestingly, vRNPs from influenza B viruses were able to activate IRF3-mediated IFN responses during the early time points and this activation was dependent on encapsidation of the vRNP complexes. This suggests that influenza A viral RNA is better hidden or more tightly packed into vRNP particles than influenza B viral RNA, which apparently is more exposed or more easily unfolded from the vRNP particles and recognized by cellular RNA receptors more efficiently. Our results indicate that in human DCs influenza B virus activates host innate immune responses at early stages of infection. Influenza A virus, instead, has an ability to avoid or delay the activation of host innate immune responses likely giving this virus an advantage over influenza B virus to downregulate host interferon responses.

SPB1 / SPB2 - VIRUS HOST INTERACTION/PATHOGENESIS/TRANSMISSION

B113P

Transmission of influenza to health-care workers in hospitals - could aerosol generating procedures play a role?

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Introduction

Many national authorities recommend that Healthcare workers (HCW) wear respiratory protective equipment (RPE) when treating H1N1 (2009) influenza patients undergoing suspected aerosol generating procedures (AGPs). These procedures have been specified by WHO and include positive pressure ventilation, bronchoscopy and tracheostomy. However, there is limited evidence that AGPs actually generate infectious aerosols. The requirement for use of RPE places demands on hospitals for the provision of single use facemasks, fit testing and training in their use. The aim of this study is to assess the level of exposure of HCW to aerosols of H1N1 during a range of 'high risk' procedures carried out on H1N1 patients and to provide evidenced based guidance for the use of RPE.

Materials and Methods

After ethical approval was obtained and methodologies validated, air sampling was undertaken in hospital environments in which patients infected with H1N1 were receiving these 'high risk' procedures.

A variety of air samplers were used to assess temporal and spatial dispersion of H1N1 aerosols near patients and HCWs. Air samples were fractionated into the following aerodynamic particle size ranges, <3.3µM, 3.3-6µM and >6µM. Sensitive qRT-PCR (calibrated using a copy number based standard curve) was used to detect influenza in samples after concentration by centrifugation.

Results

Of 57 suspected H1N1 cases sampled, 39 (68 %) were confirmed as being influenza positive from respiratory tract swabs. Out of a total of 492 samples, 37 air samples (7.5%) contained detectable levels of influenza, 9 of these air samples were <3.3µM in aerodynamic size, 16 air samples were in the 3.3-6µM range and 12 air samples were >6µM. The median number of viral copies/litre in each size range were 9608.9 (SD=22823.8), 4418.8 (SD=26751.6) and 5091.1 (SD=106193) respectively. Initial analysis has shown that the procedures most often linked to positive air samples were: mouthcare, bronchoscopies and procedures associated with chest physiotherapy (e.g. bagging, nebulisation, induced coughing and suctioning).

Conclusions

These results indicate that HCW are occasionally exposed to aerosolized influenza virus clouds when treating H1N1 patients. It is, however, difficult to definitively link aerosol production to any one specific procedure as sample sizes are low, and there is variability in the procedures undertaken and between patients. However, it is possible to assign risk levels to "aerosol generating procedures" on the information gathered by this project on aerosol production.

YOUNG SCIENTIST

SPB1 / SPB2 - VIRUS HOST INTERACTION/PATHOGENESIS/TRANSMISSION

B114P

Virus shedding and environmental deposition of influenza

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Introduction

As pandemic mitigation strategies have been developed over recent years it has become very clear that influenza transmission is an area that is poorly understood and hotly debated. The biggest controversy relates to whether influenza is mainly transmitted by touching virus deposited on surfaces, or by droplets or bioaerosols in the air. Furthermore, little is known about the extent to which virus is deposited by infected individuals into the environment and whether deposited virus has the ability to infect new hosts, i.e. whether it remains viable. This study was conducted to collect data on patients who had 2009 A(H1N1) infection.

Materials and Methods

Primary objectives were to describe virus shedding and investigate virus deposition in a subject's immediate environment (on fomites and in the air). Adults and children, both in hospital and from the community, who had symptoms of infection, were enrolled over 2 influenza seasons (2009/10 and 2010/11) and followed up for a maximum of 12 days. Information about symptoms was collected and samples were taken including nose swabs and swabs from surfaces and objects (fomites) around patients (e.g. door handles, remote controls). Samples of air were obtained using a two-stage cyclone bioaerosol sampler. Samples were tested using polymerase chain reaction (PCR) to detect virus genome and immunofluorescence to detect viable virus.

Results

105 subjects were followed up; 48 were infected with 2009 A(H1N1), 7 had influenza B. The following results pertain to H1N1 infection. On average, subjects were enrolled 2.2 days after symptoms began and were followed up for 6.3 days. 24/48 (50%) subjects received an antiviral (all oseltamivir); hospital cases 20/23 (87%), community cases 4/25 (16%). A rapid antigen test had a sensitivity of 37% and a specificity of 100%. The mean duration of virus shedding (by PCR) was at least 5.9 days (some cases were PCR positive on their last day of follow up).

The following results are currently only available for the first year of study (H1N1 subjects = 19); full results will be presented at the meeting. The mean duration of viable virus shedding (in 12 subjects who tested positive by immunofluorescence) was 4.7 days. Over 30% of subjects remained potentially infectious for at least five days. Virus shedding was not always greatest when an subject had their peak symptoms. 0.5% of all community and none of the hospital fomite swabs revealed virus on surfaces. 5 subjects had samples of the air around them collected and virus was detected by PCR from 4. Some of the air particles in which virus was detected were small enough to be inhaled and deposited deep in the lungs.

Preliminary Conclusion

Despite some limitations caused by the small number of subjects recruited, important observations have been made. The finding that over 30% of infected subjects had infectious virus in their noses for 5 days or more has infection control implications. The data generated to date suggest that contact transmission of pandemic influenza via fomites may be less important than hitherto emphasised; whereas transmission via bioaerosols at short range may be possible, meaning that high level personal protective equipment (PPE) might be needed by healthcare workers when attending patients with pandemic influenza. Although the data remain uncertain, further work should be performed to consolidate these findings as they have important potential implications for the protection of healthcare workers and the formulation of advice to households, nationally and internationally.

No conflict of interest

YOUNG SCIENTIST

SPB1 / SPB2 - VIRUS HOST INTERACTION/PATHOGENESIS/TRANSMISSION

B115P

Influenza a virus ns1 differentially redistributes and activates heterotypic pi3k complexes

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The multifunctional NS1 protein of influenza A virus performs a broad range of functions within the infected cell, notably contributing to evasion of host antiviral responses and overall viral fitness. Among this plethora of activities, one of the less understood is the activation of class IA phosphoinositide-3-kinases (PI3K) by directly interacting with the inhibitory subunit p85ß. Class I PI3K are heterodimeric enzymes composed of a regulatory subunit (mainly p85α or p85ß) and a catalytic subunit, p110, with three isotypes designated α, ß and δ. Here we describe the use of a bimolecular fluorescence complementation assay (BiFC) to assess the effect of NS1 on the distribution of heterotypic PI3K complexes with different p110 isotypes. Upon coexpression with NS1, the PI3K complexes show different phenotypic and functional patterns that correspond to various known signaling pathways in which PI3K is involved. Some of these pathways are different from the canonical PI3K-Ptds(3,4,5)P_3-Akt signaling cascade, suggesting a broader range of functional outputs for NS1 activation of PI3K than those considered up to date. We postulate that the variety of p85/p110 heterodimers present in infected cells may greatly determine the biological effect of NS1-mediated activation of PI3K, and thus propose that a PI3K isotype-specific elucidation of the pathways activated this way is needed to fully and clearly understand why and how influenza virus tweaks this crucial signaling chokepoint. Overall, our findings may lead to a better understanding of the effects of influenza virus infection in PI3K-regulated pathways, as well as to provide a tool to selectively track and study functionally different protein complexes that share some of their components.

SPB1 / SPB2 - VIRUS HOST INTERACTION/PATHOGENESIS/TRANSMISSION

B116P

Quantitation of cytokine mRNA levels in ferrets following influenza infection

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Following infection with influenza virus, innate immunity drives a rapid inflammatory response to the invading pathogen. This is complemented by the antigen-specific adaptive immune response where lymphocytes recognize viral antigens and use different effector mechanisms to clear the infection. Analysis of cytokine profiles can enable characterization of this immune response at the sites of infection. Highly pathogenic influenza viruses typically show altered cytokine profiles compared to low pathogenic viruses (Cameron J Virol 2008, 82:11308). Oseltamivir treatment reduces cytokine levels in nasal lavage fluids in clinical trials (Hayden JAMA 1999, 282:1240). Thus cytokine profiling may provide valuable information on the quality and level of the immune response, and virulence and pathogenesis of different influenza viruses.

In vivo analysis of novel human influenza viruses often uses the ferret model as they can easily be infected with most human influenza viruses and display similar symptoms as humans. Infection in ferrets is measured by viral load in the respiratory tract, transmission events and clinical symptoms. Seroconversion and secretory IgA are also indicative of infection or an immune response following vaccination. However, a more detailed analysis of the quality and level of the immune response is limited by reagents available for the ferret. Cross-reactive antibodies can identify some lymphocytes and a small number of cytokines have been measured by real time PCR and arrays. Thus the aim of this study was to develop a high-throughput real time RT-PCR assay to quantify cytokine m-RNA profiles in the respiratory tract of influenza-infected ferrets.

Ferret-specific primer/probe sets were designed to target conserved regions from cytokines of the innate and adaptive immune responses. Control primer/probe sets for housekeeping genes have also been designed and tested. DNA gene target standards have been developed through RT-PCR and cloning. The assay has been optimized to maximize sensitivity and specificity. We have also determined the most suitable methods for RNA stabilization, residual DNA removal, and cDNA preparation from our ferret samples. We anticipate this assay will enable analysis of the immune response during an experimental influenza infection in ferrets or following a novel vaccine trial. Data would enable more sensitive analysis of the severity of an infection and the potential for modification of the immune response to prevent further infections.

SPB1 / SPB2 - VIRUS HOST INTERACTION/PATHOGENESIS/TRANSMISSION

B117P

Excessive Innate Immune Response and Mutant D222G/N in evere A (H1N1) Pandemic Influenza: A Prospective Observational Cohort Study

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Aim

Explore the role of viral factors and immune response in patients with severe pandemic pdmH1N1 illness without significant co-morbidity.

Materials

Seven patients with documented pdmH1N1 influenza, bilateral chest X-rays infiltrates, and in need of mechanical ventilator support were consecutively recruited. Eight age- and gender-matched healthy individuals served as controls.

Results

Four patients were viremic, and sequence information from two showed a mutant D222G/N pdmH1N1. Microarray analyses of peripheral blood leukocytes suggested a marked activation of granulocytes, but no up-regulation of inflammatory cytokine mRNA. Patients with severe pdmH1NI had a marked degree of systemic complement activation, and in contrast to the lack of cytokine mRNA up-regulation in blood leukocytes, plasma levels of a broad range of inflammatory mediators, including IP-10, and mediators involved in pulmonary remodelling were markedly elevated. Viremic patients with mutant virus were characterized by particularly high IP-10 levels, and the most pronounced degree of complement activation.

Conclusions

These data suggest a link between pdmH1N1 viremia and mutant virus, and excessive and maladaptive immune response, reflected in activation of complement, granulocyte, a broad range of inflammatory cytokines, and mediators involved in pulmonary remodelling, possibly contributing to the tissue damage seen in patients with severe pdmH1N1 infection.

SPB1 / SPB2 - VIRUS HOST INTERACTION/PATHOGENESIS/TRANSMISSION

B118P

QTL mapping identifies host genetic factors determining susceptibility to influenza A infection

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Influenza A virus leads to variable disease severity across the human population caused by genetic polymorphisms of the hosts. Using the mouse as model organism for influenza A infection, we recently showed that the genetic background strongly influences the response to influenza A by infection of different inbred strains. The inbred mouse strain DBA/2J was highly susceptible compared to the C57BL/6J strain. The LD50 to the mouse-adapted Influenza A virus (PR8) in DBA/2J was more than 1000-fold lower than that in C57BL/6J. In order to identify host sequence variants modulating susceptibility, we performed QTL (quantitative trait loci) mapping using a large family of strains derived from DBA/2J and C57BL/6J. Both parental strains, F1 hybrids (B6D2F1), and 53 BXD strains were infected with influenza A H1N1 (A/PR/8/34). The infection was monitored for 13 days. Different traits, including the mean time of death, survival, and body weight were used for QTL mapping. By using the WebQTL module of GeneNetwork (www.genenetwork.org), a significant QTL was mapped on chromosome 5 for all traits. In addition, three suggestive QTLs were localized on chromosomes 16, 17 and 19. Currently, the identified chromosomal loci are under further investigation to identify candidate genes contributing susceptibility and resistance to influenza A infection.

SPB1 / SPB2 - VIRUS HOST INTERACTION/PATHOGENESIS/TRANSMISSION

B119P - Virus-host interaction / pathogenesis / transmission

Differences in HA receptor binding and NA substrate specificities of high and low pathogenic avian influenza viruses

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Background

The viral glycoproteins, HA and NA, expressed on the surface of influenza virions play an important role in determining pathogenic properties of this virus. Influenza virus infection is initiated by specific interactions between the viral HA and terminal sialic acid-containing molecules of cell surface receptors mediating the virus absorption on the target cells. The NA cleaves off the terminal sialic acid residues from the host cell promoting the release of virus progeny and preventing the formation of virion aggregates at the budding site. The functional balance between HA and NA is prerequisite of successful influenza virus replication. Human infections caused by highly pathogenic avian influenza viruses emphasize a need for the assessment of factors that allow adaptation of avian viruses (AIV) to humans. Here, in order to clear a possible role of HA and NA as well as their balanced action in pathogenic properties of AIV, we evaluated HA receptor binding and NA substrate specificities of high and low pathogenic avian influenza (HPAI and LPAI) viruses of H5N1, H5N9, H5N2, H7N7 and H9N2 subtypes for a set of synthetic sialooligosaccharides which are analogues of the natural influenza virus receptors.

Methods

Receptor binding specificity of HA was investigated in a direct binding assay as described previously (Matrosovich et al., 2000). In brief, 96-well plates were coated with purified virus for 16 h at 4°C. After that biotinylated sialyloligosaccharides in TN buffer containing neuraminidase inhibitor were added. Following the addition of Strept-POD and ABTS substrate solution, optical density at 405 nm was determined. The affinity constants (Kaff) were determined as sialic acid concentration (µM-1) at the point Amax/2 on Scatchard plots.

The fluorescent assay for studying the substrate specificity of neuraminidase was described earlier (Mochalova et al., 2005). The method based on the a quantitative separation of neutral fluorescent-labeled product from negatively charged fluorescent-labeled uncleaved substrate using anion exchanger microcartridges. Fluorescence was measured at 485/535 nm. Virus NA specificity for each sialoside was calculated as the slope of the starting linear region of the Vo versus So curve (Vo - initial rate of the desialylation, So - initial substrate concentration).

Results

HA receptor binding and NA substrate oligosaccharide specificity are usually defined as ability of the viral HA or NA to distinguish the type of bond between terminal neuraminic acid and galactose residues. HPAI viruses displayed a higher (2-12 times) HA affinity and higher (2-8 times) NA hydrolytic efficiency than LPAI viruses towards trisaccharides Neu5Acα2-3Galß1-4GlcNAcß (3'SLN) and Neu5Acα2-3Galß1-3GlcNAcß (SiaLec). Furthermore, HAs of HPAI and LPAI viruses differed in their ability to discriminate non-fucosylated and fucosylated glycans, namely, 3'SLN vs. Neu5Acα2-3Galß1-4(Fucα1-3)GlcNAcß?(SiaLex). When receptor binding specificity was plotted as 3'SLN/SiaLex ratio, the values for all HPAI viruses ranged between 3 - 5 whereas for LPAI viruses these values were only about 2 or lower. We hypothesize that the optimal ratio of affinities towards 3'SLN and SiaLex is an important modulator of pathogenicity.

Conclusion

In summary, this study presents combined data on HA and NA specificity of HPAI and LPAI viruses. A combination of both methods allows for a rapid monitoring of changes in the OS specificity of both HA and NA. Correlation of these data with mutational analyses of the glycoprotein genes promise to be a powerful tool for the prediction of new pandemic strains.

SPB1 / SPB2 - VIRUS HOST INTERACTION/PATHOGENESIS/TRANSMISSION

B120P

Role of the cellular adar1 protein in influenza a virus infection

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The nucleolus is a dynamic hub of post-transcriptional RNA processing in eukaryotic cells. The influenza NS1 protein has previously been seen to localise to nucleoli, though its role there is not clear. However, we recently found that influenza A virus infection induces the relocalisation of adenosine deaminase acting on RNA 1 (ADAR1) to the nucleolus. ADAR1 is an RNA-editing enzyme that converts adenosine residues to inosine (A-to-I). Due to the interpretation by cellular machinery of inosine as guanosine, there is potential that ADAR1 activity may affect the influenza virus genome; either negatively through hypermutation, or positively via a specific coding sequence alteration as seen with hepatitis delta virus. Evidence for non-specific editing of influenza RNAs has been reported, but the significance of this has not been demonstrated. Indeed, in other virus families such as vesicular stomatitis, measles, HIV and hepatitis delta viruses, ADAR1 has an apparently pro-viral role.
We find that NS1 is both necessary and sufficient to induce nucleolar relocalisation of ADAR1. Analysis of viruses expressing mutant forms of the NS1 protein showed that proteins with lesions in the RNA binding domain or TRIM25 interaction site failed to induce this relocalisation. We also show by GFP-trap pull-down assays that ADAR1 interacts with influenza NS1 and the viral polymerase subunits in infected cells, but not with viral ribonucleoprotein complexes (vRNPs). However, virus replication in cells treated with siRNA against ADAR1 was reduced by 3-fold, not supporting the hypothesis that ADAR1 is a restriction factor for influenza A virus.
We conclude that the influenza A virus NS1 protein induces the nucleolar relocalisation of ADAR1 and that this interaction is to the benefit of the virus. Further work is underway to test mechanistic hypotheses for how this might operate.

SPB1 / SPB2 - VIRUS HOST INTERACTION/PATHOGENESIS/TRANSMISSION

B121P

Host specificity of influenza A viruses: phenotypic and genotypic comparison of pandemic A/H1N1(2009) variants isolated from pigs and other mammals

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The epidemiological surveillance of swine influenza viruses (SIV) in recent years in France showed co-circulation of enzootic European H1N1 and H1N2 strains, with an increasing amount of new reassortants between these two lineages. Following its emergence in humans in 2009, variants of the pandemic H1N1 influenza virus (H1N1pdm) of porcine origin were isolated from pigs in metropolitan and oversea France, as well as from a domestic cat. Some H1N1pdm variants were responsible for a severe disease in their animal host, while other infections remained unapparent or mild.

The objective of this work was to define the genotype and phenotype of H1N1pdm variants isolated from pigs (symptomatic or not), cat and human in order (i) to identify specific molecular determinants that would correlate to virological characteristics after transmission from human to these other mammalian hosts, (ii) to define if these characteristics are associated with a specific pathogenesis in the host, and (iii) to compare these characteristics to those of enzootic H1N1 and reassortant SIV.

The different H1H1pdm isolates were characterized antigenically and genetically, and their replication capability and their plaque formation ability were studied in vitro using a pig trachea cell model, NPTr, at different temperatures (37 or 40°C) and compared to the usual MDCK cell culture model for mammalian influenza viruses.

All the H1N1pdm strains studied were antigenically very close, but they showed some genotypic differences according to the host species of origin. Phylogenetic analysis on the HA and NA genes also showed that these strains form a new distinct phylogenetic cluster, with a great similarity, where they are not gathered according to their original host.

Despite efficient replication at 37°C in NPTr and MDCK cells, the different strains were distinguished mainly by their growth capability at 40°C in NPTr cells. Kinetic studies showed the inability of the human H1N1pdm isolate to grow in NPTr cell culture at 40°C and a slower viral replication of swine H1N1pdm-like viruses isolated from symptomatic pigs, as compared to viruses isolated from asymptomatic ones, suggesting that adaptation to the swine host rapidly occurred for some H1N1pdm isolates.

Interestingly, the lack of growth of the human H1N1pdm strain in NPTr cell culture at 40°C was partially restored when this isolate was previously amplified in embryonated eggs, but not in MDCK cells. This underlines the importance in the choice of the substrate used for virus isolation/amplification because of its influence on the viral phenotype.

In addition, unlike some enzootic H1N1 SIV, the human H1N1pdm and swine H1N1pdm-like viruses were unable to form plaques on NPTr at 37 and 40°C, whereas they displayed a low potential to form plaques on MDCK cells. It is noteworthy that the H1N1pdm-like viruses and H1N1 SIV viruses we compared all share the NA glycoprotein that was introduced into H1N1pdm.

Altogether, these results suggest that H1N1pdm isolates acquired specific properties depending on the host to which they were transmitted. Larger comparisons of H1N1pdm isolates from different hosts might help to define host-specific signatures and degrees of adaptability, and will provide useful data to understand the molecular mechanisms underlying the adaptation of influenza A viruses to new hosts.

SPB1 / SPB2 - VIRUS HOST INTERACTION/PATHOGENESIS/TRANSMISSION

B122P

The impact of silent nucleotide changes in the influenza A virus genome on virus replication in ducks

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Adaptation to the host organism is crucial for influenza viruses upon zoonoses. Numerous amino acid substitutions in a variety of virus genes – most notably HA, NA, and PB2 – have been identified as determinants of host-range, virulence, and transmission. The impact of silent changes, nucleotide substitutions that do not affect amino acid composition, is less clear. It has been shown that the genomes of avian and human influenza viruses have characteristic codon usage that is significantly biased (own unpublished data and Greenbaum et al. 2009). Such silent nucleotide substitutions may influence virus replication by e.g. affecting transcription and post-transcriptional processes, altering RNA secondary structure, facilitating the usage of particular host tRNAs ("codon optimization"), and preventing recognition by the innate immune system.

We hypothesize that silent nucleotide substitutions in the influenza virus genome are important for virus adaptation to its host species. To test this hypothesis, we constructed recombinant avian H7 influenza viruses in which 2 gene segments – PB1 and NA – were derived from either human or avian viruses. In addition, we used variants of these PB1 and NA gene segments that had identical amino acid yet distinct nucleotide sequences. We inoculated 28-day-old Pekin ducks (Anas platyrhynchos) with the four viruses. Tracheal and cloacal swaps were taken daily and necropsy was done on day 3 and day 7. Lung slides were scored for infiltrate and presence of heterophiles.

No significant differences were observed in viral titers between groups of ducks in tracheal swabs, cloacal swaps, trachea, lung and colon on day 3 and day 7. Histologically, there were no differences in lung tissues of the four groups of birds. RNA of the input virus and virus from the last positive sample was isolated and nucleotide sequences of the PB1 and NA genes were compared, but no mutations that point towards nucleotide adaptation were detected after one passage in Pekin ducks. Therefore, at present there is no evidence that the nucleotide bias in avian vs human influenza virus genomes has a direct impact on virus replication in ducks.

SPB1 / SPB2 - VIRUS HOST INTERACTION/PATHOGENESIS/TRANSMISSION

B123P

Investigating the survival of influenza in an aerosol form – re-visiting the goldberg drum in a containment setting

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Introduction

The debate regarding the contribution of the aerosol route to the transmission of influenza is ongoing, mainly due to the difficulty in proving definitively that transmission via the aerosol route is occurring. However, evidence for the role of aerosol transmission for influenza is growing; for example the aerosol infectious dose for influenza is reportedly lower than the intranasal infectious dose, numerous studies have reported finding influenza in the air around infected patients and historical studies show that persistence of influenza viability in an aerosolized form may be considerable at low humidities.

Materials and Methods

An influenza virus aerosol viability testing capability under containment conditions has been constructed at Porton which can be adopted for use with any emerging influenza strain and any novel agent which is suspected of being transmitted via the aerosol route. This consists of a unique, modified contained Goldberg drum and Henderson apparatus, capable of maintaining aerosols at pre-defined relative humidities for prolonged periods of time, therefore allowing for sequential sampling of the pathogen of interest at a range of representative environmental conditions.

Results

Results obtained will be used to inform infection control procedures, fed into mathematical models, and will allow a greater understanding of this controversial area of aerosol science.

Conclusions

This capability will allow the potential for aerosol transmission of emerging pathogens to be elucidated, and allow the development of evidence based infection control risk assessments.

SPB1 / SPB2 - VIRUS HOST INTERACTION/PATHOGENESIS/TRANSMISSION

B124P

High prevalence of A/H1N1 (2009) viruses associated with severe outcomes expressing HA-222D/G

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Background

A/H1N1 (2009) viruses cause sporadically very severe disease including fatal clinical outcomes. In the viral haemagglutinin (HA) gene a D222G substitution was detected with significant frequency in fatal and severe cases. The D222G mutation could cause a shift from sialic acid-α2,6-galactose (α2,6 receptors) to the mixed α2,3/α2,6 receptors specificity which might increase binding to α2,3 receptors and may influence virus tropism and severity of disease. Furthermore, A/H1N1 (2009) viruses were described with heterogeneous expression of amino acid 222 (222D/G/N). In this study, the evolution of A/H1N1 (2009) viruses circulating in Germany in 2010/11 was analyzed, especially the heterogeneity of the amino acid 222.

Materials and Methods

Viral specimens from mild and severe cases including fatal cases were collected in the season 2010/11 and RNA was extracted from original material (nasal swabs and bronchoalveolar lavage samples). For characterization of A/H1N1 (2009) viruses, the HA genes were cycle sequenced and further studied. According to phylogenetic analysis, PSQ assays were developed for identification of co-circulating A/H1N1 (2009) variants and for characterization of amino acid 222.

Results

Phylogenetic analysis of HA genes (n=72) revealed co-circulation of 72% of England/142/10-like viruses which could be characterized by S185T substitution and 4 additional variants (4-8%) which were identified by N125D, S183P, A186T and I216V substitution. A similar distribution of co-circulating variants was detected by PSQ analysis (n=88). Further on, PSQ analysis of viruses received from mild (n=40) and severe cases (n=57) resulted for mild outcomes 2,5% of 222E and 97,5% of the wild-type 222D.

Viruses from severe cases showed a high increase of a heterogeneous HA-222 expression compared to the previous season. About 54% of those viruses were characterized by a 222D/G mixed population, 3% were 222D/G/N variants and 42% represented the 222D wild-type. Regarding severe clinical outcomes, the occurrence of such mutants was higher in bronchoalveolar lavage samples (65%) than in nasal swabs (42%).

Conclusion

A/H1N1 (2009) viruses with D222G substitution or a heterogeneous HA-222 expression were associated with severe clinical outcomes. In the influenza season 2010/2011 pure D222G variants were absent but a tremendous increase of variants with heterogeneous expression of HA-222 (222D/G) compared to the previous season was observed. These results may confirm the hypotheses that this quasispecies might emerge in the course of adaptation of viral receptor specificity along the heterogeneous upper and lower respiratory tract. Thus, the heterogeneous HA-222 population could be able to cause more severe disease in the lung based on D222G substitution.

YOUNG SCIENTIST

SPB1 / SPB2 - VIRUS HOST INTERACTION/PATHOGENESIS/TRANSMISSION

B125P

Glutamic acid at position 196 of the NS1 protein is associated with both a block in IRF3 activation and the virulence of a HPAI H5N1 virus in mice

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Highly pathogenic avian influenza (HPAI) H5N1 viruses have spread across Asia, Europe, and Africa. More than 500 cases of H5N1 virus infection have been reported in humans, with a high case fatality rate. One of the major determinants of influenza A virus virulence is the NS1 protein, which functions in several ways to defeat the cellular innate immune response. Different HA subtypes of influenza A virus differ in the ability of their encoded NS1 proteins to inhibit the activation of IRF3 and interferon-beta transcription in infected cells. Sequence alignment of NS1 proteins revealed that the identity of the amino acid at position 196 of NS1 is associated with the regulation of IRF3 activation, specifically lysine (K) is associated with IRF3 activation; and glutamic acid (E) is associated with the inhibition of IRF3 activation.

Aims

The goal of this study is to evaluate the contribution of the NS1 protein to the virulence of highly pathogenic H5N1 viruses in mice and determine the correlation with IRF3 activation, a function that is associated with the identity of the amino acid at position 196 of the NS1 protein.

Methods

We generated a series of reassortant viruses bearing an intact, chimeric or mutant NS1 gene from various influenza A virus subtypes on the backbone of A/Vietnam/1203/04 (VN/04) H5N1 virus. We assessed the ability of these viruses to inhibit IRF3 activation in mammalian cells and evaluated their virulence in BALB/c mice.

Results

Reassortant H5N1 viruses containing NS1 genes from seasonal and pandemic H1N1 viruses, A/Texas/36/91(TX/91) and A/California/04/09 (CA/09) were highly virulent in mice. In contrast, reassortant viruses possessing NS1 genes from the seasonal H3N2 A/Udorn/72 (UD/72) and A/Wisconsin/67/05 (WI/05) viruses were restricted in replication in the lungs of mice. The NS1 proteins of the virulent viruses contain E at position 196 of NS1, whereas the NS1 proteins of the attenuated viruses contain K at this position. Using H5N1 viruses encoding a chimeric NS1 protein derived from UD/72 or VN/04, we found that the difference in pulmonary replication of the H5N1 reassortant viruses was mediated largely by the C-terminal region of the effector domain of NS1. To determine the role of the amino acid at residue 196, we generated two mutant H5N1 viruses: one with a point mutation E196K in the VN/04 NS1 protein (VN/04 E196K) and the other with the UD/72 NS1 protein with a K196E mutation (UD/72 K196E). A single mutation from E to K at position 196 of VN/04 NS1 resulted in significant attenuation in mice, whereas a K196E mutation of UD/72 NS1 enhanced the virulence in mice. The level of pro-inflammatory cytokines, such as IL-1a and TNF-a, was increased in the lungs of mice infected with viruses encoding E at position 196 of the NS1 protein. We showed that the effect of IRF3 activation correlated with virulence in mice; IRF3 was activated and the viruses were restricted in replication in mice if the NS1 genes were derived from UD/72, WI/05, and VN/04 E196K that encoded K at residue 196, while viruses containing NS1 genes from VN/04, TX/91, CA/09, and UD/72 K196E that encoded E at 196 inhibited IRF3 activation and were virulent.

Discussion/Conclusion

Our findings show that E at position 196 in the NS1 protein plays an important role in blocking IRF3 activation during VN/04 H5N1 influenza virus infection and is a virulence determinant of H5N1 viruses in mice.

YOUNG SCIENTIST

SPB1 / SPB2 - VIRUS HOST INTERACTION/PATHOGENESIS/TRANSMISSION

B126P

Differential Host Determinants Contribute to the Pathogenesis of 2009 Pandemic H1N1 and Human H5N1 Influenza A Viruses in Experimental Mouse Models

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Influenza viruses are responsible for high morbidities in humans and may, eventually, cause pandemics. Here, we compared pathogenesis and host innate immune responses of a seasonal H1N1, two 2009 pandemic H1N1 and a human H5N1 influenza virus in experimental BALB/c and C57BL/6J mouse models. We found that both 2009 pandemic H1N1 isolates studied (HH05 and HH15) were low pathogenic in BALB/c ($logMLD_{50}$>6 p.f.u.) but displayed remarkable differences in virulence in C57BL/6J mice. HH15 was more virulent ($logMLD_{50}$=3.5 p.f.u.) than HH05 ($logMLD_{50}$=5.2 p.f.u.) in C57BL/6J mice. In contrast, H5N1 influenza virus was more virulent in BALB/c ($logMLD_{50}$=0.3 p.f.u.) than C57BL/6J ($logMLD_{50}$=1.8 p.f.u.). Seasonal H1N1 influenza revealed marginal pathogenicity in BALB/c or C57BL/6J mice (logMLD50>6 p.f.u.). Enhanced susceptibility of C57BL/6J mice to pandemic H1N1 correlated with a depressed cytokine response. In contrast, enhanced H5N1 virulence in BALB/c mice correlated with an elevated pro-inflammatory cytokine response. These findings highlight that host determinants responsible for the pathogenesis of 2009 pandemic H1N1 influenza viruses are different from those contributing to H5N1 pathogenesis. Our results show for the first time that the C57BL/6J mouse strain is more appropriate for the evaluation and identification of intrinsic pathogenicity markers of 2009 pandemic H1N1 influenza viruses which are “masked” in BALB/c mice.

SPB1 / SPB2 - VIRUS HOST INTERACTION/PATHOGENESIS/TRANSMISSION

B127P - Virus-host interaction / pathogenesis / transmission

Introduction of adaptive mutations in the polymerase subunit PB2 of A/Hamburg/05/2009 (H1N1) increase in vitro polymerase activity and pathogenicity in the mouse model

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Introduction.

Adaption of an avian influenza virus to a mammalian host involves a convergent evolution of the polymerase. Over the years, several critical positions for an adaption of the polymerase have been identified. In the 2009 pandemic H1N1 virus these critical positions in the PB2 and PA subunits still show the avian signature. It was therefore of interest to find out if adaptive mutations in these positions alter the pathogenicity of the virus in mammals.

Material & Methods.

After setting up a reverse genetic system for the pandemic A/H1N1-virus, we introduced the known adaptive mutations E627K, D701N and S714I and corresponding double mutations in the PB2-subunit of A/Hamburg/05/2009 (H1N1) and analyzed the polymerase activity in mammalian (HEK293T) cells. Recombinant viruses were rescued and tested for pathogenicity in mice.

Results.

All adaptive mutations showed an increase in polymerase activity *in vitro*. While the single substitutions did not alter pathogenicity in the mouse model, there was a significant increase detectable after infection with the double mutant viruses.

Conclusion.

These observations indicate that adaptive mutations may further increase the pathogenicity of the pandemic H1N1 virus. Thus, its pandemic and pathogenic potential may not be exhausted yet.

SPB1 / SPB2 - VIRUS HOST INTERACTION/PATHOGENESIS/TRANSMISSION

B128P

Cellular transcriptional profiling in lung epithelial cells infected by different subtypes of influenza A viruses reveals a strong down regulation of the p53 pathway

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Background:

Influenza infections impact on the host cell homeostasis via the combination of the virally induced alterations and hijacking of molecular machineries/metabolic pathways and the cellular antiviral response triggered by intracellular signalling cascades. The development of High throughput 'Omic' studies has increased our understanding of viral-host interactions and numerous in vitro and in vivo studies have described modified host gene expression related to viral infection.

We recently compared the cellular gene expression profile of human lung epithelial A549 cells infected by five different influenza A viruses (H1N1, H3N2, H5N1, H5N2 and H7N1). This shared signature was exploited to find new molecules, which act on host metabolic pathways to bring about an antiviral effect against several subtypes (1).

Methods

We used the same transcriptomic data set to analyze and compare host pathways and cellular response during infections with each subtype. We observed that the transcriptomic signatures were systematically associated with pathways linked to cell growth and cell death. We decided to focus of our investigation further on the p53 pathway by RT-qPCR and western blot analysis.

Results

A global inhibition of the p53 pathway was detected during infection. A down-regulation of mRNA expression was observed for the main regulators of the p53 protein stability during infections with H3N2, H5N1, H5N2 or H7N1 and a significant decrease was further observed for p53 mRNA itself in H5N1 infected cells. In contrast, a relative increase of p53 protein level was observed for all viruses, suggesting viral modulation of p53 protein stabilization and activation. Furthermore, numerous p53 transcriptional targets, such as p21 or 14-3-3, were also down regulated at both the mRNA and protein levels during infection by all influenza viruses.

In addition, comparison of the viral production yields in the HCT116 p53 -/- and p53 +/+ cellular models have shown that p53 protein can slow down viral production, supporting the hypothesis that the virally-induced global down-regulation of the p53 pathway could lead to a cellular state, which favours influenza replication.

Discussion:

Our results reveal influenza infection on impacts p53 and associated pathways at transcriptomic and proteomic levels. Further studies are required to characterize putative interactions of p53 with viral proteins, as recently reported for NS1 (2). The global down-regulation of the p53 pathway we report could lead (i) to the inhibition of cell apoptosis and (ii) to the cell cycle progressionas recently reported (3).
In conclusion, these results have underlined the p53 pathway as an important player in influenza replicative cycle. Further investigations are now needed to discriminate the cellular antiviral response from the viral hijacking of the p53 pathway

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SPB1 / SPB2 - VIRUS HOST INTERACTION/PATHOGENESIS/TRANSMISSION

B129P

Pathogenesis and transmission of canine influenza (H3N2) virus in ferrets

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1. Introduction

Canine influenza virus (CIV) is an emerging pathogen that causes acute respiratory disease in dogs. Evidence of canine influenza infection in pet dogs, a primary companion animal for humans, raises the possibility that dogs may provide a new source for transmission of novel influenza A viruses to humans. Thus, knowing the inherent possibility of infection and transmission of CIV in humans is important for executing appropriate public health responses. We have therefore characterized the pathogenesis and transmissibility of H3N2 CIV in the ferret (*Mustela putorius furo*) model, which is a suitable animal model for influenza A virus infections in humans.

2. Materials and methods

To determine pathogenicity, nine adult male ferrets were inoculated intranasally with A/canine/Korea/LAM412/07 (H3N2) with a titer of $10^{7.0}$ EID_{50}/ml. Ferrets were monitored for changes in body temperature and weight, and the presence of clinical signs. Nasal washes were collected every other day for at least 9 days post-infection (p.i.). At 3 days p.i., tissues were collected from three ferrets for examination of viral load profile and histopathologic examination. For direct contact transmission experiments, two adult male ferrets were inoculated intranasally with CIV as described above. At 1 d.p.i., two naive ferrets were housed in the same contaminant cage. Nasal washes were collected every other day for at least 9 days p.i. and 11 days post-exposure (p.e.). Serum samples were collected before and 14 days after exposure and analyzed by hemagglutination inhibition assay.

3. Results

All the infected ferrets exhibited clinical signs of disease, including lethargy, sneezing, ruffled fur, and decreased interest in food. The mean maximum weight loss was 13% for the infected ferrets. The infected ferrets shed high peak mean titers of infectious virus in nasal washes as early as day 1 p.i. that were sustained for 5 days p.i. In examination of viral load profile, virus was also detected in trachea and lung from infected ferrets. Gross examination of the lungs revealed focal to multifocal mild consolidation in infected ferrets. In direct contact transmission experiments, CIV efficiently transmitted via direct contact to one of the contact ferrets that shed virus as early as day 2 p.e. The infectious virus was recovered from nasal washes, and seroconversion was detected in one of the contact ferrets.

4. Conclusions

In this study, we demonstrated that the replication and pathogenicity of H3N2 CIV in ferrets. Our results indicated that H3N2 CIV replicates efficiently in both the upper and lower respiratory tract of ferrets, is associated with moderate clinical signs and pathological changes. These results are in agreement with observations in mammals, in which extensive virus replication in the respiratory tract of the 2009 A (H1N1) pandemic influenza viruses has been observed.

Furthermore, we confirmed that H3N2 CIV is transmitted efficiently between ferrets via direct contact. Although H3N2 CIV preferentially recognizes avian influenza virus binding receptor (SAĐ 2,3-gal), these results suggest that the virus may have the ability to efficiently replicate and transmit in humans, which have been shown to physiologically resemble ferrets in terms of the expression and distribution of sialic acid receptors in the respiratory tract.

In conclusion, we have shown in this study that H3N2 CIV is able to replicate in the respiratory tract of ferrets and to transmit efficiently to direct contacts. Considering these characteristics of H3N2 CIV infection in ferrets and epidemics of the virus in pet dogs, the public health threat of H3N2 CIV cannot be overemphasized. Our findings highlight that continued monitoring of H3N2 CIV is needed to investigate the potential for the emergence of a novel human pathogen.

YOUNG SCIENTIST

SPB1 / SPB2 - VIRUS HOST INTERACTION/PATHOGENESIS/TRANSMISSION

B130P

Adaptation of low pathogenic avian influenza viruses to a high growth phenotype in primary human tracheo-bronchial epithelial cells

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Introduction.

Two major barriers to the pandemic spread of avian influenza viruses are the inability of many strains to productively infect humans and, among those strains that do occasionally infect humans, a lack of human-to-human transmission. Our understanding of what host-specific adaptations are required to allow viral growth in and transmission among humans remains incomplete. While the receptor binding specificity of the hemagglutinin protein and certain features of the viral polymerase complex are known to have a strong impact, other viral factors are likely to play a role.

Materials & Methods.

With the aim of identifying a more complete set of adaptive changes that allows the efficient replication of an avian influenza virus in a human substrate, we have adapted two low pathogenic avian influenza viruses to growth in fully differentiated human tracheo-bronchial epithelial (HTBE) cells. Three independent lines of A/duck/Alberta/35/76 (H1N1; dk/AB/76) and A/duck/Ukraine/63 (H3N8; dk/Ukr/63) viruses were passaged ten times to yield a total of six independent P10 populations.

Results.

Prior to adaptation, dk/AB/76 and dk/Ukr/63 viruses showed restricted growth phenotypes in HTBE cells and a lack of contact transmission among guinea pigs. In contrast, following ten serial passages in HTBE cells, the kinetics of virus amplification and peak titers attained by dk/AB/76-P10 and dk/Ukr/63-P10 populations were very similar to those of the human seasonal A/Panama/2007/99 (H3N2) virus. Toward identification of genetic changes that support this robust growth in HTBE cells, the genome sequences of clonal isolates derived from each of the six P-10 populations are currently being determined. The transmissibility of the P-10 populations in the guinea pig model is furthermore being explored.

Conclusions.

We have generated variants of dk/AB/76 and dk/Ukr/63 viruses that are insensitive to host restriction in HTBE cells. We anticipate that characterization of these viruses will reveal novel mechanisms responsible for the constraint of avian influenza virus growth and transmission in mammalian hosts.

SPB1 / SPB2 - VIRUS HOST INTERACTION/PATHOGENESIS/TRANSMISSION

B131P

Proteomic analysis of 2009 pandemic H1N1 and seasonal H1N1 influenza-infected A549 cells by stable isotope labelling with amino acids in cell culture

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Introduction

Influenza A virus is one of the world's major uncontrolled pathogens causing seasonal epidemics as well as global pandemics. This was evidenced by the recent emergence, and now prevalence, of pandemic H1N1 2009 swine origin influenza A virus from which 18,449 people worldwide have been confirmed to have died from infection since April 2009.

Methods

In this study quantitative proteomics were used to investigate and compare the changes in the host cell proteome of human lung carcinoma-derived A549 cells infected with either 2009 pandemic nvH1N1 A/California/04/09 or seasonal H1N1 A/Solomon Islands/03/06. Stable isotope labelling with amino acids in cell culture (SILAC) coupled with bioinformatic Ingenuity Pathway Analysis allowed the detailed resolution of cellular pathways that are potentially activated/ suppressed in virus-infected cells.

Results

A549 influenza cell infection was confirmed by immuno-fluorescence and Western blot analysis. Western blot analysis of selected cellular proteins was also used to confirm protein abundance measured by proteomic analysis. Bioinformatic analysis indicated that most changes in the cellular proteome were common to cells infected with either pandemic H1N1 or seasonal H1N1 influenza A virus. Several global pathways were affected such as decreased abundance of proteins involved in cell cycle regulation and lipid metabolism.

Conclusions

Taken together, both quantitative proteomics and other transcriptomic approaches can be used to identify potential cellular proteins whose functions in the virus life cycle could be targeted for chemotherapeutic intervention as well as determining mechanisms of viral pathogenesis.

SPB1 / SPB2 - VIRUS HOST INTERACTION/PATHOGENESIS/TRANSMISSION

B132P

Studies on viral factors and host immune response involved in disease severity of pandemic influenza H1N1 infection.

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Introduction

Infections caused by the pandemic H1N1 2009 influenza virus range from mild upper respiratory tract symptoms to fatal disease. An inadequate innate immune response seems to be associated to severe disease, probably due to different host factors, but viral factors might as well play a role in this response. In particular, D/G mutant strains in position 222 in the hemagglutinin have been proposed as being more often associated with severe disease in the lower respiratory tract. In this study, we stimulate blood cells (whole blood) from healthy donors with different strains of H1N1 influenza virus and monitor the expression of several genes involved in host immune response.

Methods and Results

Blood cell stimulation is performed on whole blood from 6 healthy donors, with three different virus isolates; a seasonal H1N1 (A/New-Caledonia), and two strains of the pandemic virus H1N1 2009, one with D and one with G at the amino acid position 222 of the hemagglutinin gene.

After incubation at 37 degrees with gentle agitation, total cellular RNA is isolated from virus-stimulated blood, and transcribed into cDNA using oligo(T) primer. PCRs are then performed, using commercial Gene Expression Assays (Applied Biosystems), with primer and probes for the following target genes; DEFA4, Elastase, Lactoferrin, Myeloperoxidase, MMP8, TIMP 1, TIMP 3, IL-1beta, IL-17, IL-18, IL-10, IL-6, TNFalfa, CXCR3 and IP-10, in addition to house-keeping genes for normalisation of the results.

Results from these experiments will be presented.

SPA2: VACCINES: CURRENT AND NOVEL APPROACHES

A229P

The rationale for quadrivalent influenza vaccines

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Introduction

Influenza A/H1N1, A/H3N2, and B viruses have circulated and caused disease in humans on a global basis since 1977. Accordingly, licensed seasonal influenza vaccines have contained 3 strains, 1 from each A subtype and 1 type B virus. However, since 1985, 2 antigenically distinct lineages of influenza B viruses have circulated globally. As only 1 lineage can be selected for inclusion in current trivalent influenza vaccines, the vaccines have provided limited immunity against strains of the other lineage. Our objective was to review the available data supporting the rationale for quadrivalent influenza vaccines (vaccines containing 2 A and 2 B strains).

Results

According to data from the European Influenza Network, from 2001–2002 through 2010–2011 (excluding the 2009–2010 pandemic), on average, 23% (range: 1%–60%) of influenza samples annually were due to influenza B. US Centers for Disease Control and Prevention (CDC) surveillance data from the same seasons were similar, with a season average of 24% (range, <1%–44%). Studies of severe influenza disease have shown that influenza B causes significant morbidity and mortality in all ages, although its incidence relative to influenza A appears to be highest among older children and young adults. Medically attended illnesses in both children and adults due to influenza A and B are generally similar in regards to symptoms, severity, and rates of influenza-related complications. The principal differences observed across studies are that influenza B disease in children is more commonly associated with myalgia, myositis, and leukopenia and less commonly associated with rhinorrhea. Before 1985, there was a single lineage of influenza B in global circulation, which was the precursor to the subsequent Yamagata lineage. The Victoria lineage appears to have emerged in China by 1975 and began circulating globally in 1985. The Victoria lineage dominated global circulation from 1987–1989, followed by Yamagata dominance in the 1990s, and subsequent re-emergence of the Victoria lineage in 2001–2002. From 2001–2002 to the present, both lineages have cocirculated each season at varying levels, and predictions regarding which B lineage will predominate in an upcoming influenza season have been no better than chance alone, correct in only 5 of the 10 seasons from 2001–2011. Studies have shown limited to no immunologic cross-reactivity between the 2 B lineages. Consequently, seasonal influenza vaccines could be improved by inclusion of influenza B strains of both lineages. A US CDC analysis suggested that use of quadrivalent vaccines in the United States during the 2001–2008 seasons would have been beneficial in each season, cumulatively resulting in approximately 2.1 million fewer cases of influenza, 20,000 fewer hospitalizations, and 1200 fewer deaths. Manufacturing capacity for seasonal influenza vaccines has increased sufficiently to supply quadrivalent influenza vaccines, and methods to identify the influenza B strains to include in such vaccines are in place.

Conclusions

Quadrivalent formulations represent a next logical step for seasonal influenza vaccines. Because 2 antigenically distinct influenza B lineages have been circulating since 1985 and the predominant influenza B lineage has been unpredictable in recent years, quadrivalent vaccines would more accurately reflect the current epidemiology of influenza and would allow vaccination campaigns to more effectively protect their target populations. Multiple manufacturers have initiated clinical studies of quadrivalent influenza vaccines. Data from those studies as well as epidemiologic data regarding the burden of influenza B infections will determine the safety, effectiveness, and benefit of utilizing quadrivalent vaccines for the prevention of seasonal influenza disease.

Sponsored by MedImmune, LLC.

SPA2: VACCINES: CURRENT AND NOVEL APPROACHES

A201P -

HA1 Vaccine Produced In E. coli Against Pandemic H1N1 & H5N1 Viruses Form Functional Oligomers That Cause Hemagglutination And Generate Cross-Protective Immunity In Ferrets

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Introduction

Impending influenza pandemic requires global vaccination to prevent large scale mortality and morbidity, but traditional influenza vaccine production is too slow for rapid responses.

Materials and Methods

Recombinant hemagglutinin globular domain (rHA1) from H5N1 (A/ Vietnam/1203/2004) and pandemic H1N1 (A/California/04/2009) were produced in *E coli* and purified under controlled redox refolding conditions. These rHA1 proteins were then analyzed in multiple biophysical, functional, structural, immunogenicity and wild type influenza virus challenge assays.

Results

Importantly, the rHA1 contained functional oligomers without addition of exogenous trimerization sequence. These oligomers form rosettes which composed of 4-6 trimers as observed by electron microscopy. Site directed mutagenesis mapped the trimerization signal to the HA1 N-terminal residues. The purified HA1 proteins bound cell surface receptor, agglutinated human red blood cells and elicited potent neutralizing antibodies against homologous and heterologous pandemic influenza viruses. Ferrets vaccinated with the oligomeric rHA1, but not N-terminus-deleted, monomeric HA1 or monomeric HA0 ectodomain were fully protected from lethality and weight loss following challenge with homologous and heterologous wild type highly pathogenic H5N1 viruses (study 1) and from weight loss and fever associated with wild type pandemic H1N1 infection. Protection was associated with a significant reduction in viral loads in the nasal washes of homologous and heterologous virus challenged ferrets.

Conclusion

This is the first study that describes the presence of an N-terminal oligomerization sequence in the globular domain of influenza virus hemagglutinin. Our findings suggest that functional oligomeric rHA1-based vaccines can be produced efficiently in bacterial systems and can be easily produced shortly after emergence of new influenza strains with pandemic potential for global usage.

YOUNG SCIENTIST

SPA2: VACCINES: CURRENT AND NOVEL APPROACHES

A202P -

Vaccines: current and novel approaches
Development of a universal influenza H5N1 vaccine based on the neutralizing epitopes of Hemagglutinin

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Introduction

The continued circulation of H5N1 strains of influenza A virus in poultry, and recent sporadic outbreaks in humans, serve as a warning of a future influenza pandemic. The control of infection with current H5N1 vaccines has limited efficacy against heterologous strains or phylogenetically variant clades of H5N1 in part due to variation in the HA sequences, particularly within the neutralizing epitope region. However, the development of a universal vaccine based entirely on HA of an influenza virus still feasible, if the variation or conservation of neutralizing epitopes among the HPAI H5N1 virus clades can be identified. Such a vaccine strain selection will lead to a broad range of protection against most H5N1 lineages. Methods: We have mapped the major neutralizing epitopes and identified key amino acids at position 138, 155, 189, or 223 were involved in the formation of major neutralizing epitopes of H5N1 virus hemagglutinin. Based on our epitope distribution analysis, we have selected three different H5N1 strains, A/Vietnam/1203/2004 (clade 1.0), A/Indonesia/CDC669/2006 (clade 2.1) and A/Anhui/1/2005 (clade 2.3), to collectively represent the variations with in the major antigenic epitopes of almost 99% of all H5N1 lineages, including both human and avian viruses. HA proteins of selected strains were individually expressed on the baculovirus surface (BacHA) with the immediate-early 1 (ie1) promoter, and the vaccine formulation was evaluated in a mouse model. Results: The subcutaneous immunization of adjuvanted trivalent-BacHA significantly induced higher hemagglutination inhibition titer and neutralization antibody titers, which efficiently neutralized 100 TCID50 of heterologous H5N1 strains from various clades (clade 1.0, clade 2.1, clade 2.2, clade 4.0, clade 7.0, and clade 8.0) compared to adjuvanted monovalent-BacHA or inactivated RG-H5N1 vaccine. Also, trivalent BacHA vaccine was able to protect 100% of the mice against challenge with three different clades (clade 1.0, clade 2.1, and clade 7.0) of H5N1 strains compared to monovalent-BacHA or inactivated whole viral vaccine. In addition, adjuvanted trivalent-BacHA provided complete protection against clade 7.0 H5N1 virus without any infection symptoms. However, mice vaccinated with adjuvanted inactivated whole viral vaccine or monovalent-BacHA showed a higher loss of body weight and provided only 66.6% or 83.3% protection against 5MLD50 of clade 7.0 H5N1 challenge. This indicates the inability of monovalent vaccines to confer protection against diverse H5N1 subtypes, which might be due to the variation within the antigenic determinants (such as neutralizing epitopes) of different virus subtypes. Conclusions: The present findings revealed that the selection of vaccine strains based on the variations within the neutralizing epitopes among the subtypes will help prevent infection mediated by newly emerged H5N1 mutants. The vaccine formulation used in this study was produced rapidly without any high biocontainment facilities or tedious protein purification needed for mass production of the vaccine.

SPA2: VACCINES: CURRENT AND NOVEL APPROACHES

A203P

Candidate M2e-based vaccines against human and avian influenza viruses, produced in plant: immune response and protection

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Introduction

A number of scientific groups in recent years are developing a broad-spectrum vaccine candidates based on the extracellular domain of the M2-protein (M2e) of influenza virus A. The sequences of M2e remain remarkably stable across all influenza human isolates since 1933 and are differed from avian influenza viruses (H5N1, H5N2) in 3-5 a.a. and A/California/04/09 (H1N1) in 4 a.a. Transgenic plants are one of the expression systems for successful producing of foreign proteins and can be source of vaccine proteins. The aim of this investigation was to study the immunogenicity and protection of the two versions of M2e-based influenza candidate vaccines produced in plant.

Material and methods:

For the first candidate vaccine (HBc-M2e) hepatitis B virus core protein (HBc) was used as a carrier for M2e avian (H5N2 A/Duck/Potsdam/1402-6/1986). The hybrid protein (linkage of M2e to N-terminus of HBc) was expressed in plants by the use of recombinant plant viral vector. For the second candidate vaccine (TMV-M2e) tobacco mosaic virus (TMV)-based vector was used for expression of consensus sequence M2e of human influenza A (linkage of M2e to the coat protein of TMV). Hybrid proteins or chimeric virions were extracted from inoculated or systemic leaves of Nicotiana benthamiana after agrobacterial infiltration of pA7248AMV_M2eHBcsynt or pBin-TMV-M2e-ser. Immunization-challenge experiments involved 4 groups of 12 male Balb/c mice each. Three intraperitoneal immunizations were administered with 2-week intervals. Both candidate vaccines were injected at dose of 50 µg per mouse with incomplete Freund adjuvant. Control mice received PBS. Blood samples were collected from ventral tail vein two weeks after last immunization. Antibody titers (IgG, IgG1, IgG2a) against synthetic M2e (M2e human, M2e A/California/04/2009, M2e H5N1) were determined by ELISA and those against native M2e were determined on MDCK whole cell ELISA. Immunized mice were challenged with 1LD50 of A/Chicken/Kurgan/05/2005 R.G. (H5N1) or A/PR/8/34 (5LD50) and A/California/04/2009 (5LD50) viruses. Animals were monitored daily for 2 weeks for survival, weight loss and clinical manifestation of disease.

Results

The mean titer of anti-M2e IgG to M2e H5N1 in mice, immunized with HBc-M2e, was 51 200, and administration of HBc-M2e led to dominating IgG2a response ($p \leq 0.01$). Mouse serum bound specifically to A/Chicken/Kurgan-infected MDCK cells, and mice were protected from lethal challenge with heterologous A/Chicken/Kurgan/05/2005 R.G. (90% survival). After administration with TMV-M2e IgG titers against M2e human and M2e A/California/04/2009 were 273 000 and 200 000 respectively. In that case the difference between IgG1 and IgG2a titers was not significant. It was shown that mouse serum bound specifically to PR8-infected MDCK cells. Mice were protected from lethal challenge with homologous A/PR8/34 (90% survival), but partially protected from challenge with heterologous A/California/04/2009 (45% survival). All groups of immunized mice experienced disease symptoms that were significantly milder ($p \leq 0.05$) and weight loss that was less expressed ($p \leq 0.05$) than naive mice.

Conclusion

Anti-M2e antibodies, generated after immunization of HBc-M2e and TMV-M2e, bound to linear epitopes of corresponding synthetic peptides and could recognize native M2e on the surface of MDCK, infected with influenza virus. Provocation of IgG2a dominant antibody by HBc-M2e indicated that Th-1 response was elicited. Chimeric virions TMV-M2e didn't mediate the dominating synthesis of one of the two IgG subclasses (IgG1, IgG2a) and probably promote the formation of balanced Th1-Th2 response. The differences in the type of immune response of two investigated candidate vaccines may be due to the difference of carrier proteins. Immunization with HBc-M2e and TMV-M2e fully protected mice from a lethal challenge with homologous influenza virus and partly protected from a heterologous lethal challenge and significantly reduced weight loss and clinical symptoms.

SPA2: VACCINES: CURRENT AND NOVEL APPROACHES

A205P

Influenza strain A/HK/1/68/162/35 as potential unified vaccine donor

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Introduction

Two kinds of influenza A virus are currently used as backbond when vaccine reassortants are prepared. Particularly, high-yielding virus A/PuertoRico/8/34 (PR/8/34) is used as backbond for inactivated vaccines. Live vaccines are obtained by means of two attenuated donor strains: A/Leningrad/134/17/57 (H2N2) in Russia and A/AnnArbor/6/60-ca (H2N2) in USA.

The purpose of the present study was to develop an influenza virus strain as unified donor of internal genes, which would make it possible to obtain vaccine reassortants for both inactivated and live vaccines. The study had in view to characterize the reassortants derived from new donor strain.

Material and methods.

The desired high-yielding cold-adapted virus strain was generated from virus A/Hong Kong/1/68 (A/HK/1/68) through two series of passages in embryoned eggs (EE): first at 33°C and second at 25°C. Complete sequencing of internal genes of A/HK/1/68/162/35 was conducted using "BigDye Terminator Cycle Sequencing Kit". To obtain the reassortants, EE were co-infected by both donor strain and "wild" influenza virus. The reassortants were characterized genetically by amplification of defined regions within each of the six internal genes and neuraminidase (NA) gene by PCR, and identification of the products by restriction enzyme analysis. We checked cold-adapted (ca) and temperature-sensitive (ts) phenotype of the reassortants, and the identity of haemagglutinin (HA) by haemagglutination inhibiting (HI) test.

The following biologic properties of donor virus and its reassortants were examined: genetic stability; infectivity in eggs; pathogenicity to lab animals; HA titre; antigenic specificity with HI test.

Results

The strain named A/HK/1/68/162/35 (H3N2) was created based on virus A/HK/1/68 that caused 1968/69 pandemic. Research Institute of Influenza received it in 1968 from World Influenza Center (London).

It was demonstrated in the Institute that these varieties of A/HK/1/68 that undergone more than 15 passages on EE, had low pathogenicity (no more than 2% volunteers gave reaction of medium strength). However their immunogenicity remained high (sero-conversions were 72-77% after immunization).

Attenuation of the strain resumed few years ago. There have been eventually 162 passages on EE at 33°C and 35 passages at reduced temperature of 25°C. The resulting strain A/HK/1/68/162/35 shows infectivity in eggs of 9.0 lg to 9.5 lg and well-defined indicators of attenuation, such as cold-adaptability and temperature-sensitivity. The virus growth was 8.5 lg at 25°C, but only 1.0 lg at 39–40°C.

Genome sequencing of initial virus (as well as its passage varieties A/HK/1/68/162 and A/HK/1/68/162/35) has demonstrated that attenuation process resulted in mutation of all genes that coded internal proteins. Cold-adaptation provoked mutations in genes PB1, PB2, PA, NP, that caused amino-acid substitutions in respective proteins. The work is underway for mapping the markers responsible for attenuation. The virus proved to be not toxic to lab animals, and has stable genome.

Three A/HK/1/68/162/35 derivates have been prepared, involving genetically modified virus A/Astana/PR8 uw RG/6:2 (H5N1), as well as two "wild" viruses A/Saint-Petersburg/48/09 (H1N1) and A/Equine/Otar/764/2007 (H3N8). Every reassortant inherited these basic properties of donor virus that are important for vaccine production, namely: high reproductive capacity of 9.3 to 9.5 lg, and the indicators of attenuation. The reassortants displayed HA titre within 512 to 2512; being not pathogenic to lab animals, they better reproduced in upper respiratory ways of mice than in their lungs.

Conclusion

New A influenza strain A/HK/1/68/162/35 (H3N2) proved to be as high-yielding as A/PR/8/34, far exceeding traditional donors that currently used for live vaccines. The strain shows stable indications of attenuation. The study has demonstrated that the donor strain can pass its advantages to reassortants. The unified donor will make it possible to cut down recourses involved in development of reassortants for both types of vaccine, thus reducing vaccine costs.

YOUNG SCIENTIST

SPA2: VACCINES: CURRENT AND NOVEL APPROACHES

A206P

Influenza-responsive CD4+ T-cells predict seroprotection in a clinical trial of a virosomal H5N1 vaccine further adjuvanted with Matrix-MTM

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Introduction

The influenza A H5N1 virus has since 2003 infected over five hundred people causing serious illness and deaths in 60% of the reported cases. Vaccination is the most important measure for protecting the population, but the H5N1 virus is poorly immunogenic and two doses of vaccine are generally required to induce a humoral response associated with protection. An immunological marker for H5N1 vaccine effectiveness is therefore needed to allow early identification of the best vaccine candidate.

Materials and methods

We analysed the results of a phase 1 clinical trial of a virosomal H5N1 vaccine further adjuvanted with Matrix-MTM, which effectively induced both humoral and T-cell responses in man. Sixty volunteers were divided into four groups of fifteen subjects and vaccinated i.m. with either 30µg HA alone or 1.5, 7.5 or 30µg HA and Matrix-MTM adjuvant (50µg). The humoral response was measured by haemagglutination inhibition (HI), microneutralisation (MN) and single radial haemolysis (SRH) assays and the T-cell response by intracellular cytokine staining of CD4+ T-cells for IL-2, IFN-Ð and TNF-Ð.

Results

We found that the vaccine effectively induced expansion of influenza-responsive CD4+ T-cells and that the Matrix-M adjuvanted groups had significantly higher responses as compared to the group receiving the virosomal vaccine alone. Interestingly, the frequencies of influenza-responsive CD4+ T-cells correlated well with the antibody responses. But, whilst a second vaccine dose was needed to induce a significant seroprotection rate in the serological assays, the T-cell responses peaked already after the first dose. Furthermore, ≥0.4% influenza-responsive CD4+ T-cells at 21 days after the first immunisation predicted a subsequent seroprotective HI and MN response (HI titres ≥40 and MN titres ≥80) at 7, 14 and 21 days after the second immunisation (Fishers association test, $p<0.009$ for both assays on all days). In contrast, a weaker association was found between day 21 CD4+ T-cell responses and subsequent seroprotective SRH titres (≥25mm2 lysis zone) measured 7,14 and 21 days after the second dose (Fishers association test, $p<0.03$, $p<0.005$ and $p<0.06$, respectively).

Conclusions

These results support early identification of CD4+ T-cell responses as indicative of an efficient vaccine response, which could have great implications for identification of vaccine low- or non-responders early on when evaluating future pandemic influenza vaccines.

YOUNG SCIENTIST

SPA2: VACCINES: CURRENT AND NOVEL APPROACHES

A207P

Evaluation of the sublingual route for administration of influenza H5N1 virosomes in combination with the bacterial messenger c-di-GMP

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Introduction

Influenza is a globally important respiratory pathogen. Vaccination remains the chief protective measure, but whereas parenteral vaccination can protect against influenza disease, mucosal immunisation may block influenza virus at the site of entry and thereby prevent both disease and further transmission. In addition, mucosal vaccines can easily be administered without use of needles, making this route very feasible for protection against future influenza pandemics in third world countries.
Intranasal (IN) vaccination effectively induces mucosal immune responses, but a drawback to this route is the association of an adjuvanted IN influenza vaccine with facial paralysis (Bells Palsy). A different mucosal administration route is therefore desirable especially for pandemic influenza vaccines, which require an adjuvant to elicit a sufficient vaccine response. Vaccination under the tongue (sublingual administration, SL) could provide a safe alternative, but requires further evaluation.

Material & methods

We therefore compared the local and systemic immune response after SL, IN and intramuscular (IM) vaccination of mice with a virosomal influenza H5N1 (NIBRG-14) vaccine. We further evaluated the different administration routes by combining the vaccine with a promising mucosal adjuvant, the bacterial second messenger bis (3',5')-cyclic dimeric GMP (c-di-GMP). The humoral vaccine-response was evaluated by measuring influenza-specific secretory IgA (sIgA) in saliva and nasal lavages and IgA, IgG, IgG1, IgG2a and haemagglutination inhibition (HI) antibodies in serum. In addition, splenocytes were stimulated with influenza *in vitro* to evaluate cytokine profiles, T-cell proliferation, CD4+ Th1-cell responses by intracellular staining for IL-2, TNF-Ð and IFN-Ð and memory B-cell responses by ELISPOT.

Results

In the groups receiving the virosomal vaccine alone, only IM vaccination induced HI antibody titres above the protective level (≥40). In contrast, all c-di-GMP adjuvanted groups had HI titres ≥40 and, importantly, IN and SL administration induced significantly higher frequencies of memory B-cells and influenza specific CD4+ T-cells as compared to the IM route. In addition, mucosal vaccination (IN or SL) induced local sIgA responses as opposed to the IM route. Remarkably, whereas all groups receiving the virosomal vaccine alone had Th2-skewed responses, in the c-di-GMP adjuvanted groups only IM vaccination induced a Th2 skewed response, whilst the mucosal routes induced a Th1 polarized response.

Conclusions

This is the first report on sublingual vaccination against a potentially pandemic influenza strain and we showed that the combination of influenza virosomes and c-di-GMP effectively induces humoral and cellular immune responses when administered sublingually. These findings demonstrate a great potential of the sublingual route for administration of vaccines and encourage more research into formulation of sublingual vaccines to protect against influenza.

SPA2: VACCINES: CURRENT AND NOVEL APPROACHES

A210P

Approaches to improve the yield of seasonal influenza B virus vaccine antigens

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For influenza vaccine manufacture it is essential to use seed viruses that consistently allow high yields of influenza haemagglutinin (HA) antigens. For influenza A HAs, yields are enhanced by the selection of high-yielding reassortant seeds that are prepared by co-infection of eggs with a high-yielding donor strain (usually the H1N1 virus, A/PR/8/34) and a specified epidemic strain. However, such a system for generating high-yielding influenza B reassortants has yet to be established and yields of egg-grown influenza B epidemic viruses are generally lower than influenza A viruses. This work aims to develop higher yielding B viruses for influenza vaccine. In this study we describe attempts to improve yields of influenza B HA antigens and viruses by cold-adaptation, classical reassortment using co-infection, and by the application of reverse genetics, using epidemic strains with HA antigens specified for inclusion in vaccines. In early studies, the kinetics of HA antigen and infectious virus in eggs were measured. Infectivity was estimated by three different assays, with titres expressed as TCID50, EID50 or PFU/ml. Different cold adaptation strategies (n=7) were used and three strains, B/Malaysia/2506/2004, B/Florida/4/2006, B/Brisbane/60/2008, were selected and grown at gradually lower temperatures in eggs. Both B/Malaysia/2506/2004 and B/Florida/4/2006 produced good HA yields and the vRNA levels were similar. However, B/Malaysia/2506/2004 produced more consistent HA yields over several passages. Yields obtained following 10 serial egg passages of influenza B viruses indicated an inconsistent growth pattern that was not lineage-specific. Yields in the three selected strains after cold-adaptation were improved only by > 50 egg passages. The application of reassortment and reverse genetics also demonstrated an inconsistent growth pattern in the influenza B virus progeny. Our results indicate that current methods to manipulate influenza B virus do not overcome growth variability and that other approaches will be required to consistently improve vaccine antigen yields.

SPA2: VACCINES: CURRENT AND NOVEL APPROACHES

A211P

Dry powder influenza vaccines for improved storage stability and exploitation of new administration routes

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Current influenza vaccines are generally formulated as aqueous solutions to be administered by intramuscular or intradermal injection or – in case of live-attenuated vaccine – as nose spray. In suspension the viral antigens are highly prone to thermal degradation making storage and distribution of influenza vaccines under cold conditions a necessity. We have studied methods to stabilize whole inactivated virus (WIV) influenza vaccine by freeze-drying and spray freeze-drying. WIV freeze-dried in the presence of inulin or trehalose as excipient was stable for at least 3 months even at a storage temperature of 40°C. Not only did the freeze-dried vaccine fully retain immunogenicity it also retained the capacity to induce a Th1-skewed immune response. This indicates that the viral RNA, essential for triggering the Th1 immune response via TLR7 engagement, was completely protected. In contrast, WIV in suspension or WIV freeze-dried without excipients rapidly lost immunogenicity at 40°C. In order to produce a vaccine that could be administered as dry powder to the lungs, freeze-dried WIV was milled or crunched. None of the tested techniques rendered particles small enough for pulmonal administration. However, spray freeze-drying was found to yield particles <10 µm suitable for this purpose. Administration of the vaccine powder to the lungs induced a transient influx of neutrophils and a decrease of macrophages. 72h post administration cell numbers had returned to base line levels. Other histological changes were mild. Two pulmonal administrations of WIV vaccine powder containing 5 µg haemagglutinin induced robust serum and lung IgG titres but nose and lung IgA was found only in some of the immunized mice. Upon live virus challenge animals immunized pulmonally showed less weight loss than and a similar reduction in lung virus titre as mice immunized by standard intramuscular injection. These results demonstrate that (spray) freeze-drying prolongs vaccine shelf-life, allows vaccine handling at elevated temperatures and yields a vaccine formulation highly immunogenic upon pulmonal administration without the use of adjuvants.

SPA2: VACCINES: CURRENT AND NOVEL APPROACHES

A212P

Mixed Administration of MF59®-Adjuvanted A/H5N1 and Seasonal Influenza Vaccines: A Robust Booster Response One Year After a One-Dose Priming Schedule

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Aims

We have previously demonstrated the feasibility of primary immunization with monovalent pandemic A/H5N1, and trivalent seasonal influenza vaccine antigens combined in an MF59®-adjuvanted tetravalent formulation. We now report the results of a controlled study to assess anamnestic responses to a booster dose of tetravalent vaccine, administered one year after priming with either one or two doses of monovalent or tetravalent MF59-adjuvanted A/H5N1 vaccines. Objectives were to compare levels of immunogenicity in response to a single booster dose of tetravalent influenza vaccine after priming with either one or two doses of MF59-adjuvanted A/H5N1 vaccine, and the assessment of reactogenicity and safety.

Methods

Eligible subjects had been primed approximately one year previously with MF59-adjuvanted A/H5N1 (A/Vietnam/1194/2004) vaccine and non-adjuvanted trivalent seasonal influenza vaccine, administered either sequentially 21 days apart, concomitantly as separate injections, or as a single injection after extemporaneous bedside mixing. At Day 382 after primary vaccination, 265 (of 405 primed subjects) healthy adult (18-40 years-old) volunteers received a single booster dose of tetravalent vaccine containing seasonal A/H1N1 (A/Solomon Islands/3/2006), A/H3N2 (A/Wisconsin/67/2005) and B strains (B/Malaysia/2506/2004), and heterologous A/H5N1 (A/turkey/Turkey/1/2005) antigen. Sera were collected pre-booster (Day 382), and 7, 14, and 21 days after booster administration for immunogenicity analysis. Antibody titres were measured by haemagglutination inhibition assay (HI; for A/H5N1, A/H1N1, A/H3N2 and B strains), microneutralisation assay (MN; for A/H5N1 only), and single radial haemolysis (SRH; for A/H5N1 only). For the analysis of A/H5N1 responses, individual priming groups were pooled into two groups according to whether subjects received one or two priming doses of A/H5N1 vaccine. Reactogenicity and safety data were collected for all subjects. Solicited local and systemic reactions, and all other adverse events (AEs) were recorded for 7 and 21 days after booster vaccination, respectively. All serious adverse events (SAEs), and AEs requiring the non-routine attention of a physician were recorded for six months.

Results

Across all study groups, three weeks after booster vaccination HI seroprotection rates for the seasonal strains were between 94 and 100% for A/H1N1, 100% for A/H3N2, and 61 to 90% for the B strain. The European licensure criteria (CHMP) for seasonal influenza vaccines were met in all groups. Two weeks after booster administration, homologous A/H5N1 (turkey/Turkey/1/2005) HI antibody geometric mean titres (GMTs) increased from undetectable levels on Day 382 to a maximum of 112 in subjects primed with either one or two A/H5N1 doses (seroprotection rate 76%). SRH results confirmed these data. Heterologous (Vietnam/1194/2004) A/H5N1 GMTs also increased from undetectable levels on Day 382 to 136 and 132 at two weeks post-vaccination in subjects primed with one or two A/H5N1 doses, respectively. The seroprotection licensure criterion was met by both groups; analysis of heterologous responses by MN assay found GMTs to increase from 16 to 351, and from 20 to 387, respectively. These data were confirmed by SRH analysis. No clinically significant differences in levels of immunogenicity were observed between subjects primed with one or two A/H5N1 doses. After booster vaccination, subjects reported local reactions including erythema, induration, and pain at the injection site (68%); systemic reactions including fever, headache, fatigue, malaise, myalgia, and arthralgia occurred in 49% of subjects. Most of these reactions were transient and mild, only 2% were severe. One non-vaccine-related serious adverse event was reported during the six month follow-up safety period.

Conclusions

One dose of MF59-adjuvanted A/H5N1 influenza vaccine, administered either alone or in combination with seasonal influenza strains, is able to prime individuals equally as well as two doses, resulting in similar levels of seroprotection after booster administration. These data support the feasibility of incorporating pre-pandemic priming into seasonal influenza vaccination programmes.

Trial registration

www.clinicaltrials.gov: NCT 00481065

SPA2: VACCINES: CURRENT AND NOVEL APPROACHES

A213P

Intradermal Influenza Vaccination: Acceptability Survey in Belgian Elderly Patients (above 60 years) during Influenza Season 2010-2011

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Background

Annual vaccination against influenza is an important public health measure to prevent flu and its complications. Unfortunately, the immune response to vaccination in adults over 60 years is lower than in young adults due to immunosenescence. This emphasizes the need for more effective and more immunogenic influenza vaccines in the elderly.
Several clinical trials have shown that intradermal (ID) administration of vaccines induces an enhanced immune response.
Sanofi pasteur pioneered in the development of a new generation of intradermal influenza vaccines. Two dosage formulations (9 µg and 15 µg) of INTANZA® are approved for use in Europe. INTANZA® 15 µg (15 µg hemagglutinin per strain) is indicated for persons aged 60 years or more and demonstrated in this age group superior immunogenicity compared to classic trivalent IM seasonal flu vaccine. In a study population of ≥ 65 years INTANZA® 15µg showed comparable immunogenicity with an adjuvanted IM seasonal flu vaccine.
In Belgium, INTANZA® 15µg was the only ID vaccine available during the 2010-2011 influenza season.

Objectives

The primary objective of the present survey was to describe the acceptability of the ID influenza vaccine among Belgian vaccinees (≥ 60 years) during the influenza season 2010-2011.
The secondary objectives were:

- to describe the opinion of Belgian general practitioners (GPs) related to influenza vaccination and the use of a new Micro-Injection System for intradermal immunization during the Belgian influenza season 2010-2011
- to describe the population characteristics of the ID influenza vaccinated subjects.

Methods

A prospective, non-interventional uncontrolled survey was conducted through questionnaires from October to December 2010. Descriptive statistics were used to summarize data collected from general practitioners vaccinating patients against seasonal influenza in routine practice and data from patients being vaccinated with INTANZA® 15µg.

Results

In total, 105 GPs participated in the survey, while 834 of their elderly patients (40% ≥75 years) completed the patient's questionnaire after signing the informed consent form. Among these patients, 94.9% received a yearly influenza vaccination during the past years and 95.5% were vaccinated last year. Only a minority (2.5%) was never vaccinated against influenza before.
Vaccinees: 97.9% of the patients were very satisfied (70.0%) or satisfied (27.9%) with the ID influenza vaccine. Main reasons for the high satisfaction rate were the fact that the injection was not very painful (60.7%), the administration was quick (40.8%) and the patient felt confident about the short and fine needle (26.5%). Moreover, 91.1% of the patients preferred the ID influenza vaccine over the IM vaccine and 98.5% of all patients would consider receiving the ID influenza vaccine next year.
GPs: 97.0% were very satisfied (78.6%) or satisfied (18.4%) of INTANZA® 15µg. None of them declared to be not satisfied. The main reasons indicated by the physicians to choose for the ID vaccine were "INTANZA® 15µg is more immunogenic" (64.8%), "is an interesting innovation" (57.1%) and "is probably better accepted by the patients" (34.3%). Finally, 87.6% of the GPs favored INTANZA® 15µg over the IM vaccine.

Conclusion

Intradermal influenza vaccination with INTANZA® 15µg is an asset for the elderly population due to its demonstrated and superior immunogenicity compared to classic influenza vaccination.
INTANZA® 15µg is highly accepted both by patients over 60 years and their general practitioners in Belgium.

SPA2: VACCINES: CURRENT AND NOVEL APPROACHES

A214P

DNA vaccine based on genes from pandemic influenza A viruses induces broad protective immunity in swine

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Introduction

The composition of current influenza protein vaccines has to be reconsidered every season and possibly reformulated to match the circulating influenza viruses, continuously changing antigenicity. Thus, influenza vaccines inducing a broad cross-reactive immune response would be a great advantage for protection against both seasonal and emerging influenza viruses. We have developed an alternative influenza vaccine based on DNA expressing different influenza proteins of pandemic origin. We have previously demonstrated cross-protective immunity in ferrets immunised intradermally (i.d.) by DNA vaccines based on the pandemic genes of the 1918 H1N1 and 1968 H3N2 viruses. DNA vaccines have generally been effective in smaller animals; however, less efficient in larger animals. In this study we investigate the influenza DNA vaccine further in a larger animal, the pig. We immunised pigs i.d. with a combination of influenza DNA vaccine components based on the pandemic 1918 H1N1, pandemic 2009 H1N1 and seasonal H3N2 genes and investigated the protection against infection with virus both homologous and heterologous to the DNA components in the vaccine.

Materials and Methods

In the first line of experiments four pigs were immunised twice i.d., three weeks apart, by either gene gun or i.d. injection followed by electroporation with an influenza DNA vaccine comprising either haemagglutinin (HA) and neuraminidase (NA) genes from the 2009 H1N1 pandemic virus or, in addition, nucleoprotein (NP) and matrix protein (M) genes from the 1918 H1N1 pandemic virus. The pigs were challenged with a heterologous H1N1 virus, A/Swine/DK/19126/93, ten weeks after the second vaccination.
In a second experiment, ten pigs were immunised twice i.d. followed by electroporation with a universal influenza DNA vaccine based on six gene constructs; the HA and NA genes of the 2009 H1N1 pandemic virus, the HA and NA genes of a 2005 H3N2 virus and the M and NP genes of the 1918 H1N1 pandemic virus. The pigs were subsequently challenged three weeks after second vaccination with homologous virus, either 2009 H1N1 virus (A/California/07/09) or 2005 H3N2 –like virus (A/Brisbane/10/07). All animals were monitored for clinical signs of infection for 14 days and virus replication was measured in nasopharyngeal swabs. We monitored for haemagglutination inhibitory antibodies (HI) both after DNA vaccination and challenge as well as influenza HA specific IgG antibodies in blood.

Results

Pigs infected with a virus homologous to the vaccine HA and NA components were well protected from infection while DNA vaccinated pigs, challenged with a heterologous virus, were able to clear the infection more rapidly than the control group. The control group also showed clinical signs of infection, like fever, not observed for the DNA vaccinated pigs. Immunisation by electroporation induced HI antibodies >40 HAU between seven and ten days after second vaccination. Heterologous virus challenge ten weeks after last immunisation was able to trigger a vaccine antibody HI response 26 times higher than in the control group. In addition the influenza DNA vaccines induced high HA specific IgG antibodies after vaccination and a recall effect was seen upon challenge with the 2009 H1N1 virus.

Conclusions

Influenza DNA vaccinated pigs were better protected against infection compared to non-vaccinated controls. Influenza DNA vaccines administrated i.d. by electroporation is able to induce broad protective immune responses even in a larger animal, in swine, against both heterologous and homologous virus challenges. The DNA vaccine induces high HI antibodies and HA specific IgG antibodies. However, high HI titres shortly after the first vaccination may not be the only factors relevant for protection. We have shown both in ferrets, previously, and now in pigs, that the influenza DNA vaccines protect from infection or rapidly clear the virus infection despite relatively low HI titres after vaccination. The ability of the DNA vaccine to limit virus shedding may have an impact on virus spread in a community and thereby diminishing the risk for epidemics and pandemics to evolve.

SPA2: VACCINES: CURRENT AND NOVEL APPROACHES

A215P

Evaluation of qualified cell lines for isolation of influenza candidate vaccine viruses: from clinical specimen to high yield reassortant

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Introduction

Recently emerged seasonal influenza viruses with new antigenic properties are used to update the composition of trivalent seasonal influenza vaccines. Currently, these viruses have to be isolated and propagated exclusively in embryonated hens' eggs. Although this host system has proven reliable and safe since its introduction in the 1950's, the low rates of virus isolation in eggs, especially for recent H3N2 viruses, may delay production. The aim of this study was to evaluate the performance of qualified cell culture systems as substrates for isolation of viruses that are suitable for vaccine manufacturing. Such information would support the use of a qualified cell line by the WHO Collaborating Centers for isolation of candidate vaccine viruses. The expected rise in the number of suitable viruses increases the likelihood of successful development of high yield reassortant viruses for vaccine production. Additionally, viruses isolated in cell culture may provide a better antigenic match to the original clinical specimen for both cell- and egg-based vaccines.

Materials and methods

clinical specimens were collected in the US and Finland during the 2008-2009 influenza season. The presence of influenza virus in clinical specimens was determined by a rapid antigen test and positive samples were sub-typed by real-time PCR. Specimens containing influenza A H1N1 (n=5) and H3N2 (n=5) and influenza B Yamagata (n=5) or Victoria (n=5) were inoculated into vaccine-qualified cell lines for virus isolation: one Vero, two adherent MDCK, and one suspension MDCK (Baxter, MedImmune, Abbott and Novartis, respectively). The HA and NA genes from the original clinical specimens and from the different culture systems were sequenced to identify genetic variation and all viruses were characterized by hemagglutination inhibition to establish their antigenic properties.

Results

Identical aliquots from two influenza A-positive clinical specimens were used to isolate A/Texas/11025/2009 (H1N1) and A/FINLAND/97/2009 (H3N2) viruses in three different qualified cell lines (A, B and C). The six isolates from cell culture were then passaged in eggs 3-5 times to improve their growth properties. Three H3N2 and three H1N1 egg-adapted viruses were obtained and submitted to two laboratories that routinely develop high-yield reassortants (HYR) for vaccine production. Reassortant viruses with the HA and NA genes from seasonal viruses were generated according to the current procedure for seasonal vaccine strains using A/PR/8/34 as a donor virus.

In total, three H3N2 reassortants were produced (X193, X193A and X195A) from A and B cell culture isolates; no reassortants were obtained for the cell line C isolate. The reassortants varied in their genetic composition: 6:2, 5:3 and 3:5 (PR8:seasonal). The HYR with the lowest number of PR8 genes displayed the highest hemagglutination (HA) titer.

Multiple H1N1 HYRs for all three cell line isolates were obtained. The selected reassortants from each cell line had 6:2 genome compositions. The HA titers for all cell-derived H1N1 HYRs were very high and implied a favorable virus yield. No difference due to the cell line used for isolation was observed.

The antigen yield of virus isolates from different cell lines was evaluated in all other cell lines to assess their compatibility. A preliminary yield assessment of the H3N2 and H1N1 reassortants in the seed evaluation laboratories of egg-based vaccine manufacturers revealed similar performance as compared to historical reassortants used for vaccine production. Further studies to confirm the suitability of cell-derived HYRs for the manufacture of vaccine in eggs will be discussed.

Conclusions

the results demonstrate that utilization of qualified MDCK cell lines could enhance the availability of suitable influenza viruses for vaccine production, and thus the timely update of vaccine composition.

SPA2: VACCINES: CURRENT AND NOVEL APPROACHES

A216P

The human potential of a recombinant candidate influenza vaccine produced in tobacco plants

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Introduction

The expression of high concentration proteins in tobacco plants enables rapid cloning and expression of the desired antigen and growth to high concentrations in limited biomass. Producing recombinant haemagglutinin (HA) antigens in tobacco plants is one of the highly promising next generation vaccine production systems developed to overcome the capacity limitations of today's influenza vaccine production. In this study we have evaluated a recombinant HA antigen, HAC1, derived from the 2009 pandemic H1N1 (pH1N1) virus A/California/7/2009 as an immunogen.

Material and methods

Volunteers vaccinated with oil-in-water adjuvanted pH1N1 vaccine provided blood samples at days 0, 7, 14 and 21 and at 3, 6 and 12 months post vaccination. Serum and lymphocytes were used to study the recognition of the HAC1 antigen by human pH1N1 specific antibodies and to investigate the ability of HAC1 to be recognised by B- and T-lymphocytes *in vitro*.

Results

By ELISA and ELISPOT analysis HAC1 was shown to be recognised by serum antibodies and antibody secreting cells (ASCs), respectively, from the vaccinated individuals. The multiplex cytokine assay showed that HAC1 significantly induced secretion of IL-2 and IFN-Ð from PBMCs *in vitro*. Intracellular staining and multiparametric flow cytometry showed that HAC1 induced CD4+ T-cells secreting either single or multiple Th1 cytokines.

Conclusions

In summary we conclude that the HAC1 protein has shown good vaccine potential and should be combined with a safe and effective adjuvant for further testing in clinical trials. Our results further indicate that the production of viral antigens in plants is a good strategy for future production of human influenza vaccines.

SPA2: VACCINES: CURRENT AND NOVEL APPROACHES

A217P

A lentiviral vector vaccine harnesses the alveolar macrophage for T-cell mediated cross-strain immunity against influenza

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Background

Generating memory T-cell responses to conserved influenza epitopes is a promising approach for universal cross-strain vaccination. However, a major limitation of systemic immunization is the delay between infection and the recall of sufficient memory T-cells in the lung to control viral replication. This permits development of clinical illness and an ample window for onward transmission. Also, highly virulent pandemic strains may overwhelm the host whilst the local T-cell recall response is nascent.

Aims

Using a lentiviral vector (LV) vaccine, we aimed to generate strong and sustained lung mucosal T-cell immunity that can quickly eliminate infected cells and suppress viral replication soon after inoculation.

Methods and Results

Here we describe an intranasal LV vaccine expressing influenza X31 nucleoprotein (NP) that generates very high pulmonary mucosal antigen-specific CD8 T-cell frequencies (averaging 35%) in balb/c mice. Intranasal boosting after subcutaneous vaccination confers complete protection against 100% lethal doses of mouse-adapted H1N1 A/PR/8/1934 influenza, without clinical signs or weight loss. It also confers complete protection against the recent pandemic H1N1 strain A/Eng/195/2009 which shares 91% NP amino acid sequence homology with PR8/X31. Protection can be induced rapidly (within 2 weeks) and is preserved for at least 4 months post- vaccination. A single intranasal administration of LV to unvaccinated mice 10 weeks after non-lethal influenza challenge boosted mucosal antigen-specific CD8 T-cells (from 4% to 30%) and conferred complete protection against subsequent lethal challenge with a different strain. This scenario is highly relevant to vaccination in the human domain where prior exposure is common.

Lentiviruses have a well-established tropism for macrophages and this is key to the success of this approach. Intranasal administration of a single dose of LV transduced the majority (>80%) of alveolar macrophages (AM). Adoptive transfer of *in vivo* transduced AM induced protective mucosal antigen-specific CD8 T-cell recall responses in subcutaneously primed mice, comparable to direct intranasal boosting of mucosal T-cell responses with LV.

Consistent with the low inherent immunogenicity of LV, we found that co-expression of antigen presenting cell (APC) maturation/activation factors together with antigen is also crucial for effective T-cell priming or recall. We have identified two such activators of equivalent efficacy: 41BB ligand (41BBL, which matures dendritic cells (DC) via a reverse-signaling domain) and viral FLICE-like inhibitory protein (vFLIP) from Kaposi's sarcoma herpes virus (a potent activator of NFKB). Transduction of balb/c DCs with LV expressing these activators significantly up-regulated expression (between 2.5- to 5-fold) of ICAM-1, CD40, CD80, CD86, PDL-1 and MHC II. Subcutaneous vaccination with these LV resulted in significantly larger, polyfunctional CD8 T-cell memory pools, greater Th-1 CD4 T-cell responses and superior protection against lethal PR8 challenge compared with LV expressing X31 NP alone (81% vs 13%).

The adjuvanticity of 41BBL and vFLIP was also evident *in vitro* upon induction of CD8 T-cell memory recall responses against NP by co-incubation of LV-transduced, autologous monocyte-derived DCs with PBMCs from healthy volunteers.

Conclusion

We have shown that AM are an accessible and abundant population of APCs that can be readily harnessed by LV vaccines for the generation of an unusually high degree of protective mucosal T-cell immunity. Subversion of the default immunoregulatory role of AM by LV-encoded activators seems to be pivotal to the success of this strategy. This method of manipulating lung mucosal T-cell responses represents a powerful and novel approach to universal cross-strain influenza vaccination.

SPA2: VACCINES: CURRENT AND NOVEL APPROACHES

A218P

Simplified oil-in-water emulsions for use with H5N1 pandemic influenza vaccines

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Introduction

There have been 322 confirmed deaths due to H5N1 (and 552 cases), according to the WHO, which have occurred between the first reported case in 2003 and April of 2011 (World Health Organization, www.who.int/csr/disease/avian_influenza). Although H5N1 has not caused a pandemic thus far, if the virus acquires the ability to spread more efficiently from human-to-human, this scenario could occur. To prepare for such a pandemic, vaccines are currently being developed and stockpiled. Responses to the HA protein from H5N1 are significantly enhanced by the use of an adjuvant and could therefore allow dose sparing of the H5N1 vaccines.

Aims

We are currently developing different oil-in-water (o/w) emulsions for inclusion with different types of H5N1 vaccines such as recombinant H5 (rH5) proteins and those that form virus like particles (VLPs). One of our strategies involves the use of simplified emulsions that still possess the ability to generate strong immune responses in mice, while enhancing the stability and durability of the adjuvant under temperature extremes.

Methods

Stability studies (particle size) were performed on the adjuvants used in the H5N1 vaccines. Immune responses were also performed on C57BL/6 mice immunized with an H5N1 vaccine plus different o/w emulsions. We characterized the immune response by measuring (a) antigen-specific endpoint antibody titers from the sera of immunized mice, (b) Hemagglutination inhibition (HAI) responses against the vaccine virus strain and to a different H5N1 virus, (c) long-lived rH5-specific plasma cells derived from the bone marrow using an enzyme-linked immunosorbent spot (ELISPOT) assay, and (d) antigen-specific cellular cytokine responses from rH5-stimulated splenocytes (IL-5 and IFN-gamma ELISPOTs).

Results

Herein, we show that two component o/w emulsions are as effective as a MF59-like o/w emulsion. We found that both humoral and cellular responses generated with vaccines containing these two component emulsion systems closely matched those that were generated with the standard o/w formulations. Furthermore, in addition to the vaccine generating HAI titers against the virus strain (clade 1; A/Vietnam/1203/2004) we showed that the rH5 vaccines mixed with these emulsions were capable of inducing cross-reactive HAI titers against a different virus clade (clade 2.3.4; A/Anhui/1/2005).

Conclusion

These data suggest that a safe, stable, simplified and inexpensive adjuvant can be produced for application to current H5N1 vaccines, which could greatly expand vaccine supply in the event of a pandemic.

Abbreviations

o/w (oil-in-water), HAI (hemagglutination inhibition), rH5 (recombinant H5), VLP (virus like particle), enzyme-linked immunosorbent spot assay (ELISPOT)

SPA2: VACCINES: CURRENT AND NOVEL APPROACHES

A219P

Acceptability of Intradermal influenza vaccine -Intanza 15mcg- among nurses

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Introduction

In 2009, the first intradermal vaccine against influenza (Intanza®, Sanofi Pasteur MSD) was approved by the European Medicines Agency (EMA). Intanza® has shown a high potential to generate immune responses (1) and, thanks to an innovative injection system (i.e. easy to inject, microneedle, needle protective shield), it might facilitate the acceptance of the vaccine, by nurses and vaccinees, thereby helping to improve vaccination coverage. Two dosage levels have been licensed: 9 µg per strain for adults 18–59 years of age, and 15 µg per strain for persons 60 years of age and older.(2) In clinical trials prior to its license, Intanza® acceptance by patients was good (3). However, no data has been published concerning satisfaction of nurses with the new vaccine. The vaccine has been recently introduced in Melilla for patients over 65 years old, offering the opportunity to evaluate its acceptance.

Objective

To evaluate the satisfaction of nurses with the new intradermal vaccine and its microinjection system, during the 2010 Influenza vaccination campaign in the City of Melilla.

Material and methods

Descriptive cross-sectional observational study. Nurses involved in the vaccination campaign were invited to voluntarily and anonymously answer a closed survey.

Results

- 41 surveys have been conducted in 8 vaccination centers that have used the vaccine (2.961 administered doses)
- 95.1% consider that the administration of the vaccine was easy with the microinjection system and 95.1% of respondents declared to be satisfied or very satisfied with the injection system used.
- 90.2% of responders were satisfied with the needle autoshielding system.

The most appreciated feature of the vaccine was biosafety (shielding system) for 63.4% of nurses and ease of administration for 26.8%.
As for the perception of local reactions at the injection site, 70.7% considered them little/not at all annoying and 80.5% considered them very/completely acceptable. 44% of respondents believe that the new vaccine has been useful to increase coverage in his area.
97.5% of respondents are satisfied/very satisfied with the intradermal vaccine and 97.5% of respondents stated preference for intradermal vaccination for the following vaccination of their patients.

Conclusions

The level of satisfaction among nurses, with the new microinjection system was high, as well as their willingness to use the intradermal vaccine for the following vaccination of their patients, consistently with previous acceptability studies in patients.

Nearly half of respondents think that the new vaccine has improved vaccination coverage. Specific studies designed to assess the magnitude of this effect are desirable.

Nurses particularly valued the needle shielding system. This type of biosafety mechanisms would be desirable in flu vaccines, especially after the coming into effect of the directive 2010/32/UE on prevention from sharp injuries in the hospital and healthcare sector.

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SPA2: VACCINES: CURRENT AND NOVEL APPROACHES

A220P

Mummy flu – serology

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Abstract

We present the serological findings of this two part observational study to investigate vertically acquired passive immunity in babies of mothers vaccinated against pandemic specific A/California/7/2009 H1N1 during pregnancy.

Introduction

The 2009-10 pandemic amply displayed the key vulnerability of some individuals and risk groups to influenza. Although overall the impact was less severe than at first feared, the outbreak was notable for causing serious illness and death in some, including pregnant women.
Achieving acceptance of immunisation can be problematic, particularly during pregnancy when there is specific concern about intervening medically unless the benefit outweighs the risk.
This research was undertaken to investigate the transfer of passive humoral immunity to the unborn child of mothers immunised against pandemic specific A/California/7/2009 H1N1. An additional component (reported separately) examined the degree and duration of protection against acquisition of influenza from the passively acquired immunity during the first year of life.
Verification that humoral immunity is transferred would inform policy and promote immunisation to pregnant women as a means of protecting their infant in both seasonal influenza and future pandemic incidents.

Methods

104 pregnant women were enrolled, who had (77 (74%)) or had not (27 (26%)) already been vaccinated against A/California/7/2009 H1N1 (as part of the national immunisation programme with a single dose of AS03 adjuvanted vaccine) and were admitted for delivery at one of three hospital sites in the East Midlands (United Kingdom) during winter 2009 -10.
After delivery, venous cord blood samples were obtained to determine the serological status of the infant to A/California/7/2009 H1N1. Laboratory staff, blinded to vaccination status, determined immune status with haemagglutinin dilution and microneutralisation assays. The primary endpoint was the proportion of cord blood specimens with haemagglutination inhibition (HI) titres ≥ 1:40.
The infants were then followed up until March 2011 to assess the protective effect and duration of maternally provided immunity. This is reported separately (abstract Mummy Flu – PCR)

Results

Samples were obtained at delivery at a median interval of 42 days (range, 1 to 108) post immunisation. Evidence of seroprotection (HI titre >1:40) was found in 58 (75.3%) samples from vaccinated women and 5 (18.5%) unvaccinated women (P<0.0001).
Overall geometric mean haemagglutination inhibition antibody titres were 142.6 (95% CI 93.3-217.4) in samples from all immunised women and 9.1 (5.6-14.7) from all unimmunised women (P<0.0001). Evidence of seroprotection was found in 52 of 67 (77.6%) vaccinees from as early as day 8 post immunisation.

Conclusions

Maternal immunisation with AS03 adjuvanted monovalent A/California/7/2009 H1N1 rapidly provides passive humoral immunity at titres consistent with clinical protection in three-quarters of infants. This immunity may protect the infant from influenza early in life when prevention and treatment choices for infection are restricted (because vaccine or antiviral drugs are either not licenced or ineffective in this age group).

These results inform policy decisions and clinicians in the advocacy of influenza immunisation during pregnancy in seasonal and epidemic / pandemic situations and assist pregnant women in making informed choices in such circumstances.

Funding: Department of Health, England, via the National Institute for Health Research (NIHR).

SPA2: VACCINES: CURRENT AND NOVEL APPROACHES

A221P

HPAI H5N1 VLP vaccine provides protective immunity in chickens and a strategy for the differentiation of infected from vaccinated animals

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Introduction

Currently, Asian lineage HPAI (H5N1) has become widespread across three continents and become endemic in Southeast Asia in poultry. In order to control the HPAI H5N1 outbreaks, several strategies have been applied including vaccination, stamping-out, quarantine, and movement control. Although vaccination against HPAI in poultry is still a controversial topic and has been discouraged, it has been recommended as a part of HPAI control strategies since mass culling has not proven to be successful in HPAI H5N1 endemic regions.

HPAI H5N1 vaccines for poultry should prevent clinical disease and death, induce resistance to infection. Moreover, vaccines for poultry also need to allow DIVA (Differentiating Infected from Vaccinated Animals) strategy and be applicable to wide range of avian species. In this study, we developed HPAI H5N1 VLP vaccine and evaluated its immunogenicity and protectivity in specific pathogen free (SPF) chickens. Furthermore, VLP vaccine was assessed for DIVA strategy by using nucleocapsid protein (NP) coated enzyme linked immunosorbent assay (ELISA) test, which may provide a useful method for sero-surveillance in vaccinated flocks.

Material & methods

HPAI H5N1 VLP was produced by infecting SF9 cells with recombinant baculovirus encoding HA, NA, and M1 protein of A/chicken/Korea/Es/03 (H5N1). Culture supernatant containing VLP were formalin treated for baculovirus inactivation and concentrated by ultrafiltration. VLP vaccines were prepared by emulsifying the escalating HAU (2^8, 2^9, and 2^10) of culture supernatant with Montanide ISA70. To evaluate immunogenicy and portectivity of HPAI VLP vaccines, three groups of 5-week-old SPF chickens were immunized intramuscularlly with VLP vaccines of escalating antigen doses. Three weeks after a single immunization, serum sample were collected for HI test and chickens were intranasally challenged with 100ul of 10^6EID50/ml of A/chicken/Korea/Es/03 (H5N1). Clinical signs were monitored daily for 2wk post-challenge. Oro-pharyngeal and cloacal swab samples were collected and analyzed for viral shedding by rRT-PCR at 3,5, and 7 days postchallenge. To differentiate VLP vaccinated from infected chicken, NP-coated ELISA test was performed.

Results

Three weeks after a single immunization, all groups of VLP-vaccinated chickens showed significant levels of virus-specific antibodies. Although, some chickens immunized with VLP vaccine shed virus with the low CT value, excretion of challenge virus in VLP-vaccinated chickens was significantly reduced in both oro-pharyngeal and cloacal swabs compared to mock-vaccinated chickens. Moreover, regardless of VLP dose, all VLP-vaccinated chickens were protected against lethal challenge of HPAI, whereas all mock-vaccinated birds were severely affected and died within 2 or 3 days post-challenge (MDT=2.4days).

To verify that VLP vaccines could be used as a part of DIVA strategy, serum samples from both VLP-vaccinated/ uninfected and VLP-vaccinated/ infected chickens were tested using NP-coated ELISA. As expected, all serum samples from VLP-vaccinated/ uninfected chickens were negative by the NP-cELISA, whereas serum samples from VLP-vaccinated/ infected chickens were all positive for antibody response against influenza A NP protein.

Conclusions

Efficacy of VLP vaccines against HPAI H5N1 have been demonstrated in several mouse and ferret studies. In this study, we first described the immunogenicity and protectivity of VLP vaccines against HPAI infection in chickens. VLP vaccines were highly immunogenic and could confer protective efficacy against HPAI in chickens even without costly purification steps. Furthermore, using commercial NP-coated ELISA tests, VLP-vaccinated chickens could be differentiated from vaccinated/infected chickens.

In conclusion, HPAI H5N1 VLP vaccine induced protective immune responses against HPAI H5N1 infection and allowed DIVA strategy, indicating that VLP vaccination in poultry could be effectively used as a part of HPAI H5N1 control strategy that does not interfere with sero-surveillance in vaccinated animals.

SPA2: VACCINES: CURRENT AND NOVEL APPROACHES

A222P

Dominant gene constellation selected through classical reassortment and its impact on vaccine yields

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Introduction

A retrospective analysis of potential vaccine seed reassortants over 8 years indicated that in over 50% of cases viruses contained the PB1 gene in addition to the HA and NA genes of the seasonal isolate. In this study we used reassortants of A/Udorn/307/72 (H3N2) virus (Udorn) and high growth A/PR8/8/34 (H1N1) virus (PR8) to investigate the dominant gene constellation resulting from this combination of viruses and its impact on vaccine yield.

Materials & methods

Classical reassorting between PR8 and Udorn was performed to confirm the preferential selection of particular viruses in our model. Reassortants and reverse-genetics derived viruses were amplified in multiple eggs from a constant dose of infectious virus and the allantoic fluid was analysed by HA content (haemagglutination assay) and particle number (qRT PCR of vRNA).
Transcriptional and replicative abilities of viruses were measured by qRT PCR to determine the copy number of matrix vRNA and mRNA in infected MDCK cells. Viral protein levels in infected cells and allantoic fluid were analysed by western blot and relative protein content quantified by densitometry.

Results

Classical reassortment between the PR8 and Udorn viruses resulted in preferential inclusion of Udorn PB1 with its HA and NA. However, analysis of reverse-engineered viruses showed the predominant virus with Udorn PB1, PR8(Ud-HA,NA,PB1), produced eight-fold fewer viral particles and two-fold less HA units (HAU) compared to the virus containing PR8 PB1, PR8(Ud-HA,NA). This suggests that inclusion of Udorn's PB1, which leads to a hybrid polymerase, results in a less-fit virus. The data also indicated a four-fold higher HAU:particle ratio when Udorn's PB1 was included. This altered growth phenotype was not observed when Udorn PB2 or PA rather than PB1 were included.
No difference in the ability of the polymerase complexes to produce mRNA or vRNA was observed between the two viruses indicating the replicative and transcriptional activities of the polymerase was not responsible for differences in virion production. However, differential expression of viral proteins was observed in MDCKs infected with the two viruses. A PR8(Ud-HA,NA,PB1) infection resulted in higher HA:matrix protein ratio compared to PR8(Ud-HA,NA). This observation supported analysis of allantoic fluid which showed the PR8(Ud-HA,NA,PB1) had a four-fold higher HA:NP protein ratio compared to PR8(Ud-HA,NA). These data together indicated that the inclusion of Udorn's PB1 resulted in higher HA density in the virion.

Conclusions

PR8(Ud-HA,NA,PB1) was a relatively poor growing virus but dominated over the better growing PR8(Ud-HA,NA) following classical reassortment. vRNA and mRNA production by the two polymerase complexes was similar and could not account for the difference seen in yields of the two viruses. In contrast, differences in viral protein expression levels and ratios of viral proteins observed in infected cells could result in limited virus assembly and explain differences in both virus yield and HA density of the virion.
In this study, we show that the inclusion of a seasonal PB1 rather than PB1 of the high growth parent in a model vaccine seed virus results in a four-fold higher HA content per virion yet it produces eight-fold less virions resulting in a two-fold lower yield of vaccine. Further investigation into the mechanism of selection of the poorer growing virus during classical reassortment is required to understand how to optimise seed viruses for higher vaccine yields.

SPA2: VACCINES: CURRENT AND NOVEL APPROACHES

A223P

Mummy Flu - PCR

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Background

Women in their second and third trimesters of pregnancy are at high-risk of severe influenza complications, particularly during pandemic seasons. Similarly, adverse effects of influenza have been observed in perinatal and early neonatal periods and increasing evidence suggests that young children <2 years of age are at greater risk of developing complications and dying from the infection than older ones. During the 2009-2010 A/H1N1 flu pandemic, there were up to 3 times more hospitalized children <5 years of age than any other age groups, and a number of pregnant women required ITU admission, further giving evidence of morbidity from influenza infection in these groups.
Vaccination is the best available method for the prevention of an influenza infection and immunized pregnant women have been shown to have a decreased risk of infection and complications from the virus. Moreover, immunization during pregnancy with a trivalent, non-adjuvanted seasonal vaccine has been shown to provide maternal antibodies to infants until 6 months after birth, reducing the child's risk for influenza infection and influenza-like illness (ILI). However, vaccination of pregnant women has only recently been recommended in the UK and uptake in the US, where it has been in practice for several years, has consistently been low despite favourable risk/benefit reports.

Aim

To provide further evidence of the benefits to children of mothers immunized against flu while pregnant, by investigating the incidence of influenza-like illness within the first 16 months of life.
Methods
Pregnant women who fit either the vaccinated or unvaccinated eligibility criteria and who were expected to deliver during the study period at one of three selected area hospitals in the East Midlands, UK were enrolled into the study. At time of delivery, venous cord blood samples were collected to assess the babies' immune status. Following delivery until 31 March 2011, nasal swab samples were collected from neonates exhibiting ILI symptoms for respiratory infection testing by RT-PCR, to establish the efficacy of protection.

Results

A total of 104 pregnant women were enrolled into the study; 77 (74%) participated in the national vaccination programme and received an ASO3-adjuvanted, monovalent A/H1N1/09 vaccine, while 27 (26%) did not. Eighty percent of babies born to vaccinated mothers developed antibodies (reported separately: see abstract Mummy Flu - serology). Swab samples were received from 59 neonates, 45 of whom were born to vaccinated mothers. Of these 45, four tested positive for pandemic A/H1N1/09, ten to 12 months after birth; 30 developed ILI within the 1st 6 months of life and 16 had a co-infection at least once in 16 months. Of those born to unvaccinated mothers, one tested positive for A/H1N1/09 after 13 months; 11/14 developed ILI within the 1st 6 months, and 6/14 had a co-infection at least once in 16 months. Chi-square analysis suggests that vaccination with ASO3-adjuvanted, monovalent A/H1N1/09 has no effect on co-infection and there was not enough data on babies from unvaccinated mothers to determine statistical significance of ILI in both groups.

Conclusion

Immunization of pregnant women with an ASO3-adjuvanted, monovalent A/H1N1/09 vaccine provides neonatal humoral immunity. However, more data are needed to demonstrate the benefits of vertical immunity in relation to ILI.

Funding: Department of Health, England, via the National Institute for Health Research (NIHR).

SPA2: VACCINES: CURRENT AND NOVEL APPROACHES

A224P

Pulmonary administration of monophosphoryl lipid A-adjuvanted whole inactivated virus influenza vaccine formulated as dry powder successfully induces IgA and IgG.

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Aim

Intramuscular injection is the most widely used route for the administration of inactivated influenza vaccines. These needle-based vaccines induce production of serum antibodies thus not providing initial protection against virus in the respiratory tract. Earlier studies from our group have shown that whole inactivated virus (WIV) vaccine, processed to dry powder by spray freeze-drying and administered via the pulmonary route induces production of substantial amounts of IgG but little IgA. Here we investigated whether adjuvantation of powder WIV with monophosphoryl lipid A (MPLA) enhances mucosal IgA and systemic IgG responses as compared to non-adjuvanted WIV after pulmonary administration.

Methods

Different concentrations of MPLA were analysed in combination with WIV for NFkB activation using the RAW Blue cell reporter cell line and were Spray Freeze-Dried (SFD) using inulin as excipient. The resulting vaccine powder was characterised for virus structure, particle size, surface area, morphology, moisture content and binding to hemagglutinin. After characterization, adjuvanted and unadjuvanted vaccines were administered to female BALB/c mice. A dry powder insufflator was used for powder vaccine delivery while liquid vaccine was administered using a micro-sprayer. Subunit vaccine delivered by intramuscular (i.m.) injection was used as positive control. Total IgA in nose and bronchioalveolar lavages (BAL), IgG, IgG1, IgG2a in serum and IgG in BAL were evaluated using ELISA.

Results

WIV structure and antigenic epitopes were preserved after spray freeze-drying as assessed by electron microscopy and heamagglutination assay, respectively. On RAWBlue cells, MPLA showed a similar level of NFkB activation as the liquid formulations indicating that spray freeze-drying did not compromise the biological activity of MPLA. A combination of WIV and MPLA stimulated NFkB production more strongly than either WIV or MPLA alone. Upon pulmonary vaccination MPLA-adjuvanted liquid and powder vaccine induced stronger serum and lung IgG responses than unadjuvanted vaccines. Liquid and SFD WIV showed biased IgG1-skewed immune response. However, adjuvantation with MPLA reduced the skewing by stimulating production of IgG2a thereby decreasing the IgG1/IgG2a ratio. IgA responses in the lungs to both vaccines were significantly increased for the MPLA-adjuvanted vaccines. However, MPLA had no effect on IgA responses in the nose which were very low for liquid vaccine but more robust for dry powder vaccine.

Conclusion/Discussion

Our results show that MPLA can act as an adjuvant for pulmonally administered vaccine boosting mucosal and systemic IgG responses as well as IgA responses to WIV at the site of immunization, the lungs. Moreover, MPLA adjuvantation stimulated the production of IgG2a, an antibody subclass earlier shown to be important for protection. Of the formulations tested, SFD WIV MPLA rendered the highest antibody responses and was able to induce IgA responses in the nose, the port of entry of the virus. These results imply that immunization with adjuvanted dry powder vaccine may be an alternative route for influenza vaccination in the future.

SPA2: VACCINES: CURRENT AND NOVEL APPROACHES

A225P

Comparison of influenza subunit and virosome vaccines as platform for the saponin-derived adjuvant GPI-0100

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Introduction:

Adjuvants can enhance immune responses in immune-compromised individuals and can contribute decisively to antigen dose sparing when vaccine amounts are limited as in case of a pandemic. We earlier showed that GPI-0100 is an excellent adjuvant for influenza subunit vaccine (Liu et al, Vaccine 2011). Since GPI-0100 contains a hydrophobic aglycone backbone we wondered whether the effects of the adjuvant could be further enhanced by using influenza virosomes, reconstituted viral membrane envelopes, as vaccine platform. Here we evaluate whether GPI-0100 adjuvanted virosomes are more effective than adjuvanted subunit vaccine in stimulating influenza-specific immune responses in terms of both antigen and adjuvant dose sparing.

Methods:

6-8 week old Balb/c mice were immunized intramuscularly on day 0 and day 20 with different doses of A/PR8 (H1N1) influenza subunit or virosomes (8, 40 or 200 ng hemagglutinin) alone or in combination with varying doses of GPI-0100 (5 or 15 μg). The immunized mice were challenged with PR8 virus at 200 TCID50 on day 27 and were sacrificed on day 30. Serum samples were collected on day 20, 27 and 30 for pre-boost, pre-challenge and post-challenge antibody response evaluation. ELISA and hemagglutination inhibition (HAI) assays were performed to determine the quantity, quality and functionality of the vaccine-elicited antibodies. Spleen and lung samples were collected on day 30 upon termination for the evaluation of influenza-specific cellular immune responses and lung virus titers, respectively.

Results:

Mice immunized with GPI-0100-adjuvanted virosomes developed higher hemagglutination inhibition titers than subunit-immunized mice. In addition, at a very low antigen dose (8 ng), GPI-0100 adjuvanted virosome elicited better influenza specific antibody responses than the adjuvanted subunit, in terms of speed, quantity and quality. At 8 ng antigen dose, most of the mice receiving the adjuvanted virosomes readily developed detectable IgG titers 20 days after the 1st immunization. The titers were further boosted after the 2nd immunization and were significantly higher than those developed in mice receiving the adjuvanted subunit vaccine. In particular, influenza-specific IgG2a and cellular immune responses, which play important roles in virus clearance, were much more strongly induced by the adjuvanted low dose virosome vaccine than by subunit. In the challenge experiments, both adjuvanted subunit and virosome vaccine fully protected mice against virus growth in the lungs at antigen doses of 40 and 200 ng. However, at 8 ng antigen only the adjuvanted virosomes provided full protection.

Conclusions:

GPI-0100-adjuvanted influenza virosomes showed better antigen sparing capacity than the adjuvanted subunit vaccine. Remarkably, 8 ng of adjuvanted virosomes were sufficient to elicit strong immune responses and provide protection from virus growth in the lungs. Further characterizations of GPI-0100 adjuvanted virosomes are ongoing to understand the mechanisms for their superiority in immune stimulations and why such effects are less prominent with higher antigen dose.

SPA2: VACCINES: CURRENT AND NOVEL APPROACHES

A226P

Virosomal adjuvanted influenza vaccine Inflexal®V: an integrated analysis of immunogenicity and safety in annual update studies in adults and elderly

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Background and aims

As requested by the European legislation, the annual relicensing of influenza vaccines is granted based on the demonstration that the yearly recommended strain composition of the vaccine fulfills the immunogenicity and safety criteria in a small number of healthy adults and elderly, as set by the CHMP (1). Inflexal®V has consistently fulfilled those criteria in all relicensing studies throughout the years.
We carried out an integrated analysis of the last ten studies in order to assess the overall immunogenicity and safety performance of Inflexal®V in adults and the elderly and publicly account for the suitability of Inflexal®V in the control of influenza.

Methods

The studies were designed according to the CHMP criteria and carried out in compliance with GCP. The vaccine composition fulfilled the requirements of the yearly WHO/EMA strain recommendations. A minimum of 110 eligible volunteers per study were stratified into two age groups (adults between 18 and 60 years and elderly aged over 60 years). Humoral immunogenicity was analyzed and results assessed according to the CHMP criteria in blood samples drawn at baseline and 22±2 days after vaccination with a single dose of Inflexal®V (0.5 ml). Solicited local and systemic adverse events (AEs) were observed during the vaccination day and for 3 days after. Unsolicited AEs were observed throughout the study. The integrated analysis was compiled from the individual datasets of ten annual update studies and was performed using a generalized linear mixed modeling procedure, which accounts for clustering of the results in years and proper probability distribution.

Results

The integrated analysis considered the annual update studies* (2000 - 2010) and included 1'127 subjects. Immunogenicity results obtained from the per-protocol population (N = 1'101) are presented in the table below. Inflexal®V consistently fulfilled all CHMP criteria and performed considerably above the CHMP threshold values in both age groups. As expected from previous literature reports (2), seroconversion rates were significantly higher in previously non-vaccinated subjects compared to those previously vaccinated.

	SEROPROTECTION			SEROCONVERSION			GMT fold increase		
	ALL‡ (%)	Adults (%)	Elderly (%)	ALL‡ (%)	Adults (%)	Elderly (%)	ALL‡ (%)	Adults (%)	Elderly (%)
N	1'101	551	550	1'101	551	550	1'101	551	550
EMA criterion		70%	60%		40%	30%		2.5	2
A/H1N1	93	96	89	64	71	57	11	17	7
A/H3N2	94	94	94	61	64	58	8	9	7
B	92	94	91	58	66	49	6	9	5

‡ adults and elderly

Reactogenicity data were available for all 1'127 subjects. Two thirds of the pooled subjects did not report any local AEs, and over 90% of the pooled subjects did not report any systemic AEs. All local or systemic AEs resolved within 1 day after the day of vaccination. The most frequently reported AE was pain (29%), which was mostly mild (painful on touch), and only 0.7% of all subjects reported severe pain (spontaneously painful). Gender/age pattern was reflected in the reporting of local pain, as follows: Female Adults>>Male Adults/Female Elderly>>Male Elderly.

Conclusions:

- Inflexal®V triggers a powerful immune response demonstrated consistently across the years and has a good safety record in both adults and elderly.
- The strong immune response in the elderly is also important to notice due to the recognized immunosenescence in this age group.

*No study was performed in 2003 as vaccine composition was the same as in previous year.

(1) Committee for Medicinal Products for Human Use. Note for guidance on harmonisation of requirements for influenza vaccines. CPMP/BWP/214/96 . 12-3-1997. http://www.emea.europa.eu/pdfs/human/bwp/021496en.pdf Last accessed May 2011.

(2) Goodwin K, Viboud C, Simonsen L. Antibody response to influenza vaccination in the elderly: a quantitative review. Vaccine 2006; 24(8):1159-1169.

SPA2: VACCINES: CURRENT AND NOVEL APPROACHES

A227P

Immune response in unprimed children aged 6 - <36 months after vaccination with Inflexal®V, a virosomal adjuvanted influenza vaccine

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Background

High influenza morbidity is reported most frequently in paediatric populations. Annual influenza immunisation is recommended by the WHO for healthy children aged 6 - 23 months and for all children above 6 months of age with chronic conditions [1]. According to the European Commissions Guideline on the Core SPC for trivalent influenza vaccines, the clinical data in children 6 – 35 months are limited. In the currently recommended vaccination schedule dosages of 0.25ml or 0.5ml have been used. For children 6-36 months of age who have not previously been vaccinated, a second dose is recommended after at least four-week interval. The dose given should be according to the approved dosage for the respective vaccine [2].

Methods

An open, randomized, parallel, multi-centre study was conducted, using WHO recommended influenza strains for the 2010/2011 Northern Hemisphere season, in 197 unprimed children aged 6 - <36 months stratified into two groups. In one group (N = 98) the children were administered two half-doses (2x0.25 ml) of Inflexal®V according to a 0/4 week schedule and blood samples were drawn at baseline, 4 weeks and 6 months after the second vaccination. In the other group (N = 99) the children were administered a full Inflexal®V dose (1x0.5 ml) and blood samples were drawn at baseline, 4 weeks and 7 months after the vaccination. Immunogenicity was analyzed and results assessed according to the CHMP relicensing criteria for adults [3]. Solicited local and systemic adverse events (AEs), as well as unsolicited AEs, were also evaluated.

Results

The preliminary results of the immunogenicity assessments obtained at 4 weeks after the last vaccination with Inflexal®V are presented in the table below‡. All three immunogenicity criteria were met for all strains in both study groups. The immunogenicity parameters between a full-dose and the two half-doses were comparable and not statistically significantly different. Both groups of unprimed children achieved a strong immune response despite the immature immune system recognized in this age group. No significant increase in local and systemic adverse events was observed after vaccination with a full dose compared to the two half-dose regime. No cases of febrile convulsions were reported for any subjects enrolled in the study.

	SEROCONVERSION (%)			GMT fold increase		
	Inflexal®V 1 x 0.5 ml	Inflexal®V **2 x 0.25 ml**	p value	Inflexal®V 1 x 0.5 ml	Inflexal®V 2 x 0.25 ml	p value
A/H1N1	98	99	p=0.567	19.6	25.5	p=0.025
A/H3N2	97	99	p=0.317	24.6	31.6	p=0.042
B	86.9	92.9	p=0.164	14.7	12.8	p=0.210

‡ Results based on ITT population

Conclusions

This study shows that an alternative administration schedule of influenza vaccination with Inflexal®V in unprimed children aged between 6 and <36 months could also trigger an immune response that fulfils the CHMP criteria.

1. WHO, Position paper influenza vaccine, 2005, available at: http://www.who.int/immunization/wer8033influenza_August2005_position_paper.pdf last accessed April 2011.
2. Core SPC for trivalent influenza vaccines, CMDh/128/2003/rev, September 2009
3. The Committee for Medicinal Products for Human Use. Note for guidance on harmonisation of requirements for influenza vaccines. CPMP/BWP/214/96. Issued 12 march 1997.

SPA2: VACCINES: CURRENT AND NOVEL APPROACHES

A228P

Development of immune response after vaccination with the virosomal adjuvanted influenza vaccine Inflexal®V

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Background and aims

Humoral protection against influenza infection is mainly conferred by antibodies to the surface virus antigens. The immune response to influenza infection and influenza vaccines is complex and not yet completely elucidated. Better understanding on how the immune response evolves after vaccination could allow for predictions of the onset and the duration of the efficacy induced by the influenza vaccine. The current study evaluated the early dynamics of the immune response induced by Inflexal®V in adults and elderly.

Methods

The study was designed according to the CHMP [1] guidelines and was carried out in compliance with Good Clinical Practice. The vaccine composition was produced according to the WHO/EMA strain recommendations for the 2010-2011 North Hemisphere season. A total of 108 eligible volunteers were stratified into two age groups (adults between 18 and 60 years, and elderly aged over 60 years) and vaccinated with a single dose of Inflexal®V (0.5 ml). Blood samples were drawn at baseline and 8, 15 and 22 days after vaccination.

Results

Analysis of the immunogenicity results for the adults and elderly revealed that the immune response was initiated rapidly after vaccination and that the CHMP criteria for relicensing of influenza vaccines were reached: two weeks after vaccination with Inflexal®V, two out of three criteria were met for each strain in adults, while in the elderly group all three criteria were met for the A strains and one out of three criteria for the B strain. Rates of seroprotection and seroconversion, as well as the geometric mean titers (GMTs), rapidly increased during the two weeks after vaccination in elderly as shown in the tables 1, 2 and 3.

Table 1.
Evolution of the seroprotection rates in elderly after vaccination with Inflexal®V (CHMP criteria: seroprotection achieved in >60% of the vaccinees)

SEROPROTECTION (%)			
Day	0	8	15
A/H1N1	18.2	45.5	70.9
A/H3N2	27.3	49.1	69.1
B	27.3	47.3	58.2

Table 2.
Evolution of the seroconversion rates in elderly after vaccination with Inflexal®V (CHMP criteria: seroconversion reached in >30% of the vaccinees)

SEROCONVERSION (%)		
Day	8	15
A/H1N1	16.4	54.5
A/H3N2	12.7	30.9
B	5.5	20

Table 3.
Evolution of the GMT fold increase in elderly after vaccination with Inflexal®V (CHMP criteria: >2 GMT fold increase)

GMT FOLD INCREASE		
Day	8	15
A/H1N1	2.3	4
A/H3N2	1.9	3.1
B	1.7	2.3

Conclusions

This study demonstrated that after vaccination with a single dose of Inflexal®V (0.5 mL) CHMP criteria for immunogenicity were met two weeks after vaccination.

1. The Committee for Medicinal Products for Human Use. Note for guidance on harmonisation of requirements for influenza vaccines. CPMP/BWP/214/96. Issued 12 march 1997.

SPA2: VACCINES: CURRENT AND NOVEL APPROACHES

A229P

The rationale for quadrivalent influenza vaccines

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Introduction

Influenza A/H1N1, A/H3N2, and B viruses have circulated and caused disease in humans on a global basis since 1977. Accordingly, licensed seasonal influenza vaccines have contained 3 strains, 1 from each A subtype and 1 type B virus. However, since 1985, 2 antigenically distinct lineages of influenza B viruses have circulated globally. As only 1 lineage can be selected for inclusion in current trivalent influenza vaccines, the vaccines have provided limited immunity against strains of the other lineage. Our objective was to review the available data supporting the rationale for quadrivalent influenza vaccines (vaccines containing 2 A and 2 B strains).

Results

According to data from the European Influenza Network, from 2001–2002 through 2010–2011 (excluding the 2009–2010 pandemic), on average, 23% (range: 1%–60%) of influenza samples annually were due to influenza B. US Centers for Disease Control and Prevention (CDC) surveillance data from the same seasons were similar, with a season average of 24% (range, <1%–44%). Studies of severe influenza disease have shown that influenza B causes significant morbidity and mortality in all ages, although its incidence relative to influenza A appears to be highest among older children and young adults. Medically attended illnesses in both children and adults due to influenza A and B are generally similar in regards to symptoms, severity, and rates of influenza-related complications. The principal differences observed across studies are that influenza B disease in children is more commonly associated with myalgia, myositis, and leukopenia and less commonly associated with rhinorrhea. Before 1985, there was a single lineage of influenza B in global circulation, which was the precursor to the subsequent Yamagata lineage. The Victoria lineage appears to have emerged in China by 1975 and began circulating globally in 1985. The Victoria lineage dominated global circulation from 1987–1989, followed by Yamagata dominance in the 1990s, and subsequent re-emergence of the Victoria lineage in 2001–2002. From 2001–2002 to the present, both lineages have cocirculated each season at varying levels, and predictions regarding which B lineage will predominate in an upcoming influenza season have been no better than chance alone, correct in only 5 of the 10 seasons from 2001–2011. Studies have shown limited to no immunologic cross-reactivity between the 2 B lineages. Consequently, seasonal influenza vaccines could be improved by inclusion of influenza B strains of both lineages. A US CDC analysis suggested that use of quadrivalent vaccines in the United States during the 2001–2008 seasons would have been beneficial in each season, cumulatively resulting in approximately 2.1 million fewer cases of influenza, 20,000 fewer hospitalizations, and 1200 fewer deaths. Manufacturing capacity for seasonal influenza vaccines has increased sufficiently to supply quadrivalent influenza vaccines, and methods to identify the influenza B strains to include in such vaccines are in place.

Conclusions:

Quadrivalent formulations represent a next logical step for seasonal influenza vaccines. Because 2 antigenically distinct influenza B lineages have been circulating since 1985 and the predominant influenza B lineage has been unpredictable in recent years, quadrivalent vaccines would more accurately reflect the current epidemiology of influenza and would allow vaccination campaigns to more effectively protect their target populations. Multiple manufacturers have initiated clinical studies of quadrivalent influenza vaccines. Data from those studies as well as epidemiologic data regarding the burden of influenza B infections will determine the safety, effectiveness, and benefit of utilizing quadrivalent vaccines for the prevention of seasonal influenza disease. Sponsored by MedImmune, LLC.

SPA3 - PANDEMIC AND EPIDEMICS

A301P

Influenza-like illness in general practice in Norway: Clinical course and attitudes towards vaccination and preventive measures during the 2009 pandemic

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Objectives.
To document clinical characteristics of influenza-like illness (ILI), reported use of health preventive measures and attitudes towards vaccination among patients with ILI in general practice during the pandemic in 2009.

Methods.
This is a cross-sectional survey in general practice. Patients who were identified as having ILI during the peak of the influenza pandemic activity in Norway, were eligible for inclusion in the study. A questionnaire was sent 2-4 weeks after patients visit to GP with ILI diagnosis during October to December 2009, from GP practices in a county in Norway. A sample of responders older than 18 years also had a blood test to check for serological response to the pandemic H1N1-virus.

Results.
Questionnaires were sent to 1324 patients, and 357 (27%) were returned. Fever (91% vs. 49%, P<0.01), cough (85% vs. 73%, P=0.016) and GI-symptoms (58% vs. 38%, P<0.01) were more frequent in the age group <18 years compared to older patients. Serological H1N1 responses were analysed in 72 patients; 34 cases (47%) were positive (HAI titres >40). There were no statistically significant differences in symptoms between seropositive and seronegative patients. Women reported better adherence to personal protective measures, such as hand wash and cough etiquette than men. Women were also more concerned about possible adverse effects of the pandemic influenza vaccination than men.

Conclusions.
Discrimination between influenza and other viral upper respiratory tract infections is difficult in daily clinical practice, even during an influenza pandemic. A gender difference was found in reported precautions to prevent influenza.

SPA3 - PANDEMIC AND EPIDEMICS

A302P

Surveillance and characterisation of influenza viruses among patients with influenza-like illness in Bali, Indonesia: preliminary findings

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Introduction

With high densities and close proximity of humans, poultry and pigs, along with its status a popular tourist destination, the island province of Bali in Indonesia is a potential hotspot for mixing of influenza viruses from different geographic regions and host species. Moreover, while Indonesia continues to report the majority of avian influenza outbreaks worldwide, research on the molecular ecology and evolution of influenza in the country has been severely limited.

Materials and Methods

The Molecular Epidemiology of Influenza A in Bali project ("BaliMEI") aims to conduct five years of active surveillance for influenza viruses among patients presenting with influenza-like illness at health facilities across Bali. Nasopharyngeal swab samples are screened for Influenza A and B, and Influenza A-positive samples are subtyped and tested for genetic markers of resistance to oseltamivir, using PCR based assays. Sequencing and phylogenetic analysis of samples will take place at a later stage.

Results

To date, we have tested 256 patients (mean age = 19y, range= <1 to 75y) who presented with influenza-like illness between July 2010 and April 2011. Of these, 31 (12.1%) tested positive for Influenza A, and 24 (9.4%) tested positive for Influenza B. Initial findings suggest different temporal patterns in circulation of Influenza A and B in Bali, with Influenza B dominating in July 2010 through September 2010, and Influenza A dominating from December 2010 through April 2011. Positive rates for both Influenza A and B were slightly higher among patients 16 years and older (Flu A = 15.6%; Flu B = 11.5%), compared with patients under 16 years (Flu A = 9.0%; Flu B = 7.5%). Of 11 Influenza A isolates subtyped to date, all were identified as pandemic H1N1, except for one which was identified as seasonal H1N1.

Conclusions

Further details on the methodologies, lessons learned, and initial results from the first year of sampling will be discussed. The findings from the preliminary phase of this project have implications for guiding and building capacity for influenza research in low- and middle-income countries.

SPA3 - PANDEMIC AND EPIDEMICS

A303P

Investigations of the seroloprevalence status of Australians against pandemic and seasonal influenza from 2008-11.

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Serological studies complement epidemiological data by detecting symptomatic, asymptomatic and unreported cases as the majority of people seroconvert following infection with influenza virus. Exposure to virus can be detected by haemagglutination inhibition or virus neutralization assays. Four fold rise in titre from acute to convalescent bleeds or titres of 32/40 and above for HI assays on single bleeds are generally used to identify those people that have had an influenza infection or vaccination. Following the emergence of a novel A(H1N1) influenza virus in 2009 (H1N1pdm), serological studies were performed on discarded plasma samples from healthy adult Australian blood donors to ascertain the burden of infection with pandemic influenza. These studies were continued through 2010 and showed a reduction in the seropositive proportion of the population across all collection sites in Australia, except Brisbane, over the 2010 winter.

Since then (October 2010) until April 2011, Australia has experienced an unseasonably large number of laboratory confirmed cases of influenza A cases over the summer-autumn period. This increased activity has been especially pronounced in the northern states of Queensland (eg Brisbane and Townsville) and the Northern Territory (eg Darwin). At present this phenomenon is unexplained, but may be related to variable weather conditions, the consequences of flooding, the emergence of an antigenically distinct strain of influenza or a decline in population immunity. The latter may have arisen from waning antibody levels following exposure or vaccination to H1N1pdm or to seasonal influenza A viruses.

To investigate this observation further, HI assays using H1N1pdm and H3N2 viruses have been performed to determine the population antibody titres to influenza A viruses in late 2010 (November) and early 2011 (April) and compared to 2008/9 data. Discarded plasma samples have been collected from healthy Australian blood donors in two collection sites in northern Australia (Brisbane and Townsville) and a reference collection site in southern Australia (Melbourne) across the age spectrum of the donor population. Antigenic drift in circulating strains of influenza is assessed by virological surveillance. Both currently circulating and older H1N1pdm and H3N2 viruses have been used as a source of antigen. This serological evaluation during a period of unseasonal influenza activity may provide new insights into the influence of host population immunity on influenza strain circulation and dominance.

SPA3 - PANDEMIC AND EPIDEMICS

A304P

Influenza Epidemics in Shenzhen, a Subtropical Chinese City, 2003-2009

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Introduction

A sentinel hospital-based surveillance for influenza-like illness was established in 13 provinces of China in 2000 and subsequently expanded to 30 provinces in 2005. Shenzhen, a subtropical city located in Southern China, established its own influenza surveillance system in 1995 and joined the national surveillance network in 2003. This study describes the epidemiological and virological surveillance data collected in Shenzhen during 2003-2009, with a focus on influenza A/H3N2 in year 2007.

Methods

Influenza-like illness (ILI) was monitored weekly in 29 study sites in the city in 2003-2009, including 7 CDC virology laboratories and 22 sentinel practices located in general hospitals, schools, community health-care centers, poultry farms and live markets. Nasopharyngeal swabs were randomly collected on a daily basis; influenza viruses were isolated by culture, the viral RNA extracted, and the HA1 region of the hemagglutinin was sequenced. For 2007, the year in which the most isolates were available (n = 131), phylogenetic trees were constructed, including 871 global background sequences during 2006-2007 and using the Maximum Likelihood approach implemented in PhyML v.3.412 with 1000 bootstrap replications.

Results

In total, 728,824 ILI cases were recorded during 2003-2010, representing 6.5% of all consultations. Peak ILI activity occurred in the summer, between May and August. Of the ILI cases, 17,197 were randomly selected for virus identification and sequencing, of which 2337 (13.6%) tested positive for influenza virus. Laboratory-confirmed influenza infections were reported throughout the year but also predominated during May-August, with a peak in June or July, consistent with ILI patterns. Influenza B and two subtypes of influenza A co-circulated in the city in 2007: 59.8% A/H3N2, 3.4% A/H1N1, and 36.8% B). The A/H3N2 virus predominated in the first half of 2007, but the B/Yamagata lineage became the dominant strain in July. Phylogenetic analysis of A/H3N2 in 2007 revealed multiple introductions of the influenza virus into Shenzhen during this year, including two major clades that circulated globally. One clade mainly circulated in Shenzhen before April 2007 and was phylogenetically related to viruses isolated in China, Japan, and South Korea. The second clade represented viruses that predominantly circulated between April-August 2007 and were related to viruses that circulated during the winter epidemic of 2006-2007 in Europe, North America, and other parts of Asia.

Conclusion

Influenza displayed a clear seasonal pattern in Shenzhen during 2003-2010, with peak activity occurring in June and July. This pattern differs from that observed in the neighboring city of Hong Kong, which experiences two influenza peaks within the year, with predominant activity in winter months, Jan-Feb. Our results demonstrate the complexity of seasonal influenza patterns in tropical and subtropical regions, and the need to enhance viral surveillance in these areas. Combining epidemiological and phylogenetic analysis may further elucidate the seasonal patterns of influenza virus circulation in Southeast Asia and globally. Additional phylogenetic analyses of influenza sequence data from 2003-2010 are underway.

Abbreviations: Influenza-like illness (ILI)

SPA3 - PANDEMIC AND EPIDEMICS

A305P

Evaluation of a multi-faceted influenza vaccination implementation strategy for health care workers in hospitals

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Introduction Influenza prevention in hospitals by vaccination is thought to reduce transmission to vulnerable patients and sick leave by personnel. Since uptake of the vaccine is low in health care workers (HCW) in the Netherlands we developed a multifaceted intervention program and evaluated its short- and long-term effects over two influenza seasons.

Methods We conducted a randomized controlled trial among the eight University Medical Centers (UMC) in the Netherlands. Prior to the trial in 2008 a questionnaire study was performed to determine the predictors of influenza vaccine uptake among health care workers. Based on the predictors a multi-faceted implementation strategy was developed and implemented in the intervention group. After the 2009/2010 pandemic H1N1 season and the 2010/2011 regular season we evaluated uptake of the vaccine among health care workers. Baseline and outcome data were collected using webbased questionnaires filled out by health care workers of selected risk departments. Absenteeism rates were also collected via the departments of occupational health. Further, we collected data from two selected risk departments (internal medicine and the pediatric ward) of each UMC on the number of flu cases in patients, the duration of hospitalization, admission at the intensive care unit and mortality. A clustered analysis will be performed using a generalized estimation equation model.

First results After the first season, 2255 webbased questionnaires were sent out. 679 HCW (30%) filled out the questionnaire. In all, 74% of both intervention and control UMC participants were vaccinated with the pandemic vaccine. Uptake of the second pandemic vaccine was a little higher, though not statistically significant, in the intervention group than in the control group (63% vs. 57%). Currently, data on vaccine uptake for the second season and clinical outcome data are being collected and will be presented at the congress.

SPA3 - PANDEMIC AND EPIDEMICS

A306P

The Norwegian Influenza Cohort Study: NorFlu

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Background and Aim

The Norwegian Influenza Cohort Study (NorFlu) is a prospective population-based pregnancy cohort study conducted by the Norwegian Institute of Public Health. The study was set up to capture pregnant women during the influenza A H1N1 pandemic of 2009/ 2010, aiming to study pregnancy outcomes, maternal health and childhood development following exposure to the pandemic; including the study of immune responses after infection and/or vaccination.

Study population and data collection:

Participants were recruited from four hospitals in Norway from February 2010 through September 2010, 41.2% of the invited women consented to participate and the cohort included 3228 mothers giving birth to 3278 children. Participants had their last menstrual period (LMP) between June 1st and December 1st 2009. Recruitment was during pregnancy week 28-40. All participants had given birth by September 25th 2010. Non-pregnant controls (327) were recruited to NorFlu among participants in another large pregnancy cohort study in Norway, the MoBa study. Blood samples were obtained from mothers and children (umbilical cord) at birth and from non-pregnant women in October 2010. Maternal samples include DNA, RNA, serum, plasma and peripheral blood mononuclear cells (PBMC). The umbilical cord samples include DNA, RNA and plasma.

Two questionnaires were administered at inclusion, one addressing general health and pregnancy, the other influenza, vaccination, anti-viral medication, symptoms of disease and suspected adverse events following vaccination or medication. Non-pregnant women answered similar questionnaires. Follow-up will be conducted by questionnaires at regular intervals until the child reaches 16 years of age. Pregnancy outcomes are obtained through linkage to the Medical Birth Registry of Norway (MBRN). Linkage to other national health registries may also be performed (ie the Norwegian vaccination registry), and will lay the ground for the following sub-projects:

Planned and ongoing sub-projects

Mjaaland, S., Oftung, F et al: Cellular immunity to pandemic influenza: risk factors for severe infections, and implications for preventive measures.

Hungnes, O., Kilander, A. et al: Prevalence of antibodies to the pandemic influenza virus in mothers and children in the NorFlu study.

Michaelsen, T et al.: IgG and IgG subclass immune response after vaccination and/or infection with pandemic flue H1N1v09.

Tambs, K et al: Influenza during pregnancy and childhood mental development.

Preliminary Results

Overall 14.9% of the women reported having had influenza in pregnancy. 56.5 % of the participating women were vaccinated against H1N1, and the majority among these 83.3 %, were vaccinated while pregnant. Linkage to the MBRN for pregnancy outcomes is still in process. Analyses of antibodies to the pandemic influenza virus is being performed for all maternal samples, and for a sub-sample of the children.

Acknowledgement:

We are grateful to all the participating families in Norway for taking part in this ongoing cohort study.

SPA3 - PANDEMIC AND EPIDEMICS

A307P

Transmission of 2009 pandemic H1N1 in households: a systematic review

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Introduction

During the 20th Century, large community-based household studies provided invaluable data on pandemic and non-pandemic influenza transmission. Household studies allow for the characterization of the transmission of influenza in a well-defined setting, and can provide information on the full spectrum of illness profiles associated with influenza virus infection. We conducted a review on household transmission studies conducted during the 2009 pandemic which report a household secondary attack proportion (SAP) associated with pH1N1, a commonly used measure of transmissibility.

Methods

Using a systematic search strategy we identified 144 titles for screening, of which 34 full length articles were assessed for eligibility and 25 were finally included in the review. To be included, studies must report a SAP or sufficient data to retrieve a SAP based on either virologic confirmation, clinical diagnosis, or self-reported signs and symptoms. Influenza-like illness (ILI) was defined as the presence of fever plus cough or sore throat. SAPs stratified by age group were extracted, as well as data on illness patterns and estimated serial intervals.

Results

Households were surveyed (via telephone, post, or internet) in 60% (15/25) of studies, while home visits were conducted in 36% (9/25) of studies. In 48% (12/25) of studies virologic specimens were collected, and serologic specimens were collected from household contacts in 12% (3/25). In meta-analysis, a combined SAP of virologically confirmed pH1N1 cases from 11 studies was 11.1% [95%CI: 6.3%-17.0%, I^2=95.3%], and in a subset of 8 studies was 20.0% [95%CI: 12.0%-29.4%, I^2=87.7%] in children, and 9.0% [95%CI: 2.8%-18.0%, I2=96.7%] in adults. SAPs for ILI were slightly higher and displayed similar heterogeneity between studies. Point estimates of the mean serial intervals ranged between 2.6-3.9 days. Among virologically confirmed household contacts, cough was the most commonly reported symptom while fever was reported in approximately half of the cases.

Conclusions

Studies consistently found that children appeared to be relatively more susceptible to infection than adults. There was substantial heterogeneity in SAP estimates between studies, potentially associated with study design as well as the timing of the sampling during the epidemic. Some of the studies with highest SAPs were conducted during periods of peak pH1N1 activity when risk of infection from the community would have been highest. Whereas household data conveniently permit objective and comparative assessment of transmission in the unique natural experimental setting, our review demonstrates various pitfalls and suggests a number of useful points for consideration when planning future studies of household transmission for interpandemic or pandemic influenza.

SPA3 - PANDEMIC AND EPIDEMICS

A308P - Pandemic and Epidemics

Surveillance of Severe Acute Respiratory Infection in Belgium through a sentinel network of hospitals

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Aims

The Belgian National Influenza Center (NIC) monitors influenza epidemics through a network of sentinel general practitioners. During the 2010-2011 season, the NIC has extended for the first time the surveillance to other respiratory viruses and to hospitalized cases of severe acute respiratory infections (SARI) with the objectives (1) to provide timely epidemiological and virological data on SARI, (2) to determine the role of the different respiratory viruses in SARI and (3) to characterize virus strains isolated from most severe SARI cases.

Methods

A pilot network of hospitals located in the three regions of the country (n=7) was set up and surveillance was carried out from week 40/2010 to week 18/2011. Pediatric and adult's emergency wards collected both clinical data and nasopharyngeal swabs of patients which corresponded to the SARI case definition and were expected to be admitted to the hospital for 24 hours or more.

Swabs were collected in transport medium and sent to the laboratory by post. Real-time PCRs were used to detect 9 respiratory viruses: Influenza A, Influenza B, RSV-A, RSV-B, human metapneumovirus (hMPV), rhinovirus and parainfluenza virus 1, 2, and 3. When diagnosed, influenza, viruses were submitted for subtyping (A(H1N1), A(H3N2), A(H1N1)2009) for influenza A and determination of the lineage (Yamagata or Victoria) for influenza B. Sequencing was realized for a subset of influenza and RSV samples.

Results

From week 40/2010 to week 18/2011, 551 swabs were collected by the hospital network. Patient age was known for 499 samples which were classified by age group (<5, 5-14, 15-44, 45-64, >65 years old). Most samples were collected from patients below 5 years old (n=211).

Of 499 samples, 246 were positive for at least one virus (49,3%); including 91 influenza (18,2%), 85 RSV (17,0 %), 47 rhinovirus (9,4%), 11 hMPV (2,2%) and 12 parainfluenza virus (2,4%).Fifteen co-infections were detected, mostly with rhinoviruses. Among influenza isolates, 10,4% were type A and 7,8% type B. Subtyping of influenza A revealed that 47 out of 48 (98%) were the pandemic influenza A(H1N1)2009 variant and one was an A(H3N2) influenza virus. Influenza B viruses belonged mainly to the Victoria lineage (27/31) but 4 belonged to the Yamagata lineage. Influenza positive samples increased from week 52/2010 to reach 53% of positivity in week 4/2011. RSV was detected from week 47/2010 to week 14/2011 mainly in children. Rhinovirus was present throughout the whole surveillance period in all age groups but levels of hMPV and parainfluenza viruses remained low.

In the age group < 5 years, RSV was the most detected virus (37%) with 60% of RSV A, while the proportion of influenza was 9.5% only. The highest proportion of influenza positive samples (43%) were in children attending schools (5-14 years old), with a co-circulation of influenza A and B.

Discussion :

The surveillance of SARI has been highly recommended after the 2009 pandemic. This study focused on the surveillance of severe infections caused by respiratory viruses including influenza and confirmed that the SARI case definition is not specific to influenza. Indeed, in this study both RSV and influenza were most detected viruses in samples from SARI patients. Interestingly, influenza B played a significant role in hospitalisations. The Influenza findings are consistent with clinical and virological data collected by the sentinel network of general practitioners.

SPA3 - PANDEMIC AND EPIDEMICS

A309P

Persistence of the influenza A(H1N1) virus pandemic in water and on non-porous surface.

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Influenza A virus survival in different environmental conditions is a key element for the implementation of hygiene and personal protection measures by health authorities. As it is dependent on virus isolates even within the same subtype, we studied the survival of the H1N1 pandemic (H1N1pdm) virus emerging in 2009 and the seasonal A/New Caledonia/20/99 (H1N1) virus strain, in water and on non porous surface. Both viruses were subjected to various environmental parameters over time and tested for infectivity using a microtitre endpoint titration. Viruses were put in water at different temperatures and with different salinity levels, for up to 600 days. Watch glasses were used to mimic smooth surfaces. Genomic RNA concentration was also determined to evaluate the integrity of the viral genome. In water, at medium and low salinity levels and 4°C, H1N1pdm virus survived between 195 days for 35 parts per thousand (ppt) of salt and 1097 days for 0 ppt of salt. However, the A/NewCaledonia/20/99 virus strain survived no more than 40 days at 35 ppt of salt, suggesting that H1N1pdm strain is more stable than seasonal H1N1 virus in liquid environment. Increasing temperature and salinity had a strong negative effect on the survival of both viruses, which remained infectious no more than 1 day at 35°C and 270 ppt of salt. On smooth nonporous surface, the H1N1pdm virus retained its infectivity for at least 7 days at 35°C and up to 66 days at 4°C. The H1N1 viruses have thus the ability to persist in water and on glass surface for extended periods of time, even at 35°C. Additional experiments also suggest that external viral structures in direct contact with the environment are mostly involved in this virus loss of infectivity.

SPA3 - PANDEMIC AND EPIDEMICS

A310P

Epidemiological evidence of the effects of school closures on influenza outbreaks: systematic review

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Introduction

The World Health Organization recommends that school closure be considered as part of a mitigation strategy during an influenza pandemic; this intervention has been used in many settings, including during the 2009 pandemic. However, there has been uncertainty about the effects of school closure on the transmission of influenza.

Materials and methods

We searched Medline and Embase for papers, published before 31 December 2010, which presented data on seasonal or pandemic influenza outbreaks coinciding with periods of school closure (either school holidays or closures in response to the outbreak). Additional papers were identified from the reference lists of the identified articles, handsearching key journals, and from the authors' collections. The epidemic curve and dates of school closure were extracted for each study.

Results

2053 papers were identified through Medline and Embase, of which 46 were eligible for inclusion in the review. A further 13 papers were identified from other sources, resulting in 59 studies being included in the review: 23 for seasonal and 36 for pandemic influenza (5 from the 1918 pandemic, 1 from 1968, and 30 from 2009). Studies were available from Europe (15), Asia (17), Australasia (6) and North (16), South (2) and Central (3) America. Data most frequently referred to schoolchildren or to the general population; data specifically on other age groups were available less often. School closure was frequently followed by a decline in influenza incidence, but it was often unclear how much the intervention contributed to this. This was largely because schools were often closed late in the outbreak (e.g. after the peak) but also in some cases because of changing levels of ascertainment during the outbreak. However, the effect was sometimes reversed when schools reopened, supporting a causal role for school closure in reducing incidence. Heterogeneity between studies made it difficult to determine how specific factors (e.g. the timing and duration of closure) influence the effectiveness of the intervention.

Conclusions

School closures appear to have the potential to reduce influenza transmission, but there is limited evidence available to define specific determinants of the effects of school closure. Consequently, the optimum school closure strategy (e.g. the ideal length of school closure) remains unclear. Quantifying the effects of school closures on transmission could be improved if future epidemiological studies used active ascertainment and consistent case definitions throughout the study period, and assessed age-specific incidence in other age groups besides schoolchildren. Comparisons between studies may then help to identify features which influence the effectiveness of school closures.

SPA3 - PANDEMIC AND EPIDEMICS

A311P

1957 and 1968 pandemics: World Health Organization influenza program and surveillance network response and post-pandemic improvements

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The aim of this poster it to analyze the responses given by the World Health Organization influenza program and the influenza surveillance network to public health crisis caused by the 1957 and 1968 influenza pandemics.
The WHO influenza program was established in 1947 while the network of laboratories has been functioning since 1952. Concerns about high mortality associated to the 1918 influenza pandemic, the unpredictable variability of influenza viruses, the lack of knowledge about the control of the disease, and the recently developed influenza vaccines encouraged the creation of a program dedicated to the study and control of influenza.
The 1957 and 1968 pandemics provided a unique opportunity for the study of influenza given that new concepts and tools developed by virologists and epidemiologists could be put in practice.
The theoretical approach used in the construction of this poster can be inscribed in the history of public health. This approach emphasizes different measures taken for the control and prevention of epidemics. Literature review and documents published by the WHO constitute the sources for writing this paper.

The analysis is divided in three parts:

The first part describes the organization of the WHO influenza program and the surveillance network at the time of the pandemics, its structure and its composition, the group of experts working on influenza and the international conferences held after the pandemics.
The second part analyses actions followed by the WHO influenza program and the worldwide network of laboratories during the 1957 and 1968 influenza outbreaks. Three main issues concerning the identification of the new virus strains, the surveillance and the evolution of the pandemics, and the production of influenza vaccines are considered.
The third part explores the post-pandemic improvements related to epidemiological surveillance, laboratory techniques, basic and applied research on influenza, the revision of the system of nomenclature for influenza viruses, influenza vaccines research and development, and animal influenza virus surveillance.
In 1957, for the first time during a pandemic, applying concepts of modern virology, virus strains were isolated and recognized as a new variant of A type. Laboratories all over the world followed the progress of both pandemics, even if epidemics originated in a region not covered by the program. It was regarded that a better influenza surveillance required the expansion of the network, the improvement of the quality of the epidemiological information and the use of simpler and more accurate diagnostic methods.
Experiences of these pandemics confirmed the need for conducting further research on influenza. Following the 1968 pandemics, the concepts of classification and the earlier antigenic changes within the human influenza A viruses were re-examined. The description of hemagglutinin and neuraminidase antigens was included in the nomenclature of influenza virus.
In 1957, difficulties in the massive production of vaccines restricted their application to selected occupational groups. In 1968, even if problems in production persisted, influenza vaccines began to be considered as a public health measure for the entire population. Vaccine production jumped from artisanal to industrial large-scale form. Since then, significant progress in manufacturing methods has been developed. As influenza vaccines effectiveness needed to be improved, pandemics constituted unique opportunities for large-scale trials of influenza vaccines.
The 1957 and 1968 epidemics represent a definitive conceptual and practical rupture concerning the knowledge of influenza virus and the control of the disease. Henceforth, it was recognized that pandemics could be controlled and prevented by intensive surveillance and by the development of effective vaccines. From these events on, global surveillance and the production of great amounts of vaccine in a short period of time has constituted a major challenge for public health response to influenza epidemics.

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Epidemiological characteristics of laboratory confirmed fatal influenza cases during the 2009-2010 and 2010-2011 influenza seasons in Georgia

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Aim To describe the epidemiological characteristics of laboratory confirmed fatal influenza cases occurred during two influenza seasons in Georgia.

Methods All laboratory confirmed influenza associated fatal cases registered during the 40th to 17th weeks of 2009-2010 and 2010-2011 seasons were assessed for epidemiological and clinical characteristics. Influenza virus infection was confirmed by real time RT-PCR. The patients' data were collected from hospital medical records.

Results During the 2009-2010 influenza season 33 fatal cases were related to pandemic A(pH1N1) virus. Median age of the lethal cases was 32 year (range 14-70). 51% versus 40% of fatal outcomes occurred in 30 to 64 compared to 15 to 29 year age groups. No single death was confirmed in children aged < 5 years. From all lethal cases 16 (48.5%) suffered with underlying conditions and 8 (24.2%) were pregnant women. The leading pre-existing conditions were diabetes and chronic viral hepatitis; other co-morbidities included asthma, tuberculosis, anemia, chronic renal disease and etc. In 17 % of the lethal cases antiviral medications were not received and in 65% their administration was delayed.

In 2010-2011 season, 33 out of the total 53 laboratory confirmed influenza deaths, 33 were associated with pandemic influenza A(pH1N1), 19 with influenza B and one with both viruses. The median age of fatal cases attributed to pandemic influenza A(pH1N1) was 45.5 (range 2-85) compared to 73 (range 31-95) in case of influenza B. Among fatal cases associated with pandemic influenza A(pH1N1) the highest mortality (62%) was observed in 30-64 year age group while for influenza B the peak death rate (65%) was identified in patients above > 65 years. Underlying medical conditions were reported in 27 (79%) and 18 (90%) of deaths related to pandemic influenza and influenza B respectively. Diabetes, cardiovascular diseases and obesity were dominant pre-existing conditions. In addition, 3 fatal outcomes occurred in pregnant women: 2 associated with pandemic influenza A(pH1N1) and 1 with influenza B. Majority of the lethal cases (79%) were treated with antivirals, but none had received them within the first 48 hours of illness.

None of the fatal cases were vaccinated against pandemic influenza virus in both seasons.

Conclusions Mortality data of the two influenza seasons demonstrate that relatively young adults were at risk of pandemic influenza A(pH1N1) associated death compared to seasonal influenza B with most fatal cases observed in elderly population. Although the minority of the lethal cases occurred in previously healthy people, persons with underlying medical conditions and pregnant women represent increased risk groups and their vaccination should be a public health priority. Early antiviral treatment will contribute to reduction of lethal outcomes.

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A seasonal influenza vaccine booster after priming with whole virus cell-derived H1N1 pandemic influenza vaccine is highly immunogenic in children

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This Phase 1/2 open-label, randomized clinical study investigated the safety and immunogenicity of a whole virus Vero cell-derived H1N1 pandemic influenza vaccine (strain A/H1N1/California/07/2009) in a total of 340 healthy infants, children and adolescents aged 6 months to 17 years. Subjects were stratified into 4 age strata (A: 9-17 years, B: 3–8 years, C: 12–35 months, D: 6–11 months) and randomized (1:1) to receive two vaccinations of either the 3.75µg or 7.5µg vaccine dose at a 21 day interval. A booster vaccination with a seasonal trivalent virosomal influenza vaccine for the season 2010/2011 was administered one year after the first vaccination to approximately 30 subjects who had previously received the 7.5µg dose in each of the four age groups.

CHMP defined seroprotection criteria determined by HI assay were met 21 days after the second vaccination in subjects aged 12-35 months, 3-8 years and 9-17 years in both the 7.5 µg and 3.75 µg dose groups and with the licensed 7.5 µg dose in infants aged 6–11 months. Seroconversion rates met the CHMP criterion (>40%) after both the first and second vaccination with the 7.5 µg dose in the 9-17 years (72% and 98.0%) and 3-8 years age groups (48% and 96.1%), and after the second vaccination in young children aged 12-35 months (91.8%) and infants 6-11 months (78.9%). GMT increases were: 6.7 and 14.2 in the 9-17 years olds, 3.2 and 15.6 in the 3-8 year olds, 2.9 and 16.0 in young children aged 12-35 months and 2.3 and 11.1 in the 6-11 months age group after the first and second vaccination, respectively. MN and SRH assay results confirmed the immunogenicity of the vaccine in the investigated age groups. High seroprotection rates were maintained through 6 and 12 months after primary vaccination. The booster vaccination with the seasonal trivalent influenza vaccine induced a strong booster response to the A/California/07/2009 strain, reaching 100% seroprotection and high levels of antibodies in all four age strata as shown in HI, MN and SRH assays.

Safety data suggest that the whole virus Vero cell-derived vaccine is well tolerated in all four age groups including infants and young children. No dose effect was observed and no vaccine related SAEs were reported. The booster vaccination was also well tolerated.

In summary, these data confirm the suitability of Baxter's H1N1 whole virus cell-derived pandemic influenza vaccine for use in the pediatric population showing that a 2-dose primary vaccination schedule induces a memory response in an essentially naïve population that can be effectively boosted with the A/H1N1/California/07/2009 component of a seasonal virosomal influenza vaccine.

YOUNG SCIENTIST

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A314P

Estimating Age-Specific Global Infection Rates for the 2009 Influenza Pandemic Influenza: a Meta-Analysis of H1N1pdm Serological Studies

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The true global impact of the 2009 influenza pandemic (H1N1pdm) is not well understood. Here we estimate age-specific cross-reactive antibodies to H1N1pdm virus and rates of infection of H1N1pdm during the first year of the pandemic using data from published and unpublished sero-epidemiologic studies of H1N1pdm.

Upon request, investigators provided sero-positivity results stratified in harmonized age groups (0-4, 5-19, 20-44, 45-64, and ≥65 years old). Studies were evaluated based on when sera was collected. Regardless of study design, all sera collected prior to April 2009 were deemed prepandemic sera for which baseline age-specific cross reactive antibodies to the H1N1pdm virus were estimated. Studies which included sera that were collected prior to widespread community transmission were evaluated separately as prepandemic sera to calculate age-specific rates of cross-reactive antibodies and compared to pre-pandemic sera collected prior to April 2009. H1N1pdm infection rates were estimated for studies in which sera was collected at two time periods: prior to April 2009 and after the pandemic wave(s) were over. Finally, age-specific H1N1pdm seropositivity was calculated among sera collected during time periods which coincided with a decline in H1N1pdm transmission or when transmission was clearly over within the country

Twenty-seven published and unpublished studies from 20 countries were eligible for inclusion and provided data for this study. The overall pooled pre-pandemic prevalence of cross-reactive antibodies varied significantly by age with the highest age-specific rates found among persons ≥65 years old. Similarly, the overall prevalence of post-pandemic H1N1 antibodies varied significantly by age.

Despite limitations of serological evidence and geographic representativeness, the systematic review study offers an insight of the impact of the 2009 influenza pandemic in its first year.

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A315P

Cost savings of utilizing celvapan H1N1 as a pandemic flu prevention strategy in Sweden

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Introduction

CELVAPAN (Influenza vaccine (H1N1)v(whole virion, Vero cell derived, inactivated)) is a pandemic influenza vaccine containing the inactivated pandemic influenza virus H1N1. It has been shown to be safe and effective, and two doses are given by injection at least three weeks apart. CELVAPAN is manufactured using Vero cell technology, which allows for faster pandemic vaccine production compared to traditional egg-based manufacturing. A prospective study conducted in Jersey, Channel Islands, examined the efficacy of CELVAPAN in a pediatric and adult population. OBJECTIVE: Using the Jersey efficacy data as a baseline, we examined the potential economic benefit of adopting CELVAPAN as compared to an egg-based vaccine in a representative country such as Sweden using economic modeling methods.

Materials And Methods

An Excel-based model was developed to document the benefits of utilizing CELVAPAN with respect to total additional prevented cases and the inpatient-, primary care-, and productivity-related cost-savings associated with these prevented cases. Literature-based costs associated with hospitalizations and physician visits in Sweden were used in the model. Published average wage data in Sweden were used to calculate productivity costs.

Results

When compared to adopting an egg-based strategy, CELVAPAN can prevent an additional 5476 cases of pandemic influenza if solely used as a pandemic strategy in Sweden. These prevented cases translate to a cost-saving of 197,123 SEK (Swedish krona) (US$31,239) in inpatient costs, 10,951,294 SEK (US$1,735,549) in primary care costs, and 5,475,647 SEK (US$867,772) in overall societal costs (lost productivity).

Conclusions

The benefit of faster production time for Vero cell derived vaccines such as CELVAPAN may translate to additional cases prevented and overall cost-savings if utilized as the primary pandemic flu strategy for Sweden.

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A316P

A case-control study on influenza A(H1N1)2009 infection in the First Few 100 cases and close contacts: results and lessons learned from the Netherlands

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Introduction Rapid collection of detailed data of the first few hundred (FF100) cases and their close contacts (as exposed but healthy controls) was included in the comprehensive assessment of the Dutch pandemic preparedness plan. This enabled us to perform a case-control study, assessing patient characteristics and risk factors for experiencing a symptomatic influenza A(H1N1)2009 infection and providing insight into transmission. We set out to evaluate the FF100 approach in terms of the feasibility during the 2009 pandemic and the added value compared with alternative data sources available.

Material & methods

Cases and contacts were recruited using the national mandatory notification system and the Dutch sentinel influenza surveillance system. Contacts were considered as controls matched for exposure to influenza A(H1N1) 2009 virus. Virological and serological sampling was scheduled at days 0, 5, 10 and 30. We assumed that persisting high titers in first and following blood samples of controls indicated exposition to the same source as the case, and that seroconversion indicated secondary transmission. Both cases and controls were asked to complete a questionnaire, extracting information about demographics, medical history, and exposure to influenza A(H1N1) 2009 virus. We assessed to what extent timely and novel data were generated compared to other available data sources.

Results

In May-December 2009, a total of 68 cases and 48 controls were included in the study. Underlying non-respiratory diseases were significantly more common among cases compared to controls (OR_{adj}=9.7; 95%CI: 1.6-57.9), while a protective effect was found for frequent (>8 times/day) hand washing (OR_{adj}=0.4; 95%CI: 0.2-0.9). Seroconversion was found for 7/30 controls (23%), and persisting high titers for 4/30 controls (13%). The labour-intensive study design resulted in slow and limited recruitments of cases and contacts.

Conclusions

The findings of an increased risk for symptomatic infection in people with underlying non-respiratory disease, a protective effect of frequent hand washing and a secondary transmission rate of approximately 20% gave new insights in transmission risks and possible interventions for improved control. While our design resulted in novel findings, the FF100 approach lacked timeliness and power due to slow and limited recruitment. For future pandemics we suggest to pool data from several countries, to enable collecting sufficient data in a relatively short period.

The members of the Dutch ZonMW influenza A(H1N1) 2009 consortium are:

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Introduction Rapid collection of detailed data of the first few hundred (FF100) cases and their close contacts (as exposed but healthy controls) was included in the comprehensive assessment of the Dutch pandemic preparedness plan. This enabled us to perform a case-control study, assessing patient characteristics and risk factors for experiencing a symptomatic influenza A(H1N1)2009 infection and providing insight into transmission. We set out to evaluate the FF100 approach in terms of the feasibility during the 2009 pandemic and the added value compared with alternative data sources available.

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vaccinating children to help manage the pandemic influenza

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Introduction

Jersey is a small island with a population of 92,000 (including 18,000 children) with close links to the UK and Europe. It has a 230 bedded acute hospital and a small ITU.

Early arrival of A/H1N1 with a rapid single wave outbreak overwhelming medical facilities in the face of a high occupancy of medical facilities in the UK was considered a serious threat requiring mitigation.

Methods

We used enhanced containment (public hygiene, antiviral treatment of probable/confirmed cases and close contacts, a 48 hr voluntary exclusion from schools of children returning from abroad) pending rapid deployment of vaccine (once available) focusing on school children (super-spreaders) in addition to those in the classical risk groups and health care workers. School children were vaccinated over 6 working days as flu activity was on the ascent. Activity was monitored by screening all influenza like illness for A(H1N1)v.

Results

In total there were 706 confirmed cases - 673 of these occurring from the first local confirmed case to 9 days after vaccination of children. Vaccine uptake was 82% in children 6 months to 18 years. Vaccine efficacy in children was 100% (95% C.I. 70.7-100%) (1). The proportion of cases prevented in the general population is estimated to range from 17.4-28.3% (1)

Conclusion

The results suggest that the rapid deployment of flu vaccine in children has an important role in the management of influenza outbreaks even when used whilst activity is escalating. The results also suggest that preemptive vaccination of this group would modulate outbreaks in the general population.

1 Rolland M, Haeberer M,Seyler T. Impact of the pandemic influenza vaccinationcampaign in Jersey 2009-2010 ESCAIDE, Lisbon

SPA3 - PANDEMIC AND EPIDEMICS

A318P

Comparison between the age specific clinical impact of pandemic and seasonal influenza viruses in terms of hospitalisation and mortality

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Introduction

Over the last 100 years we have seen four influenza pandemics, each of which has a distinct age profile in its epidemiology. The pandemic H1N1 in 2009 was no exception. The reasons for these specific signatures and how they compare to seasonal disease is important for human health and disease prevention. This study looks at past pandemics and uses recent data to explore this age specific pattern and compares this with the impact of seasonal disease.

Methods

Historical data, derived from national mortality statistics for past pandemics of the last 130 years, is compared with Pandemic H1N1 data on mortality and hospitalisation collected prospectively in 2009 and 2010. This contemporary data is compared with that derived from a case-control study carried out in Wales in 1999/2000, the last time seasonal Influenza caused significant disease in the UK. The data was used to explore the age based differences between individual pandemics and how these compare to seasonal disease. Conclusions will be drawn in relation to prior population exposure and antibody prevalence.

Results

The results demonstrate how each pandemics has its own distinct demographic signature, but the overall trend, in attack rate and sequelae, is towards the young and those with no prior experience to similar H and N antigens.

Conclusion

This study sheds new light on the reasons for some of the specific mortality curves of previous pandemics and highlights, not only what is known about the relationship between population antibody prevalence and disease, but also what we still have left to learn.

Conflict of interest:

Advisory board:: I have acted in an advisory capacity for Sanofi Pasteur MSD and Roche from time to time in the past;

SPB3 - VIRUS STRUCTURE & REPLICATION

B301P

D225G mutation in haemagglutinin of pandemic Influenza H1N1 (2009) virus enhances virulence in vitro and in vivo

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Aims

Though the majority of infections by the pandemic influenza H1N1 (2009) virus is mild, a higher mortality occurs in young adults with no risk factors for complications. Some of these severe cases were infected by virus with an aspartate to glycine substitution at 225 position (D225G, H3 numbering) in the haemagglutinin (HA). Previous studies with the highly virulent 1918 pandemic H1N1 virus suggested that such substitution was associated with a dual binding specificity of virus for both a2,3 and a2,6 linked sialic acid receptors on host cells. This study aimed to investigate whether the D225G mutant may cause more severe disease with its increased predilection for the lower respiratory tract, where the a2,3 sialic acid receptor is more prevalent.

Methods

We obtained a mutant virus after four sequential passages in lungs of BALB/c mice with a wild type pandemic influenza A H1N1 (2009) virus. One plaque purified mutant virus had a single non-synonymous D225G mutation in the HA gene. We tested virulence of this mutant in vitro and in vivo.

Results

This mutant was more toxic to chick embryo and produced a viral load of about two log higher than that of the wild type parental virus during the first 24 hours. Pathogenicity test showed that the mean 50% lethal dose in mice (MLD50) was reduced from over 2×10^6 plaque forming units (PFU) with the parental virus to just 150 PFU with the mutant virus. The survival of mice challenged with the mutant virus was significantly decreased when compared with the parental virus ($P<0.0001$). Significantly higher viral titres and elevated proinflammatory cytokines in lung homogenates of mice infected with the mutant virus were found, which were compatible with severe histopathological changes of pneumonitis. The only consistent mutation in the genomes of viral clones obtained from dying mice was D225G substitution.

Conclusion

This is the first study that corroborated the clinical findings that a single D225G mutation in haemagglutinin of the pandemic H1N1 (2009) virus is sufficient to confer adaptation and enhances virulence in chick embryo and mice.

SPB3 - VIRUS STRUCTURE & REPLICATION

B302P

The different functions of PI3K during influenza virus replication

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Aim

Upon influenza A virus infection (IAV) a variety of signalling cascades are activated that are primarily induced by the cell to mount an antiviral response, however, are in part also exploited by the virus to support its replication. Recently, the phosphatidylinositol-3-kinase (PI3K) could be added to a growing list of seemingly antiviral-acting cellular factors that are misused by the virus to support its propagation. To elucidate the role of PI3K in IAV-infected cells we focussed on the diverse regulatory mechanisms and functions of this kinase.

Methods

The methods used include infection and stimulation experiments in cell-culture, siRNA-based knock-down experiments, immunofluorescence- and western-blot based IAV entry assay, creation and analysis of recombinant influenza viruses in *in vitro* and *in vivo* experiments, etc.

Results

During IAV infection the cellular PI3K is activated by diverse mechanisms at different time points during the replication cycle. Depending on the time and mode of activation the kinase fulfils different functions in the infected cells. While PI3K was actually shown to induce antiviral activity via the activation of the interferon-regulatory factor 3, recent results further revealed virus supportive functions of the kinase. We could show that an immediate and transient activation of PI3K occurs during virus attachment and is required for efficient virus uptake. Here we identified receptor tyrosine kinases, such as the epidermal growth factor receptor as mediators of IAV induced activation of PI3K. At later time points of the replication cycle PI3K is again activated, however in a more sustained fashion dependent on the expression of the viral non-structural NS1 protein. This activity seems to inhibit premature apoptosis. It was demonstrated by us and others that PI3K activation occurs upon direct interaction of the NS1 protein to the regulatory subunits of PI3K p85 alpha and beta. Several reports proposed that two *src homology* (SH)-binding motifs within NS1 (aa89 [YXXXM] and aa164-167 [PXXP]) may mediate binding to p85 beta. Our work confirmed this observation. Further, mutant viruses of the NS1(Y89F) showed differences to wild-type viruses with regard to their replication fitness. More detailed analysis suggested that besides expression of the NS1 there are alternative virus-induced mechanisms to activate PI3K. Here we demonstrate that this additional inducer is viral RNA (vRNA), which accumulates during infection. Furthermore, novelties of IAV induced PI3K functions *in vitro* and *in vivo* will be presented.

Conclusion

The PI3K emerged as a seemingly antiviral acting cellular factor that is misused by IAV at different times of infection regulating diverse mechanisms to ensure efficient virus replication. Therefore, PI3K may is a potential cellular target for antiviral interventions against IAV infections.

No conflict of interest

YOUNG SCIENTIST

SPB3 - VIRUS STRUCTURE & REPLICATION

B303P

Structural and functional characterisation of an influenza virus RNA polymerase-genomic RNA complex

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The replication and transcription of influenza A virus are carried out by ribonucleoproteins (RNPs) containing each genomic RNA segment associated with nucleoprotein monomers and the heterotrimeric polymerase complex. These RNPs are responsible for virus transcription and replication in the infected cell nucleus. Here we have expressed, purified, and analyzed, structurally and functionally, for the first time, polymerase-RNA template complexes obtained after replication *in vivo*. These complexes were generated by the cotransfection of plasmids expressing the polymerase subunits and a genomic plasmid expressing a minimal template of positive or negative polarity. Their generation *in vivo* was strictly dependent on the polymerase activity; they contained mainly negativepolarity viral RNA (vRNA) and could transcribe and replicate *in vitro*. The three-dimensional structure of the monomeric polymerase-vRNA complexes was similar to that of the RNP-associated polymerase and distinct from that of the polymerase devoid of template. These results suggest that the interaction with the template is sufficient to induce a significant conformation switch in the polymerase complex.

SPB3 - VIRUS STRUCTURE & REPLICATION

B304P

MDCK cell line with inducible allele B NS1 expression propagates delNS1 influenza virus to high titres.

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Influenza A virus lacking the non-structural NS1 gene (delNS1) are considered for use as live attenuated vaccines in both human and animals. Furthermore, vaccines based on such viruses allow to serologically differentiate between naturally infected and vaccinated-only animals, which improves the surveillance of outbreaks of avian influenza among poultry. However, due to their low virus replication efficiency, commercial delNS1 vaccine production in embyonated eggs or cell culture is constrained. The aim of our work was to develop a cell substrate, based on the Madin-Darby canine kidney (MDCK) cell line, that recombinantly expresses NS1 to complement the absence of this protein during infection with delNS1 virus. Since previous studies showed that transient NS1 expression induces apoptosis, we used an inducible expression system based on the prokaryotic Tet repressor protein.

Two MDCK Tet-on Advanced cell lines were obtained that showed inducible production of the allele B NS1 protein, originating from the avian influenza strain A/turkey/Wisconsin/68 (H5N9). Upon induction, both cell lines expressed the NS1 protein to about 1000-fold lower levels than influenza virus-infected MDCK cells, as revealed by Western blot analysis. For use as model virus in this study we developed a delNS1 virus using a background of A/Puerto Rico/8/34 (H1N1), in which the gene encoding NS1, but not the gene encoding NEP was removed from the 8th RNA segment. The HA and NA originated from A/turkey/Turkey/1/05 (H5N1) and the polybasic cleavage site was removed from HA. Despite the reduced NS1 expression levels, induction of NS1 expression in these MDCK cell lines increased delNS1 virus titres to high levels, comparable to those obtained with an isogenic virus strain containing an intact NS1 gene. The infectious virus titres increased 244 to 544-fold.

NS1 facilitates virus replication in many different ways; most importantly by inhibition of the type I interferon (IFN) mediated antiviral response of the cell. NS1 also limits processing and export of cellular mRNA, regulates viral genome replication and transcription and nuclear ribonucleoprotein export. Furthermore, NS1 is involved in both the induction and inhibition of apoptosis. Contrary to previous findings, recombinant NS1 expression did not induce apoptosis nor did it affect cellular growth rate. Moreover, NS1 production did not significantly inhibit virus-induces IFN-beta production. This discrepancy with previous findings could be due to use of allele B NS1 since most previous studies were done using allele A NS1.

The low amount of recombinant NS1 produced is sufficient to restore delNS1 virus replication in both MDCK cell lines. Since IFN induction was not inhibited, it appears that the low yield of delNS1 virus in normal MDCK cells could be due to the loss of another NS1 function, such as regulation of viral genome replication and transcription. The results of this study show new insights into the actual NS1 function that is limiting replication of delNS1 virus and may help in development of a commercially feasible delNS1 virus vaccine production process.

SPB3 - VIRUS STRUCTURE & REPLICATION

B305P

Does segment 8 of influenza a virus encode a negative-strand polypeptide?

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The segmented, single-stranded RNA genome of influenza A virus is packaged into virions as negative sense viral RNA (vRNA). To date, all twelve confirmed influenza A proteins are encoded in the positive sense and translated from mRNA transcribed from vRNA. A hypothetical open reading frame (ORF) exists on segment 8 of the influenza A genome in the opposite sense to, yet overlapping with, the established NS1 and NEP proteins expressed from that segment. Accordingly, the putative polypeptide that may be expressed is termed the negative strand protein (NSP). Previous bioinformatics analyses have identified multiple potential variants of NSP, differentiated by ORF length, and have also suggested a correlation between NSP length and host range, with avian forms of influenza containing a severely truncated form.

Using a reverse genetics system for A/PR/8/34 (PR8) influenza A strain, we generated mutant viruses containing short (15kDa), intermediate (18kDa; as in the WT virus) and long (24kDa) forms of PR8 NSP, as well as one with early stop codons that severely truncate the ORF as found in avian viruses. All viruses replicated to similar titres in MDCK cells and produced similarly-sized plaques. The synthesis and distribution of the major viral structural proteins as well as the plus-strand segment 8 polypeptides NS1 and NEP were also not affected by alterations to the NSP ORF. NSP produced by *in vitro* translation was successfully immunoprecipitated by an NSP-specific antibody. However, no specific product could be immunoprecipitated from virus-infected cells, or detected by western blotting. We conclude that if NSP is indeed expressed by influenza A virus, it is not significant for virus replication in vitro and nor is it expressed to high levels.

No conflict of interest

SPB3 - VIRUS STRUCTURE & REPLICATION

B306P

influenza a virus ns1 protein is required for efficient segment 7 mrna nuclear export

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The RNA genome of influenza A virus is replicated and transcribed in the cell nucleus, necessitating nuclear export of viral vRNA and mRNA, respectively. Although influenza vRNA export is well studied less is known about how the viral mRNAs are exported from the nucleus. The NXF1-dependent mRNA export pathway has been shown to be involved in the export of some segments but how the viral mRNAs are recruited to this pathway is unknown.

Using a minireplicon transfection system to recreate viral RNPs with single vRNA templates, the requirements for segment 4 and 7 mRNA export were studied. Fluorescence *in situ* hybridization (FISH) showed that under these circumstances, segment 4 mRNA accumulated in the cytoplasm, as seen in virus infected cells. However, segment 7 mRNA was largely retained in the nucleus, suggesting an additional viral protein was needed for its export. Addition of further viral proteins identified this as NS1 and analysis of NS1 mutants showed that intact RNA-binding and effector domains were required for export activity. Use of an intron-specific probe confirmed that NS1 promoted efficient export of the unspliced M1 transcript. Nuclear export of segment 7 mRNA was also found to be defective in cells infected with NS1 mutant viruses. Protein analyses showed a reduction in both M1 and M2 protein expression in cells infected with the NS1 mutant viruses, but mRNA analysis did not show significant differences in the proportion of spliced segment 7 mRNA species, suggesting that export is independent of splicing.

We conclude that NS1 is required for efficient export of M1 mRNA, potentially by acting as an adaptor protein between the viral RNA synthesis machinery and cellular export pathway.

SPB3 - VIRUS STRUCTURE & REPLICATION

B307P

Functional and evolutionary importance of high-order RNA structures encoded in the influenza virus genome

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Secondary and tertiary RNA structures play fundamental roles in the replication of RNA viruses. In addition to the protein-coding properties, RNA genomes code for the RNA structures that regulate various processes such as gene expression, RNA replication, processing, translation and packaging into virions. Such structures are conserved during virus evolution, being an important constraint in genome variability, but may also evolve to produce diverse motifs. The influenza A virus genome consists of eight negative-sense RNA segments. While the pivotal role of the base-pairing interactions between the 5'- and 3'-termini of segments ("panhandle" structures) in replication, transcription and RNA packaging is well-established, less is known about the possible structures in other genome regions.

As a first step to search for these conformations we scanned available sequences for strongly conserved secondary structure elements. This bioinformatic search was conducted in a stepwise way, starting from structure predictions for representative sequences followed by extensive sequence and structure comparisons over available sequences from various virus strains. A number of conserved structures were revealed in several segments, including their coding regions.

Existence of some of the suggested structures is supported by nucleotide covariations, which also indicate a functional significance of the foldings. Interestingly, some base pairs and structures seem to be specific for certain hosts and/or virus subtypes. Apparently, these structures may also undergo lineage-specific conformational transitions (for instance, a single substitution can change a pseudoknot/hairpin equilibrium in the NS segment mRNA). The structures are further supported by experimental data such as in vitro probing, NMR spectroscopy and mutagenesis. In particular, the probing of model oligonucleotides mimicking the NS RNA structures validates the proposed equilibrium and its shift in the mutated sequences. Furthermore, preliminary reverse genetics experiments on the most stable and conserved structures in segments 7 and 8, with destabilized structures versus restored folding in compensatory double mutants, show that the structures are functional for the virus. The presented examples confirm that influenza virus RNA folding is an important factor in virus replication and evolution.

SPB3 - VIRUS STRUCTURE & REPLICATION

B308P - Virus structure & replication

Lethal combinations of packaging signal mutations suggest a genome assembly checkpoint during influenza a virus morphogenesis

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Influenza A virions generally incorporate eight separate ribonucleoprotein complexes (RNPs) containing one copy of each of the genomic segments and this is thought to be achieved through the operation of *cis*-acting segment-specific packaging signals contained in the terminal regions of the viral RNAs (vRNA). The mechanism by which these signals function is uncertain, but studies have shown that their disruption can lead to drops in virus replication of ~ 10 – 1000 fold.

We performed a systematic analysis of pairwise combinations of packaging mutations in different segments and, surprisingly, we found that most, but not all double mutants exhibited a synergistic reduction of over 10,000-fold in virus growth, with certain combinations resulting in a lethal phenotype. Since purely random packaging of any eight RNPs would be predicted to produce around 1 in 400 infectious particles, the extent of the defect observed suggests that this mechanism is not available as a fall-back option after disruption of specific packaging, and that therefore a checkpoint likely exists to prevent assembly of virions with grossly defective genomes.

To test these hypotheses, we are currently studying several highly attenuated viruses containing lesions to packaging signals of different segments. Analysis of double mutant-infected cells has shown that they were able to express abundant levels of viral protein and vRNA. Further examination has suggested that the block to efficient virus replication occurs after nuclear export of the genome but prior to membrane scission during virus budding. Moreover, it has been possible to isolate double mutant pseudo-revertant viruses from serial passage that maintained the initial lesions in packaging regions but recovered growth properties. Interestingly, none of the suppressor mutations were located in the previously identified packaging signals. Further analysis will help us understand how influenza A virus can overcome disruption of specific packaging mechanism.

SPB3 - VIRUS STRUCTURE & REPLICATION

B310P

Dissection of the influenza A virus endocytic routes reveals macropinocytosis as an alternative entry pathway

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Influenza A virus (IAV) enters host cells upon binding of its hemagglutinin glycoprotein to sialylated host cell receptors. Whereas dynamin-dependent, clathrin-mediated endocytosis is generally considered as the IAV infection pathway, some observations suggest the occurrence of an as yet uncharacterized alternative entry route. By manipulating entry parameters we established experimental conditions that allow the separate analysis of dynamin-dependent and -independent entry of IAV. Whereas entry of IAV in phosphate-buffered saline could be completely inhibited by dynasore, a specific inhibitor of dynamin, a dynasore-insensitive entry pathway became functional in the presence of fetal calf serum. This finding was confirmed with the use of small interfering RNAs targeting dynamin-2. In the presence of serum, both IAV entry pathways were operational. Under these conditions entry could be fully blocked by combined treatment with dynasore and the amiloride derivative EIPA, the hallmark inhibitor of macropinocytosis, whereas either drug alone had no effect. The sensitivity of the dynamin-independent entry pathway to inhibitors or dominant-negative mutants affecting actomyosin dynamics as well as to a number of specific inhibitors of growth factor receptor tyrosine kinases and downstream effectors thereof all point to the involvement of macropinocytosis in IAV entry. Consistently, IAV particles and soluble FITC-dextran were shown to co-localize in cells in the same vesicles. Thus, in addition to the classical dynamin-dependent, clathrin-mediated endocytosis pathway, IAV enters host cells by a dynamin-independent route that has all the characteristics of macropinocytosis.

SPB3 - VIRUS STRUCTURE & REPLICATION

B311P

Stabilization of the influenza virus a h1n1 hemagglutinin by in silico mutagenesis

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Introduction

Hemagglutinin (HA) is the receptor-binding and membrane fusion glycoprotein of influenza viruses, and is also the target for neutralizing antibodies. As any protein, the stability of HA is based on covalent and non-covalent interactions. Because the position of the residues that form sulfur bridges is conserved, the variation in stability between diverse HA is related with non-covalent interactions between monomers. The HA of the pandemic influenza virus 2009 presents structural instability, thus restraining its application as purified protein. For that reason, in this work we introduced, *in silico*, punctual amino acid mutations in the stem of the HA to increase the protein's stability.

Materials & methods

HA amino acid sequences from diverse A (H1N1) influenza viruses, were obtained from the *Influenza Virus Resource* of the NCBI. Multiple sequence alignments were carried out with Clustal X 2.0.11 and MAFFT v6; the consensus sequence was created with BioEdit 7.0.0. Mutations and structural analysis were performed with VMD 1.8.7. Molecular dynamics simulations were done with NAMD 2.7.

Results

Analysis of the multiple sequence alignments showed eight mutations along the stem portion of the HA that may cause new favorable interactions intra- or inter-monomer. To further study their effect in the protein stability, we introduced punctual mutations into the structure of the 2009 pandemic HA (PDB: 3M6S), and carried out a two-nanosecond simulation at high temperature (600 K). The mutated HA presented a general improvement in secondary structure stability, when compared with the wild-type HA simulated at the same temperature. Moreover, the mutated HA displayed a global reduction in RMSD values per residue, indicating more rigidity in this protein and, consequently, a stabilization of the HA. Unlike the study of Huang and cols. (2003) where they looked for amino acids that destroy interactions between subunits; we found substitutions which appear to stabilize HA by creating new interactions in the protein.

Conclusions

We found several mutations in the stem portion of the pandemic HA with a stabilizing effect in the protein. Currently, our workgroup is performing experiments aimed to confirm these findings.

SPB3 - VIRUS STRUCTURE & REPLICATION

B312P

Analysis of influenza virus replication in human nasopharyngeal and oropharyngeal cells

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Aims

Influenza virus causes a respiratory tract infection worldwide. The nasopharynx is a major site for the initial viral infection and replication of influenza virus before systemic spread. In the clinical diagnosis of influenza, nasal aspirates and sputum specimens are collected to detect the presence of influenza virus; however, there is limited scientific evidence to support this statement. The purpose of this study is to compare the efficiency of viral production between two kinds of cells and determine the host factors that enhance influenza virus production after infection. The goal of our ongoing research is to develop cells for rapid production of large amounts of influenza virus.

Methods

We collected the clinical swab specimens from patients with influenza-like symptoms and compared the RNA copy numbers of influenza virus among nasal cavity, nasopharynx, and oropharynx swab specimens. To analyze the kinetics of influenza virus propagation in human nasopharyngeal cells (HNC) and human oropharyngeal cells (HOC), we quantified the amount of viral RNA sequentially in these cells and in the culture supernatants of both cells using real-time RT-PCR. Finally, we compared the gene expression profiles to determine cellular host factors that affect the efficiency of influenza virus replication in HNC and HOC using genome-wide microarray analysis.

Results

Quantitative real-time PCR analysis revealed that there was a significant positive correlation between nasopharynx (log10 5.33 copies/ml) and either of nasal cavity (log10 4.23 copies/ml, p=0.043) or oropharynx (log10 3.7 copies/ml, p=0.005) swab specimens between 12 and 24 hours after the onset of high fever (maximum temperature?38?). Influenza virus rapidly replicated in HNC at 12-24 hours post-infection in vitro, and the amount of intracellular viral RNA was significantly more than HOC. Furthermore, the titers of released extracellular viruses from infected HNC were significantly higher by 1-2 logs compared to HOC. We found that a variety of cellular host factors were differently expressed in HNC and HOC. These factors were classified to categories such as transcription factors, membrane proteins and cytoskeleton-relating proteins.

Discussion/Conclusion

HNC showed higher efficiency of influenza virus replication than HOC, and many differently expressed genes between HNC and HOC were identified. It is suggested that a part of these genes listed here would be key determinants of the efficiency for influenza virus infection. The nature of HNC to replicate influenza virus more rapidly than HOC could contribute to higher detection sensitivity of influenza virus in clinical specimen from nasopharyngeal site than from oropharyngeal site. Although further study is needed to identify the key cellular factors that enhance influenza virus infection, the mechanisms which enable the cells to produce more amount of influenza virus will be applicable to development of better cell substrates for cell-culture-based influenza vaccines.

SPB3 - VIRUS STRUCTURE & REPLICATION

B313P

Structural determinants of binding in the virulence-associated substitution D222G in hemagglutinin of 2009 pandemic H1N1 influenza

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Introduction

The frequency of detection of the D222G mutation in the hemagglutinin (HA) protein of the 2009 pandemic influenza A/H1N1 virus (pdmH1N1) has been significantly higher in cases with severe pneumonia. This mutation is of special interest, as it has also been described as the single change in HA between two strains of the ?Spanish? 1918 A/H1N1 virus that alter glycan specificity. The introduction of D222G in the 1918 virus results in increased binding to avian glycans (α2,3-SAs) and reduced binding to human glycans (α2,6-SAs) in glycan arrays. The pdmH1N1 virus with D222G has also been found to bind more abundantly to avian receptors yet does not have the associated loss in binding to receptors present on human cells.

Aim

To investigate this potential discrepancy in binding of the two different H1N1 virus mutants to α2,6-SAs.

Methods

A model of the structure of HA from the A/Netherlands/602/200 strain of the 2009pdm H1N1 influenza virus was built based upon the crystal structure of HA of the H1N1 1918 A/SouthCarolina/1/18 virus. The D222G mutation was introduced into both of the A/Netherlands/602/2009 and A/SouthCarolina/1/18 structures. An α2,6-linked sialic acid was docked into the binding site of each strain based upon the experimentally determined crystal structure of swine 1930 H1N1. Several strategies were employed to explore the docking of the glycan within the binding pocket, altering both glycan and protein sidechain conformations within the binding pocket. Molecular dynamics simulations and global optimization by basin-hopping were used to produce structures of the complex for strains with and without D222G. The interactions between α2,6-SA and residues that are known to be involved in binding or residues which are different between A/SouthCarolina/1/18 and A/Netherlands/602/2009 were monitored throughout the simulations.

Results

For A/SouthCarolina/1/18, α2,6-SA was observed to occupy two distinct conformations, which we refer to as modes 1 (53% of structures) and 2 (46% of structures). These modes differ in the interactions formed between α2,6-SA and HA, due to a change in the position of the galactose sugar of the glycan. A/Netherlands/602/2009 was also observed to adopt both binding modes 1 (55% of structures) and 2 (27% of structures). However, unlike A/SouthCarolina/1/18, many intermediate structures were also observed. The most common interactions seen in the simulations of WT strains involved the sidechains of D187 and K219, and both the sidechain and backbone of D222. For A/Netherlands/602/2009, additional interactions are seen with the sidechains of K130 and K142. For A/SouthCarolina/1/18 -D222G, there were fewer interactions with the sidechains of G222 and K219. These are key to the stability of mode 2 which is seen to be almost entirely lost in A/SouthCarolina/1/18 -D222G. For A/Netherlands/602/2009-D222G, a decrease of mode 2 was also observed in the simulations but to a lesser extent. Additional interactions with the sidechains of K130, K142 and E224 which are not present in A/SouthCarolina/1/18 -D222G were able to partially maintain this mode.

Conclusion and Discussion

The loss of key interactions between α2,6-SA and HA of A/SouthCarolina/1/18-D222G offers an explanation for the reduced binding seen for A/SouthCarolina/1/18-D222G. Genetic differences between A/SouthCarolina/1/18 and A/Netherlands/602/2009 provide additional interactions which prevent the destabilization of mode 2 in A/Netherlands/602/2009-D222G. We propose that it is these differences that allow A/Netherlands/602/2009-D222G to maintain binding to α2,6-SA.

SPA4 - ANTIVIRALS AND RESISTANCE

A401P

Cross-Protective Effect of Antisense Oligonucleotide Developed against the Common 3' NCR of Influenza a Virus Genome

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Influenza A virus (IAV) continues to exemplify its unpredictable disposition and rapid evolution causing pandemics costing nearly a million lives and considerable economic losses. Novel antiviral approaches are required to achieve global knockdown of the viral genome expression for effective control of virus replication. Antisense technology offers small interfering RNAs (siRNAs) as potential antiviral agents. Each of the eight segments of IAV contains evolutionarily conserved non-coding 5' and 3' ends, crucial for viral replication. M1, NS1 and PB1 genes of the IAV were cloned and a single siRNA against 3' non-coding region of the genome was designed .The efficacy of siRNA was analysed with three different IAV strains (A/Puerto Rico/8/34, A/New Caledonia/20/99, A/ Udorn/307/72) both ex-vivo (MDCK cell line) and in-vivo (Balb/c mice). Viral plaque assay and RT-PCR analysis showed 60% inhibition of virus replication as compared to untreated controls. The efficacy of siRNA was confirmed by immunoblotting. Mice survival assay also exhibited a dose dependent protection by the common siRNA against the virus. Thus, we demonstrate that a single siRNA designed by us is capable of providing protection against multiple strains of influenza virus.

SPA4 - ANTIVIRALS AND RESISTANCE

A402P

Identification and characterization of peptides and neutralizing scFv's targeting Influenza virus entry by the phage display technology

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Introduction

Influenza A is one of the most severe threats to human health and animal welfare. Outbreaks of highly pathogenic avian Influenza virus H5N1 and the human H1N1 virus occur worldwide requiring the development of new antiviral strategies. Virus entry inhibition represents an attractive target for therapeutic or prophylactic interventions. Therefore, we designed several targets predicted to be involved in Influenza virus entry based on computational analysis for the selection of peptides or scFV fragments with neutralizing capacity by phage display.

Methods

Targets for the biopannings were Influenza pseudovirions and receptor-binding domains (RBD) of human and avian HA. For selections three random peptide phage libraries were used. After the biopanning process selected phages were tested for specific binding by ELISA and for their inhibitory effects in neutralization assays based on Influenza pseudovirions. In addition a phage immune library from an Influenza-vaccinated person will be generated to identify also binding and neutralizing scFv fragments targeting epitopes relevant for H1N1 Influenza virus entry.

Results

So far, retroviral particles pseudotyped with H5N1 or H1N1 Influenza surface glycoproteins were generated and investigated for their infectivity in single-round infection assays. An in vitro neutralization assay based on Influenza pseudotyped viruses was established with appropriate controls. Further, phage display screenings with H5N1 pseudovirions were performed and inserts from selected phages were determined. Neutralisation studies with synthetic peptides and the expression of the different receptor-binding domains as well as the construction of the immune phage library are currently in progress.

Conclusion

We could identify and generate the experimental tools to select and functionally analyze peptides or antibody fragments from phage libraries that represent potential virus entry inhibitors. At present, the characterization of recently identified peptides is ongoing.

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SPA4 - ANTIVIRALS AND RESISTANCE

A403P

Assessment of the antiviral properties of recombinant porcine SP-D against Influenza A viruses in vitro

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Introduction

The emergence of influenza viruses resistant to existing classes of antiviral drugs raises concern and there is a need for novel antiviral agents that could be used therapeutically or prophylacticaly. Surfactant protein D (SP-D) belongs to the family of C-type lectins which are important molecules of the innate immune system with antimicrobial activity. In the present study we assessed the antiviral properties of recombinant porcine SP-D (RpSP-D) against influenza viruses (IAV) *in vitro*.

Materials and methods

The recombinant pSP-D and human SP-D were produced in HEK293 cells. We assessed the capacity of SP-D to neutralize 30 IAV of the H1N1, H3N2 and H5N1 subtypes from various hosts (human, swine and avian) in the hemagglutination inhibition assay and in an infection reduction assay. Inhibition of virus attachment to epithelial cells of ferret and human respiratory tract by SP-D was also studied. The effect on neuraminidase activity was also assessed using two recombinant neuraminidases (NA1 and NA2) proteins.

Results

Using the selected viruses it was shown by hemagglutination inhibition assay, that RpSP-D was more potent than recombinant human SP-D and that especially the highly assembled form of SP-D had the strongest antiviral activity. RpSP-D was broadly reactive and neutralized a variety of H1N1 and H3N2 influenza A viruses, including 2009 pandemic H1N1 viruses. RpSP-D was also able to inhibit infection of MDCK cells with various IAVs in a dose-dependent fashion. Using NA inhibition assay we were able to demonstrate that RpSP-D also inhibited the activity of NA to a certain extent. Using tissue sections of ferret and human trachea, we demonstrated that recombinant RpSP-D prevented attachment of seasonal H1N1 and H3N2 virus to receptors on epithelial cells of the upper respiratory tract.

Conclusion

In conclusion, the results obtained in the present study showed that RpSP-D has potent antiviral activity against IAV in vitro. RpSP-D had broad neutralizing activity against IAV which were neutralized differentially based on their subtype and species from which they originated. The antiviral effect is mostly mediated by binding of SP-D to the viral HA preventing it from binding to the host cells receptors as was exemplified by virushistochemistry using ferret and human trachea tissue. This study justifies further evaluation of RpSP-D as an antiviral drug against IAV. Future investigations will aim at the delivery of RpSP-D and assessing its antiviral properties in vivo.

SPA4 - ANTIVIRALS AND RESISTANCE

A404P

Antiviral activity of usnic acid derivates against influenza A(H1N1) 2009 virus

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Introduction

Despite success in chemotherapy and vaccine prophylaxis, influenza remains a poorly controlled infection causing annual epidemics and pandemics. Usnic acid (UA), a dibenzofuran originally isolated from lichens has been shown previously to act as a growth regulator in higher plants. In humans, it can possess anti-inflammatory, antimitotic, antineoplasic, antibacterial, and antimycotic activities. The aim of this study was to evaluate anti-influenza properties of some derivatives of usnic acid.

Material and methods

We had tested 15 compounds, derivatives of usnic acid; all were synthesized in Novosibirsk Institute of Organic Chemistry. Derivatives of both (+) and (-) isomers of UA were included into the study. Pandemic influenza virus A/California/7/09 (H1N1)v was grown in MDCK-cells in a presence of compounds. After 48 hours of incubation virus yield was evaluated in hemagglutination test. The toxicity of compounds was tested in microtetrazolium test (MTT). Based on the results obtained, the 50% cytotoxicity dose (CTD_{50}), 50% effective dose (ED_{50}) and their ratio – selective index (SI) were calculated.

Results

In general, derivates of UA showed moderate activity against pandemic influenza. Among 15 studied compounds six had SI (selective index) more then 10. The most potent compound of this group is compound 608 (SI=12.7). Restricted analysis off structure-activity relationship revealed that (+) and (-) isomers of UA possess different antiviral activity, (-) isomers being approximately 1.5-4 - fold more potent.

Conclusions

These data suggest that derivatives of usnic acid can be considered as an affordable and prospective group of compounds to be used for prevention and/or treatment of influenza.

SPA4 - ANTIVIRALS AND RESISTANCE

A405P

Antiviral activity of amino derivatives of benzimidazol against influenza A(H1N1) 2009 virus

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Introduction

Influenza remains a poorly controlled infection causing annual epidemics and pandemics and at the same time the number of effective flu drugs is limited and. Besides their wide application leads to an increase in resistant strains. This fact highlights the need to develop new tools against the flu. In recent years, numerous studies have been conducted regarding antiviral properties of substances of natural origin. From another hand the mostly used in clinical practice are compounds of synthetic origin. Particular attention should be given to derivatives of benzimidazole, because those substances have a wide spectrum of biological activity.

Material & methods

We have tested 18 amino derivatives of benzimidazole, all were synthesized in Souther Federal University. Pandemic influenza virus A/California/7/09 (H1N1) was grown in MDCK cells in a presence of compounds. After 48 hours of incubation virus yield was evaluated in hemagglutination test. The toxicity of compounds was tested in microtetrazolium test (MTT). Based on the results obtained, the 50% cytotoxicity dose (CTD_{50}), 50% effective dose (ED_{50}) and their ratio – selective index (SI) were calculated.

Results

The results obtained demonstrated that value of CTD_{50} of compounds under investigation varied in a rouge of 6-1000 microgram/mL and their SI's – were from 1to 83. Among 18 studied compounds 10 (55%) had SI more then 10.

Conclusion

In general, amino derivatives of BI showed relatively high activity against pandemic influenza and can therefore be recommended for further development for prevention and/or treatment of influenza.

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The proton translocation domain of cellular vacuolar ATPase provides a cellular target for the treatment of influenza A virus infections

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Cellular vacuolar ATPases (v-ATPase) play an important role in endosomal acidification, which is a critical step in influenza A virus (IAV) host cell infection. We investigated the antiviral activity of the v-ATPase inhibitor Saliphenylhalamide (SaliPhe) and compare it to several older v-ATPase inhibitors Concanamycin A, Bafilomycin A1 (BafA), and Archazolid B targeting the subunit c of the V_0 sector.

An in vitro assay was set up that allowed to quantify the anti-influenza effect of v-ATPase inhibitors by measuring GFP fluorescence of a reporter IAV. This data was combined with cytotoxicity testing to calculate selectivity indices (SI). Data were validated by testing v-ATPase inhibitors against wild type IAV strains including pandemic A/Hamburg/01/2009, Oseltamivir resistant A/Luxembourg/572/2008 and highly pathogenic A/Chicken/Nigeria/BA211/2006 *in vitro* and mouse adapted A/PR/8/34 *in vivo*. During mouse in vivo experiments multiple parameters were recorded to monitor evaluate disease progression including body weight, temperature and viral titers in the lung.

In vitro SaliPhe blocked the proliferation of pandemic and multidrug resistant viruses at concentrations up to 51-fold below its cytotoxic concentrations for GFP modified virus. Identical efficacy was observed against A/Hamburg/01/2009 and Oseltamivir resistant A/Luxembourg/572/2008 with an SI of 48 and 61 respectively. The efficiency against H5N1 was reduced to a SI of 8.5. All other tested v-ATPase inhibitors were virtually ineffective, except Bafilomycin A1 with SI ranging between 7 and 8 for all tested influenza viruses.

At essentially untoxic concentrations of 21 mg/kg/day, SaliPhe protected 62.5% of mice against a lethal challenge of a mouse adapted influenza strain, while even toxic concentrations of BafA showed essentially no protection (SaliPhe vs. BafA $p < 0.001$).

Interestingly, the survival outcome of this experiment could not be improved by increasing the amount of SaliPhe per injection, extending duration of treatment or decreasing the dose of virus used for infection but in all cases survival in the SaliPhe treated group was significantly better than mock treated control groups.

Viral infiltration of lungs was also examined by immunohistochemistry using an anti-matrix antibody. Virus infected and mock-treated mice showed massive viral infections of the bronchiolar epithelial cells at the time of death (or euthanasia). No staining was visible in mock infected negative control mice. In the surviving SaliPhe-treated mice the virus was essentially cleared.

Four days post-infection 3 mice of each treatment group were sacrificed to determine viral titers in the lung. Although, the SaliPhe-treated mice had over 90% reduction in virus titer than the mock treated mice with only 3 mice tested, this was not statistically significant ($p = 0.176$). Lungs of BafA and mock treated mice showed very similar viral titers in both groups.

Our results show that a distinct binding site of the proton translocation domain of cellular v-ATPase can be selectively targeted by a new generation v-ATPase inhibitor with reduced toxicity to treat influenza virus infection including multiresistant strains. Treatment strategies against influenza that target cellular proteins are expected to be more resistant to virus mutations than drugs blocking viral proteins.

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A407P

Increased detection of a novel A(H1N1) 2009 influenza variant with reduced oseltamivir and zanamivir sensitivity

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Introduction

The emergence and global spread of an oseltamivir-resistant seasonal influenza A(H1N1) variant containing a H275Y neuraminidase (NA) mutation, demonstrated the potential for drug resistant influenza viruses to arise and spread within the community in the absence of drug selective pressure. Since the start of the pandemic, the oseltamivir resistant H275Y mutation has only been detected on rare occasions in A(H1N1) 2009 community specimens (<1%), with little evidence of community transmission. Other NA mutations in A(H1N1) 2009 viruses with mildly reduced oseltamivir and/or zanamivir susceptibility have been reported, but their detection has been very rare and has occurred mostly in immunocompromised individuals under long-term NAI treatment. Here we report the identification and increased rate of detection of a novel A(H1N1) 2009 variant with reduced oseltamivir and zanamivir susceptibility in community specimens from the Asia-Pacific region.

Materials and Methods

Specimens and isolates submitted to the WHO Collaborating Centre for Reference and Research on Influenza (WHOCCRRI), Melbourne, as part of the WHO Global Influenza Surveillance Network were analysed for oseltamivir and zanamivir susceptibility after culturing using a fluorescence-based neuraminidase inhibition assay. Viruses with reduced NAI sensitivity were sequenced using standard techniques and novel NA mutations were investigated further by reverse genetics.

Results

Prior to December 2010, NAI sensitivity analysis of over 2900 A(H1N1) 2009 influenza viruses from the Asia-Pacific region detected only 23 H275Y oseltamivir resistant viruses (0.8%), with the remaining strains being fully sensitive to both oseltamivir and zanamivir. However since December 2010, 17 out of approximately 250 A(H1N1) 2009 Asia-Pacific viruses (6.8%) have contained a S247N NA mutation that confers a 5-fold reduction in oseltamivir sensitivity and a 2-fold reduction in zanamivir sensitivity compared to the mean IC_{50} of sensitive viruses. The majority of the S247N variants detected to date have been from either northern Australia or Singapore, but the variant has also been detected in Western Australia and Brunei. Of the 28 A(H1N1) 2009 strains from northern Australia sampled during 2011, 9 (32%) have contained the S247N mutation, while 7/y (z%) strains from Singapore have contained the S247N mutation. In Western Australia, a S247N variant infected an immunocompromised patient who was hospitalised, treated with oseltamivir in ICU, but ultimately died. A sample taken five days after commencing oseltamivir treatment contained a virus with dual S247N+H275Y mutations that had an oseltamivir IC_{50} over 7000-fold higher than sensitive viruses and over 10-fold higher than seen in a virus with the H275Y mutation alone. The NAI sensitivity of the single S247N and dual S247N+H275Y mutants has been confirmed using site-directed mutagenesis and reverse genetics experiments.

Conclusions

The S247N mutation that is located in the NA enzyme active site has been reported previously to mildly reduce NAI sensitivity in seasonal H1N1 viruses and highly pathogenic H5N1 viruses. The effect of the S247N mutation on the in vivo efficacy of either zanamivir or oseltamivir is not currently known. Pharmacokinetic data would suggest that the maximum drug levels easily exceed the observed IC50 values of the S247N mutant, although it is noteworthy that recent clinical studies have demonstrated a reduced oseltamivir efficacy for normal influenza B viruses which have IC_{50} values only 5-fold higher than that of the S247N variants. Detection of the highly resistant dual S247N+H275Y variant raises concerns that, if the S247N variant spreads globally, then additional NA mutations may cumulatively decrease NAI sensitivity to levels where NAI efficacy is reduced to a clinically significant level.

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Efficacy of a single intravenous administraion of laninamivir (an active metabolite of inavir®) in influenza virus infection mouse model

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Introduction

Inavir® (laninamivir octanoate) has been approved to complete treatment of influenza by a single inhalation in Japan. Laninamivir octanoate is hydrolyzed to an active metabolite laninamivir, a potent novel neuraminidase (NA) inhibitor and retains as laninamivir in a respiratory tract for a long time. It is suggested that one reason why a single inhalation of laninamivir octanoate is enough for treatment of influenza is a long retaining of the laninamivir, another a tight binding of laninamivir to neuraminidases of influenza viruses including H1N1, H3N2 and B subtypes. Peramivir is a drug to complete treatment by a single intravenous infusion which might be due to a tight binding ability to N9 neuraminidase. In this paper, we determined whether a single intravenous administration of laninamivir is effective in a influenza virus infection model of mice.

Materials & Methods

Mice (Balb/c, female, 5w) were infected with influenza virus A/PR/8/34 (H1N1) and laninamivir, zanamivir or peramivir was admistered to the mice intravenously once daily for 5 days at 3 mg/kg or once at 3-30mg/kg. The survival of mice were monitored for 20 days. The survival rate and the median survival times (MST) were calculated by the Kaplan-Meier method. The efficacy of NA inhibitors against saline was analyzed by a log-rank test based on the joint ranking method. The multiplicity adjustment was performed by the Bonferroni method for each compound. The comparison of efficacy of laninamivir with that of zanamivir or peramivir was carried out by Cox regression analysis and verified by Wald test.

Results

The repeated intravenous administrations of laninamivir and peramivir at 3 mg/kg gave the survival rate of 93.3% and 100%, respectively, which were statistically significant ($p<0.0001$) against saline group. Zanamivir showed only 20% of survial rate under the same experimental conditions. The MSTs of laninamivir, peramivir and zanamivir were >20, >20 and 8.0 days, respectively.

The single intravenous administration of the 3 NA inhibitors showed dose-dependent life-prolonging effects and they were significant at 30 mg/kg ($p<0.0001$) compared to saline group. The MSTs of laninamivir, peramivir and zanamivir at the dose were >20, >20 and 12.5 days, respectively. The life-prolongling effect of laninamivir was similar to that of peramivir ($p=0.9881$) and significantly superior to zanamivir ($p=0.0268$).

Conclusion

It was suggested that a single intravenous administration of laninamivir show significant efficacy in mouse infection model like peramivir. This might be due to a tight binding to neuraminidase of influenza virus. A single infusion of laninamivir could be enough to complete treatment of influenza in human like peramivir (Rapiacta®) at a same dosing regimen.

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Monitoring oseltamivir-resistance influenza viruses in New Zealand

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Introduction

The neuraminidase inhibitors play an essential role in managing infections caused by pandemic and seasonal influenza. The potential emergence of oseltamivir resistance can disable our defences against seasonal and pandemic influenza. National antiviral susceptibility surveillance provides evidence for guiding clinical management and public health strategies to control influenza. This study describes antiviral susceptibility monitoring during 2006-2010 in three different settings: the availability of oseltamivir in New Zealand without a prescription since 2007; and the global emergence of the oseltamivir-resistant seasonal A(H1N1) strain in 2008 and the co-circulation of oseltamivir-sensitive pandemic A(H1N1) and oseltamivir-resistant seasonal A(H1N1) viruses in 2009.

Methods

Through the national influenza surveillance system, influenza viruses were systematically collected during 2006-2010. A fluorometric neuraminidase inhibition (FNI) assay was used to test for antiviral susceptibility to oseltamivir. A molecular sequencing method was employed to detect the presence of the H275Y mutation.

Results

We tested n = 1795 influenza viruses isolated from 2006 to 2010 to track the development of oseltamivir-resistance in the context of these events. No significant evidence was found to suggest "over-the-counter" availability of oseltamivir would give rise to resistance, but a clear importation of the global 2008 resistant seasonal A(H1N1) virus was documented 9 months after being first reported in Europe in January 2008. This oseltamivir-resistant strain became the predominant strain early in the 2009 influenza season and was displaced by the emergence of the pandemic A(H1N1) 2009 virus. Of 817 pandemic influenza A(H1N1) viruses tested, no evidence of oseltamivir-resistance was found. Interestingly, 1.1% (n=1044) co-infections of oseltamivir-sensitive pandemic A(H1N1) and oseltamivir-resistant seasonal A(H1N1) viruses were detected with an intermediate resistance phenotype by the FNI assay.

Conclusion

This study demonstrates that national antiviral susceptibility surveillance in New Zealand is essential for effective public health interventions, clinical management and pandemic response.

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Effectiveness of Oseltamivir Treatment in Human Influenza A (H5N1) Infections: An Updated Analysis

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Aims

To examine the effectiveness of oseltamivir in the treatment of human influenza A (H5N1) infections from around the world.
Methods The Avian Flu Registry is a global observational study of clinical and treatment outcomes of influenza A (H5N1) infection in humans. Data are collected via voluntary reporting using a structured case report form from medical records, published case reports, and reports from other clinical records and governmental agencies.

Results

386 cases from 12 countries are included in this analysis: 68 cases from medical records in Azerbaijan, Hong Kong SAR, Nigeria, Pakistan, Thailand, and Turkey; 225 cases from various sources in Egypt and Indonesia, and 93 cases from detailed case publications from Bangladesh, Cambodia, China, and Vietnam. All cases have laboratory confirmation of infection with H5N1 influenza, with nearly all data coming from WHO-approved laboratories. Median age was 18 years (range 1 – 67), and 46% of cases were male. The overall survival rate was 42.2%.

Most cases who were treated with an antiviral received oseltamivir. Only about 8%% of cases were able to receive oseltamivir within two days after symptom onset and 70% received oseltamivir within seven days after symptom onset. Among those who received at least one dose of oseltamivir, the survival rate was 53.5%, compared with 24.1% of cases who did not receive any antiviral therapy ($p < 0.0001$). The greatest survival benefit was seen when oseltamivir was started close to symptom onset. Cases who received treatment within three days after symptom onset were 3-4 times as likely to survive as those who did not (n=12 treated within one day of symptom onset; relative risk of survival (RR)=3.89, 95% CI 2.47-6.13, $p<.001$); n=22 treated between 2-3 days after symptom onset, RR=3.09, 95 CI=2.03-4.69; $p<.001$). Although treatment benefit declined with time thereafter, there is still evidence of reduction in the risk of death if treatment is started within seven days after symptom onset (n=39, RR =1.35, 95 CI 0.84-2.17).

Discussion/Conclusion

Despite this registry having the largest collection of patient-level data on avian influenza, like any study, it faces the challenge of small numbers when looking at subgroups of interest. Nonetheless, these data show that oseltamivir is an effective treatment for highly lethal H5N1 infection, particularly when given within 3 days after the onset of symptoms. Prompt identification and early treatment of cases improves survival. Initiating antiviral treatment more than 8 days from symptom onset is unlikely to improve the chance of survival.

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Virological response to standard and double dose oseltamivir in patients with pandemic (H1N1) 2009 influenza: a prospective randomised trial

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Aim

Prospective data on the virological response to antiviral therapies in patients with pandemic (H1N1) 2009 influenza are limited. This study (NCT00949533) evaluated the response to standard and double dose oseltamivir regimens in infected adults and children. The emergence of antiviral resistance was also investigated.

Methods

This prospective, unblinded, randomised study was performed at three clinics in Brazil (two in São Paulo and one in Curitiba) between April 2009 and August 2010. All patients were aged ≥5 years, presented within 48 hours of the onset of influenza symptoms (fever ≥37.5°C and at least one respiratory symptom), and had a positive rapid test result for influenza. Adult patients were randomised to receive standard (75mg) or double dose (150mg) oseltamivir b.i.d. for 5 days, and children received equivalent recommended weight-based unit doses. Influenza signs and symptoms were recorded by the patient on Days 1 and 5 using a questionnaire. Tolerability was assessed by solicited adverse event (AE) reporting. Nasal and oropharnygeal swabs were taken on Days 1 and 5 and tested for pandemic (H1N1) 2009 by RT-PCR. Positive samples were cultured for phenotypic analysis by chemiluminescent neuraminidase inhibition assay (NAStar Kit, Applied Biosystems). Virological parameters evaluated at Days 1 (baseline) and 5 were the proportion of patients shedding virus, viral load and the proportion of patients with resistant viruses.

Results

Thirty-seven patients (mean ± SD age: 21.6 ± 11.1 years) were randomised to receive standard (19 patients, including 6 children <14 years old) or double dose (18 patients, including 5 children <14 years old) oseltamivir. At baseline, virological and clinical characteristics of infection were similar in the two groups. By Day 5, the proportion of patients who tested positive for pandemic (H1N1) 2009 was substantially reduced in both groups, and all cultures were negative (**Table**). Viral loads were also reduced to negligible levels (**Table**). No significant difference in the virological response to the two regimens was observed at Day 5 ($p>0.05$). Compared with baseline frequencies, influenza signs and symptoms were substantially and similarly reduced in both groups by Day 5 ($p>0.05$). No resistant viruses were detected. AEs were reported by eight patients (42.1%) in the standard dose group and by seven patients (38.9%) in the double dose group; no serious AEs or deaths occurred.

Conclusions

Viral shedding and viral load were rapidly and substantially reduced in patients with pandemic (H1N1) 2009 influenza who received standard or double dose oseltamivir regimens. Responses were similar in the two groups. No resistant viruses were detected.

Virological parameter	Standard dose (n=19)		Double dose (n=18)	
	Baseline	Day 5	Baseline	Day 5
RT-PCR positive for pandemic (H1N1) 2009, n (%)	19 (100)	5 (26.3)	18 (100)	6 (35.3)
Viral load, mean ± SD (genomes/µl)	130403.2 ± 247715.4	133.7 ± 122.7	72258.1 ± 144075.7	82.2 ± 87.3
Culture, n (%)				
Positive	19 (100)	0 (0)	18 (100)	0 (0)
Negative	0 (0)	19 (100)	0 (0)	17 (100)
Total patients	19	19	18	17

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Incidence of natural and drug-selected resistance to the neuraminidase inhibitors over 3 years: findings from the Influenza Resistance Information Study (IRIS)

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Aim

In response to the emergence of naturally-occurring neuraminidase inhibitor (NAI)-resistance among seasonal influenza A/H1N1 viruses in 2008, a global observational trial (the Influenza Resistance Information Study or IRIS [NCT00884117]) was initiated to study the epidemiology of NAI resistance in treated and untreated patients with influenza. We present results from season 1 and 2 and preliminary results from season 3.

Methods

Patients in the Northern and Southern hemispheres (US, France, Germany, Poland, Norway, Hong Kong, Australia) with influenza-like illness and / or a positive rapid test result for influenza were enrolled. Throat / nose swabs were collected on Day 1, 3 (self-swab), 6 and 10. Swabs were tested for influenza A and B viruses and subsequently subtyped and tested for NAI resistance (N1 position 275 and N2 positions 119 and 292) by real-time RT-PCR. Influenza-positive samples collected on Day 1, 6 or 10 were cultured and subsequently sequenced (HA, NA and MA) and phenotypically tested for NAI resistance. Clinical information, including scoring of seven influenza symptoms (scale: 0 [absent], 1 [mild], 2 [moderate], 3 [severe]), was recorded on diary cards by the patient (Days 1–12). Symptoms were also assessed by the investigator at each visit.

Results

In season 1 and 2, 663 patients who were influenza RT-PCR positive and for whom typing was available were enrolled (first patient in: 29 December 2008; 603 influenza A [47 seasonal H1, 64 H3, 492 pandemic H1] and 60 influenza B). Influenza symptoms, as assessed by baseline total symptom score (mean: 11.0) and temperature (mean: 38.2°C), were mild to moderate. NAIs were prescribed in 343 (52%) of the influenza RT-PCR positive individuals (23 seasonal H1 [49%], 35 H3 [55%], 264 pandemic H1 [54%], 21 influenza B [35%]); all of the seasonal H1 strains (last strain detected: 21 September 2009) were genotypically (NA 275Y) and phenotypically (mean IC_{50} 141 ± 45) resistant to oseltamivir and sensitive to zanamivir (mean IC_{50} 0.5 ± 0.2). No genotypic or phenotypic NAI resistance was observed during NAI therapy in H3 or influenza B infected patients. Genotypic NAI (NA 275Y) resistance emerged during oseltamivir treatment in three patients who were infected with the pandemic H1 virus (1% of the pandemic H1 cases who were prescribed oseltamivir alone or in combination with another antiviral). In all three cases, the resistant variant could be detected at a single time point only, viral loads were low and viruses could not be cultured or phenotypically studied. All three patients cleared the virus by Day 6. In season 3, more than 900 influenza RT-PCR positive individuals were enrolled. Up to 18 March 2011, virological data on 216 H3 and 315 pandemic H1 influenza patients were available. Genotypic NAI resistance was detected in 10 oseltamivir-treated patients; two had 292R in H3 and eight had 275Y in pandemic H1. There were no cases with evidence for NAI resistance at baseline.

Conclusions

In accordance with global epidemiological data, naturally NAI-resistant seasonal influenza A/H1N1 viruses disappeared following the emergence of the 2009 pandemic H1N1 virus. In season 1 and 2, resistant viruses emerged only rarely during treatment, were present at low viral loads and cleared rapidly in this community based population. A sub-study of IRIS is now ongoing to collect information on the emergence of NAI-resistance in immunocompromised patients.

Conflict of interest

Employee: Regina Dutkowski, Ann Nist, Xiao Tong and Klaus Klumpp are employees of Roche.; Commercially-sponsored research: Martin Schutten, Hoffmann-La Roche Ltd. Arnold Monto, Sanofi.; Other substantive relationships:: Martin Schutten has been a consultant for Hoffmann-La Roche Ltd. Charles Boucher has been a consultant for GSK, a research contractor and scientific advisor for Merck, and a scientific advisor for Roche. Bruno Lina has been a board member, consultant, and scientific advisor for Roche, DMC for clinical trials for Biocryst, a board member for GSK France and a consultant for SANOFI-Pasteur. Arnold Monto has had an advisory/consultant role for GSK, Novartis, Roche and Biocryst, and received honoraria from GSK, Novartis, Roche and Biocryst. Jonathan Nguyen-Van-Tam been a collaborator and scientific advisor for Roche, a grant investigator, scientific advisor and participated in a speaker's bureau for GSK, a trainer, scientific advisor and participated in a speaker's bureau for Baxter AG, and a scientific advisor and grant referee for Solvay. Any remuneration for such activities ceased in or before September 2010. Richard Whitley has been on the Board of Directors of Gilead Sciences, a consultant for Chimerix and receives NIH support for studies on influenza.;

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Oseltamivir plus zanamivir or oseltamivir plus amantadine bi-therapies compared to oseltamivir monotherapy in the treatment of the A(H1N1) 2009

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Introduction

The recent emergences of "oseltamivir-resistance in 2007" and of the new influenza A(H1N1) variant in 2009 have increased the need of information about the in vivo antiviral efficacy and tolerance of combination therapy with approved anti-influenza agents.

Aim

The clinical assay "COMBINA" aimed to evaluate the in vivo antiviral efficacy and tolerance of the oseltamivir plus zanamivir or oseltamivir plus amantadine bi-therapies compared to the oseltamivir monotherapy.

Methods

During the circulation of the A(H1N1) 2009 in France, adults with influenza-like-illness for less than 42 hours and with a positive influenza A rapid test diagnosis were randomized by general practitioners in 3 groups of antiviral treatments: oseltamivir plus zanamivir (group O+Z), oseltamivir plus amantadine (group O+A) or oseltamivir in monotherapy (group O).

The patients had a nasal wash just before the beginning of antiviral treatment (day 0) and on days 1, 2, 3 and 4. A nasal swab was also performed on days 5 and 7. Virological response was assessed by the quantification of influenza A M gene in all of the samples with a real time RT-quantitative PCR adjusted or not to the cell content estimated by the quantification of the GAPDH gene. The detection of the H275Y mutation by an allele specific RT-PCR was also performed on the nasal wash sampled after 3 days of treatment to detect the potential emergence of oseltamivir-resistance.

Results

Analysis was possible for 38 patients randomized in O+Z (n= 12), O+A (n= 14) and O (n= 12) treatment groups. The treatment was well tolerated and only one patient (group O+A) had post-prandial gastralgia. Considering a threshold of 3 $\log_{10}$ cgeq / µl, the virological response after two days of treatment was obtained for 2 patients (16,7%), 5 patients (35,7%) and 3 patients (25%) in the O+Z, O+A and O groups respectively. The mean viral load decrease per day was around 0.9 to 1 $\log_{10}$ cgeq / µl whatever the treatment group.

The presence of the H275Y mutation in the neuraminidase was detected only for one patient in the O+A group at days 3, 4 and 5 of treatment.

Discussion/Conclusion

In adults with an influenza A(H1N1) 2009 infection, the combination therapies oseltamivir plus zanamivir or oseltamivir plus amantadine were well tolerated. However, we could not detect any significant difference between the treatment groups in the in vivo antiviral efficacy. The oseltamivir plus zanamivir or oseltamivir plus amantadine bi-therapies are not superior to the oseltamivir monotherapy and should not be recommended in clinical practice for the treatment of A(H1N1)2009 infection.

YOUNG SCIENTIST

SPA4 - ANTIVIRALS AND RESISTANCE

A414P

Continued emergence and changing epidemiology of oseltamivir-resistant H1N1 (2009) influenza, UK 2010/11

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Introduction

Following the extensive global use of Neuraminidase inhibitors (NI), particularly oseltamivir, for pandemic influenza treatment and prophylaxis, low rates of oseltamivir resistance have been reported worldwide, with a total of 447 resistant cases having been recognised, from over 20,000 tested [WHO, 8th April 2011]. The majority of resistance was detected in severely immunosuppressed individuals or hospitalised patients sampled post-treatment, although several clusters involving limited person-to-person have been recognised. Consequently, restriction of NI use to certain clinical risk groups has been advocated in the UK during the winter of 2010 by national guidance (NICE).

Methods

We screened a large number of A/H1N1(2009) positive clinical specimens from community and hospital sources, for the most common cause of oseltamivir resistance, the H275Y mutation in the neuraminidase gene (N=5500 from May 2009 to April 2010, N=3500 May 2010-April 2011). Clinical follow up was performed on resistant cases to identify the risk factors associated with NI resistance.

Results

The prevalence of resistance has doubled during the study period; In the 2009/10 period 45 resistance cases were detected (0.8%) as opposed to 56 cases in the 2010/11 period (1.6%, $p<0.001$). The prevalence of community-acquired resistance cases was 1% in 2010/11 as opposed to nil in 2009/10. Moreover, a change in the risk factors associated with resistance has been noted. In 2009/10, severe immunosuppression (24 cases: 53%) and a history of drug treatment (11 cases: 24%) were the key risk factors for oseltamivir resistance, similar to the global situation and a link to oseltamivir therapy could not be established in only 5 cases (11%). Contrary to that, during 2010/11 oseltamivir resistance has increasingly been detected in epidemiologically unlinked individuals with no known exposure to oseltamivir (16 cases: 28%, $p<0.05$). These unexposed resistant cases have been detected mainly in the hospital (87% of cases) but also the community setting (13% of cases) and in dispersed geographical locations in the UK. The frequency of severe immunosuppression among 2010/11 resistant cases was lower (15 cases, 27%, $p<0.01$).

Conclusions

Over 2010/11 the epidemiology of oseltamivir resistant A/H1N1(2009) has changed significantly, involving both an increase in prevalence, community circulation and possible risk factors. While the number of cases infected with a resistant strain who have been detected in the community remain low, it is likely to have epidemiological significance given that no such cases were detected in 2009/10. These data suggest low level circulation oseltamivir resistant (H275Y) A/H1N1(2009) in 2010/11. The significantly lower frequency of immunosuppression as an underlying risk factor for resistance in 2010/11 may be explained in part by the heightened awareness to the possible emergence of resistance due to the H275Y mutation, resulting in appropriate and timely use of zanamivir in this patient population, as advocated by national UK guidance.

Mindful of the emergence of transmissible oseltamivir resistant seasonal influenza A/H1N1 strain in 2007/8 which spread globally, the data presented here may represent an early warning of virus evolution supporting transmissible oseltamivir resistant AH1N1(2009). Our surveillance findings imply the need for urgent studies to evaluate possible underlying compensatory mutations among resistant strains and underpin the need for continuous surveillance for antiviral resistance, particularly among high-risk groups, which is crucial to inform national and international antiviral policies.

SPA4 - ANTIVIRALS AND RESISTANCE

A415P

Pyrosequencing as a tool for surveillance of molecular markers associated with resistance to neuraminidase inhibitors in influenza A(H1N1)pdm09 viruses

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The 2009 pandemic caused by the A(H1N1)pdm09 influenza virus highlighted the need for antiviral medications. There are two classes of drugs available in the U.S. for influenza control: M2 blockers (adamantanes) and the neuraminidase inhibitors (NAIs). However, since A(H1N1)pdm09 viruses are reassortants that acquired M and NA gene segments from a Eurasian adamantane-resistant swine influenza virus, therapeutic options are limited to the NAIs - oseltamivir and zanamivir. During the 2009 pandemic, the investigational NAI peramivir was also available under the Emergency Use Authorization. The rapid emergence and global spread of oseltamivir-resistant seasonal H1N1 viruses in previous years led to the awareness that increased antiviral drug use for treating A(H1N1)pdm09 infections could lead to an emergence of drug resistance. A nucleotide substitution (C?T) resulting in a replacement of Histidine (H) with Tyrosine (Y) at residue 275 (H275Y) in the N1 neuraminidase (corresponds to H274Y in N2 amino acid numbering) is an established marker of resistance to oseltamivir and peramivir, although this substitution does not affect susceptibility to zanamivir. It has also been shown that substitutions in other amino acid residues of the N1 neuraminidase can reduce the susceptibilities to NAIs. For instance, in seasonal H1N1 and H5N1 viruses, changes at residues V116, I117, Q136, D199 (D198), and I223 (I222) are associated with reduced susceptibility to NAIs . Judicious determination of drug susceptibility through laboratory surveillance is vital and assists with making informed decisions about antiviral drug use and the management of influenza.

Methods

In response to an increased need for drug resistance monitoring, the pyrosequencing assay was implemented to detect the oseltamivir resistance-conferring H275Y substitution in the A(H1N1)pdm09 viruses. More recently, the testing has been expanded to include residue I222 since its replacements with R, K or V, alone or in a combination with H275Y, have been reported in specimens collected from A(H1N1)09 infected patients. Pyrosequencing was conducted with the PyroMarkQ96 ID (Qiagen) platform on clinical samples and/or virus isolates. Drug resistant targets were analyzed in sequence identification (SQA) or single nucleotide polymorphism (SNP) mode. In addition to routine surveillance, the Centers for Disease Control (CDC) provided diagnostic testing for the H275Y marker in A(H1N1)pdm09 viruses collected from severely ill patients with a clinical suspicion of drug resistance. A minor genetic variant in the mixture was reported only when present at ≥10%.

Results

Since April 1, 2009, a total of 6030 A(H1N1)pdm09 viruses have been screened for routine surveillance of the H275Y substitution in the neuraminidase. H275Y was detected in 75 (1.2%) samples. Additionally, a total of 134 diagnostic specimens from patients with a clinical suspicion of drug resistance were tested. Of those, 59 (44%) carried the H275Y substitution. Due to the detection of changes at residue 223 in some H1N1pdm09 viruses, this target was recently added to routine surveillance. One hundred eight A(H1N1)pdm09 positive clinical specimens and virus isolates have been screened for changes at I223. Dual substitutions H275Y/I223R, H275Y/I223K, or H275Y/I223V were detected in viruses collected from four patients treated with oseltamivir. Such dual changes were associated with enhanced resistance to NAIs in the phenotypic assays. In order to increase drug resistance surveillance capacity, the standard operating procedures provided by the CDC were put into operation by several public health laboratories in the U.S. and were also posted on the World Health Organization's website.

Conclusion

Pyrosequencing is used for rapid screening of sequence information in order to detect known molecular markers of antiviral drug resistance. Overall, A(H1N1)pdm09 viruses remain susceptible to NAIs. However, sporadic cases of oseltamivir-resistant A (H1N1)09 virus infections have been reported globally. Reported detection of H275Y in viruses collected from patients with no exposure to oseltamivir is a concern and indicates a need for enhanced surveillance. The ability to empirically determine appropriate NAI drug use will depend on regional antiviral resistance testing gathered from surveillance programs.

SPA4 - ANTIVIRALS AND RESISTANCE

A416P

Emergence of D151 neuraminidase variants in cell culture affects drug susceptibility of influenza A/H3N2 viruses

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Introduction

Monitoring the susceptibility to neuraminidase (NA) inhibitors (NAIs) is an integral part of influenza virus surveillance. Viruses are tested for functional (phenotypic) NA inhibition as well as in genotypic assays. In contrast to genotypic testing, which can be directly applied to clinical specimens, functional NA inhibition assays require viruses to be propagated in cell culture, such as MDCK/ATCC cells. However, it has previously been shown that propagation of seasonal A/H1N1 viruses in cell culture can result in the emergence of NA variants with amino acid substitutions at either D151 or Q136. Moreover, changes at D151 have been reported in A/H3N2 viruses, although their effect on drug susceptibility has not been fully ascertained. Here, we assessed the correlation between changes at D151 and susceptibility of A/H3N2 viruses to NAIs zanamivir and oseltamivir.

Methods

Influenza A/H3N2 viruses were recovered from clinical specimens and passaged in MDCK/ATCC. In addition, a subset of viruses was recovered and passaged in MDCK/SIAT1 and Caco2 cells. Pyrosequencing method using a customized order of nucleotide dispensation was applied to detect the presence of NA variants and assess their proportion in the virus sample. The baculovirus-insect cell expression system was used to produce recombinant protein of both D151 and G151 NA variants of the A/Perth/16/2009 virus. The chemiluminescent NA inhibition assay (NA-Star) was used to determine the concentration of NAI needed to inhibit 50% of NA activity (IC_{50} value) for zanamivir and oseltamivir.

Results

To assess genetic variance at D151, 231 MDCK/ATCC grown viruses collected from 2009-2011 from different geographic locations were analyzed by pyrosequencing. The analysis revealed the presence of the NA variants G, N, or V at 151, either alone or together with the wild type D151, in 84% of the tested viruses. Of note, the viruses, in which the NA variants were detected, exhibited a 5 to 80-fold increase in the zanamivir IC_{50} values. When present by itself, the N151 virus variant had IC_{50} values similar to those of the D151 (wild type) viruses. In contrast, the V151 virus variant demonstrated elevated IC_{50} for both zanamivir (≥400-fold) and oseltamivir (≥7-fold). A greater proportion of the G151 variant in the virus population was associated with a greater zanamivir IC_{50} value. Furthermore, when the G151 and D151 recombinant NA proteins were compared, the G151 variant showed a ≥200-fold elevation in zanamivir IC_{50} and no changes in oseltamivir IC_{50}. Interestingly, only the wildtype D151 variant was detected in the matching clinical specimens (n=50) tested by pyrosequencing indicating the selective pressure exerted by cell culture. To explore the potential utility of other cell lines, a subset (n=14) of influenza A/H3N2 clinical specimens were propagated in MDCK/ATCC, MDCK-SIAT1 and Caco-2 cells in parallel. The MDCK/ATCC-grown viruses contained mixed variants (D/G or D/G/N) after two passages while the same viruses grown in MDCK/SIAT1 cells contained no changes after three passages. Nevertheless, extensive passaging in MDCK/SIAT1 cells also led to the accumulation of G151 and N151 variants in the virus preparations. Passage in Caco-2 cells led to the accumulation of N151 variants, which did not affect IC_{50} values, although not all viruses were recovered in this cell line (9 of 14).

Conclusions

Influenza A/H3N2 viruses propagated in MDCK/ATCC cells often acquire changes at D151 in the NA and this can result in their reduced susceptibility to NAIs. To minimize the adverse effect of this phenomenon on drug susceptibility monitoring, we suggest propagating A/H3N2 viruses in the MDCK/SIAT1 cell line and restricting the number of passages to 2-3 when possible.

SPA4 - ANTIVIRALS AND RESISTANCE

A417P

Clinical effectiveness of long-acting neuraminidase inhibitors in children: intravenous peramivir and inhaled laninamivir

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Introduction

In addition to oseltamivir and zanamivir, the two newly approved long-acting neuraminidase inhibitors, the intravenous drug peramivir and the inhaled drug laninamivir, were used in Japan during the 2010-2011 season, bringing to four the total number of neuraminidase inhibitors currently being used in hospitals and clinics nationwide. The purpose of this study was to evaluate the effectiveness and safety of peramivir and laninamivir in children with influenza virus infection.

Methods

During the 2010-2011 season there was a mixed influenza epidemic in Japan caused by pandemic A (H1N1) 2009 (abbreviated H1N1/09) and by A (H3N2) and B. Effectiveness was evaluated by comparing the duration of fever after the initiation of neuraminidase inhibitor treatment in an oseltamivir-treated group (mean age, 3.9 years), a peramivir-treated group (mean age, 4.4 years), and a laninamivir-treated group (mean age, 8.0 years) in all of which neuraminidase inhibitor treatment was initiated within 48 hours after the onset of illness. Laninamivir was administered in the form of a single inhalation on the first day of treatment, and peramivir (10 mg/kg) was administered intravenously on the first day of treatment. Laninamivir and oseltamivir were used to treat outpatients, and peramivir was used to treat inpatients.

Results

In influenza A virus infection, the mean duration of fever was 33 hours in the oseltamivir group (N=40) and 28 hours in the laninamivir group (N=26). The mean duration of fever was significantly longer in influenza B, i.e., 51 hours in the oseltamivir group (N=12) and 66 hours in the laninamivir group (N=28). There was no significant difference between the oseltamivir group and laninamivir group in duration fever after the start of treatment. However, both drugs were significantly less effective against influenza B virus infection than influenza A virus infection. The mean duration of fever after the start of treatment was only 22.9 hours in the patients with H1N1/09 infection in the peramivir group (N=10). No severe adverse events were reported in in any of the patients.

Conclusion

Oseltamivir and laninamivir were equally effective in reducing the febrile period of children with influenza A virus infection, and both drugs were less effective against influenza B. Peramivir was clinically effective against H1N1/09.

SPA4 - ANTIVIRALS AND RESISTANCE

A418P

Aprotinin, a protease inhibitor, as a drug against influenza

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Introduction

Research efforts are focusing on development of new antiviral chemotherapeutic approaches that target either influenza virus replication itself or host factor(s) that is critical to influenza replication. Host mediated influenza hemagglutinin (HA) cleavage critical for activation of virus infectivity is such a chemotherapeutic target. Influenza pathogenesis develops through a “vicious cycle”: host proteases activate progeny virus which amplifies replication and stimulates protease activities in infected host.
Material & methods. Aprotinin, a 58 amino acid polypeptide from bovine lung, is one of a family of the host-targeted antivirals that inhibits serine proteases involved in influenza virus activation.

Results

This drug was shown to suppress virus HA cleavage and multicycle reproduction of human and avian influenza viruses including pandemic influenza virus H1N1 with a single arginine in the HA cleavage site. Additionally, serine protease inhibitors, including aprotinin, are known to target a number of host mediators of inflammation and down regulates their levels in virus-infected hosts. Aprotinin is a generic drug approved for intravenous use to treat pancreatitis and limit post-operative bleeding.

Conclusion

As an antiinfluenzal compound, aprotinin can be delivered by two routes: (i) a middle-particle aerosol has been used for local respiratory application in mild-to-moderate influenza and (ii) an intravenous administration is proposed for severe influenza to provide both an antiviral effect and a decrease in systemic inflammation.

SPA4 - ANTIVIRALS AND RESISTANCE

A419P

Impact of different oseltamivir treatment regimens in the otherwise healthy or immunocompromised influenza infected patient: insights from a modelling study

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Introduction

Several studies have proven oseltamivir to be efficient to reduce viral titre and symptoms intensity. However the effectiveness of oseltamivir strongly depends on the delay between infection (or onset of symptoms) and first antiviral intake. The effectiveness of oseltamivir can also be compromised by the emergence and further spread of drug-resistant viruses.

Material and Methods

We therefore explored these interactions in silico, using a pharmacokinetic (PK) model of oseltamivir and a virus kinetics / symptoms dynamic (VKSD) model fitted to experimental human infection data. We used a "population approach" that allowed us to compute average population as well as individual predictions. We modelled the emergence of resistant virus as mutations occurring randomly and proportionally to the number of infected cells. The selective pressure exerted by oseltamivir on resistant viruses was modelled through the difference of IC50 for susceptible and resistant viruses. We studied the effect of the dose, of the time of first intake, of the intake frequency and of immunodeficiency on influenza infection, illness dynamic and resistant virus emergence and selection.

Results

With the recommended treatment regimen i.e. 75 mg bid during 5 days, administration prior to symptom onset, provided both virological and clinical efficacies above 95%. During the incubation period, the virological and clinical efficacies decreased to 50% and 30% respectively and resistant virus emergence was maximal. After symptom onset, the virological and clinical efficacies decreased progressively until 2% and 1.7% respectively three days after symptom onset. The proportion of subjects shedding resistant virus fell below 1% when the first intake was after symptom onset. A daily administration and a shortened period of administration were associated with an increased risk of resistance emergence and selection. Immunodeficiency was associated with a substantially increased proportion of subjects shedding resistant virus and a dose effect was found on efficacy and resistance emergence.

Conclusions

Our model provides a global picture of the effect of oseltamivir, would they be positive or negative. It indicates that oseltamivir regimen should be carefully chosen and adapted to the subject immunological status. The recommended regimen for prophylaxis should moreover be avoided as it increases the risk of resistance emergence and that immunocompromised subjects should be treated with higher doses in order to combine an increased efficacy with a low risk of resistance emergence.

SPA4 - ANTIVIRALS AND RESISTANCE

A420P

Treatment of experimental influenza infection with 2-(imidazol-4-yl) ethanamide pentanedioic-1,5 acid (Ingavirin).

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Introduction

The influenza A virus is a highly infective agent that causes acute pulmonary diseases. In serious cases, influenza A leads to severe pneumonia, which is particularly fatal in young children and the elderly, in patients with cardiopulmonary diseases and renal malfunctions. In case of 2009 pandemic, in addition to these groups, the virus appeared additionally dangerous for patients with obesity and pregnant women. Due to the danger influenza represents to the human population, there is a need for search and development of new effective antivirals for prevention and treatment of influenza infection. We demonstrate here a protective effect of the low-molecular weight compound Ingavirin (2-(imidazol-4-yl) ethanamide pentanedioic-1,5 acid) against lethal influenza virus infection caused by A(H1N1)2009 or A(H3N2) viruses in mice.

Materials and methods

White mice were infected with either A(H1N1)2009 or A(H3N2) influenza virus. Ingavirin was applied orally in a dose 3-45 mg/kg body weight. Mortality, weight loss, infectious titer of the virus in lungs and lung and cell morphology were monitored in the groups of Ingavirin-, oseltamivir- and placebo-treated animals.

Results

Oral application of both Ingavirin and reference compound Tamiflu led to reduction of infectious titer of the virus in the lung tissue (from $10^{5.1}$ EID_{50}/20 mg tissue in placebo- treated animals to $10^{3.5}$ and $10^{2.6}$ EID_{50}/20 mg after treatment with Ingavirin and Tamiflu, respectively). Treatment with Ingavirin also resulted in prolongation of the life of the infected animals, normalization of their weight dynamics throughout the course of the disease, lowering of mortality of treated animals compared to a placebo control and normalization of lung tissue structure reducing he degree of lung edema and inflammatory infiltration. As was shown by morphological analysis of the cells of bronchial epithelium layer, infecting with influenza virus resulted in the death of approx. 60% of cells on day 3 p.i. Application of Ingavirin led to prevention of cell death and restored the ratio of live/dead cells to the values close to those of intact animals. In addition, electron microscopy analysis revealed the restriction of viral morphogenesis in the presence of Ingavirin both on early (nuclear) and late (virus progeny budding) stages decreasing the number of intranuclear virus- specific inclusions and pattern and number of budding virions, respectively.

Conclusion

Based on the results obtained, Ingavirin should be considered as an important part of anti-influenza prophylaxis and therapy, in particular in severe cases of the disease.

SPA4 - ANTIVIRALS AND RESISTANCE

A421P

Measuring the fitness cost of the oseltamivir resistance mutation H275Y in the NA gene of pandemic H1N1 from early and late waves of the outbreak.

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Introduction

The H275Y mutation that confers oseltamivir resistance to the NA genes of influenza viruses has in most instances been associated with a fitness cost to the enzyme and to the virus itself. Consequently, where the mutation has arisen following drug use, it has not transmitted onwards from the infected patient. In 2007-2008 however, seasonal H1N1 viruses with the H275Y mutation emerged and became the predominant strain by the end of that season. The reason for the apparent advantage conferred by drug resistance is not completely elucidated. However it is clear that the HA and NA genes of influenza virus must attain a balance between the affinity of binding of HA to sialic acid receptor and the efficiency of receptor digestion by the NA enzyme. Thus we speculate that as these genes evolve, differences in the balance may arise that make it more or less likely that resistance to neuraminidase inhibitor drugs will emerge and predominate.

Material and methods

We have observed sequence changes in the HA and NA genes of the pandemic H1N1 virus since it first emerged in 2009 during its circulation in humans in the subsequent second and third waves of the pandemic in the UK. Here we asked whether the H275Y mutation affects virus fitness in a complex culture of human airway epithelium (HAE), arguably the most authentic model of human influenza virus replication and spread available. Viruses that differed only the NA gene were generated by reverse genetics and infected into HAE cultures in competition assays. Using single nucleotide position pyrosequencing and allele frequency analysis we determined whether the H275Y mutation affected virus growth in this system.

Results and conclusions

In the context of a first wave strain we found a small fitness cost conferred by H275Y that was statistically significant. The effects of HA and NA mutations that have arisen on this outcome were further investigated.

YOUNG SCIENTIST

SPA4 - ANTIVIRALS AND RESISTANCE

A422P

Multidrug resistant 2009 a/h1n1 influenza clinical isolate with a neuraminidase i223r mutation retains its virulence and transmissibility in ferrets

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Introduction

Only two classes of antiviral drugs, neuraminidase inhibitors and adamantanes, are approved for prophylaxis and therapy against influenza virus infections. A major concern is that influenza virus both becomes resistant to these antiviral drugs and spreads in the human population. The new influenza A/H1N1 virus subtype is already resistant to adamantanes. Recently, a novel neuraminidase I223R mutation was identified in an A/H1N1 virus showing class resistance to the neuraminidase inhibitors oseltamivir, zanamivir and peramivir. However, the ability of this virus to cause disease and spread in the human population is unknown. Therefore, this clinical isolate (NL/2631-R223) was compared with a well-characterized reference strain (NL/602).

Materials and methods

Influenza A/NL/2361-R223 was isolated from an immune compromised patient who was subsequently treated with oseltamivir and zanamivir. Recombinant virus with a single I223R mutation was obtained by reverse genetics. Replication kinetics was performed by inoculation of MDCK cells with virus at an MOI of 0.01 followed by virus titration of samples taken at 6, 12, 24 and 48 hours post inoculation. Virus pathogenicity was studied in the ferret model. Groups of 6 ferrets were inoculated intratracheally (10^6 $TCID_{50}$) with control or mutant virus. Throat and nose swabs were collected daily to determine virus excretion from the upper respiratory tract. The animals were weighted daily as indicator of disease and observed for clinical signs. Three ferrets from each group were euthanized and necropsied at days 4 and 7. Trachea and lung samples were collected to study virus distribution and patho-histology. Virus transmission was studied in a ferret aerosol transmission model. On day 0, ferrets (4 or 2 animals) were housed individually in transmission cages and inoculated intranasally (10^6 $TCID_{50}$) with control of mutant virus. On day 1, naïve female ferrets were individually placed in a transmission cage adjacent to an inoculated ferret, separated by two stainless steel grids. Nasal and throat swabs were collected daily from the inoculated and exposed groups to study virus transmission. Virus titers in the collected swabs were determined by means of endpoint titration in MDCK cells.

Results

In vitro experiments showed that NL/2631-I223R replicated as well as NL/602 in MDCK cells. In a ferret pathogenesis model, body weight loss was similar in animals inoculated with NL/2631-R223 or NL/602. Also, pulmonary lesions were similar at day 4 post inoculation. However, at day 7 post inoculation, NL/2631-R223 caused milder pulmonary lesions and degree of alveolitis than NL/602. This indicated that the mutant virus was less pathogenic. Both NL/2631-R223 and a recombinant virus with a single I223R change (recNL/602-I223R), transmitted among ferrets by aerosols, despite observed attenuation of recNL/602-I223R *in vitro*.

Conclusions

The I223R mutated virus isolate has comparable replicative ability and transmissibility, but lower pathogenicity than the reference strain based on these in vivo studies. This implies that the new influenza A/H1N1 virus subtype with an isoleucine to arginine change at position 223 in the neuraminidase has the potential to spread in the human population. It is important to be vigilant for this mutation in influenza surveillance and to continue efforts to increase the arsenal of antiviral drugs to combat influenza.

SPA4 - ANTIVIRALS AND RESISTANCE

A423P

Detection of an influenza B strain with reduced susceptibility to neuraminidase inhibitor drugs.

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Since 1999, the neuraminidase inhibitors (NAIs), oseltamivir and zanamivir have played an essential role in the prophylaxis and treatment of influenza infection. The role of neuraminidase (NA) is to remove sialic acid from receptors present on the surface of the host cell. In the absence of NA activity, the ability of the progeny virions to spread to uninfected cells is compromised. The NAIs act by binding to the viral NA active site, thus preventing the release and spread of progeny virions. The residues forming the NA active sites are highly conserved among all A and B influenza viruses. Decreased susceptibility to NAIs can develop as a result of substitution at the residues forming the enzyme active site. There have been reports of in vivo resistance for both influenza type A and B viruses. Here we report the isolation of an influenza B virus designated B/Ontario/RV75-11/2010 with reduced susceptibility to both oseltamivir and zanamivir. B/Ontario/RV75-11/2010 showed a 14-fold lower susceptibility to oseltamivir and an 18-fold lower susceptibility to zanamivir compared with wild type control, B/Hong Kong /36/2005. The virus isolate contained a new G109E amino acid substitution in the neuraminidase. The recovery of the influenza B virus with the new G109E substitution which affects susceptibility to two drugs available for treatment of influenza B virus infections, highlights the importance of monitoring NAI- susceptibility using functional (phenotypic) assays. This is crucial for the implementation of proper treatment and infection control measures.

SPA4 - ANTIVIRALS AND RESISTANCE

A424P

Nanobodies with in vitro neutralizing activityprotect mice against h5n1 influenza virusinfection

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Introduction

Influenza A virus infections impose recurrent and global disease burden. Although a number of small drug antiviral therapeutics against influenza is available, their impact is limited, in particular in cases of severe complications resulting from influenza. We assessed the protective potential of monovalent and bivalent llama-derived immunoglobulin single variable domains (VHH domains or VHHs), called Nanobodies® targeting H5N1 hemagglutinin (HA).

Material & Methods

Two llamas were immunized with recombinant A/Vietnam/1203/2004 H5N1-HA. H5N1-HA–specific Nanobodies were obtained by phage display library, selected by panning and expressed in E. coli and administered intranasally to female BALB/c mice (SPF, 7–9 weeks old). The NIBRG-14 virus used in this study is a reassortant of A/Vietnam/1194/2004 (H5N1) and A/PR/8/34 (H1N1) viruses and has been mouse-adapted. NIBRG-14 escape viruses were plaque purified in presence of H5N1-HA–specific Nanobodies, and the HA gene cloned and sequenced.

Results

Intranasal administration of Nanobodies effectively controlled homologous influenza A virus replication. Administration of Nanobodies before challenge strongly reduced H5N1 virus replication in the lungs and protected mice from morbidity and mortality after a lethal challenge with H5N1 virus. The bivalent Nanobody was at least 60-fold more effective than the monovalent Nanobody in controlling virus replication *in vivo*. In addition, Nanobody therapy after challenge strongly reduced viral replication and significantly delayed time to death. Epitope mapping, based on in vitro escape virus selection and sequence analysis of the HA coding segment, revealed that the VHH Nanobody binds to antigenic site B in H5 hemagglutinin.

Conclusions

Prophylactic and therapeutic use of H5N1-HA–specific Nanobodies protect mice against lethal challenge with homologous H5N1 virus. The Nanobodies are small, stable, and simple to produce, they are a promising, novel therapeutic agent to treat or prevent influenza.

SPA4 - ANTIVIRALS AND RESISTANCE

A425P

Preparation of Commercial Quantities of a Hyperimmune Human Intravenous Immunoglobulin Preparation against Pandemic H1N1 Influenza

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Background

The recent H1N1 pandemic provided an opportunity to conceptually assess the possibility of rapidly providing a "hyperimmune" human immunoglobulin (H-IVIG) to an emerging infectious disease, in useful quantities with respect to public health. Commercial scale H-IVIG production from plasma collected from donors convalescent from or vaccinated against pandemic influenza A (H1N1) virus is described.

Study design and methods

A special protocol was implemented for the collection, processing, and shipment of plasma from previously qualified Source plasma donors, self-identifying as convalescent from or vaccinated against H1N1 influenza. A licensed IVIG manufacturing process was utilized for the preparation of two commercial lots of approximately 50 Kg 10% human IVIG preparation in total. The H1N1 hemagglutination inhibition and neutralization antibody titers of the resulting H-IVIG preparations were determined and compared with standard preparations.

Results

wenty six plasma collection centers participated in the protocol. Donor enrollment exceeded 300 donors per week, and within 30 days of protocol deployment plasma was being collected at a rate of over 2,000 L/week. Manufacture of both H-IVIG lots was unremarkable and both lots met the requirements for commercial release and the bulk of the product was distributed in normal commercial channels. Examination of plasma pools and final IVIG product confirmed pandemic H1N1 antibody titers substantially higher than those collected before the emergence of the pandemic H1N1 virus.

Conclusions

This work demonstrates the feasibility of producing a H-IVIG preparation at large scale relatively rapidly, with a significant enrichment in antibodies to the H1N1 influenza, achieved by donor self-identification.

SPA4 - ANTIVIRALS AND RESISTANCE

A426P

Examination of the antiviral and adjuvant activity of a novel anti-influenza peptide.

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Introduction

We previously demonstrated that a 20 residue peptide (EB, RRKKAAVALLPAVLLALLAP) from the FGF-4 signal sequence inhibits influenza in vitro and in vivo by preventing attachment to cells. EB peptide's antiviral activity was broad-spectrum, inhibiting multiple influenza A subtypes as well as influenza B. Here, we have identified the minimal and optimal peptide sequence that retained antiviral activity and identified truncated peptides with alternate mechanisms of action. Additionally, we further report on consensus EB's mechanism and the exploitation of this phenomenon for use as an influenza vaccine adjuvant.

Materials & Methods

To examine minimal sequence, a library of peptides with serial deletions of a single residue from either the N- or C-terminus was synthesized and tested for antiviral activity by hemagglutination inhibition and plaque reduction assay. Electron microscopy and sediment density ultracentrifugation were utilized to study the structural impact of peptides on influenza virions. To examine adjuvant activity, 6 wk old BALB/c mice were primed and boosted with EB supplemented, whole virion vaccine and challenged with H5N1 and H1N1 subtypes to assess protection. Immune correlates of protection were examined by hemagglutination inhibition, virus specific antibody ELISA and IFN-γ ELISpot.

Results

Our truncation library identified 11 peptides within full length EB that retained antiviral activity. The minimal sequence, peptide $B10^{NP}$ (RRKKLAVLLALLA), possessed 50% inhibitory and effective (IC_{50}, EC_{50}) values similar to concensus (5 µM vs 7 µM respectively). An additional variant, peptide $B7^{NP}$ (RRKKVALLAVLLALLA), had an EC_{50} value 20 fold lower (0.3 µM) as compared to consensus and displayed virucidal properties.

Further analysis of full-length EB demonstrated that it aggregates influenza virions, and this aggregation leads to decreased attachment. EB-induced virus aggregates were readily engulfed by phagocytic cells in vitro. In vivo, mice vaccinated with a suboptimal dose of whole virus vaccine containing EB peptide had reduced morbidity, improved viral clearance, and faster recovery than mice receiving vaccine alone. This response was retained when vaccinating against H1N1 or the poorly immunogenic H5N1 subtype. No increase in virus-specific antibodies was observed, however, cell-mediated immunity was enhanced as demonstrated by ≈ 4 fold increase in interferon-γ production from splenoctyes.

Conclusions

These studies enhance our knowledge of the novel influenza antiviral EB peptide by identifying truncated variants that display lower IC_{50}, $EC_{50\ values}$, and alternate mechanisms of action. Additionally, EB peptide may be utilized as a vaccine adjuvant by virtue of its virion aggregating activity. Unlike many adjuvants, EB primarily enhances cell-mediated immunity, a phenomenon often deficient in non-replicating vaccines. Increased resistance to current antivirals and poor immunogenicity to the H5N1 subtype vaccines highlight the urgent need to identify novel strategies for treatment and prevention of influenza infection. EB peptide and its truncated variants offer the potential to expand our arsenal of influenza antivirals, as well as enhance existing influenza vaccines.

SPB4 - ECOLOGY AND EPIDEMIOLOGY OF ANIMAL INFLUENZA

B401P

The importance of the Central Asian waterbodies in the circulation of HPAI viruses.

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Materials and methods.

Sample collection.

We were sampling cloacal and tracheal swabs. Two samples were taken from each bird. All samples were stored and transferred according to OIE protocols.

Virus isolation

Viruses were isolated by chicken embryo inoculation (CEI).

PCR and Sequencing

Commercial kit was used for HPAI H5N1 identification (Amplisens, Russia), sequencing was made with original primer sets.

Introduction

HPAI H5N1 were described in South East Asia since 1997 and were circulating in the region. These viruses were no spreading westwards to the other part of the world. The situation has changed, when in 2005 HPAI H5N1 viruses were somehow transmitted into Qinghai Lake, where they have caused a huge outbreak in bar-headed geese population. And after that these viruses started their spread all over the world.

Results

The first influenza A (H5N1) outbreak in Russia was reported in summer 2005 in Western Siberia, in the Chany lake (Lipatov et al., 2007). With migratory wild birds, virus spread westward across Eurasia and as far west as England and West Africa. Analysis showed that strains isolated in Russia in 2005 were related to H5N1 viruses caused an outbreak among wild birds at Qinghai Lake in China in Spring 2005. Subsequently, the Qinghai-like (clade 2.2) HPAI H5N1 lineage was detected in wild birds and poultry in many countries. The source of these introductions, while still debated, is likely through bird migration. Interestingly enough numerous wild birds' species were involved in Russia outbreaks (swans, gulls, grebes, spoonbills, sparrows and others). HPAI H5N1 viruses were also isolated in 2006 at the Chany lake. In June 2009, an outbreak of HPAI was recorded in wild birds in Mongolia and on the Uvs-Nuur Lake in Russia. Phylogenetic analysis showed that viruses belong to clade 2.3.2. These viruses were close to viruses isolated in Mongolia and at Qinghai Lake few weeks before. But this outbreak did not cause a virus spread all over the world. We suggest that wild birds brought the virus to Uvs-Nuur Lake from outside the country. And the most possible source of this introduction was Qinghai Lake.

We hypothesized that bodies of water like the Qinghai Lake, Chany lake and the Uvs Nuur Lake may play an important and outstanding role in the circulation of avian influenza so we suggested enhancing of surveillance program in this area and therefore we continued to study new outbreaks thoroughly (Sharshov et al., 2010).

Our hypothesis was fully confirmed in June 2010. An outbreak of HPAI was recorded in wild birds at the Uvs Nuur Lake. Phylogenetic analysis of the hemagglutinin (HA) gene showed a close relation to strains isolated during outbreaks at the same location in 2009, at the Qinghai Lake in 2009 and 2010 (Gao et al. 2010) and in Mongolia in 2010 as all of them fall into clade 2.3.2. We can suppose that these strains originally appeared around 2009 at the Qinghai Lake and later in May 2010 caused an outbreak in Central Mongolia (Sakoda et al., 2010). In June 2010 dead birds were found at the Uvs Nuur Lake and viruses with 98-99% identity to Mongolian strains were isolated. The short time between the outbreaks of these closely related strains support our hypothesis of a direct connection between Qinghai and Uvs Nuur lakes and establish this region as one possible route for virus transmission from South-East Asia to Western Siberia and then to Europe.

Recent detection of clade 2.3.2 H5N1 viruses during outbreaks in Romania and Bulgaria (Reid et al., 2010) confirm that detection of these viruses in big Eurasian lakes is very important factor of its successful spread all over the world.

SPB4 - ECOLOGY AND EPIDEMIOLOGY OF ANIMAL INFLUENZA

B402P

Genetic analyses of avian influenza viruses in Mongolia, 2007–2009, and their relationships with Korean isolates from domestic poultry and wild birds

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Introduction

The present study was conducted to monitor wild birds based on concern that they could disseminate avian influenza virus (AIV) between Mongolia and Korea, which shares the same migratory flyway. Therefore, the main objectives of this study were to survey AIVs circulating in wild birds in Mongolia and to analyze their genetic characterization and relationships with Korean isolates from domestic and LBM poultry as well as wild birds.

Materials and Methods

Fresh fecal samples from wild birds were collected during May–October to survey for AIV in Mongolia between 2007 and 2009. A barcoding system utilizing mitochondrial DNA of bird feces was employed to determine host species. The sequence data were aligned using the AlignX multiple sequence alignment in the VectorNTI Advance program and phylogenetic tree was constructed using the neighbor-joining method within Clustal X version 1.83, with 1000 bootstrapping replicates.

Results

Of 1,528 feces samples analyzed, 21 low pathogenic avian influenza viruses were isolated from 2007–2009. Nineteen AIV-positive feces samples were identified as Anseriformes by DNA barcoding. The most frequently isolated subtype was H3 (61.9%) and the most prevalent HA/NA combination was H3N8 (52.4%). Phylogenetic analysis was performed to assess their genetic relationships with those of domestic poultry and wild birds in Korea. The H3 and H7 surface genes belonged to the Eurasian lineage and clustered together in a group with Korean wild birds and poultry. Most N8 genes clustered phylogenetically with viruses isolated in Eurasia, whereas one of the Mongolian virus and some Korean viruses belonged to the North American lineage. The PA of the internal gene was not distinguishable from the H5N1 highly pathogenic avian influenza viruses of the Gs/GD-like lineage.

Conclusions

The results suggest that the presently obtained Mongolian AI isolates have evolved with genetically multiple genotypes and are closely related to those of AI viruses in poultry as well as wild birds of Korea. Therefore, LPAI viruses as well as HPAI viruses in both countries should be monitored closely to understand their epidemiological relationships, and further surveillance should continue through the international cooperation with countries sharing common migratory flyways.

SPB4 - ECOLOGY AND EPIDEMIOLOGY OF ANIMAL INFLUENZA

B403P

Molecular epidemiology of highly pathogenic avian influenza viruses isolated from domestic poultry and wild birds in South Korea between 2010 and 2011

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Introduction

On December 7, 2010, H5N1 highly pathogenic avian influenza (HPAI) virus was isolated from a healthy mallard captured at the Mankyung River in South Korea. During December 30, 2010 ~ April 6, 2011 in South Korea, several outbreaks of highly pathogenic avian influenza (H5N1) were confirmed among domestic poultry and wild birds of various species. We had experienced three times of H5N1 HPAI virus outbreaks in 2003~2004, 2006~2007 and 2008. This study was conducted to characterize of new HPAI virus in 20010~2011 to compare with the previous HPAI viruses by identification, isolation, genetic analysis.

Material & Methods

Oropharyngeal swab, cloacal swab, feces from poultry and wild birds and organ samples from dead birds were collected to diagnosis of HPAI all over the country. For virus isolation, 9- to 11-day-old embryonated chicken eggs were used and the isolated viruses were subtyped using RT-PCR. Gene sequencing and phylogenetic analysis were carried out. The intravenous pathogenicity index (IVPI) was confirmed according to the OIE manual.

Results

Between December 2010 and April 2011, a total of 52 cases among domestic poultry and a total of 20 cases from wild birds were diagnosed H5N1 HPAI. HPAI viruses from the captured healthy mallard and dead wild birds had never been isolated in previous HPAI cases. Fourteen viruses were selected, respectively, from poultry isolates and wild bird isolates for genetic analysis, and all eight gene segments from each of the influenza viruses were sequenced. A phylogenetic analysis showed that all of the viruses were of the same virus type and that the hemagglutinin (HA) gene was clustered with that of clade 2.3.2 viruses.
A/duck/Korea/Cheonan/2010(H5N1), the first isolates from poultry, was distinct from the HPAI viruses responsible for previous outbreaks in South Korea (A/chicken/Korea/ES/2003 (94.2%), A/chicken/Korea/IS/2006 (93.3%) and A/chicken/Korea/Gimje/2008 (97.3%), whereas closely related (99.2-99.8%) to H5N1 isolates found in Mongolia, China and Russia in 2009 and 2010. The IVPI index was 3.0, meaning that all chickens died within 24 hours.

Conclusions

The clade 2.3.2 H5N1 strains isolated in Eastern Asia in 2009-2010, have distinctive gene constellation unlike previous clade 2.3.2 viruses. In other words, the phylogenetic evidence suggests that polymerase acidic proteins (PA) of these viruses was similar to that of clade 2.5 HPAI viruses, but the other viral genes were originated from viruses that caused outbreaks in South Korea, Japan and Russia in 2008. The sources of the past three HPAI outbreaks in South Korea were not yet clear, but 2010-2011 outbreak are a good example which of the H5N1 viral transmission by migratory birds. In addition, this transmission has caused serious economic damage on poultry farms and has affected to outbreaks among the permanent resident birds. It showed interspecies transmission to clarify the importance of avian hosts in the ecology of influenza viruses.

SPB4 - ECOLOGY AND EPIDEMIOLOGY OF ANIMAL INFLUENZA

B404P - Ecology and Epidemiology of Animal Influenza

Avian Influenza H5N1 at the Human-Animal Interface in Egypt

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Introduction

Five years after the onset of avian H5N1 influenza in Egypt, no signs of its eradication are visible. In 2009-2010, Egypt reported 68 new human cases as well as continuous outbreaks among poultry and became the new epicenter for H5N1 infections.

Methods

In order to better understand the situation in Egypt, we conducted an epidemiological and molecular analysis among the human cases and carried out active surveillance in various geographic regions and poultry production sectors to detect influenza viruses among poultry.

Results

The onset of new human cases peaked annually during the winter and spring months. Most cases were less than 18 years old (62%) and females (60%). The overall case-fatality rate was 34% and significantly increased by age. There was a significant difference between the case-fatality rates among females and males. We observed a significant drop (p=0.004) in case fatality rate in 2009 (10%) as compared to higher rates (36%-56%) in other years. Hospitalization within 2 or 3 days after onset of symptoms significantly decreased mortality. Molecular analysis showed that variations do occur among viruses isolated from birds as well as from humans in Egypt, and these mutations were especially noted in 2009 viruses. Between August 2009 and July 2010, 5562 swabs were collected from poultry and 5% tested positive for influenza A virus by RT-PCR. All positive samples were of H5 hemagglutinin subtype suggesting that Egyptian poultry are a reservoir for a high burden, but low diversity of influenza A viruses. We were able to detect H5N1 viruses in all production sectors, but commercial farms were a significant reservoir. There was no clear seasonal pattern for H5N1 activity.

Discussion:

Our data clearly demonstrate that H5N1 viruses will continue to be a public health burden and that many sectors of the Egyptian population are continually exposed to the virus.

SPB4 - ECOLOGY AND EPIDEMIOLOGY OF ANIMAL INFLUENZA

B405

Web-based data management system to support surveillance of Molecular Epidemiology Influenza A in Bali, Indonesia

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The Molecular Epidemiology of Influenza A in Bali project ("BaliMEI") is conducting five years of active surveillance of influenza A among patients presenting with influenza-like illness (ILI) at 21 health facilities across all 9 districts in Bali. This project collects data from each patient via validated questionnaires, resulting in at least 750 ILI swabs per year and 3150 ILI swabs at the end of the study. This project collects data via validated questionnaires on clinical epidemiology, viral testing, and field epidemiology for each patient.

To manage such data for a long time and to allow multiple accesses by relevant individuals, BaliMEI web-based database management system was developed. This system uses SQL language, MySQL DBMS for managing data, and PHPMyAdmin for Graphical User Interface. The database is uploaded in a secured university internet network system. Access to data is controlled by uniquely coded password, by which different users can access relevant sections of the database. As additional safeguard, unique study identification numbers are given to identify study patients on data forms without transmitting patients' names or other identifying information over the internet. The data enterer records all of the data questionnaires on Web-based data entry forms, and the system allows data enterers, project coordinators, researchers, and collaborators to monitor research activities development in "real time". The system is bilingual (Indonesian and English) and provides information in simple data presentation which shows case distribution in a map. All information on demographic variables, clinical observation, relevant exposures, travel patterns, medication, laboratory testing, and field epidemiology investigation report are recorded in the database, whose summary can be downloaded in a professional report format.

Currently 256 ILI cases have been inputted with their data complexity from the aforementioned facilities. The system has been accessed by researchers, local health authorities, and international collaborators for disease surveillance and research monitoring purposes, particularly the current location and time distribution of influenza A (pandemic H1N1 and seasonal H1N1) and influenza B. Due to the current outbreak of H5N1 on poultry in several districts, this system is intensively used for accessing the monthly report by the local health authority to monitor potential transmission from animal to human. This web-based database system is being integrated to influenza disease registry as part of the effort from Ministry of Health to establish the Influenza WHO Collaborating Center.

SPB4 - ECOLOGY AND EPIDEMIOLOGY OF ANIMAL INFLUENZA

B406P

Experimental inoculation of chickens with gull-derived low pathogenic avian influenza virus subtype H16N3 causes limited infection

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The infectivity, transmission and pathogenicity potential in chickens of avian influenza virus (AIV) subtype H16N3, isolated from European herring gull (Larus argentatus), was examined. Nineteen six-week-old commercial Lohmann White chickens were inoculated intranasally with 1 x 10^6 50 % egg infectious dose and clinical signs, humoral immune response, virus shedding, virus transmission and pathological changes in the respiratory tract were studied. Oropharyngeal and cloacal swabs were collected for viral RNA detection by real-time RT-PCR (rRT-PCR). Sera were collected and examined for H16-specific antibodies using hemagglutination inhibition test. Tissue samples from the nasal cavity, trachea and lung were collected at post-mortem examination for histopathology and viral RNA detection by rRT-PCR. In one bird bilateral serous nasal discharge was observed at 2 days post inoculation (DPI) and viral RNA was detected in oropharyngeal swabs at 2 and 4 DPI. Viral RNA was also detected from the oropharynx of an additional bird at 5 DPI. Moreover, H16-specific antibodies were detected in sera from these two birds at 14 and 21 DPI. No viral RNA was detected from cloacal swabs, and no virus transmission between virus-inoculated chickens and non-inoculated contact chickens was observed. Tissue samples from the nasal cavity, trachea and lung were negative for viral RNA and no gross or histopathological lesions were observed in the virus-inoculated birds. These results indicate that gull-derived AIV subtype H16N3 causes only limited infection in chickens under experimental conditions.

SPB4 - ECOLOGY AND EPIDEMIOLOGY OF ANIMAL INFLUENZA

B407P

Prevalence of Adamantane-Resistance Gene in Swine Influenza Viruses in Taiwan

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Introduction

There are two classes of anti-influenza virus drugs. One class includes neuraminidase inhibitors which can be used to treat influenza A as well as B virus infections. Aminoadamantane drugs (amantadine and rimantadine) belong to the second class – the ion channel blockers of matrix protein 2 (M2) for influenza A virus infections. Amantadine-resistant strains had been found in avian and swine, as well as human, influenza viruses. High prevalence of amantadine resistance among circulating porcine influenza A viruses in European countries (112 viruses; mainly from Germany) and other Asian country/area (8 viruses from Hong Kong SAR, China) collected between 1981 and 2006 was observed. All 5 swine influenza H1N1 viruses obtained from China during 2004 to 2008 was adamantine resistance. Sequence data of M genes from the influenza virus sequence database (Influenza Virus Resource [http://www.ncbi.nlm.nih.gov/genomes/FLU/FLU.html], accessed on 21 July 2008) indicated that 38 strains of 201 swine influenza viruses are resistant to amantadine in large-scale sequence analysis. In this study, the prevalence of amantadine-resistant strains in swine influenza viruses in Taiwan was investigated.

Materials & Methods

Ninety two viral isolates of 4 subtypes (H1N1, H1N2, H3N1 and H3N2) collected from 1992 to 2010 including swine isolates of pandemic H1N1 influenza 2009 viruses in Taiwan were studied. Full-length sequence of M genes was determined from DNA product following RNA extraction of viral isolates and reverse transcription-polymerase chain reaction. Sequence data was compiled by Lasergene Sequence Analysis Package Software (version 9.0; DNASTAR). Five known molecular markers of the M2 protein associated with amantadine (and rimantadine) resistance: L26F, V27A, A30V (or A30T), S31N, and G34E were analyzed. Phylogenetic tree based on multiple alignment of M2 amino acid sequences were constructed. Case histories of 92 studied viruses were used to interpret temporal and spatial distributions of amantadine-resistant strains in Taiwan.

Results

Forty-six (50% of total) viral isolates can be referred as resistant strains because at least one of 5 molecular markers was observed in amino acid sequence of M2 protein. The first one resistant strain in our viral collections was a H1N2 virus isolated in 1999. Resistant strains are widely spread over Taiwan (included the Kinmen and Matsu islets). Eleven of 17 H1N1 (64.7%), 31 of 61 H1N2 (50.8%) and 4 of 8 H3N2 (50%) viruses are resistant, whereas all 6 H3N1 viruses were sensitive. Only one H1N1 virus in 2007 had two resistance markers (L26F and S31N) and the remaining 45 resistant viruses harbored single S31N mutation. Quite interestingly, phylogenetic tree of H1N1 viruses revealed that all of early type (Iowa/1930-like) classical swine H1N1 viruses are sensitive in one cluster whereas almost of recent type (Iowa/1985-like) classical swine H1N1 viruses (except the earlier isolate in 1992) are resistant in the other cluster. In addition to S31N mutation, one additional possible marker (R77Q) could be found in M2 protein of all swine isolates of pandemic H1N1 influenza 2009 viruses and newly H3N2 reassortant progeny which contained matrix protein gene from pandemic H1N1 influenza 2009 viruses in Taiwan.

Conclusions

Our findings suggested high prevalence of aminoadamantane drugs-resistant influenza viral strains in Taiwanese pig herds and drug-resistance gene might be distributed increasingly into any subsequent viruses via reassortment. Biological significance revealed from amantadine resistance in molecular epidemiology of Taiwanese swine influenza viruses should be more concerned and investigated in detail. It highlighted further that molecular analyses of M2 protein are greatly needed to be incorporated in routine characterization of any newly emerging viral isolates or variants to improve swine influenza surveillance in Taiwan.

SPB4 - ECOLOGY AND EPIDEMIOLOGY OF ANIMAL INFLUENZA

B408P

The GISAID EpiFlu™ Influenza Database - Curation of the Data

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Background:

In response to growing needs of the global influenza community to share genetic sequences and associated epidemiological and clinical data, the GISAID initiative launched in May 2008 the EpiFlu™ database as a tool for sharing and analyzing such data. The EpiFlu™ database is publicly accessible (www.gisaid.org) and has introduced a unique sharing mechanism that protects the rights of the data submitter while facilitating further research and the development of vaccines and policies for drug use. It enshrines the principle of acknowledging the contributions of all participants to sustain a collaborative ethos throughout the influenza community. All users identify themselves and agree not to attach any restrictions to the data, to acknowledge both the originator of the specimen and the submitter of the data, and to seek collaborations with the data providers. With this distinctive mechanism GISAID's EpiFlu™ database provides an alternative to current public-domain databases. As of May 11, 2011, GISAID's EpiFlu™ database comprised 194,244 nucleotide sequences from 56,673 isolates; approximately 20% of them (36,935 nucleotide sequences from 15,102 isolates) were submitted directly to GISAID's EpiFlu™. With the majority of the latter available only in the GISAID's database, EpiFlu™ emerged as the world's most comprehensive collection of influenza sequence data.

Methods and Results

As of January 2011, the Federal Republic of Germany is the official host for the EpiFlu™ database. Three German institutions are engaged in development and maintenance of the database: the Max Planck Institute for Informatics (MPII) is responsible for the development of the software, the Federal Office for Agriculture and Food (BLE), hosts the GISAID portal, and the Friedrich-Loeffler-Institute (FLI) performs quality control and data curation.

The quality of the data is crucial for detailed analyses of huge molecular datasets. With the rapidly rising volume of sequence data, a systematic and scalable procedure for data curation is becoming more and more essential. Thus, bringing forward the effectiveness and quality of data curation is an important aspect of the GISAID EpiFlu™ database. GISAID data curation comprises a two-stage process with automatic and manual annotation steps. During submission, the sequence is checked via an automatic procedure for the correct annotation of segment designation, virus type, virus subtype and lineage. The assignment of open reading frames and an examination for completeness of the segment sequence are also facilitated by an automatic process. The automatic tasks are accomplished by a sequence of BLAST searches and alignments against specific reference datasets. If the output of this protocol differs from the original annotation, the submitter will be informed about both the extent of and the character of the required change. Subsequently, the submitter is responsible for the release of the sequence to the GISAID community. After release, data are manually inspected in a second curation phase. The metadata of each submission are monitored for completeness and plausibility. Entries are checked for the correct assignment of isolate names or the origin of the sequence. If there is any need for correction, the curator enters into a respective dialog with the submitter and requests amendments. In addition, the curator monitors the quality of the submitted sequences regarding to the correct use of the IUPAC code and the absence of ambiguities. The data curator also monitors the availability of sequences and encourages those possessing long-time unreleased sequences to share their data promptly with the GISAID community.

Conclusion

The meticulous curation of the GISAID EpiFlu™ database enhances data quality and consequently the scientific exploitation of the influenza sequence collection. The GISAID team will continue to develop and improve programs and procedures for analysis and annotation, and will provide effective tailor-made solutions for the influenza scientific community.

YOUNG SCIENTIST

SPB4 - ECOLOGY AND EPIDEMIOLOGY OF ANIMAL INFLUENZA

B409P

The ecology and risk of highly pathogenic avian influenza in Bangladesh

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The agro-ecology and poultry husbandry of the south Asian and south-east Asian countries share common features, however, with noticeable differences between countries. Hence, the ecological factors associated with highly pathogenic avian influenza (HPAI-H5N1) risk are expected to differ between Bangladesh and e.g. Thailand and Vietnam. The primary aim of the current study was to explore the ecological factors associated with the risk of HPAI outbreaks at subdistrict level in Bangladesh. Secondary, we explored the performance of two different statistical modeling approaches for unmeasured spatially correlated variation.

We performed an ecological study at subdistrict level in Bangladesh with 138 subdistricts with outbreaks between 2007 and 2008, and 324 subdistricts with no outbreaks. Risk factors investigated included natural and human made components associated with the spread of H5N1 in different countries in previous studies. We applied a generalized linear mixed model to explore the relationship of the risk factors with the disease occurrence. The influence of spatial clustering of the ecological data was modeled using 1) an intrinsic conditional autoregressive (ICAR) model at subdistrict level, and 2) a multilevel model at district level, respectively. Backward elimination was used to obtain the final model. We checked for possible interactions and confounding in the model.

Ecological factors associated with risk of HPAI-H5N1 infection in both models were household density, staging area of migratory birds, live bird markets, highway network and river network. No interaction and confounding were observed. Results suggest that ecological factors are linked to the risk of HPAI subtype H5N1 outbreaks at subdistict level of Bangladesh. The ecological risk factors of the HPAI in Bangladesh are different from those found in Thailand and Vietnam. The most important difference is that ducks are identified as an important factor in the south-east Asian countries, but not in Bangladesh. This finding is not surprising, since duck husbandry is very different in Bangladesh where ducks are reared in marshy areas with the low poultry density. Another important finding is that the river network is playing an important role along the side of the road network in Bangladesh in spreading the virus.

The present study revealed that the ecological factors can vary among the countries. We, therefore, recommend careful consideration of generalization of ecological study over different countries

SPB4 - ECOLOGY AND EPIDEMIOLOGY OF ANIMAL INFLUENZA

B410P

Introduction of a new clade of highly pathogenic avian influenza A/H5N1 into wild birds in Bangladesh

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Introduction

In January-February 2011 Bangladesh reported widespread outbreaks of highly pathogenic avian influenza (HPAI) A/H5N1 in domestic poultry. During the same period, there were several reports of crow die-offs throughout the country. We jointly investigated crow deaths identified in collaboration with the Department of Livestock and the Department of Forestry of Bangladesh to understand the cause and nature of the outbreaks.

Methods

We investigated die-offs in two crow roosts, one during 17-23 January 2011 in Potuakhali, a southern small town and another in the capital city of Dhaka, during 13-15 February 2011. The sites are 150 kilometers apart. In Potuakhali, the crows roosted in isolated orchards surrounded by dense human settlement, and in Dhaka, the crows roosted in a city park. The total number of healthy, sick and dead crows in both roosts were counted at two time points. We also interviewed the residents living under the crow roost in Potuakhali to estimate frequencies of crow deaths. We collected oropharyngeal and a cloacal swab samples from dead or sick crows found under the roosts and pooled them in viral transport medium, one pool per crow. We performed real-time reverse transcription polymerase chain reaction (rRT-PCR) to identify the influenza A M gene and the H5 subtype of HA. Viruses were isolated from an aliquot of the swab samples and hemagglutinin (HA) and neuraminidase (NA) genes were sequenced to identify their origin and markers of pathogenicity.

Results

We estimated that 1500 crows roosted at the Potuakhali outbreak site. Of them, 17 (1%) appeared sick and 57 (4%) were found dead during the investigation. In Dhaka city, about 900 crows roosted in a park. Of them, five (1%) appeared sick and 39 (4%) were found dead during the investigation. Both house crows (Corvus splendens) and large-billed crows (Corvus macrorhynchos) that were identified sick during the investigation died within 24 hours. We observed that when a crow got sick or died, other crows pecked on it or scavenged the carcass. All 34 swab samples tested positive for influenza A/H5 by rRT-PCR. We isolated virus from four out of seven crow samples used for virus cultivation: three from Potuakhali and another from Dhaka. Sequence analysis of the HA and NA genes indicated that the four isolates were influenza A/H5N1 and the HA contained the multibasic cleavage site motif (PQRERRRKR*G) characteristic of HPAI. Preliminary phylogenetic analysis of the HA gene shows these viruses are nearly identical to each other and are most closely related to clade 2.3.2 viruses found predominately in wild birds with an increasing geographical distribution throughout Southeast Asia, the Far East and Eastern Europe. The isolates we identified had closest nucleotide sequence identity (98.5%) with A/chicken/Vietnam/NCVD-398/2010 (H5N1) virus.

Conclusion

H5N1 viruses identified in poultry from 2007 to early 2009 in Bangladesh belonged to clade 2.2 and the commercial poultry distribution chain played a vital role in its spread. Our findings suggest introduction of a new H5N1 clade into Bangladesh which causes high pathogenicity in wild birds and is likely capable of infecting poultry and humans. Since both clades share common hosts and reservoirs, surveillance is important to identify whether they co-circulate or replace one another. Bangladesh continues to be at risk for introduction of new influenza viruses. Informing and educating the population may help avoid direct contact with sick and dead wild birds and promote sanitary disposal to limit human and poultry infection.

SPA5 - HOW TO EVALUATE VACCINE EFFECTIVENESS AND EFFICACY?

A501P

MF59 adjuvant affects antibody epitope repertoire, augments affinity maturation and neutralization antibody titers to Swine-origin H1N1 and Avian H5N1 influenza viruses

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Introduction

Rapid response against pandemic influenza viruses requires development of effective vaccines. Oil-in-water adjuvants were found to significantly increase virus neutralizing titers, heterosubtypic immunity, and afforded dose sparing. However, the complete impact of these adjuvants on antibody repertoire and affinity maturation was not investigated before.

Material and Methods

We employed Whole Genome Fragment Phage Display Libraries (GFPDL) and surface plasmon resonance (SPR) to elucidate the effects of MF59 adjuvant on the quantity, diversity, specificity, and affinity of human antibody responses to avian influenza H5N1 in adults and to swine origin-H1N1 vaccine in toddlers, young children and adults.

Results

Using GFPDL and SPR, we found that the oil-in-water adjuvant (MF59) selectively enhanced epitope spreading from HA2 to HA1 in the hemagglutinin (HA) and to neuraminidase, when compared with unadjuvanted or aluminum-adjuvanted inactivated H5N1 vaccines in terms of increased antibody titers as well as more diverse antibody epitope repertoire. Furthermore, a 2-3 fold increase in the binding avidity of antibodies to properly folded HA1 was measured in SPR, which correlated with broadening of cross clade neutralization. A similar expansion of HA1 epitopes recognition and increased binding avidity was observed in sera from pandemic H1N1 immunized individuals receiving the MF59 adjuvanted vaccine compared with unadjuvanted vaccine. Importantly in the most naïve population (12-35 month), MF59 enhanced serum antibody affinity as measured by increased 7M urea resistance and 10-fold drop in binding off-rate constants using SPR. A close correlation between inferred serum antibody affinity and virus-neutralizing titers was demonstrated ($r > -0.8$).

Conclusion

Thus, MF59 quantitatively and qualitatively enhances functional antibody responses to HA-based vaccines by improving both epitope-breadth and binding affinity, demonstrating the added value of such adjuvants for influenza vaccines.

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SPA5 - HOW TO EVALUATE VACCINE EFFECTIVENESS AND EFFICACY?

A502P

Development of enzyme linked immunoassays for the quantitation of influenza haemagglutinin: an alternative method to single radial immunodiffusion

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Review of the 2009 pandemic highlighted the need to develop alternate assays and reagents to quantify vaccine antigen in order to facilitate influenza vaccine formulation in a more expeditious manner. The current method for measuring influenza HA content, single radial immunodifusion (SRID) is considered labour intensive, time consuming and relies on matched standardised reagents.

Aim:

The aim of this work was to develop an alternative assay; a capture and detection enzyme linked immunoassay (EIA) using strain specific monoclonal antibodies for the quantitation of H1, H3, H5, and B influenza HA.

Method:

Monoclonal antibodies (MAbs) were selected by haemagglutination inhibition assay. Each MAb was also conjugated with horse-radish peroxidase and the assay optimised as a capture detection EIA for the quantitation of HA antigen and compared to the standard antigen quantitation assay. Standard antigens were used to quantify HA in samples. Single radial immunodiffusion assay was basd upon the method described by Willimas et al 19801.

Results

The selection of the monoclonal antibodies has allowed these assays to be applicable for multiple seasons reducing the need to update reagents on a seasonal basis. The nature of this assay of capture and detection by the same monoclonal antibody quantifies native, trimeric and higher order complex immunologically relevant HA. The EIA assays correlated with the standard assay for quantitation of influenza HA antigen (SRID assay), yet are more sensitive, accurate and have a higher throughput. The EIA assay correlated with SRID in stability trails over a 6 month period.

Conclusion

We have demonstrated an alternative assay that is suitable for vaccine quantitation and stability assessment. In many cases, this EIA approach would lead to earlier availability of both seasonal and pandemic vaccines and could be standardized between laboratories.

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SPA5 - HOW TO EVALUATE VACCINE EFFECTIVENESS AND EFFICACY?

A503P

Live attenuated influenza vaccine provides comparable protection in children upon initial vaccination and revaccination

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Background:

In the European Union (EU), an Ann Arbor strain live attenuated influenza vaccine (LAIV) was recently approved for eligible children 2 through 17 years of age. In a two-year, double-blind, placebo-controlled study of previously unvaccinated children 12 to 35 months of age, subjects who received 2 doses of vaccine or placebo in year 1 were re-randomized in year 2 to a single dose of vaccine or placebo. This design enabled an assessment of the efficacy of multiple two-year vaccination regimens (year 1/year 2 treatment: LAIV/LAIV, LAIV/placebo, placebo/LAIV). Although the overall study results have been previously described, strain-specific results for year 2 have not. In both study years, there were significant cases of influenza illness caused by A/Panama/2007/99-like (H3N2), a strain antigenically similar to the A/H3N2 component of the vaccine in both years. As a result, it is also possible to compare LAIV efficacy against A/H3N2 across study years.

Objective:

To evaluate the efficacy of LAIV in children against the same influenza strain upon initial vaccination and revaccination.
Methods In year 1, the efficacy of 2 doses of LAIV compared with placebo against culture-confirmed A/H3N2 illness was calculated. In year 2, efficacy against A/H3N2 illness was calculated for the 3 treatment groups (year 1/year 2 treatment: LAIV/LAIV, LAIV/placebo, placebo/LAIV).
Results The efficacy of 2 doses of LAIV in year 1 was 81% (95% CI: 69, 89). In year 2, the efficacy of LAIV/LAIV compared with placebo/placebo was 86% (95% CI: 71, 94), demonstrating sustained efficacy upon revaccination. The efficacy of a single dose of LAIV in year 2 was evaluated in 2 comparisons: in previously unvaccinated children (placebo/LAIV), efficacy compared with placebo/placebo was 58% (95% CI: 25, 77); and in previously vaccinated children (LAIV/LAIV), efficacy compared with LAIV/placebo was 65% (95% CI: 22, 85). Thus a single dose in year 2 had comparable efficacy in previously vaccinated and previously unvaccinated children. Year 2 also enabled evaluation of the second-season efficacy of year 1 vaccination in 2 comparisons: LAIV/placebo efficacy compared with placebo/placebo was 61% (95% CI: 35, 77); and LAIV/LAIV efficacy compared with placebo/LAIV was 67% (95% CI: 24, 87). Thus LAIV vaccination in year 1 continued to contribute to protection in year 2.

Conclusions

In young children, LAIV provided comparable protection against influenza with initial vaccination and revaccination. LAIV also provided two-year protection against influenza illness caused by an antigenically similar strain. These data support the annual use of LAIV in previously unvaccinated and previously vaccinated children. These data also highlight the value of multiyear influenza vaccine efficacy studies with year 2 re-randomization for evaluating efficacy upon initial vaccination and revaccination.
Sponsored by MedImmune, LLC.

YOUNG SCIENTIST

SPA5 - HOW TO EVALUATE VACCINE EFFECTIVENESS AND EFFICACY?

A504P

Modified Vaccinia virus Ankara (MVA): a potent platform for pandemic influenza vaccines

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Introduction

Traditional influenza vaccines contain inactivated viruses and have been around for over 50 years. They form the cornerstone of the seasonal influenza vaccination campaigns that effectively counter-act upon the yearly influenza epidemics. In contrast the establishment of an effective vaccination campaign against a pandemic outbreak of influenza is a complex challenge. Pandemic influenza vaccine development suffers from many hurdles that complicate the production, distribution and timely availability of sufficient amounts of vaccines with good immunogenicity. Several of the these hurdles have been overcome by the use of reverse genetics for vaccine strain construction, the availability of cell-culture based production platforms and the development and registration of several adjuvants. However, there is still room for improvement which was clearly exposed during the vaccination campaign following the pandemic outbreak of new A/H1N1 virus in 2009. Many countries received their ordered vaccines (too) late or did not receive sufficient amounts, while other countries could not afford a large-scale vaccination campaign. To address the hurdles in pandemic influenza vaccine development new vaccine platforms are explored and under development. A promising platform is that of viral vector vaccines such as adenoviral vectors and poxviral vectors. In the last category, Modified Vaccinia virus Ankara (MVA) is a prominent candidate.

MVA is a replication deficient poxvirus, originally tested as safe alternative for the traditional poxvirus vaccine. Due to loss of over 30kb genetic material after serial passages in CEF cells, the deletion sites in the viral genome can be used for the incorporation of foreign genes. This makes MVA an attractive candidate to function as a vector vaccine. It is being evaluated as a vaccine platform for different infectious diseases and as a therapeutic vaccine. These studies are in various phases of preclinical and clinical evaluation. The available data demonstrate that the vector is strongly immunogenic, even in the presence of pre-existing immunity to the vector.

Materials & Methods

In order to function as an influenza vaccine, the hemagglutinin (HA) gene can be cloned in the MVA-genome through homologous recombination. This was done for the H5 gene of influenza virus A/Vietnam/1194/04, a wildtype highly pathogenic avian influenza virus that has been studied extensively in animal models. We have studied the MVA-H5 vaccine in depth in a mouse model and a non-human primate model.

Results

Initially a two dose immunization regimen with MVA-H5 was tested in C57Bl6/J mice and it induced protective antibody responses that resulted in sterile immunity in these animals against the homologous and heterologous strains. As a follow-up study the two dose regimen was tested in cynomolgus macaques in which it proved to be safe and it induced sterile immunity against the homologous and heterologous challenge viruses.

Subsequently, we determined in a dose escalation study in mice the minimal dose to induce protection against challenge infection and explored the possibility to induce protection with a single immunization. Mice that were immunized once with a relatively high dose ($10e^8$ pfu) or twice with a low dose ($10e^5$ pfu) developed antibodies and were protected against challenge infection with the homologous or heterologous influenza A/H5N1 virus.

Conclusions

Thus MVA-H5 is a safe and strongly immunogenic vaccine and, in combination with the other favourable properties of MVA , it is a promising candidate as a future pandemic influenza vaccine platform.

SPA5 - HOW TO EVALUATE VACCINE EFFECTIVENESS AND EFFICACY?

A505P

Influenza vaccine effectiveness in the valencian community (spain) in 2010 – 2011 season

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Background :

Seasonal flu vaccination campaign in the Valencian Community started on the 40/2010 week (September 27 to October 3), 8 weeks prior to the epidemic wave (48/2010 to 7/2011 weeks). This campaign is addressed to risk groups between 6 months and 59 years of age, and all the adults over 60 years of age.

The surveillance system used to monitor the seasonal influenza collects the data from the cases declared with suspected influenza through the electronic medical records (SIA), with the aim of knowing the vaccine status of each case, the system also shares information with the Vaccine Information System (SIV).

Methods

In order to estimate the influenza vaccine effectiveness in the 2010 – 2011 season, we used the screening method (comparing the vaccine proportion between the cases and the population), all the suspected cases reported by SIA during the epidemic wave were included. We used Epidat 3.1 for the statistical analysis.

Results

The adults over 64 years of age had the highest vaccine coverage (52%), followed by 15 to 64 years of age (6%), the latter was the group of 14 years of age (2.6%).

A total of 44,069 cases were notified with suspected influenza, 1,798 (4%) of them were vaccinated during the campaign.

The vaccine effectiveness is higher among adults, particularly the group over 64 years of age (table 1).

Table 1. Cases notified by SIA from 48/2010 to 7/2011 weeks, relative risk and vaccine effectiveness

	Vaccinated		No Vaccinated			
Age (years)	Cases	Rate per 10^5	Cases	Rate per 10^5	**RR (95% CI)**	**VE % (95% CI)**
<4	98	37.79	4,430	1708.1	0.84 (0.68 ; 1.84)	17 (-0.84 ; 32)
5 a 14	247	52	12,349	2599.75	0.74 (0.66 ; 0.85)	25 (15 ; 34)
15 a 64	902	25.91	24,141	693.33	0.57 (0.53 ; 0.61)	43 (39 ; 47)
≥ 64	551	67.74	1,351	166.1	0.46 (0.34 ; 0.41)	62 (59 ; 66)

Discussion :

The vaccine effectiveness is age-dependent, in other words, it increases progressively with the age. This is because the vaccine coverage is higher with the age; therefore the cases younger than 4 years of age have lower vaccine coverage, and higher incidence rate.

On the other hand the vaccine effectiveness in adults, over 64 years of age particularly, has higher effectiveness; this is because they have higher vaccine coverage.

The vaccine coverage variability by age groups is because in younger than 60 years of age tackles only to risk groups and is not reaching to all the vulnerable population.

SPA5 - HOW TO EVALUATE VACCINE EFFECTIVENESS AND EFFICACY?

A506P

Safety and immunogenicity of a quadrivalent inactivated influenza vaccine (QIV) containing two A and two B strains among persons >=65 years of age

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Introduction

Two distinct B influenza lineages (Victoria and Yamagata) have co-circulated worldwide for over a decade, making it difficult to predict which will predominate during a given season. Consequently, the B strain selected in the spring for trivalent inactivated influenza vaccine (TIV) often fails to match the B strain that predominates in the fall. Among elderly persons, type B is the second most common cause of influenza-related morbidity and mortality after H3N2. QIV has been developed to address the frequent mismatches by incorporating a strain from each B lineage. A previous study demonstrated the safety and immunogenicity of QIV in adults < 65 years of age. This study was designed to evaluate QIV in an older population.

Materials & Methods

In a double-blind, controlled, multicenter study, 675 persons ≥ 65 years of age were randomized 1:1:1 to receive one intramuscular dose of 2010-2011 TIV (containing B/Brisbane/60/2008 [Victoria lineage]), investigational TIV (containing B/Florida/04/2006 [Yamagata lineage]), or QIV (containing both B strains). All 3 vaccines contained the same H1N1 (A/California/07/2009) and H3N2 (A/Victoria/210/2009) strains. Safety was monitored for 21 days after vaccination. Blood specimens for immunogenicity (hemagglutination inhibition [HI] assay) were collected pre- and 21 days post-vaccination.

Results

Mean age: 72.4 to 72.8 years; female: 53.8% to 57.3%; White: 87.6% to 91.1%. Post-vaccination geometric mean titers (GMTs; 1/dil), seroprotection rates (≥1:40; SP), and seroconversion rates (4-fold rise; SC) are shown below.

	QIV (N=220)				2010-2011 TIV (N=219)			Investigational TIV (N=221)		
	H1N1	H3N2	B1	B2	H1N1	H3N2	B1	H1N1	H3N2	B2
GMT	231	501	73.8	61.1	269	291	57.9	271	360	54.8
SP (%)	91.4	100.0	77.7	73.2	91.3	95.4	71.7	91.9	95.9	67.4
SC (%)	65.9	69.1	28.6	33.2	66.7	55.7	18.7	72.9	62.9	31.2

B1: B/Brisbane/60/2008 [Victoria lineage]; B2: B/Florida/04/2006 [Yamagata lineage]
The HI antibody response to each A and B strain in QIV was non-inferior to the response with each respective strain in TIV (data from the 2 TIVs pooled for the A strains) based on GMT ratios (QIV/TIV: H1N1, 0.85; H3N2, 1.55; B1, 1.27; B2, 1.11). Seroconversion rates were non-inferior based on differences in rates for H3N2 and the 2 B strains, but not for H1N1 (QIV minus TIV: H1N1, -3.89%; H3N2, 9.77%; B1, 9.91%; B2, 1.96%). When comparing QIV to the TIV not containing the respective B strain, GMTs were significantly higher for B1 and superior for B2 (QIV/TIV: B1, 1.75; B2, 2.14) and seroconversion rates were superior for both B1 and B2 (QIV minus TIV: B1, 20.0%; B2, 24.1%).

Solicited injection-site reactions were reported at comparable rates after QIV (33.5%), 2010-2011 TIV (29.5%), and investigational TIV (24.0%); solicited systemic reactions also were reported at similar rates in all 3 groups (QIV, 24.6%; 2010-2011 TIV, 24.1%; investigational TIV, 20.9%). Unsolicited events occurring within approximately 21 days after vaccination were reported at similar rates in all 3 groups (QIV, 12.4%; 2010-2011 TIV, 10.7%; investigational TIV, 10.2%). None of 3 serious adverse events was considered related to any vaccine.

Conclusions

These data, together with data from a similar previous study in younger adults, indicate that the addition of a second B lineage strain to influenza vaccine does not adversely affect the safety or immunogenicity profile of QIV compared with TIV. QIV has the potential to be a useful alternative to TIV and offers the possibility of protection against both B lineages, including among older adults who experience high rates of complications and mortality due to influenza.

SPA5 - HOW TO EVALUATE VACCINE EFFECTIVENESS AND EFFICACY?

A507P

Impact of intranasal live attenuated influenza vaccine on influenza-associated economic burden in European and Israeli children

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Background:

An intranasal live attenuated influenza vaccine (LAIV) was recently approved in the European Union for eligible children 2 through 17 years of age. Three previous randomized studies evaluated LAIV efficacy in children in Europe and Israel. Study 1 examined LAIV versus placebo in 2000–2002 in 1784 children 6–35 months of age attending daycare; study 2 examined LAIV versus trivalent inactivated influenza vaccine (TIV) in 2002–2003 in 2187 children 6-71 months of age with recurrent respiratory tract infections; study 3 examined LAIV versus TIV in 2002–2003 in 2229 children 6–17 years of age with asthma. Although primary analyses of safety and efficacy have been reported for each study, prospectively collected economic burden outcomes associated with influenza illness have not been described.

Objective:

To describe the impact of LAIV versus placebo and TIV on influenza-associated economic burden in European and Israeli children

Methods

In the studies, multiple economic burden outcomes were collected for all symptomatic respiratory illnesses after illness resolution. Influenza was confirmed by viral culture. Incidence among LAIV versus placebo or TIV recipients was compared for each economic burden endpoint collected.

Results

In study 1 (6–35 months of age), the influenza illness incidence for placebo vs LAIV recipients was 13.4% vs 1.9% and 30.9% vs 4.4% in years 1 and 2, respectively, yielding vaccine efficacies of 86% (95% CI: 76, 92) and 86% (95% CI: 79, 91). The incidence of influenza-associated missed daycare, missed parental work, any medication or antibiotic use, any medication use, any antibiotic use, and unscheduled provider visits among each group in year 1 was 10.5% vs 1.7%*, 7.2% vs 0.8%*, 11.6% vs 1.6%*, 9.3% vs 0.9%*, 6.6% vs 0.7%*, and 8.6% vs 1.2%*, respectively, and in year 2 was 27.3% vs 3.6%*, 12.9% vs 1.3%*, 27.6% vs 4.1%*, 26.7% vs 4.1%*, 11.8% vs 1.4%*, and 10.4% vs 0.8%*, respectively (*=statistically significant). In years 1 and 2, LAIV recipients missed 272 and 923 fewer days of daycare per 1000 children than placebo recipients (P<0.001); parents of LAIV recipients missed 185 and 319 fewer days of work per 1000 children (P<0.001).

In study 2 (6–71 months of age), the incidence of influenza illness was 5.8% vs 2.8% for TIV vs LAIV recipients, respectively, for a relative efficacy of 52% (95% CI: 30, 68). The incidence of influenza-associated missed daycare/school, any medication or antibiotic use, any medication use, any antibiotic use, unscheduled provider visits, and overnight hospitalizations among each group was 4.4% vs 1.4%*, 4.7% vs 2.2%*, 4.5% vs 2.2%*, 1.8% vs 0.4%*, 0.9% vs 0.6%, and 0.1% vs 0.0%, respectively. LAIV recipients missed 140 fewer days of daycare/school per 1000 children than TIV recipients (P<0.001).

In study 3 (6–17 years of age with asthma), the incidence of culture-confirmed influenza illness was 6.6% vs 4.5% for TIV vs LAIV recipients, respectively, for a relative efficacy of 32% (95% CI: 1, 54). The incidences of influenza-associated missed school/work, any medication or antibiotic use, any medication use, any antibiotic use, unscheduled provider visits among each group were 5.8% vs 3.7%*, 5.1% vs 3.1%*, 4.5% vs 2.9%, 1.6% vs 1.0%, and 1.1% vs 0.7%, respectively; there were no influenza-associated hospitalizations. LAIV recipients missed 76 fewer days of school/work per 1000 children than TIV recipients(P=0.02).

Conclusions

In European and Israeli children, LAIV reduced influenza illness and the associated economic burden relative to placebo and TIV. These data highlight the potential public health benefit of LAIV in eligible European and Israeli children and may facilitate future economic modeling of the impact of the LAIV relative to TIV.

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SPA5 - HOW TO EVALUATE VACCINE EFFECTIVENESS AND EFFICACY?

A508P

Efficacy of live attenuated seasonal and pandemic influenza vaccine in school-age children: A randomized controlled trial

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Background:

A novel pandemic influenza A(H1N1) virus emerged in North America in early 2009 and rapidly spread worldwide. Monovalent pH1N1 vaccines were licensed later in 2009 based on preliminary studies demonstrating their immunogenicity and safety. In this study we report the efficacy of live attenuated monovalent pH1N1 vaccine and 2009-10 seasonal influenza vaccine in a randomized double-blind placebo-controlled trial.

Methods

We enrolled 703 children aged 7-11 from 27 primary schools in Hong Kong. Each child was randomly allocated in the ratio 3:2 to receive one dose of live attenuated monovalent pH1N1 vaccine or saline placebo between November 2009 and January 2010, followed after 3-10 weeks by one dose of live attenuated trivalent 2009-10 seasonal influenza vaccine or saline placebo in the same ratio. Children were followed up through September 2010 with biweekly telephone calls and symptom diaries. Seasonal and pandemic influenza infections were confirmed by virologic testing by RT-PCR of nose and throat swabs collected during acute respiratory illness episodes. ClinicalTrials.gov number: NCT00981513.

Results

Overall, 30 children had confirmed influenza including 3 (0.43%) pH1N1, 10 (1.4%) seasonal A(H3N2), and 17 (2.4%) influenza B. There were no significant differences in incidence rates of pH1N1, A(H3N2), acute respiratory illness or influenza-like illness between study arms, but receipt of the seasonal influenza vaccine was associated with a significant reduction in risk of influenza B ($p<0.001$). Vaccine efficacy against confirmed pH1N1 infection associated with receipt of the monovalent pH1N1 vaccine was 65% (95% confidence interval, CI: -213, 94). Vaccine efficacies against confirmed seasonal influenza A(H3N2) and B infection associated with receipt of the seasonal influenza vaccine were 31% (95% CI: -124, 79) and 93% (95% CI: 67, 99) respectively. No serious adverse events were reported following vaccination, and the only statistically significant differences in frequency of reported events between vaccines and placebo were in headache after pH1N1 vaccine, and nasal congestion after seasonal vaccine.

Conclusions

Vaccine efficacy was consistent with other studies of the monovalent pH1N1 vaccine and seasonal influenza vaccines. Lower vaccine efficacy against seasonal A(H3N2) was likely associated with substantial antigenic drift in the variant A/Perth/16/09 (H3N2)-like virus that was prevalent in Hong Kong during the study period. Our study was underpowered to provide precise estimates of vaccine efficacy due to low incidence of influenza A viruses during the study period.

SPA5 - HOW TO EVALUATE VACCINE EFFECTIVENESS AND EFFICACY?

A509P

Immunogenicity and safety of intradermal influenza vaccinein adults and elderly in Korea

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Introduction

The immune response in the elderly is comparatively lower than in younger adults, highlighting the need for more immunogenic vaccines for this population. Reports have shown the intradermal (ID) route is highly effective for various vaccines. We performed this study to evaluate immunogenicity and safety of ID influenza vaccine (IDflu™) in Korea.

Material & Methods

This study was a multi-center open-label randomized controlled trial conducted in 6 centers in Korea (ClinicalTrial.gov number. NCT01215669.). Total 240 subjects were randomized in 4 groups with each 60 subjects; Group 1: 18 to 59 years were vaccinated with the IDflu™ 9 μg; Group 2: 18 to 59 years were vaccinated with the intramuscular (IM) Vaxigrip™ 15 μg; Group 3: 60 years and over were vaccinated with the IDflu™ 15 μg; Group 4: 60 years and over were vaccinated with the Vaxigrip™ 15 μg, Northern Hemisphere 2010-2011 formulation. All subjects received a single dose of vaccine, and provided blood samples for immunogenicity assessment at pre-vaccination and at the 21th day post-vaccination. Immunogenicity was evaluated using the hemagglutination inhibition (HAI) antibody test. The three criteria were evaluated as defined in the recommendations as per EMA guidance.

Results

In the 18 to 59 years group, three criteria were met for each of the three strains (A/H1N1, A/H3N2 and B) in the group administered with the 9 μg IDflu™, and in the group administered with the 15 μg Vaxigrip™ (Table 1). In the 60 years or older group, three criteria were met for each of the three strains in the group administered with the 15 μg IDflu™, and in the group administered with the 15 μg Vaxigrip™ (Table 2). No particular safety concern was raised during the study.

Conclusions

The ID influenza vaccine had the good immunogenicity and safety comparable with IM route in also both adults and elderly in Korea. Because the lower dose has the similar efficacy compared with IM vaccine in adults and the method of injection is convenient, the IDflu™ can be used with good compliance.

Table 1. Summary of HAI Antibody Response for Each Strain – 18 to 59 Years Group.

	18 to 59 years 9µg ID (IDflu™)				18 to 59 years 15µg IM (Vaxigrip™)			
	EMA requirements	H1N1	H3N2	B	EMA requirements	H1N1	H3N2	B
Seroprotection (≥40 [1/dil]): n(%) (95% CI)	>70%	55 (91.7) (81.6; 97.2)	60 (100.0) (94.0; 100.0)	60 (100.0) (94.0; 100.0)	>70%	60 (100.0) (94.0; 100.0)	59 (98.3) (91.1; 100.0)	60 (100.0) (94.0; 100.0)
Geometric mean of individual ratio (95% CI)	>2.5	22.8 (14.8; 35.1)	32.4 (20.9; 50.1)	5.4 (3.6; 8.0)	>2.5	29.3 (19.8; 43.5)	23.8 (15.1; 37.5)	6.6 (4.6; 9.6)
Seroconversion rate or significant increase: n (%) (95% CI)	>40%	49 (81.7) (69.6; 90.5)	52 (86.7) (75.4; 94.1)	31 (51.7) (38.4; 64.8)	>40%	55 (91.7) (81.6; 97.2)	52 (86.7) (75.4; 94.1)	36 (60.0) (46.5; 72.4)

Table 2. Summary of HAI Antibody Response for Each Strain – 60 Years or Older Age Group.

	≥ 60 years 15 µg ID (IDflu™)				≥ 60 years 15 µg IM (Vaxigrip™)			
	EMA requirements	H1N1	H3N2	B	EMA requirements	H1N1	H3N2	B
Seroprotection (≥40 [1/dil]): n(%) (95% CI)	>60%	57 (95.0) (86.1; 99.0)	60 (100.0) (94.0; 100.0)	60 (100.0) (94.0; 100.0)	>60%	53 (88.3) (77.4; 95.2)	58 (96.7) (88.5; 99.6)	60 (100.0) (94.0; 100.0)
Geometric mean of individual ratio (95% CI)	>2.0	13.1 (9.3; 18.5)	19.0 (12.8; 28.3)	6.7 (4.7; 9.7)	>2.0	25.0 (16.0; 39.0)	24.7 (15.2; 40.1)	7.0 (4.8; 10.0)
Seroconversion rate or significant increase: n (%) (95% CI)	>30%	51 (85.0) (73.4; 92.9)	50 (83.3) (71.5; 91.7)	38 (63.3) (49.9; 75.4)	>30%	48 (80.0) (67.7; 89.2)	48 (80.0) (67.7; 89.2)	39 (65.0) (51.6; 76.9)

SPA5 - HOW TO EVALUATE VACCINE EFFECTIVENESS AND EFFICACY?

A510P

Meta-analysis of the efficacy of intranasal live attenuated influenza vaccine in children 2 through 17 years of age

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Background:

In the European Union (EU), an intranasal live attenuated influenza vaccine (LAIV) was approved for eligible children 2 through 17 years of age in 2011. Nine randomized controlled clinical trials, including approximately 25,000 children aged 6–71 months and 2,000 children aged 6–17 years, have evaluated the efficacy of LAIV against culture-confirmed influenza illness compared with placebo or trivalent inactivated vaccine (TIV) in children. However, these data have not been collectively analyzed for children 2–17 years of age, the age group for whom LAIV is approved for use in the EU.

Objective:

To evaluate the efficacy of LAIV in children 2–17 years of age, using data from all available randomized, controlled clinical trials.

Methods

The meta-analysis was conducted on the per-protocol population using a fixed effects model. A log binomial model was used to calculate LAIV relative risk adjusting for study variation. LAIV efficacy relative to placebo and TIV were calculated as one minus the adjusted relative risk (RR) of culture-confirmed influenza in LAIV recipients relative to placebo and TIV recipients, respectively. The 95% confidence interval (CI) of LAIV efficacy was constructed from the 95% CI of the adjusted RR. Because classification of drifted influenza B viruses varied across studies, cases caused by drifted influenza B were analyzed in two manners: as originally classified by the studies and classifying all antigenic variants as dissimilar.

Results

The meta-analysis included 5 placebo-controlled studies (4 of which were two-season studies) and 3 single-season TIV-controlled studies that were conducted between 1996 and 2005. Compared with placebo, the efficacy of 2 doses of LAIV in previously unvaccinated children in year 1 was 83% (95% CI: 78, 87) against antigenically similar strains and 79% (95% CI: 73, 83) against all strains regardless of antigenic match. Efficacy for similar strains was 87% (95% CI: 78, 93), 86% (95% CI: 79, 91), and 76% (95% CI: 63, 84) for A/H1N1, A/H3N2, and B respectively. With drifted B strains classified as dissimilar, efficacy against similar B strains increased to 93% (95% CI: 83, 97) and overall efficacy against all similar strains increased to 87% (95% CI: 83, 91). Year 2 efficacy compared with placebo was 87% (95% CI: 82, 91) against similar strains and 78% (95% CI: 72, 82) against all strains. Compared with TIV, LAIV recipients had 44% (95% CI: 28, 56) and 48% (95% CI: 38, 57) fewer cases of influenza illness caused by similar strains and all strains regardless of match, respectively. Opposite-lineage influenza B strains, which circulated to varying degrees across studies, were a principal cause of reduced efficacy compared with placebo against all influenza strains. LAIV efficacy estimates relative to placebo and TIV for only those subjects from European countries were robust and were similar to or higher than those observed overall.

Conclusions

In children 2 through 17 years of age, LAIV has demonstrated high efficacy following 2 doses in year 1 and with a single revaccination dose in year 2, as well as greater efficacy compared with TIV. This meta-analysis gives more precise estimates of LAIV efficacy among children 2 through 17 years of age, the age group for whom the vaccine is approved for use in the EU.

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SPA5 - HOW TO EVALUATE VACCINE EFFECTIVENESS AND EFFICACY?

A511P

Effectiveness and safety of H1N1 adjuvanted inactivated split-virion pandemic influenza vaccine in HIV-1 infected patients

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Introduction

Influenza is a common respiratory infection which significantly contributes to morbidity and even mortality of HIV-infected individuals. Weakened immunity in these patients often causes prolonged replication and long-term shedding of the influenza virus (weeks to months) which may facilitate the evolution of oseltamivir-resistant strains. As vaccination against seasonal influenza proved to be effective in several studies, annual vaccination is generally recommended in HIV-infected patients. The emergence of the 2009 H1N1 pandemic influeza virus has raised concerns about the immonogenicity and safety of newly developed pandemic vaccines, especially in immunocompromised individuals. In our study, we evaluated the effectiveness and safety of an adjuvanted pandemic influenza vaccine in 34 HIV-1 infected patients.

Material and Methods

HIV-1 infected patients over the age of 18 years who had given informed consent were enrolled in the study. They were vaccinated with adjuvanted inactivated split-virion pandemic H1N1 influenza vaccine, Pandemrix®, containing 3.75 µg of hemagglutinin of A/California/7/2009 (H1N1) and adjuvant AS03, between December 4, 2009 and January 7, 2010. Two serum samples were collected from each patient - one on day 0 just before vaccination and the other on day 30±2 after vaccination.

Antibody production was tested by haemagglutination inhibition assay according to the WHO methodology. Strain A/Praha 196/09_{pdm} identified using the WHO standard serum (A/California 7/2009, kindly provided by the WHO collaborating center, NIMR, UK) and further specified by sequence analysis served as the antigen.

The titres of protective antibodies were quantified and evaluated according to the standard criteria of the European Medicines Agency´s Committee for Medicinal Products for Human Use (CHMP) for influenza vaccines (adults aged 18 - 60 years):

1. seroconversion rate – fourfold or higher increase in antibody titres between pre-vaccination and post-vaccination sera in at least 40% of the vaccinees.
2. seroprotection rate – post-vaccination titre of 1:40 or more in at least 70% of the vaccinees.
3. conversion factor – ratio of the post-vaccination geometric mean titre to the pre-vaccination geometric mean titre of 2.5 or more.

Results

Thirty-four HIV-1 infected patients were included in the study - 33 males (97%) and 1 female at the median age of 42 years (age range 27-71). The average CD4+ count was 511/µl (range 90-1151) and the median nadir CD4+ count was 337/µl (range 6-673). Twenty-six patients (76.5%) were on combination antiretroviral therapy and 23 (88.5%) of them achieved complete viral suppression. Five subjects had low titres of antibodies (<1:40) in the pre-vaccination sera. After vaccination, protective titres 1:40 or higher were found in 24 (70.5%) vaccinees and at least fourfold increase in antibody titers was detected in 29 vaccinees (85.2%). Seroconversion was not observed in 4 vaccinees (11.2%) whose antibody titres remained undetectable. The conversion factor reached 25.1. When additionally screened for antibodies 10 months after vaccination, three of four vaccinees still had antibody titres above the protective level.

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No serious adverse event was observed among the vaccinees, nine individuals (37.5%) only reported moderate pain at the application site lasting one to two days.

Conclusions

The production of protective antibodies after vaccination of HIV-1 infected individuals with a single dose of adjuvanted inactivated split-virion pandemic H1N1 influenza vaccine fully met the criteria set by CHMP for the healthy adult population. The vaccine was well tolerated, with no serious adverse events reported. It can even be expected that the protective effect will last throughout the duration of increased circulation of influenza viruses in the population. The adjuvanted inactivated split-virion pandemic H1N1 influenza vaccine proved to be effective and safe in HIV-1 infected patients.

SPA5 - HOW TO EVALUATE VACCINE EFFECTIVENESS AND EFFICACY?

A512P

Influenza vaccine effectiveness against laboratory confirmed A(H1N1)2009 infection during 2010-11season in Spain

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Aims

The recommended trivalent vaccine for the 2010-11 season included the A(H1N1) strain similar to the monovalent 2009 pandemic vaccine, in addition to the AH3 and B strains. The A(H1N1)2009 predominantly circulated until the peak of the 2010-11 epidemic wave (week 02/2011) with an increased contribution of influenza B virus since then. The influenza vaccine effectiveness (IVE) has been estimated since the 2008-09 season in Spain, using the case-control design (cycEVA). The objective of this study was to estimate the IVE against laboratory confirmed influenza A(H1N1)2009

Methods

We conducted two studies using the case-control test-negative design: an observational study (cycEVA) in 8/17 sentinel networks of the Spanish Influenza Sentinel Surveillance System (SISSS) and another one surveillance–based study (SISSS-based study) with all swabbed patients notified to SISSS. In both studies, we included influenza-like illness (ILI) patients attended by a sentinel general practitioner (GP) from week 50/2010 to 12/2011. Cases were ILI laboratory-confirmed for A(H1N1)2009 influenza virus. Controls were ILI testing negative for any type influenza. In the SISSS based study, beside the demographic and laboratory data, GPs collected information on 2010-11 seasonal vaccination, chronic conditions and pregnancy. In the cycEVA study, sentinel physicians collected additional information: influenza vaccination status for the previous season (seasonal and pandemic vaccines), smoker status, functional status, any hospitalisation for chronic conditions in the previous year and the number of outpatient visits for any reason in the previous year. We estimated adjusted odds ratios (OR) using logistic regression and computed IVE as (1-OR)*100. We included in the regression model those variables which changed the crude OR by >10%. We first conducted the analysis including all patients swabbed less than eight days, and then for those eligible for vaccination. We also restricted the analyses to patients swabbed less than four days after onset of symptoms. To check the effect of being vaccinated with both vaccines (seasonal 2010-11 and monovalent 2009 pandemic) we also carried out the analysis using a categorical variable for vaccination

Results

A total of 1160 cases and 1315 controls were included in the SISSS-based study and 570 cases and 581 controls in the cycEVA study. We identified 64 vaccinated cases in the SISSS-based study and 23 in cycEVA study. In the cycEVA study, the adjusted IVE was 50% (8;73) for all patients and 49% (-10;77) for patients swabbed less than four days. We obtained similar estimates in population eligible for vaccination, 51% (0;77) and 52% (4;76) respectively. In patients swabbed less than four days receiving only the 2010-11 trivalent vaccine, the adjusted IVE was 52% (7;76) and 74% (11;93) when both vaccines were received. We found similar results in population eligible for vaccination: 52% (4;76) and 51% (0;77), respectively. IVE preliminary results in the SISSS-based study, for patients swabbed less than four days, were in line with cycEVA study: 55% (37, 68) for all cases and 67% (35;83) in those eligible for vaccination

Discussion/Conclusion

We observed a protective effect of trivalent 2010-11 vaccination against laboratory confirmed A(H1N1)2009, lower than reported for the monovalent 2009 pandemic vaccine. Vaccination with both vaccines conferred a better protection. Similar results were obtained in population eligible for vaccination. In its third edition, cycEVA study was able to provide information about the protective effect of the influenza vaccine, useful to support decision-making in public health. Preliminary IVE estimates from the SISSS-based study are in the same line with the cycEVA ones. However, we further need to validate the utilization of surveillance data for IVE estimates, in order to include it as a component of the influenza surveillance in Spain

No conflict of interest

SPA5 - HOW TO EVALUATE VACCINE EFFECTIVENESS AND EFFICACY?

A513P

An analysis of target recipient groups for monovalent 2009 pandemic influenza vaccine and trivalent seasonal influenza vaccines in 2009-10 and 2010-11

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Introduction

Vaccination is generally considered to be the best primary prevention measure against influenza virus infection. Many countries encourage specific target groups of people to undertake vaccination, often with financial subsidies or a list of priority. To understand differential patterns of national target groups for influenza vaccination before, during and after the 2009 influenza pandemic, we reviewed and identified changes in national target groups for trivalent seasonal influenza and the monovalent 2009 pandemic influenza vaccines during 2009-10 and 2010-11.

Methods

We searched for information on national policies from websites and electronic press releases of health related governmental authorities, the published literature, and other secondary sources. Search terms in English and the official language of the countries were used. Target groups were identified as groups prioritized or subsidized to receive the influenza vaccines. Agreement statistics as measured by the AC1 metric were computed to measure the consistency of groups targeted to receive the 2009 and 2009-10 trivalent seasonal influenza (S0910) vaccines, the 2009 monovalent pandemic influenza vaccine (P0910) and the 2010 and 2010-11 trivalent seasonal influenza (S1011) vaccines.

Results

We identified target groups for 33 (S0910 vaccine), 72 (P0910 vaccine) and 34 (S1011vaccine) countries. Many countries prioritized the elderly (97%), those with chronic conditions (91%) and health care workers (70%) to receive the S0910 vaccine. Fewer countries prioritized the elderly (17%), close contacts (22%), animal contacts (3%), care home residents (1%) and care home workers (15%) to receive the P0910 vaccine compared to the S0910 vaccine while pregnant women (90%), obese persons (28%), health care workers (92%) and essential community workers (38%) were more commonly prioritized. Comparing the S0910 and P0910 vaccines, there was lowest consistency in recommendations for elderly to receive vaccine (AC1 -0.66, 95%CI -0.93, -0.36). Recommendations were generally very consistent between the S0910 and S1011 vaccines except in pregnant women, obese persons, close contacts of vulnerable individuals, and care home residents and workers. Subsidy was commonly offered in the form of free vaccine, partial subsidy, reimbursement or nation health insurance coverage. Groups subsidized were generally those prioritized to receive influenza vaccine for the seasonal influenza vaccines, over one-third of the countries provided universal subsidy to their population to receive the P0910 vaccine.

Conclusions

Differences in recommended groups between countries reflect variable objectives as well as uncertainties regarding the differential transmission dynamics, severity and mechanisms of immunity. During 2009-10, the elderly, close contacts, animal contacts, and care home residents and workers were less commonly targeted to receive the P0910 vaccine compared to the S0910 vaccine. However, the pregnant women and obese persons (who were identified as being at high risk of severe disease early in the 2009 pandemic), health care workers and essential community workers were increasingly targeted. These changes appeared to last after the pandemic in targeting the pregnant, obese, health care workers, close contacts, and care home residents and workers to receive the S1011 vaccine.

SPA5 - HOW TO EVALUATE VACCINE EFFECTIVENESS AND EFFICACY?

A514P

The physical basis for the conformational selectivity of single radial immunodiffusion-based influenza vaccine release assays

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The content of antigenically intact hemagglutinin (HA) in subunit influenza vaccines is determined by single-radial immunodiffusion (SRID) of Zwittergent-treated samples in agarose. Producing sheep antisera for SRID can delay vaccine release after seasonal or pandemic strain changes. More rapid alternative assays based on denaturing techniques cannot distinguish properly folded from mis-folded HA. We investigated the basis for the conformational selectivity of SRID. Sheep antisera have limited ability to distinguish denatured from folded HA, but denatured HA does not diffuse into an agarose gel, even in the presence of Zwittergent and the absence of antibody. Thus, in SRID sieving of Zwittergent-resistant HA separates misfolded from properly folded HA. Ultrafiltration also can remove aggregates of denatured HA. Coupling rapid sieving techniques to physical methods to determine HA identity and quantity can provide more rapid, conformation-sensitive release assays and relieve a bottleneck to the rapid distribution of pandemic and seasonal influenza vaccines.

SPA5 - HOW TO EVALUATE VACCINE EFFECTIVENESS AND EFFICACY?

A515P

Influenza vaccine induced antibody response against B influenza viruses included in the vaccine or naturally circulating during the winter season 2010/2011

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Introduction

The possibility of preventing influenza B virus infections by immunization with influenza vaccine can be particularly problematic since: a) there are two distinct evolutionary lineages (B/Yamagata and B/Victoria);
b) many reports evidenced heterogeneity among influenza B viruses circulating in the same epidemic season;
c) the antibody response to inactivated trivalent vaccines might be substantially lower against the B antigen than against type A antigen.
The aim of the study was to evaluate the immune response induced by influenza vaccine administration in elderly institutionalized people against different B influenza viruses, the vaccine strain or naturally circulating viruses in the winter season 2010/2011.

Materials and methods

Study population: 112 elderly (≥65 years) people living in two nursing homes in Umbria, Italy, were immunized with one dose of 2010/2011 trivalent inactivated MF59-adjuvanted subunit influenza vaccine (FLUAD, NOVARTIS) (A/Perth/16/09, H3N2; A/California/7/09, H1N1; B/Brisbane/60/08) in November 2010.
Viruses: the antibody response elicited by influenza vaccine was evaluated against different egg- or cell-grown influenza B viruses. The circulation of influenza viruses was monitored examining throat swabs collected from people with influenza like illness (ILI) using reverse-transcriptase-polymerase chain reaction (RT-PCR) and/or cultivation in Madin-Darby Canine Kidney (MDCK) cells. Antigenic characterization was performed by haemagglutination inhibiting (HI) test with specific post-infection sera. Genetic characterization was carried out by sequencing the HA1 domain of hemagglutinin.
Vaccine immunogenicity: vaccine immunogenicity was evaluated measuring HI antibodies titres in sera collected before (day 0) and one month (day 30) after immunization.

Results

The local influenza virus circulation was monitored in the region where the two nursing home were located examining a total of 223 throat swabs collected from people with ILI from week 47/2010 to week 17/2011. Sixty six (29.6%) were influenza positive and, among them, 40 were found to be A/H1N1 pandemic (60.6%), 24 B (36.4%) and 2 not sub-typed A (3.0%) viruses. A selected number of B virus isolates were antigenically and genetically characterized and found to be closely related to the corresponding B/Victoria-like vaccine strain of the 2010/2011 vaccine. The antibody response induced by the 2010/2011 influenza vaccine was evaluated against the B egg-grown 2010/2011 vaccine strain (B/Brisbane/60/08), four locally isolated cell-grown B/Victoria-like strains (B/Perugia/1/11; B/Perugia/3/11; B/Perugia/11/11; B/Perugia/22/11) and against an egg-grown B/Yamagata-like virus (B/Bangladesh/3333/07).
The results obtained showed that the pre-vaccination and post-vaccination HI titres against all the different B viruses examined were in most instances similar. Statistically significant increases in the numbers of seroprotected people (HI titres ≥40) and in the values of geometric mean titres (GMT) were found against all B viruses, except for seroprotection against B/Perugia/11/11. Examining the post-vaccination responses according to the criteria of the European Commission for elderly people, the values of seroprotection were higher or slightly lower than the required 60% (range 58.0-76.8%). On the contrary the requirements of mean fold increase (MFI) of GMT (≥2) and of seroconversions (≥30%) were not satisfied. The MFI ranged 1.4-1.7 and the percentages of seroconversions 9.8-19.6%.

Conclusions

The results obtained evidenced the ability of the 2010/2011 MF59-adjuvanted subunit influenza vaccine to elicit a broad antibody immune response both against B/Victoria-like viruses (the vaccine strain and three naturally circulating B viruses) and against one B/Yamagata-like virus. However, in accordance with previous data, the immune response induced against B vaccine and other different B strains was not adequate.

SPA5 - HOW TO EVALUATE VACCINE EFFECTIVENESS AND EFFICACY?

A516P

Effectiveness of vaccination against pandemic influenza A/H1N1 in a cohort of chronically ill individuals in Denmark

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The present study is the first nationwide register based cohort study analysing pandemic vaccine effectiveness (VE) in individuals with underlying illness.

The aims were to determine VE of an adjuvanted monovalent vaccine against laboratory confirmed infection with pandemic influenza A/H1N1 and influenza-related hospital admission caused by confirmed pandemic influenza A/H1N1 among all Danish individuals < 65 years of age with underlying illness.

A cohort of 388,069 individuals with at least one hospital discharge within the past five years with an illness that was expected to increase the risk of severe influenza illness was established and followed from 2 November 2009 to 31 January 2010. Information on 2009-2010 seasonal influenza vaccine status, pandemic vaccination status, vital status, laboratory confirmed influenza A/H1N1 infection, and influenza related admission to hospitals was obtained from national registers and linked to the cohort using a unique personal identifier.

Pandemic VE was estimated in a Cox proportional hazards model with pandemic vaccination status as time-dependent variable and age-group, sex, number of different chronic diagnoses, 2009-2010 seasonal vaccination status and an interaction between pandemic and seasonal vaccine status included as co-variates.

In the cohort, 79,988 (20.6%) received the pandemic vaccine while 49,435 (12.7%) received the 2009-2010 seasonal influenza vaccine and 799 individuals had a laboratory confirmed influenza A/H1N1 infection. VE against confirmed influenza A/H1N1, 15 days after one dose was 58% (95% CI, 5% to 81%) in individuals who received the 2009-2010 seasonal influenza vaccine and 54% (95% CI, 2% to 78%) after pandemic vaccine only. During the first 14 days after receiving the pandemic vaccine there was a significant difference in VE between seasonal vaccinated and non-seasonal vaccinated (P=0.036). VE 1-7 days after receiving the pandemic vaccine was 10% (95% CI, -66% to 51%) in individuals who received the seasonal influenza vaccine and -110% (95% CI, -199% to -48%) after pandemic vaccine only. In the cohort 229 individuals were admitted to hospitals due to confirmed pandemic influenza A/H1N1. VE against influenza A/H1N1 related hospitalisation 15 days after one dose was 65% (95% CI, -10% to 89%) in individuals who received the seasonal influenza vaccine and 49% (95% CI, -40% to 81%) after pandemic vaccine only.

Among individuals with chronic illness, an adjuvanted monovalent influenza A/H1N1 vaccine protected against influenza A/H1N1 infection and hospitalization, although the protective effect on hospitalization did not reach statistical significance. Furthermore, individuals who received both the seasonal influenza vaccination and the pandemic vaccination had a lower risk of confirmed influenza A/H1N1 in the first 7 days after vaccination compared to those who only got the pandemic vaccine. This finding indicated a priming effect of the seasonal vaccine.

SPA5 - HOW TO EVALUATE VACCINE EFFECTIVENESS AND EFFICACY?

A517P

Development of an ELISA-based potency assay for inactivated influenza vaccines

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Introduction

Potency of all inactivated influenza vaccines is determined using a single radial immunodiffusion (SRID) assay. This assay is not very sensitive and requires the production of large quantities of specific reagents (reference antigen and reference antiserum) for each virus strain. New potency assays, with improved accuracy and sensitivity, are needed to improve and accelerate influenza vaccine manufacture.

Materials & Methods

We describe the generation and characterization of a panel of monoclonal antibodies to the hemagglutinin (HA) of the 2009 pandemic H1N1 virus A/California/7/2009, and evaluate their potential use in quantifying the HA content in vaccines. Eleven monoclonal antibodies have been characterized using Western blot, ELISA, immunofluorescence, hemagglutination inhibition assay, microneutralization assays, and a passive protection study in mice. In addition, we explored the use of these monoclonal antibodies as capture antibodies in a sandwich ELISA to quantify the HA content in vaccines using the SRID reference antigen as a standard. As a detection antibody, we used either rabbit polyclonal serum, or monoclonal antibodies from the tested panel.

Results

The potency of vaccines from two different manufacturers was determined using the ELISA assay, and compared to the potency values obtained by traditional SRID analysis and found to be similar. Further, data obtained from analyzing vaccine samples that were exposed to elevated temperatures for extended periods of time in an accelerated stability study indicated that the ELISA was able to detect and quantify subpotent vaccines, a key requirement for an alternative potency assay. Finally, the new ELISA potency assay is considerably more sensitive than the traditional SRID assay. Due to the standardized design in a 96-well format, the novel assay could easily be automated for a high throughput at significant accuracy and precision.

Conclusion

The data indicates the feasibility of an ELISA-based method to quantify HA content in vaccines and suggests that further development might provide a suitable alternative assay to measure the potency of inactivated influenza vaccines.

SPA5 - HOW TO EVALUATE VACCINE EFFECTIVENESS AND EFFICACY?

A518P

Estimating influenza vaccine effectiveness for the circulating strain using surveillance data, from season 2006-2007 to 2008-2009, Spain

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Introduction

The Spanish Influenza Sentinel Surveillance System aims at providing timely epidemiological and virological information on influenza activity. During the influenza season (from the epidemiological week 40 of one year to epidemiological week 20 of the following year), sentinel physicians collect basic information, take swabs and notify influenza like illness (ILI) patients to the surveillance system. Starting the season 2002-2003, individual data including vaccination status has been collected for each ILI patient and included in an electronic database weekly. We aim to estimate the seasonal influenza vaccine effectiveness (IVE) during three seasons (before the pandemic one), using as outcome influenza cases - laboratory confirmed for the predominant circulating strain, in order to explore the utility of the surveillance system in providing strain-specific IVE estimates.

Material and methods :

We used the case control test-negative study design. All swabbed ILI patients reported to the surveillance system during three influenza seasons (2006-2009) were included in the study. Cases were ILI laboratory-confirmed for influenza A(H3N2), A(H1N1) or B according to the predominant circulating strain during the respective season. Laboratory confirmation was done by PCR or culture. Controls were ILI testing negative for any type of influenza. Missing data on laboratory results or sub-typing were excluded. We restricted the analysis to the epidemic period, defined as the influenza season weeks when the ILI rate overpassed the epidemic threshold, for each season. Data on age, sex, vaccination status and laboratory results were available for all seasons. We used logistic regression to calculate adjusted odds ratios for age, month of swabbing and Spanish region and their correspondent 95% confidence intervals (95% CI). IVE was computed as (1-odds ratio)*100.

Results

For the season 2006-2007, we included in the analysis 579 cases of predominant strain A(H3N2) and 421 test-negative controls. Among cases, 43 (7.43%) were vaccinated and 44 (10.5%) among controls. The crude IVE was 31% (95% CI: -9; 57) and the adjusted IVE was 30% (95% CI: -17; 49). For the season 2007-2008, co-circulation of A(H1N1) and B influenza viruses was registered, therefore we included in the analysis 296 A(H1N1) cases and 386 B cases comparing either one to 495 test-negative controls. Five (1.69%) A(H1N1) cases and 22 (5.70%) B cases were vaccinated compared to 51 (10.3%) controls. The crude IVE was 85% (95% CI: 62; 95) for A(H1N1) cases and 47% (95% CI: 10; 70) for B cases. Adjusted IVE was 79% (95%CI: 43; 92) for A(H1N1) and 53% (95% CI: 12; 74) for B virus. For the season 2008-2009 we included in the analysis 403 cases of predominant circulating strain A(H3N2) and 525 controls. A total of 43 (10.67%) cases were vaccinated and 82 (15.62%) controls. The crude IVE was 35% (95% CI: 3; 58) in the season 2008-2009 and the adjusted IVE was 58% (95% CI: 16; 73).

Conclusions

The Spanish Influenza Sentinel Surveillance System allowed estimating IVE in the studied seasons according to the predominant circulating strain. The IVE might be either underestimated or overestimated due to lack of collecting important confounding factors known to influence IVE estimates. To strengthen the surveillance system, systematic swabbing of ILI patients was introduced starting the season 2009-2010 and additional information on some confounding factors is collected. Consequently, estimating IVE will improve and will be routinely included among the activities of influenza surveillance in Spain.

SPA5 - HOW TO EVALUATE VACCINE EFFECTIVENESS AND EFFICACY?

A519P

Long-term Immunogenicity of 2009 H1N1 Influenza vaccine: Analysis Based on Vaccine Composition and Vaccination Status for Seasonal Influenza

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Introduction

Since first reports in April 2009, the pandemic influenza A/H1N1 virus spread globally and persisted for a long time. Influenza vaccines are the primary method for the control of influenza, but influenza vaccine-induced antibody is known to decline rapidly over 6 month period.

Materials & Methods

We evaluated the long-term immunogenicity of 2009 A/H1N1 influenza monovalent vaccine in adults aged 18-64 years. Serum hemagglutinin inhibition (HI) titers were determined pre-vaccination and at 1, 6, and 10 months after vaccination. These were compared according to the vaccine composition and status of seasonal influenza vaccination (at least 3 weeks before H1N1 influenza vaccination). During the pandemic, two different influenza vaccines were administered in Korea. The vaccine compositions were 3.75 μg (MF-59 adjuvanted) and 15 μg (unadjuvanted) of hemagglutinin antigen.

Results

Of the 415 subjects, 306 (73.7%) were followed up during a 10-month period. Seroprotection/seroconversion rates at 1 month post-vaccination were 82.4/74.5% (MF59 adjuvanted vaccine) and 83.3/79.4% (unadjuvanted vaccine). At six months post-vaccination, seroprotection and seroconversion rates met the EMA criteria irrespective of vaccine composition. Geometric mean titer (2009 A/H1N1) was significantly lower among seasonal influenza vaccine recipients compared to the non-recipients at 1, 6, and 10 month post-vaccination ($p < 0.05$). Of note, seroprotection rate among the seasonal influenza vaccine recipients did not met the EMA criteria (70%) six month later after immunization with 3.75-μg dose of MF59 adjuvanted 2009 A/H1N1 vaccine.

Conclusion

Both single 3.75 μg dose of MF59 adjuvanted and 15 μg dose of non-adjuvanted 2009 A/H1N1 influenza vaccines showed adequate immunogenicity up to 6 months post-vaccination for adults aged 18-64 years. Receipt of seasonal influenza vaccine showed negative influence on the immunogenicity of 2009 A/H1N1 influenza monovalent vaccine.

No conflict of interest

YOUNG SCIENTIST

SPA5 - HOW TO EVALUATE VACCINE EFFECTIVENESS AND EFFICACY?

A520P

Pandemic influenza vaccine effectiveness among adults during 2009-2010 influenza season: a population-based cohort study

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Introduction

During the pandemic caused by a new Mexican influenza virus A (H1N1) millions of doses of adjuvanted pandemic vaccines were administered. First evaluations among adults indicate adequate protection, but most studies were designed as case-control studies which are vulnerable to selection bias. We designed a cohort study using seasonality analysis to adjust for potential bias using a nationally representative database from The Netherlands. We aimed to assess the effectiveness of the new pandemic vaccine in the community on the occurrence of medically attended influenza.

Material & methods

We conducted a retrospective population-based cohort database study during the period 2009-2010 among adults aged 18 years or older. The data were collected from the Netherlands Information Network of General Practice (LINH) database records and included information on demographic and clinical risk factors, pandemic vaccination status and outcome codes. Medically attended influenza was coded as R80 according to the International Classification of Primary Care coding system. We defined the period from June 11 2009 till October 1 as the preseason outcome period and the period between November 15 and December 31 2009 as the outcome season. Pandemic vaccination status was recorded as administered if at least one vaccine was administered and absent if no vaccine was administered. The majority of the recommended primary care population received two vaccines between mid October and 15 November 2009. In The Netherlands the vaccine used for adults was from Novartis (Focetria®). The data about confounders was collected during one year preceding 2009/2010 influenza season and included information on underlying chronic conditions (cardiovascular disease, COPD or asthma, diabetes mellitus and other co-morbidities) and prior health care utilization.

Results

In total, the cohort consisted of 66,319 individuals, 18,662 (28%) of which received the pandemic influenza vaccine. There were 30,858 (46%) male subjects and 18,818 (28%) were 60 years and older. In the total sample, 11,430 (17%) were suffering from cardiovascular disease, 4,254 (6%) from diabetes mellitus, 6,315 (10%) from asthma or COPD, and 1,773 (3%) from other underlying chronic conditions. After adjustments for confounders with multivariate logistic regression, receiving pandemic vaccine reduced influenza disease occurrence by 41% (odds ratio [OR] .59; 95% confidence interval [CI]: .22-1.56) in the whole sample and by 82% in the elderly (OR .18; CI 95%: .03 -.98, $p<0.05$). There was no effect of receiving pandemic influenza vaccine in preventing influenza in 18-59 year olds, OR 1.23 (95% CI: .37-3.38), nor during the preseason.

Conclusions

Though statistical power of the study was limited, our results suggest that pandemic influenza vaccination prevented influenza, notably in elderly subjects, and support the national recommendations for vaccination by the Dutch Health Council. The point estimates of effectiveness are in line with results from cohort studies on seasonal influenza vaccines.

SPA5 - HOW TO EVALUATE VACCINE EFFECTIVENESS AND EFFICACY?

A521P

Influenza vaccination and the elderly: Interpreting the uninterpretable.

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Introduction

The comprehensive Cochrane meta-analysis of 68 studies on efficacy and effectiveness of vaccinating the elderly against influenza (Rivetti et al. 2006, Jefferson et al. 2010) unfolds the clinical evidence. Recently, another source of evidence has become available: Coudeville et al. (2010) presented a meta-analysis of studies, which linked pre-exposure anti-haemagglutinin antibody to the probability of infection. Both papers were reviewed to quantify and interpret the effects of the intervention.

Results

The Cochrane meta-analysis shows that randomized controlled trials (RCTs), though limited, provide an estimate for efficacy against infection (~60%). The number of unrandomized studies assessing disease reduction is large, but effectiveness against disease is notoriously difficult to interpret. Disease outcomes can be biased by selection imbalance and disease not caused by influenza. Indeed, many studies poorly address these key issues and may result in exaggerated or undervalued effectiveness estimates. When focusing on the studies with highest quality and sound efforts to reduce selection bias, most effectiveness estimates lie between 20% and 30% against influenza-like illness and hospitalisation for respiratory and cardiac conditions, covering many seasons with various attack rates and degrees of vaccine match. Even with 20% effectiveness, vaccination reduces the risk of morbidity for many persons, as it concerns a large part of the population. The mathematical relationship between exposition, infection and disease (see abstract Nauta & Beyer) is such that an effectiveness of ~20% is consistent with an underlying efficacy against infection of ~60%.

The authors of the Cochrane meta-analysis themselves, however, give a different interpretation: They regard the effectiveness estimates as only "modest" failing to mention that an even small relative risk reduction translates into large absolute numbers of prevented cases (Nichol 2007). They point to the low quality of many studies, large heterogeneity among studies and other inconsistencies, and conclude that the "data presented in this review are so biased as to be virtually uninterpretable." In an additional paper (Jefferson et al. 2009), even the impact of influenza, as a single entity, on morbidity and mortality in the elderly is questioned. These concerns will be discussed.

With the protection curve of Coudeville et al., vaccine efficacy against infection can be estimated from antibody titres (Nauta et al. 2009). However, the protection curve is based on studies in adolescents and non-elderly adults. A re-analysis of an RCT in the elderly (Govaert 1994) shows that the protection curve is also applicable for this age group. When large data collections of serological vaccination studies in the elderly are analysed by this approach, estimates for vaccine efficacy against infection are consistently between 60 and 65%, thus compatible with the results above. Details will be presented.

Conclusions

All together, ~60% efficacy against infection and ~20% effectiveness against disease are fair and plausible state-of-the-art results confirming the relevance of vaccinating the elderly against influenza, justifying current policy, and providing the basis for future improvements.

YOUNG SCIENTIST

SPA5 - HOW TO EVALUATE VACCINE EFFECTIVENESS AND EFFICACY?

A522P

The effectiveness of vaccination of health care workers for the protection of patients at higher risk of acute respiratory disease: a systematic review

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Introduction

Respiratory disease is a leading cause of global mortality, and influenza and pneumococcal infections are important contributors. Those most vulnerable to severe or complicated illness following respiratory infection are likely to be frequent users of health care services, and nosocomial outbreaks have been observed amongst these groups despite high vaccination coverage. Although often difficult to establish the source of infection, there is some evidence to suggest that health care workers may be important vectors. It has thus been postulated that vaccinating health care workers themselves may play a role in reducing transmission, providing indirect protection to higher risk patient groups.

Objective:

The objective of this review was to assess existing evidence for the effectiveness of vaccination of health care workers in protecting patients at higher risk of severe or complicated disease from acute respiratory infection.

Methods

We searched a number of electronic health care databases including, EMBASE, CINAHL, MEDLINE, Pubmed, Cochrane Library, J-Stage, BDSP, EASTVIEW, Index-F, eLIBRARY, WHO regional indexes, and the WHO portal of clinical trials, using a predefined, peer reviewed search strategy. In addition, attempts were made to access relevant evidence based reviews, guidelines and grey literature. A three-stage approach was used to select papers, reviewing the title, abstract and full text against eligibility criteria. Data extraction was undertaken using a pre-defined, piloted template and the risk of bias assessed at both the study and outcome level. Results were synthesised using a narrative approach.

Results

A total of 12,351 citations were identified, 11,233 being excluded following a review of the titles, 941 following a review of the abstracts and 160 at the full text stage. A total of 17 papers were included plus three additional papers identified from reference or citation tracking. Of the final 20 papers, 14 were primary research studies and 6 reports of two systematic reviews. The majority were conducted in long-term residential care settings. All included papers were at risk of bias and there was marked heterogeneity, which limited the conclusions that could be drawn. Nevertheless there appeared to be consistency in the direction of effect when measures of acute respiratory disease, clinically or laboratory defined influenza/influenza-like illness and mortality were considered.

Conclusions

Existing evidence for the effectiveness of vaccination of health care workers in providing indirect protection to those patients at higher risk of severe or complicated illness following respiratory infection is limited. The observed consistency in the direction of effect across a number of different outcome measures however, suggests there is a potential true underlying protective effect. Current evidence is largely confined to long-term residential settings and it is not clear whether similar effects would be observed amongst other high risk patient groups. As such, further high quality research is required to inform future vaccination strategy.

SPA5 - HOW TO EVALUATE VACCINE EFFECTIVENESS AND EFFICACY?

A524P

Effectiveness of MF59-adjuvanted versus non-adjuvanted seasonal influenza vaccines on hospitalizations of the elderly due to all respiratory illnesses

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Introduction

Influenza infection cause significant morbidity and mortality in the general population, most severely affecting the very young and very old. Immunosenescence in the elderly leads to suboptimal vaccination efficacy in this age group. The MF59-adjuvanted influenza vaccine, Fluad® (Novartis Vaccines) was developed to amplify the immunogenicity of non-adjuvanted seasonal vaccines, and over 50 million doses have been administered. The LIVE study reported 23% reduction in hospitalizations due to influenza or pneumonia for MF59-adjuvanted seasonal influenza vaccine compared to non-adjuvanted in adults older than 65 years. We now report on the hospitalizations outcome due to any respiratory illness, a secondary endpoint of the LIVE study.

Methods

We conducted a prospective, population-based, non randomized observational cohort study to compare the risk of hospitalization for influenza or pneumonia during the influenza season among elderly persons vaccinated with MF59-adjuvanted (Fluad®) versus non-adjuvanted, sub-unit (Agrippal®, Novartis Vaccines) influenza vaccine. The study was conducted over three consecutive influenza seasons (2006-2009) through general practitioners and local health district offices in five regional health districts in the Lombardy region of northern Italy. Data on vaccine exposure, potential confounders, and medical history were collected through questionnaires and administrative databases. Discharge diagnoses for hospitalizations for any respiratory illness (ICD9-CM codes 460 - 519) during influenza seasons were identified through administrative databases which captured all hospitalizations in the region. We conducted stratified and regression analyses to adjust for confounding, and generalized estimating equations to account for repeated vaccination. We conducted stratified and regression analyses to adjust for confounding, and generalized estimating equations to account for repeated vaccination. The outcome was the adjusted relative risk ratio for hospitalization for any respiratory illness comparing the two vaccine groups.

Results

Overall, 107,661 persons enrolled, contributing 170,988 person-seasons of observation, with 88,449 doses of adjuvanted and 82,539 of non-adjuvanted influenza vaccines administered. In Italy Fluad is preferentially recommended for high risk elderly individuals, and as vaccine distribution was not dictated by the study protocol, it was not surprising to find that subjects who received Fluad had 19% greater risk for hospitalization due to influenza or pneumonia at baseline compared with those vaccinated with Agrippal. Mean ages were 76.5 and 74.9 years in the Fluad and Agrippal groups, respectively. Analysis was focussed using the narrowest definition of the risk window for influenza-related events, which should provide the greatest specificity for the outcomes of interest. These corresponded to calendar weeks 4-7 inclusive in 2006-7, and weeks 52-4 and 1-7 for the subsequent two influenza seasons. We identified 492 cases. Applying the analysis model controlling for the principal individual confounders and propensity score based on all confounders, we estimated a reduced risk of hospitalization due to any respiratory illness for Fluad relative to Agrippal, with risk ratio of 0.79 (95% CI 0.66–0.95).

Conclusion

In the secondary analysis of this prospective, observational study over three influenza seasons in individuals 65 years of age or older, the MF59-adjuvanted influenza vaccine was estimated to reduce the risk of hospitalization for any respiratory illness by 21% compared with the non-adjuvanted vaccine. To the extent that there is residual bias, this is likely to be an underestimate.

SPA5 - HOW TO EVALUATE VACCINE EFFECTIVENESS AND EFFICACY?

A525P

Safety and immunogenicity of a whole virus, vero cell-derived h5n1 pandemic influenza vaccine in immunocompromised and chronically ill patients

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A non-adjuvanted whole virus, Vero cell-derived H5N1 pandemic influenza vaccine was previously shown to be safe and immunogenic in a healthy adult and elderly population (*Ehrlich et al. 2008*)[1]. Here we report on a study in specified risk groups of immunocompromised individuals, including transplant and HIV infected patients, and chronically ill patients aged 18 years or older. A total of 319 immunocompromised and 300 chronically ill patients received two vaccinations 21 days apart with the H5N1 vaccine containing 7.5 µg HA antigen of clade 1 A/Vietnam/1203/2004 strain. A heterologous booster vaccination with a 7.5 µg dose of clade 2.1 A/Indonesia/05/2005 strain vaccine was administered 12 to 24 months after the first vaccination to approximately 100 subjects in each of the risk groups.

The H5N1 vaccine was shown to be safe and well tolerated in immunocompromised patients and patients with chronic disease conditions with a safety profile similar to the safety profile in healthy adult and elderly subjects and that of licensed seasonal influenza vaccines. Systemic reaction rates were 28.5% after the first and 16.7% after the second vaccination in the immunocompromised individuals and 36.7% after the first and 20.1% after the second vaccination in the chronically ill patients. Local reactions occurred at rates of 12.5% and 8.4% in immunocompromised subjects and 17.0% and 13.4% in chronically ill subjects after the first and second vaccination, respectively. The most frequently reported symptoms of local and systemic reactions in both patient groups were injection site pain, fatigue and headache. Adverse reactions were predominantly mild and transient. There were no serious adverse reactions reported.

Immunogenicity was analyzed in a subset of 122 immunocompromised and 123 chronically ill subjects. A substantial immune response was observed after 2 vaccinations 21 days apart with the H5N1 vaccine with 41.5% and 64.2% of subjects achieving a neutralizing titer associated with protection (MN titer ≥ 1:20) in the immunocompromised and chronically ill patients, respectively. In addition, the H5N1 vaccine induced a strong immunological memory as demonstrated by the substantial booster response in immunodeficient and chronically ill patients 12 to 24 months after priming. Seroprotection rates (MN titer ≥ 1:20) were shown to increase from 1.5% pre-booster to 67.5% 21 days post-booster against the A/Indonesia/05/2005 booster strain in immunocompromised subjects. In the chronically ill population, seroprotection rates increased from 2.2% to 70.8% against the A/Indonesia/05/2005 strain. Against A/Vietnam/1203/2004 (the strain used in the vaccine for primary vaccination), seroprotection rates increased from 10.4% to 71.6% and from 24.7% to 77.5% in immunocompromised and chronically ill subjects, respectively.

In summary, these data demonstrate that the inactivated whole virus Vero cell-derived H5N1 influenza vaccine is immunogenic in immunocompromised and chronically ill patients. A booster dose with a heterologous H5N1 vaccine 1 – 2 years after priming results in a substantial booster response in both patient populations, emphasizing the potential role of the vaccine as a prepandemic or pandemic influenza vaccine in risk groups. Furthermore, the vaccine is well tolerated with a safety profile similar to an inactivated seasonal influenza vaccine.

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SPA5 - HOW TO EVALUATE VACCINE EFFECTIVENESS AND EFFICACY?

A527P

The mathematical relationship between influenza vaccine efficacy and effectiveness

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Introduction

In recent years, doubts have been raised about the usefulness of seasonal influenza vaccination, particularly in the elderly. Evaluated trials were mostly observational effectiveness studies with considerable heterogeneity, which makes interpretation difficult and controversial.

Methods

We studied the relationship between influenza vaccine efficacy (protection against influenza infection in the absence of mismatch) and influenza vaccine effectiveness (protection against outcomes, which may or may not caused by influenza, such as pneumonia, excess hospital admission, or excess mortality), and derived a mathematical expression for it.

Results

We note that efficacy is not dependent on temporal (seasonal) circumstances, as the probability of exposure to the virus, or the probability of infection following exposure if not vaccinated. In contrast, effectiveness is strongly dependent on these probabilities, and further on the strength of the relationship between the effectiveness outcome and influenza infection.

Conclusion

Our interpretation of the mathematical relationship is that vaccine effectiveness is a potentially misleading measure to assess the usefulness of seasonal influenza vaccination, due to its temporariness. Under certain circumstances, effectiveness studies may be used to estimate the underlying vaccine efficacy.

SPA5 - HOW TO EVALUATE VACCINE EFFECTIVENESS AND EFFICACY?

A528P

Influenza vaccine for children in a developing country: an ongoign study in India

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Aims

The burden of disease due to influenza and the effectiveness of influenza immunization is not well characterized in populations in low resource countries. We established a 3 year study to define the direct and indirect household protective effects of immunizing children with seasonal trivalent influenza vaccine (TIV) in a rural community of India.

Methods

The study began in 2009 and is a phase IV, prospective, household randomized, controlled, and blinded with children 6 mo-10 y of age vaccinated with TIV or inactivated polio vaccine. First-time vaccinees 6m-8 years were administered two doses 4 weeks apart. The primary outcome is laboratory-confirmed influenza among vaccinees and their household members. Vaccinated children and household members in 3 rural villages are under active surveillance with weekly home visits for identification of febrile acute respiratory infection (FARI). Respiratory swabs are collected from FARIs for influenza detection by realtime RT-PCR.

Results

16,911 individuals enrolled in surveillance for indirect effects (90% of eligibles). In year 1, 2979 (80.7%) of 3691 eligible children, were completely vaccinated (1 or 2 doses), 390 (10.7%) received 1 of 2 doses, and 322 (8.7%) were not vaccinated. The appearance of 2009 H1N1 required two rather the originally planned one dose of vaccine in year 2. In year 2, 85% of 3832 eligibles were immunized completely and 6% received 1 of 2 planned doses. Overall 91% received complete or partial immunization each year. From Nov 2009 - Dec 2010, influenza was detected in 1413 (16.5%) of 8580 FARI samples, 2009 H1N1 was detected in 745 (8.7%), and influenza B in 649 (7.6%). 2009 H1N1 peaked in December 2009 and subsequently almost completely disappeared, reappearing in July 2010 during the monsoon influenza season. Influenza B persisted throughout the winter, spring and summer of 2010. There was limited influenza activity in the fall of 2010. Nucleotide sequence analysis of the HA1 region of 9 2009 H1N1 viruses showed they were in clade 7 similar to other 2009 H1N1 viruses from India. Influenza B viruses (n=9) clustered with the vaccine strain B/Brisbane/60/2008.

Conclusions

A study of TIV among children in rural India is ongoing with high rates of enrollment (90%) and vaccination (91%). Year 1 of the study when pandemic 2009 H1N1emerged, only circulating influenza B matched the vaccine strain, reducing the power of the study. The pandemic also resulted in a change not only in seasonal influenza vaccine composition for 2010, but also in the number of doses to be given in the 2nd year. Antigenic changes of influenza vaccines due to drift and shift emphasize the importance of multi-year studies of influenza vaccines.

Study supported by cooperative agreement U01P000177 from the Centers for Disease Control and Prevention.

SPA5 - HOW TO EVALUATE VACCINE EFFECTIVENESS AND EFFICACY?

A529P

Persistence of immunity to a booster dose of an H5N1 influenza vaccine administered 12 months after one- or two-dose priming

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Introduction

This phase II, open, randomised study evaluated the safety and immunogenicity of a one- and two-dose prime-boost concept for a pandemic monovalent influenza (H5N1) vaccine candidate (split virus formulation adjuvanted with AS03), administered according to different vaccination schedules in adults aged 18–60 years.

Material & Methods

Adults aged 18–60 years were randomised to receive either one or two doses (21 days apart) of an AS03-adjuvanted A/Vietnam/1194/2004 (H5N1) influenza vaccine (3.75 µg haemagglutinin antigen [HA]), followed 6 or 12 months later by a homologous or heterologous subclade (A/Indonesia/05/2005 [H5N1]) booster vaccine. We focus here on the Month 12 revaccination groups only. Haemagglutination inhibition (HI) seroconversion rates (SCRs), seroprotection rates (SPRs) and geometric mean antibody titres (GMTs) were assessed on Day 21 (D21), Day 42 (D42), Month 6 (M6) and Month 12 (M12) after the primary vaccination, 7 and 21 days after the booster vaccination (M12+7, M12+21), and 6 months after the booster dose (M18). Seropositivity, booster SCRs and GMTs for neutralising antibodies were also measured at M12, M12+7, M12+21 and M18. The frequency of influenza-specific CD4+ T-cells producing at least two markers (CD40-ligand [CD40L], interleukin-2 [IL-2], interferon gamma [IFN-γ], and tumour necrosis factor-alpha [TNF-Ð]) after *in vitro* stimulation with H5N1 antigen was measured using an intracellular cytokine assay at M12, M12+7, M12+21 and M18. Serious adverse events (SAEs) and withdrawals due to AEs were recorded throughout the study period (Day 0 to M18).

Results

A total of 256 subjects were vaccinated in the Month 12 booster groups, of which 196 were included in the According-To-Protocol (ATP) cohort for immunogenicity. HI GMTs and seropositivity rates (≥10 1/DIL) against both strains ranged from 55.6–385.9 and from 77.6–97.4% at M18, demonstrating a persistence of the humoral immune response 6 months after booster vaccination. Seropositivity for neutralising antibodies (≥28 1/DIL) was ≥97.9% across groups and GMTs ranged from 423.7–1708.9 for both strains 6 months after the booster. HI and neutralising antibody GMTs against both strains were higher when a heterologous booster schedule was used. Booster SCRs for neutralising antibodies ranged from 14.9–42.1% against the A/Vietnam/1194/2004 strain and were ≥45.0% against the A/Indonesia/05/2005 strain at M18. The frequency of specific CD4+ T-cells expressing two of the activation markers increased after booster vaccination and persisted for 6 months for both strains. Higher CMI responses against both strains were observed for the two-dose primary vaccination groups 21 days after the booster. Ten SAEs were reported during the entire study period, of which 9 were considered unrelated to vaccination. There was one case of non-Hodgkin's lymphoma considered as possibly related and which led to withdrawal from the study.

Conclusions

A single-dose heterologous booster vaccine administered to subjects primed with one dose of the H5N1 influenza pandemic vaccine was effective at eliciting high and persistent cross-reactive responses and had a clinically acceptable safety profile.

SPA5 - HOW TO EVALUATE VACCINE EFFECTIVENESS AND EFFICACY?

A530P

Immunogenicity, safety and reactogenicity of an AS03-adjuvanted H5N1 split virion prepandemic candidate influenza vaccine in children aged 3-9 years

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Introduction

This phase II, randomised, open, controlled trial (NCT00502593) evaluated the safety and immunogenicity of a pandemic influenza vaccine candidate (split virus formulation adjuvanted with AS03 [tocopherol based oil-in-water emulsion Adjuvant System]).

Methods

Children aged 3–9 years of age received two doses (21 days apart) of an AS03-adjuvanted A/Vietnam/1194/2004 (H5N1) influenza vaccine containing 1.9 µg haemagglutinin antigen [HA] and $AS03_B$ (5.93 mg of tocopherol). A control group received two doses of a trivalent non-adjuvanted seasonal influenza vaccine (Fluarix™). Haemagglutination inhibition (HI) seroconversion rates (SCRs), seroprotection rates (SPRs) and geometric mean titres (GMTs) were assessed for antibodies against the vaccine strain and an H5N1 heterologous strain (A/Indonesia/5/2005) pre-vaccination (D0) and on Day 21 (D21), Day 42 (D42), Month 6 (M6), Month 12 (M12) and Month 24 (M24) after administration of dose one. SCRs and SPRs were assessed according to the US Center for Biologics Evaluation and Research (CBER) licensure criteria for influenza vaccines (95% CI SCR ≥40%, SPR ≥70%). The numbers of specific CD4+ T-cells identified after a short term in vitro stimulation by intracellular cytokine (ICS) assay were measured at D0, D21, D42, M6, M12 and M24. Solicited local and general events were recorded within the 7-day post vaccination period and unsolicited adverse events (AEs) and serious AEs were recorded until M6.

Results

In total, 51 children in the 3–5 years age group and 51 children in the 6–9 years age group were vaccinated, with 49 and 43 children included in the According To Protocol (ATP) cohort for immune persistence, respectively. At M6, the CBER criteria for SCR against the homologous strain were still met in both age strata (3–5 years: 57.1% [95% CI: 42.2%; 71.2%]; 6–9 years: 62.5% [95% CI: 45.8%; 77.3%]). SCRs declined at M12 and M24 reaching 38.3% [95% CI: 24.5%; 53.6%] in the 3–5 years group and 22.9% [95% CI: 10.4%; 40.1%] in the 6–9 years group at M24. SPRs against the homologous strain peaked at D42 in both age groups (3–5 years: 93.9% [95% CI: 83.1%; 98.7%]; 6–9 years: 100% [95% CI: 91.2%; 100%]) and declined until M24. Similarly, SPRs against the heterologous strain were highest at D42 (3–5 years: 69.4% [95% CI: 54.6%; 81.7%]; 6–9 years: 72.5% [95% CI: 56.1%; 85.4%]) and declined until M24. For both strains and in both age groups HI GMTs peaked at D42 and gradually declined until M24. The frequency of specific CD4+ T-cells producing IL-2 with another immune response marker (available only for the 3–5 years age group) peaked at D42 (median: 2078 per million CD4+ T-cells [1st and 3rd quartiles: 1119 and 3488]) and remained above prevaccination levels until M24 (median: 901 per million CD4+ T-cells [1st and 3rd quartiles: 450 and 1979]). Responses in the Fluarix™ control group were negligible. There were no marked differences between the age strata in the immune responses for any of the immunogenicity parameters considered. The incidence of unsolicited adverse events was low and the reactogenicity profile was clinically acceptable in both age groups.

Conclusions

In children aged 3–9 years, two doses of the AS03-adjuvanted H5N1 influenza vaccine at a dose of 1.9 µg HA with $AS03_B$ elicited a persistent and cross reactive immune response to 24 months post-vaccination. The vaccine was shown to have a clinically acceptable safety profile.

SPA5 - HOW TO EVALUATE VACCINE EFFECTIVENESS AND EFFICACY?

A531P

Vaccination with an adjuvanted pandemic influenza H1N1 vaccine provides rapid and long-lasting protection in healthcare workers.

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Healthcare workers (HCWs) were prioritized for pandemic vaccination during the 2009 pandemic in the majority of countries to maintain the integrity of the healthcare system. Furthermore, HCWs at the Haukeland University Hospital in Bergen, Norway are routinely offered annual influenza vaccination. In October 2009, we conducted a clinical trial in 250 frontline HCWs of this hospital to evaluate the kinetics and longevity of the immune response after vaccination with a low dose split H1N1 virus (X179a A/California/7/2009) vaccine (pH1N1 vaccine) adjuvanted with AS03. Subjects provided blood samples prior to vaccination (day 0) and 7, 14, 21 days and 3, 6, 12 and 18 months after vaccination. Pre- and post-vaccination serum samples from each individual were tested using the haemagglutination inhibition (HI) assay using the X179a virus and cross-reactivity against 3 prototype H1N1 viruses. At seven days post-vaccination 70% of the vaccinees elicited a protective antibody response (HI titre > 40) to the X179a virus. The antibody response continued to increase where at 14 and 21 days post-vaccination, 100% and 96% vaccinees, respectively had protective antibody titres. Protective antibody titres were maintained in the majority of volunteers up to 6 months post-vaccination. Twelve months after vaccination, only 53% of the vaccinees had a protective antibody titre against the X179a virus, at which point a number of HCWs opted to be vaccinated with the 2010-2011 seasonal influenza vaccine. Currently, we are evaluating serum samples taken after seasonal vaccination and also 18 months after pH1N1 vaccination in order to determine the long term immune response. In addition, as part of a sub study, HCWs that no longer had protective antibody response at 3 months after vaccination with the pH1N1 vaccine were offered a second dose of pandemic vaccine. Interestingly, out of 12 volunteers that were revaccinated, all but 1 had protective antibody titres by day 7 and this response was maintained until the annual hospital vaccination campaign which was conducted in the autumn of 2010. In ongoing studies, we are evaluating the homologous and cross-reactive memory B-cell and CD4 T-cell responses in individuals vaccinated with the pH1N1 vaccine.

In conclusion, the oil in water adjuvanted pH1N1 vaccine elicited a rapid antibody response, which was maintained in HCWs for 12 months until the start of the annual hospital influenza vaccine campaign.

YOUNG SCIENTIST

SPA5 - HOW TO EVALUATE VACCINE EFFECTIVENESS AND EFFICACY?

A532P

Patient-specific determinants of vaccine efficacy as measured by pre- and post- titer

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Introduction

Serological parameters are used to describe antibody response in populations, and are used in the assessment of vaccine efficacy and in clinical and scientific investigations on vaccinations. While these various statistics are useful in practical applications, it is important to understand how they depend on the composition of the sample population. We explore a linear model as a comprehensive description of vaccine effect on a given population, in order to make studies on repeated vaccination more reliably comparable across different years and different populations.

Aim

Age is an individual specific parameter that is commonly understood to influence average titer boost. We will test the effect of age and search for other parameters that may influence vaccine efficacy as measured by titerboosts in individuals. We will also test the effect of repeated vaccination to see if a repeatedly periodically vaccinated individual is better or worse protected than an individual vaccinated for the first time.

Method

This analysis is based on five cohort studies from three sources, two sets of young healthy adults, and three of ambulatory and residential elderly. All datasets of elderly include individuals who had been previously vaccinated.

While it is evident that post-titer values are function or pre-titer values, the homoscedasticity (homogeneity of variance) assumption necessary for ordinary least squares regression is also evidently violated as the variance of post-titer distribution diminishes as post-titer approaches maximum titer level. We use robust regression using iteratively reweighted least squares to attenuate this problem.

Results

We find that in the case of H1N1 the elderly on average have lower titers boosts than young adults even controlling for pre-vaccination titer levels. However, average titer boost is similar in the elderly and young adults for H3N2 and B.
There is variance in titer boost within each sample population that is unrelated to age. Those individuals with a stronger titer boost to one strain in one year tend to react more strongly to vaccination with other strains and in other years as well.
We can identify high-boosting and low-boosting individuals that consistently have titer boost above or below average. We show that repeated vaccination neither boosts nor diminishes the effect of any one seasonal vaccination.

Conclusion and discussion

Observed correlation between age and titer boost may generally be confounded by vaccination history, because vaccination history is either underreported for the elderly, or not taken into account. Reported correlation between age and vaccine efficacy may be spurious in the case of H3N2 and H1N1.

This finding indicates that the composition of the sample population in a vaccination efficacy test will affect vaccine efficacy results. The vaccine efficacy results of two sample populations are only comparable if the vaccination histories and the pre-titer levels of the two groups are comparable.

None of the recorded individual parameters explains why some individuals are high boosters and some individuals are low boosters, but there seems to be an unobserved individual-specific characteristic, which determines the reaction to vaccination.

YOUNG SCIENTIST

SPA5 - HOW TO EVALUATE VACCINE EFFECTIVENESS AND EFFICACY?

A533P - Vaccine effectiveness and efficacy

Clinical effectiveness of influenza vaccination for immunocompromised patients: a systematic review and meta-analysis

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Introduction

Immunocompromised patients are among the most vulnerable to severe or complicated illness and in turn are more likely to require prolonged or complicated care. Effective vaccination of immunocompromised patients against influenza is a critical need but paradoxically the immune status of such patients may also compromise their ability to respond. The objective of this study was to conduct a systematic review and meta-analysis to assess the clinical effectiveness of influenza vaccination for the prevention of influenza infection in this patient group.

Materials & methods

We searched a number of electronic healthcare databases including MEDLINE, EMBASE, CINAHL, the Cochrane Library, PubMed and WHO Regional Index Medicus Libraries using a predefined literature search strategy. Grey literature, evidence based reviews and clinical guidelines were also searched. A three-stage process was used to screen all records against the inclusion and exclusion criteria by title, abstract and then full text. Data extraction was undertaken using a pre-defined, piloted template and the risk of bias assessed at both the study and outcome level. Results were synthesised using a narrative approach and meta-analyses have been conducted where feasible. Heterogeneity between studies was assessed through calculation of I2 and publication bias was graphically represented using Begg's funnel plot and quantified using Egger's regression test. Statistical significance was assumed at the 5% level in all cases.

Results

Meta-analyses showed a statistically significant effect of preventing influenza-like illness (n = 7 studies; odds ratio [OR] = 0.24; 95% confidence interval [CI] = 0.16 to 0.34; $p < 0.01$; I2 = 22.0%) and laboratory confirmed influenza infection (n = 2 studies; OR = 0.15; 95% CI = 0.03 to 0.63; p = 0.01; $I^2 = 50.4\%$) through vaccinating immunocompromised patients compared to placebo or unvaccinated controls. We found no significant difference in the odds of influenza-like illness compared to vaccinated immunocompetent controls (n = 2 studies; OR = 0.62; 95% CI = 0.22 to 1.78; p = 0.38; $I^2 = 12.3\%$). The pooled odds of seroconversion (≥4 fold rise in haemagglutination inhibition titre) were significantly lower in vaccinated patients compared to immunocompetent controls for seasonal influenza A/H1N1 (n = 53 studies; OR = 0.55; 95% CI = 0.43 to 0.71; $p < 0.01$; $I^2 = 53.2\%$), A/H3N2 (n = 49 studies; OR = 0.55; 95% CI = 0.41 to 0.73; $p < 0.01$; $I^2 = 66.9\%$) and B (n = 46 studies; OR = 0.48; 95% CI = 0.36 to 0.62; $p < 0.01$; $I^2 = 54.3\%$). A similar trend was identified for seroprotection (≥1:40 titre post vaccination). Meta-analyses of seroconversion showed higher odds in vaccinated patients compared to placebo or unvaccinated controls although this only reached statistical significance for influenza B (n = 2 studies; OR = 9.17; 95% CI = 1.05 to 79.97; p = 0.05; $I^2 = 72.7\%$). No evidence of publication bias was present in any of the meta-analyses conducted. No consistent evidence of safety concerns or disease progression resulting in clinical sequelae after influenza vaccination in immunocompromised patients was identified.

Conclusions

Infection prevention and control strategies should include vaccination of immunocompromised patients to protect these individuals at high risk of influenza or severe illness post infection. Our data shows significant rates of clinical protection against influenza and laboratory confirmed infection are achieved through such an approach. The humoral response to influenza vaccination is lower in this patient group compared to healthy controls, although there is evidence of a trend towards increased rates of seroconversion compared to placebo or unvaccinated controls. Further research is warranted to review the effectiveness of booster doses, immunological adjuvants and the extent of immunosuppression on response to vaccination.

SPA5 - HOW TO EVALUATE VACCINE EFFECTIVENESS AND EFFICACY?

A535P

Persistence of immune response to one- or two-doses of an AS03-adjuvanted H1N1 influenza vaccine in adults

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Introduction

Data are presented from a phase III, open, randomised trial to evaluate immunogenicity after one or two doses of a monovalent H1N1 2009 influenza vaccine adjuvanted with AS03 (tocopherol based oil-in-water emulsion Adjuvant System) in adults including elderly adults.

Methods

Healthy adults were enrolled into two age groups (18–60 years and >60 years). Subjects were randomised to receive one or two doses (21 days apart) of a split-virus influenza vaccine containing 3.75 µg of A/California/2009 (H1N1) haemagglutinin antigen (HA). Safety analyses were performed on the total vaccinated cohort and the immunogenicity analyses were carried out on the According-to-Protocol (ATP) cohort for persistence at Month 6 (M6) and Month 12 (M12). Haemagglutination inhibition (HI) seroconversion rates (SCRs), seroprotection rates (SPRs) and geometric mean titres (GMTs) were assessed for antibodies against the vaccine strain at Day 21 (D21), Day 42 (D42), M6 and M12 after administration of the first dose. Serious adverse events (SAEs) and AEs of specific interest (AESIs) were recorded for the entire study period.

Results

A total of 240 subjects were vaccinated, of which 236 at M6 (135 in the 2-dose group and 101 in the 1-dose group) and 237 at M12 (135 in the 2-dose group, 102 in the 1-dose group) were included in the ATP cohort for persistence. The table shows GMTs, SCRs and SPRs for H1N1 antibodies against A/California/2009 (H1N1) at M6 and M12.

			GMT			SCR			SPR		
			Value	LL	UL	%	LL	UL	%	LL	UL
M6	18–60 years	**1-Dose**	**143.5**	96.6	213.0	**82.4**	69.1	91.6	**82.4**	69.1	91.6
		2-Dose	**214.8**	169.8	271.8	**94.0**	85.4	98.3	**94.0**	85.4	98.3
	>60 years	**1-Dose**	**51.6**	34.9	76.5	**42.0**	28.2	56.8	42.0	28.2	56.8
		2-Dose	**108.7**	85.7	137.8	**76.5**	64.6	85.9	**76.5**	64.6	85.9
M12	**18–60 years**	**1-Dose**	**64.2**	41.3	99.7	**61.5**	47.0	74.7	65.4	50.9	78.0
		2-Dose	**90.5**	69.4	118.1	**74.6**	62.5	84.5	**79.1**	67.4	88.1
	>60 years	**1-Dose**	**28.1**	19.4	40.6	**30.0**	17.9	44.6	44.0	30.0	58.7
		2-Dose	**42.3**	32.6	54.8	**41.2**	29.4	53.8	54.4	41.9	66.5

Values in bold met the EMEA guidance targets for pandemic influenza vaccine SCR (>40% for subjects aged 18–60 years; >30% for subjects >60 years of age), SPR (>70% for subjects aged 18–60 years; >60% for subjects >60 years), and geometric mean fold rise (>2.5 for subjects aged 18–60 years; >2 for subjects >60 years). For both age strata and at both M6 and M12, GMTs, SCRs and SPRs were higher in those receiving two doses of vaccine compared with those receiving one dose. Overall, at M6 and M12, GMTs, SCRs and SPRs were lower in the >60 years age group compared with those aged 18–60 years. None of the 29 SAEs reported during the study were identified as related to vaccination and no AESIs were recorded over the course of the study.

Conclusions

One or two doses of the AS03-adjuvanted H1N1 influenza vaccine induced an immune response persistent up to 12 months post-vaccination in adults >18 years; the observed immune response parameters in subjects >60 years tended to be lower. The study vaccine was considered to have shown a clinically acceptable safety profile.

SPA5 - HOW TO EVALUATE VACCINE EFFECTIVENESS AND EFFICACY?

A536P

Immune responses after boosting with a heterologous H5N1 vaccine 36 months after primary vaccination

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Introduction

An AS03 (tocopherol based oil-in-water emulsion Adjuvant System)-adjuvanted A/Vietnam/1194/2004 (H5N1) influenza vaccine has been shown to provide an effective immune response against a potential pandemic strain of influenza in adult subjects. The current study explored the persistence of the immune memory induced by the primary administration and the potential for using a heterologous A/Indonesia/05/2005 (H5N1) vaccine as a booster.

Materials & Methods

The study population comprised healthy adults who had previously received two primary doses of an AS03-adjuvanted A/Vietnam/1194/2004 influenza vaccine containing 3.75 µg of haemagglutinin antigen (HA), administered 21 days apart. Thirty-six months after primary vaccination, subjects received a booster vaccination with the same formulation but containing HA from A/Indonesia/05/2005 (subclade 2). The primary objective was to determine whether responses to boosting met the Committee for Human Medicine Products (CHMP) response criteria after 21 days – seroconversion (SCR) >40%, seroprotection rate (SPR) >70%, and geometric mean fold rise (GMFR) in antibody titre >2.5. Haemagglutination inhibition (HI), SCR, SPR and geometric mean titre (GMT) were assessed against both strains 21 days after boosting and again six months after the booster, ie, at Month 42 after administration of the first primary dose. Safety analyses were performed on the Total Vaccinated Cohort boosted at Month 36. Solicited reactogenicity from Days 0–6, unsolicited adverse events (AEs) from Days 0–30 and serious adverse events (SAEs) for the entire study period were recorded.

Results

A total of 390 subjects received booster vaccination at Month 36. By Day 21 after boosting, HI SCRs [mean (95% confidence intervals)] for A/Indonesia/05/2005 and A/Vietnam/1194/2004 strains had increased from 0% to 99.7% (98.5%, 100%) and 0% to 99.5% (98.1%, 99.9%), respectively. At Month 42 the corresponding SCRs were 96.6% (94.2%, 98.2%) and 92.1% (88.9%, 94.6%), respectively. Likewise, SPRs rose from 7.8% (5.3%, 10.9%) to 100% (99.0%, 100%) and 16.3% (12.7%, 20.3%) to 100% (99.0%, 100%), respectively and remained at 97.6% (95.5%, 98.9%) and 97.1% (94.9%, 98.5%), respectively at Month 42. At Day 21 after boosting, GMTs against A/Indonesia/05/2005 had risen from pre-booster levels of 7.0 (6.5, 7.6) to 877.5 (809.1, 951.6) and were 263.5 (238.3, 291.3) at Month 42. Corresponding GMTs against A/Vietnam/1194/2004 at these timepoints were 10.5 (9.5, 11.6), 653.1 (604.3, 705.9) and 225.4 (205.0, 247.9), respectively. SCR, SPR and GMFR met CHMP criteria at all timepoints after boosting. The most commonly observed reactogenicity response was pain at the injection site which was reported by 79.3% (74.9%, 83.2%) of subjects and was mostly of mild or moderate intensity. Overall AEs (0–30 days post booster) were reported by 21.8% (17.8%, 26.2%) of subjects; 2.3% (1.1%, 4.3%) of subjects reported Grade 3 AEs, most frequently 'influenza-like illness' (0.5% [0.1%, 1.8%]). Five AEs of special interest (potential immune-mediated diseases) were reported (2 reports of urticaria and one each of autoimmune thyroiditis, hypoaesthesia and optic neuritis). None of the SAEs reported by 12 (3.1%) subjects were considered causally related to vaccination.

Conclusions

Immunisation with split virus AS03-adjuvanted H5N1 vaccines induced an immune memory response that persisted for 3 years. This was evidenced by the fact that responses observed after heterologous booster vaccination were much higher than would be expected from a single dose vaccination. The booster responses exceeded regulatory acceptance criteria and persisted for 6 months. The vaccines were shown to have clinically acceptable safety profiles.

SPB5 - GENETIC AND ANTIGENIC EVOLUTION

B501P

Mutations in the pandemic influenza A H1N1 hemagglutinin molecule affect its antigenic properties

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Introduction

The 2009 pandemic influenza H1N1v virus has been the dominating type of influenza A virus in Finland during the 2009/2010 and 2010/2011 epidemic seasons. To the present knowledge the circulating virus has not undergone significant antigenic drifts and the seasonal influenza vaccine, containing the pandemic A/California/7/2009 as the H1N1 component, is assumed to give reasonable protection. The aim of this study was to analyze the antigenic characteristics of several pandemic influenza virus strains isolated from 2009/2010 and 2010/2011 influenza seasons by analyzing the amino acid sequences of hemagglutinin (HA), modeling the amino acid changes in HA structure and analyzing antibody responses induced by natural infection or influenza vaccination.

Material and methods

Based on the HA sequence we selected 13 different virus strains for immunological characterization. These included California-like viruses and multiple virus isolates from 2009/2010 and 2010/2011 epidemic seasons. For the analysis of the antibody responses against these viruses we selected serum samples from persons vaccinated with pandemic influenza vaccine, samples from individuals with natural infection with pandemic influenza and serum samples collected before 2009 pandemic from elderly people (>80 years of age).

Results

The analyzed viruses have two to five amino acid changes in their HA1 molecule. Analysis of the antibody levels by the hemagglutination inhibition test (HI) indicated that vaccinated individuals and people who had undergone a natural 2009 pandemic virus infection showed good immune responses against the vaccine and most of the wild virus strains. However, one to two amino acid changes in the antigenic site Sa lead to significant reduction in the ability of patient and vaccinee sera to recognize these viruses. In contrast, the antibody titers among the elderly, who had undergone natural infections in early 20th century, recognized almost all tested 2009 pandemic viruses equally well.

Conclusions

The data indicates that few amino acid changes in the major antigenic sites of the pandemic H1N1 virus HA molecule may render the virus poorly recognized by antibodies in patient and vaccinee sera.

SPB5 - GENETIC AND ANTIGENIC EVOLUTION

B502P

Genetic diversity of the influenza A(H1N1)2009 viruses identified in epidemic seasons 2009/2010 and 2010/2011 in Finland

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In Finland, the first infections caused by the influenza A(H1N1)2009 virus were identified on May 10, 2009. During the next three months almost all infections were found from patients who had recently traveled abroad. In September A(H1N1)2009 virus started to spread in the general population, leading to localized outbreaks and peak epidemic activity was reached during the weeks 43-48. During the 2010/2011 epidemic influenza cases were identified between November 2010 and March 2011. The nucleotide sequences of the hemagglutinin (HA) and neuraminidase (NA) genes from viruses collected from 172 patients were determined. The analyzed viruses represented mild and severe infections and different geographic regions and time periods. Based on HA and NA gene sequences, the Finnish pandemic viruses clustered in four groups during the 2009/2010 season and in three groups during the 2010/2011 season. Finnish epidemic viruses from season 2009/2010 and A/California/07/2009 vaccine virus strain varied from 2-8 and 0-5 amino acids in HA and NA molecules, respectively, giving a respective maximal evolution speed of 1.4% and 1.1%. The amino acid difference of HA between the Finnish 2010/2011 viruses and A/California/07/2009 virus is 5-9. There was a clear correlation with the number of amino acid substitutions and time of sample collection. Based on 3-dimensional modeling of the HA and NA structures most amino acid changes in HA and NA molecules accumulated on the surface of the molecule and were partly located in antigenic sites. Three severe infections were detected with a mutation at HA residue 222, in two viruses with a change D222G, and in one virus D222Y. Also viruses with a change of D222E were identified. All Finnish A(H1N1)2009 viruses were sensitive to oseltamivir having the amino acid histidine at residue 275 of the neuraminidase molecule. The Finnish A(H1N1)2009 viruses were still quite closely related to A/California/07/2009 vaccine virus. The nucleotide/amino acid changes within the HA or NA molecules were not clearly associated with increased epidemic potential or exceptionally high virulence. Thus, the viruses isolated from mild or lethal infections appeared to be very similar with one another and the severity of influenza disease is likely due to individual host factors. Continued laboratory-based surveillance of the influenza A(H1N1)2009 is important in order to rapidly identify drug resistant viruses and/or virus variants with potential ability to cause severe forms of infection and an ability to circumvent vaccine-induced immunity.

YOUNG SCIENTIST

SPB5 - GENETIC AND ANTIGENIC EVOLUTION

B503P

Identification of the molecular determinants of antigenic differences between H5N1 virus lineages affecting humans

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Introduction

Highly pathogenic avian influenza (HPAI) H5N1 virus was first detected in Hong Kong in 1997, and after 2003 spread over large parts of the eastern hemisphere including Southeast Asia, the Middle East, Europe and Africa. HPAI H5N1 virus has been responsible for severe outbreaks in poultry and frequent infections of wild birds, domestic mammals, and humans. So far, 552 human cases have been reported, of which 58% with a fatal outcome. Vaccines for human use will be invaluable in case sustained H5N1 virus transmission between humans will occur. Vaccine strain selection has been complicated by the genetic and antigenic diversification of H5N1 viruses since their first emergence. Currently, ten major "genetic clades" of the H5 HA have been identified, some of which have been divided into sub-clades. Most human cases to date were caused by viruses of clades 0, 1, 2.1, 2.2, and 2.3, and significant antigenic diversity has been observed between these clades. As a consequence, multiple prototype H5N1 vaccine strain candidates have been prepared by the WHO network.

Aim

We wished to study the molecular basis of the antigenic diversity of influenza A/H5N1 viruses belonging to clades 0, 1, 2.1, 2.2, and 2.3.

Methods

Representative strains for classical Eurasian H5 virus and the H5N1 clades affecting humans – clades 0, 1, 2.1, 2.2, and 2.3 – , were selected based on WHO human vaccine recommendations, and antigenic properties of these viruses were determined using the hemagglutination inhibition assay with ferret antisera. HA genes were cloned and expressed in the context of recombinant viruses. Antigenically important regions of HA were mapped by constructing HA chimeras of the different representative viruses. The exact molecular basis of antigenic differences between the clade representatives was determined using site directed mutagenesis, reverse genetics, HI assays, and antigenic cartography.

Results

Antigenic distances between clade representatives were between 3 and 8 antigenic units (8 – 256-fold difference in HI assay), and all representatives were about equidistant to the 1997 clade 0 representative virus. Analysis of HA chimeric viruses indicated that a region of 80 amino acids on the HA globular head was solely responsible for these antigenic differences. Within this region no more than five positions near the HA receptor binding site entirely determined the antigenic phenotype of these viruses.

Conclusion and discussion

We have mapped the antigenic properties of H5 viruses representative of clades 0, 1, 2.1, 2.2, and 2.3. By analysis of chimeric viruses and site-directed mutagenesis experiments we have determined the exact molecular basis of antigenic differences between these H5N1 clades. The finding that key amino acid substitutions were generally close to the receptor-binding site of HA may facilitate predicting further antigenic change in H5 viruses based on HA sequences. These results increase our understanding of H5N1 antigenic change and may aid in future vaccine strain selection and vaccine antigen design efforts.

YOUNG SCIENTIST

SPB5 - GENETIC AND ANTIGENIC EVOLUTION

B504P - Genetic and antigenic evolution

Evolutionary alterations in NA and HA genes of influenza H3N2 virus in Moscow region during 2003-2009

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Introduction

Evolutionary alterations in NA and HA genes of influenza H3N2 virus in Moscow region during 2003-2009 were studied.

Material and Methods

During influenza outbreaks of 2003 and 2009 in Moscow, H3N2 viruses were isolated from naso-pharyngeal washes of patients by propagation in human epithelial intestinal (line Caco-2) and bronchial (line Calu-3) cells. Intracellular proteolytic cleavage HA0?HA1+HA2 occurring in both cell cultures contributed to rapid virus isolation. RNA segments of neuraminidase (NA) and hemagglutinin (HA) of three H3N2 strains, isolated at the early and late stages of outbreak, were sequenced.

Results

Clinical isolates of 2009 were distinct from those ones, isolated in Moscow region in 2003, in 14 and 21 amino acids of the NA and HA genes, respectively. The NA gene was 1762 nucleotide long in distinction to 2003 isolates possessing 66 nucleotide (22 a.a.) deletion in a stalk region ("short-stalk" NA genotype). NA gene of 2009 Moscow viruses as well as 2003 ones possessed amino acid profile, which was characteristic for virus variants sensitive to oseltamivir and zanamivir. The HA of 2009 viruses contained N-glycosylation site at Asn181 (Asn165 in numbering after a signal peptide) correlating with the long-stalk NA gene. Viruses 2009 had Phe209 in the HA receptor binding center, whereas isolates 2003 possessed Ser209 that correlated with their difference in hemagglutination abilities. Phylogenetic analysis has shown that NA genes of Moscow strains 2003 and 2009 located in the same genetic clade, while their HA genes were diverged in more genetic distance and located in different clades. Distribution of viruses in the phylogenetic tree indicated that Moscow strains 2003 were not direct ancestor of Moscow 2009 virus H3N2.

Conclusion

The data show that during the period of 2003-2009 a Moscow population of H3N2 virus characterized by a "short-stalk" NA gene was exchanged for migrant virus possessing a "long-stalk" NA genotype.

No conflict of interest

YOUNG SCIENTIST

SPB5 - GENETIC AND ANTIGENIC EVOLUTION

B505P

Evidence for a difference in the evolutionary dynamics of H5N1 viruses among countries where vaccination was or was not adopted

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Introduction

Since the initial outbreaks in poultry occurring in 2006 in Egypt, Highly Pathogenic Avian Influenza (HPAI) H5N1 virus (clade 2.2) has evolved into a third order clade (clade 2.2.1) and diverged into antigenically and genetically distinct subclades. Despite the control measures taken, including mass vaccination of poultry, the virus rapidly spread among commercial and backyard flocks and it is now become endemic in this country. To better understand the dynamics of HPAI H5N1 evolution in countries that differ in vaccination policy, we undertook an in-depth analysis of those virus strains circulating in Egypt between 2006 and 2010, and compared countries where vaccination was adopted (Egypt and Indonesia) to those where it was not (Nigeria, Turkey and Thailand).

Methods

The HA gene of 313 H5N1 viruses from Egypt (39 obtained in this study), 169 from Indonesia, 87 from Turkey (all obtained in this study), 106 from Nigeria and 100 from Thailand were analyzed. Rates of nucleotide substitution per site and per year were estimated using the Bayesian MCMC approach. Positive selected sites were identified using Hy-Phy/Datamonkey package. A Bayesian skyline plot (BPS) was used to infer the population dynamics of each group of viruses in terms of changing levels of relative genetic diversity – Net, in which Ne represents the effective population size and t the generation time. Finally, Maximum Clade Credibility (MCC), neighbor-joining (NJ) and ML phylogenetic trees were estimated for the HA gene of the H5N1 viruses from Egypt.

Results

The phylogenetic trees of the HA gene revealed that two main Egyptian subclades have been co-circulating in domestic poultry since late 2007. Overall, the mean evolutionary rates of H5N1 viruses sampled from poultry in Egypt was 6.57 x 10^{-3}nucleotide substitutions per site, per year, although one of the subclades possessed and even higher substitution rate (95% HPD from 11.88 x 10^{-3} to 16.24 x 10^{-3}). In addition, Bayesian skyline plot analyses demonstrated that each subclade has a unique population dynamic history. We also found evidence for a difference in the evolutionary dynamics of H5N1 viruses among countries where vaccination was or was not adopted was noted. In particular, both evolutionary rates and the numbers of positively selected sites were systematically higher in viruses sampled from countries applying vaccination for H5N1, such as Egypt and Indonesia, compared to viruses circulating in countries which had never used vaccination (Nigeria, Turkey and Thailand).

Discussion

When properly planned and adopted, vaccination is a powerful tool for the control and eradication of avian influenza in poultry, as demonstrated by past experiences in Italy, Vietnam and Hong Kong, where it reduced economic losses and the risks for zoonotic transmission. However, if not properly applied and not coupled with careful surveillance, robust vaccine strategies and strict bio-security precautions, vaccination may contribute to the rapid evolution and antigenic change of H5N1 viruses, creating opportunities for the viruses to escape from vaccine protection. Although the direct association between H5N1 vaccination and virus evolution was difficult to establish in this study, our results suggest that inadequate vaccination practices may impact the evolutionary rate and the occurrence of mutations in H5N1 viruses circulating in poultry.

SPB5 - GENETIC AND ANTIGENIC EVOLUTION

B506P

Two epidemic seasons of pandemic influenza in Russia: molecular diagnostics and characteristics of the virus

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The aim of this study was to analyze molecular features of pandemic influenza A/H1N1 viruses in Russia during 2009-2011.

Materials and methods

Clinical (nasopharyngeal swabs, bronchoalveolar lavage), and autopsy material (fragments of the trachea, lung, bronchus, spleen) was obtained from clinics of St. Petersburg and Leningrad region and Federal Influenza Center basic laboratories. The samples were studied in real time RT-PCR using reagents and kits according to the CDC protocols, and AmpliSense kits, Central Institute of Epidemiology, Moscow. Sequencing was performed on ABI PRISM 3100-Avant Genetic Analyzer (Applied Biosystems, USA). Phylogenetic analysis was performed using Vector NTI 8.0 (Invitrogen) and MEGA 3.1 (PSU, USA), neighbor-joining and Kimura method.

Results

In the period from May 2009 to February 2010 analysis of 1,758 clinical and autopsy specimens revealed influenza A/H1N1v virus RNA in 409 patients with influenza and 163 deceased. Influenza A viruses of other subtypes and influenza B viruses were not detected. Molecular and genetic characterization of 62 pandemic strains (40 strains isolated from clinical specimens and 22 strains - from autopsy material) showed that HA and NA genes were similar to the reference strains. Most pandemic influenza Russian strains included in the analysis carried the mutations S203T in HA, N248D – in NA, I23V – in NS. Strain-specific amino acid substitutions at various positions of HA two of which located in antigenic sites of Ca and Sb were revealed in a number of Russian strains. The substitution D222G was found in HA of most influenza viruses (78%) isolated from autopsy material.

The 2010-2011 epidemic season was characterized by co-circulation of influenza viruses of three antigenic types with a predominance of influenza viruses A/H1N1v, participation of influenza B virus, and to a lesser extent - of influenza viruses A/H3N2. PCR-diagnostics of 742 clinical and 159 autopsy samples was performed. Genetic material of influenza viruses and/or other pathogens causing ARD was detected in clinical samples from 398 people (53.7% of the total surveyed) in January - March 2011. Influenza virus A RNA was detected in the clinical material obtained from 285 patients (71.6%). Influenza virus A/H1N1v RNA was detected in 185 persons (64.9%), influenza virus B RNA - in 98 patients (34,4%), influenza virus A/H3N2 RNA - in 2 persons (0.7%), indicating the flu epidemic of mixed etiology with a predominance of pandemic influenza viruses A/H1N1v. Only influenza virus A/H1N1v was detected in autopsy material from 60 patients. The 2010-2011 population of influenza virus A/H1N1v strains was genetically homogeneous and all representatives were evolutionarily related to the world dominant clade 7. Among the influenza virus A/H1N1v strains isolated in St. Petersburg we revealed strains that contained substitution A134T, S183P, and were related to the phylogenetic group (i) of clade 7, whose members were found to date in 7 countries (USA, Sweden, Norway, Finland, Great Britain, Luxembourg, Iran), as well as strains containing a substitution D97N, S185T and related to the phylogenetic group (iii) of clade 7, which was widely spread around the world in January-February 2011.

Conclusion

All the strains analyzed during 2 years had no mutations in NA determining oseltamivir resistance (H275Y) and contained a substitution S31N in the M2 protein determining the resistance of these viruses to adamantanes and characteristic of the worldwide circulating influenza strains A/H1N1v.

Molecular biological analysis revealed that the population of A/H1N1v strains is genetically homogeneous and during 2 years of circulation in the human population the pandemic virus has not undergone significant changes.

YOUNG SCIENTIST

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B507P

Evolution of H9N2 viruses: implications for human health

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Introduction

Avian influenza viruses of the H9N2 subtype are endemic in poultry populations across Asia and the Middle East and have occasionally been transmitted from poultry to mammalian species including humans and pigs. Based on the haemagglutinin gene sequence, these viruses fall into a number of genetically defined lineages, with the majority of viruses circulating in Asia belonging to two lineages – G1 and Y280 – represented by the prototype viruses A/quail/Hong Kong/G1/97 and A/duck/Hong Kong/Y280/97, respectively, which became established in domestic poultry during the mid-1990s. To explore the genetic characteristics, as well as the spatial and evolutionary dynamics of the H9N2 lineages that co-circulate in Central Asia and the Middle East, we conducted a phylogenetic analysis of whole genome sequences from H9N2 viruses sampled between 1998 to 2010 from nine Asian countries. The central aim of our study was to reveal the extent of inter- and intra- subtypic reassortment, as well as the frequency and pattern of viral gene flow. Additionally, we examined the emergence of amino acid mutations and their significance in terms of drug-resistance, adaptation to different host species, and pandemic potential.

Methods

We sequenced the complete genomes of 29 avian influenza H9N2 viruses isolated from poultry in Afghanistan, Jordan, Saudi Arabia, Iraq-Kurdistan, Iran, UAE from 2004 to 2010, as well as the partial genomic sequences of 5 additional H9N2 viruses from Iraq, Qatar, Jordan, UAE and Saudi Arabia. Sequences were aligned and compared with all the H9N2 sequences from the Middle East available in GenBank and with sequences of other subtypes. Maximum likelihood (ML) trees were estimated using the PAUP* package. Rates of nucleotide substitution per site and per year and the time of the most recent common ancestor were calculated using the Bayesian MCMC approach available in the BEAST package. Finally, we used the BaTS program to assess the overall degree of geographical structure among H9N2 viruses sampled from the Central Asian and Middle Eastern regions analyzed here.

Results

Our phylogenetic analysis revealed that four main genetic groups, namely A, B C, and D, have been co-circulating in domestic poultry in Central Asia and the Middle East. The HA, NA and M genome segments of the four groups (A to D), as well as the NP gene of group B, derive from the G1-lineage, while the remaining gene segments have been replaced through reassortment events, generating many different genotypes. Our phylogeographic analysis suggests that Eastern Asia serve as the major source for H9N2 gene segments in the Middle East and Central Asia, and that in this geographic region within-country evolution plays a more important role in shaping viral genetic diversity than between-country migration. Specific amino acid substitutions that are believed to result in increased transmissibility in mammals as well as resistance to antiviral drugs were also detected, and we identified amino acid residues under positive selection in the antigenic sites of the HA molecule, such that some H9N2 viruses could potentially differ in antigenicity. Finally, 76.4% of the viruses analyzed contain the amino acid leucine at position 226 that is responsible for human virus-like receptor specificity and critical for replication and direct transmission of H9N2 viruses in ferrets.

Discussion

The detection of increasingly large numbers of H9N2 strains showing human-like receptor specificity, combined with the wide circulation of the virus and the growing evidence for reassortment involving this subtype, emphasizes the need to constantly monitor the evolution of H9N2 viruses in poultry to better understand the potential risk for human health posed by these viruses.

YOUNG SCIENTIST

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B508P

recovery of reassortant pandemic-seasonal h1n1 influenza viruses from a clinical specimen.

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Pandemic A(H1N1) 2009 viruses appeared in March/April 2009 in Mexico and the USA, and were first introduced to New Zealand in late April 2009, immediately prior to the beginning of New Zealand's influenza season. During the 2009 influenza season in New Zealand, pandemic A(H1N1) 2009 and seasonal H1N1 viruses were co-circulating in the New Zealand population. Routine testing of ILI-samples as part of a National influenza surveillance program identified 11 patients that were co-infected with seasonal and pandemic H1N1 viruses.

Reassortment of pandemic A(H1N1) 2009 with other influenza A viruses has been identified as a risk factor for the appearance of novel viruses with altered phenotype. Of particular concern is the possibility that the pandemic viruses could acquire oseltamivir-resistance through acquisition of the neuraminidase gene segment of seasonal viruses. Reassortment events between endemic and pandemic influenza viruses have occurred in swine and in vitro in laboratory settings. To date no natural reassortant between these viruses has been reported in humans. Co-infection of patients with two different subtypes of influenza A viruses, or with influenza A and B viruses, has rarely been reported, as have naturally arising reassortants.
In order to determine whether reassortment events had taken place during patient co-infection in New Zealand in 2009, clinical samples were plaqued on Marbin-Darby canine kidney (MDCK) cells, grown on MDCK-SIAT-1 cells, and viruses genotyped by PCR. After initial identification of reassortants, two rounds of plaque-purification on MDCK cells was carried out, followed by partial sequencing of the viruses.
Reassortants of various gene combinations between seasonal and pandemic H1N1 (2009) were identified.

Table. Genotypes of five reassortants between pandemic A(H1N1) 2009 and seasonal H1N1 from a New Zealand patient*.

	PB2	PB1	PA	HA	NP	NA	M	NS
A/New Zealand/1212-a/2009	p	p	p	s	p	s	s	s
A/New Zealand/1212-b/2009	p	s	s	s	s	s	s	s
A/New Zealand/1212-c/2009	s	p	s	s	s	s	s	p
A/New Zealand/1212-d/2009	p	s	p	s	p	s	p	p
A/New Zealand/1212-e/2009	s	s	p	s	p	s	p	p

*p - pandemic A(H1N1)2009-like, s - seasonal A(H1N1)2009-like

These reassortants were recovered from a 29-year-old female patient without pre-existing conditions, who had not received the influenza vaccine before NZ's 2009 winter season. After exposure to a friend returning from Brisbane, Australia, the patient became symptomatic and experienced rapid onset of lethargy, malaise, cough, coryza, and some difficulty breathing. Upon doctor's consultation, the patient had a fever of 38.7ºC, tachycardia (108 beats per minute, resting), a blood pressure of 124/70 mmHg, and a respiratory rate of 24 breaths per minute, resting. The patient received oseltamivir within 24 hours of the doctor's consultation, as well as paracetamol, ibuprofen and salbutamol. Hospital transfer was considered but not carried out and the patient recovered fully at home. Further transmission history of this case is unknown, however, a close contact with a pre-existing condition and under oseltamivir prophylaxis did not become symptomatic.
The growth characteristics of the reassortants were compared with those of their parental strains on MDCK and normal human bronchial epithelial cells (differentiated). The pathogenicity of selected reassortant strains was then assessed *in vivo* in mice and ferrets. All reassortants identified to date contain the seasonal neuraminidase gene segment.

These data clearly show that reassortants between seasonal and pandemic viruses can be generated in humans, although there is no epidemiologic evidence that such viruses have spread to any degree.
No conflict of interest
No conflict of interest

SPB5 - GENETIC AND ANTIGENIC EVOLUTION

B509P

Mutations in the H1N1 2009 pandemic virus that adapt it for replication in human airway.

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In the UK, there have been three waves of influenza following the emergence of the new pandemic H1N1 virus (pH1N1) in 2009. In the most recent outbreak, winter 2010-2011, an increased disease burden was noted in previously healthy individuals whereas in the earlier waves many of the hospitalized patients suffered predisposing co-morbidities such as asthma or were immunocompromised (1).

In this study we asked whether genetic changes, accumulating in the virus as it adapts to humans after emerging from a porcine source, might be altering its phenotype.

The prototype virus for our study is from the first wave of the pandemic; A/England/195/09 was isolated from a mild early UK case. Subsequence viruses and sequences have been derived from the MOSAIC study which has collected viruses from individuals infected during the second and third waves of pH1N1 in the UK who experience disease severe enough to result in hospitalization. Whole genome sequencing revealed distinct genetic variation between viruses of the first, second and third waves of the pandemic. Sequence changes occurred throughout the genome including in the virus receptor binding HA protein and the RNA dependent RNA polymerase. Growth curves performed in highly differentiated human airway epithelial cultures indicate that the third wave viruses typified by isolate London 96 have enhanced ability to infect and spread through the human airway. Using a reverse genetic approach we have introduced mutations observed in the HA and NA genes of London 96 virus to test whether they are responsible for the increased replication capacity.

Although there is sporadic sequence variation in viruses from hospitalized we have not identified a single discrete virulence motif that accounts for the more severe outcome.

We conclude that H1N1 virus is evolving in its new host and some of the genetic changes will affect the biological consequence of infection with future viruses derived from this pandemic.

1. Nguyen-Van-Tam, J S, P J M Openshaw, a Hashim, E M Gadd, W S Lim, M G Semple, R C Read, et al. “Risk factors for hospitalisation and poor outcome with pandemic A/H1N1 influenza: United Kingdom first wave (May-September 2009).” *Thorax* 65, no. 7 (July 2010): 645-51.

SPB5 - GENETIC AND ANTIGENIC EVOLUTION

B510P

Epitope Mapping of the Hemagglutinin Molecule of A (H7N3) Influenza Virus by Using Monoclonal Antibodies

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Avian influenza viruses are zoonotic agents recognized as a potential threat to both veterinary and human public health. During last years infection of humans with avian influenza viruses subtypes H5, H7 and H9 has been detected on multiple occasions as a result of evolution of the viruses and acquisition of the capacity to infect humans directly without prior adaptation in mammalian hosts. In Netherlands/Germany the highly pathogenic H7N7 influenza viruses that were lethal to poultry infected the eyes of more than 80 people and killed one person. The viruses have not yet manifested effective human-to-human transmission, but the situation may change if the viruses continue to mutate and assort during an epidemic.

The purpose of this work was to obtain new MAbs to H7 subtype of avian influenza A virus.

Influenza viruses A/Mallard/Netherlands/12/00 (H7N3) was used as immunogen. The screening procedure of hybridoma cells was carried out using ELISA. Hybridomas producing specific antibodies to H7 virus were cloned by limiting dilutions in microtiter plates. As result of this investigation ten MAbs to H7N3 influenza virus were prepared as ascitic fluids and their activity was determined in ELISA with different subtypes of influenza A virus such as: human – H1N1, H2N2, H3N2 and H5N1 (vaccine strain NIBRG-14); swine – Hsw1N1; avian – H5N2, H5N3, H6N2, H6N5, H7N3, H8N4, H9N2, H11N9, H12N5, H14N5 and H15N8. All these MAbs were directed according to Western blot to influenza A(H7) virus HA1 and according to their specificity could be divided on four groups. First group (GI) of MAbs (7D11, 7H9, 9B2) interacted in ELISA test strongly with gomologous influenza virus and was found to be highly reactive (titers 1:20 000 – 1:40 000) in hemagglutination inhibition test. Second group (GII) of MAbs (8C2, *9E11*, 9G12 and 9G1) interacted in ELISA test both with influenza A (H7N3) and A (H15N8) viruses and were non reactive with other investigated influenza A viruses. Three of them (8C2, *9E11* and 9G12) showed hemagglutination inhibition activity with H7N3 and H15N8 influenza A viruses, while MAb 9G1 had not such activity. Third group (G III) of MAbs (8A3 and 9B10) interacted in ELISA test with influenza A (H7N3), A(H15N8) and A (H5N3) viruses and were non reactive in hemagglutination inhibition test with other investigated influenza A viruses. These MABs showed hemagglutination inhibition activity with A(H7N3) only but not with A(H5N3) and A(H15N8) influenza A viruses. The fourth group (GIV) of MAb (7G10) was unique. This MAb interacted in ELISA only with avian subtypes but did not react with swine and human (including H5N1) influenza A viruses.

For determination of the neutralizing epitopes in structure of hemagglutinin of influenza A(H7) virus number of MAbs were used to select the escape mutants of the virus. The HA1 genes of EMs were sequenced and predicted amino acid sequences were compared with original virus. The study found that EMs had the following substitutions: EM 9B2 - Q83L and A168D, EM 7H9- R139S, 7D11 - A168D (G I), EM 8C2 - G207E, EM 9E11 - K202N, EM9G12 – K202M (G II), EM 9B10 – E100G and G151E (G III).

YOUNG SCIENTIST

SPA6 - VACCINE SAFETY

A601P

Generation and characterization of laiv strains against potentially pandemic a(h2n2) influenza viruses

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Introduction

Influenza A(H2N2) viruses have not circulated in the human population since 1968 and the decline of herd immunity increases their pandemic potential. People under 40 years of age would lack specific aquired immunity to H2N2 antigens. Therefore the development of H2N2 virus vaccine candidates is a priority for influenza pandemic preparedness. Human influenza viruses which circulated at the end of H2N2 interpandemic period had diverged into two lineages with substantially distinct antigenic properties. We chose representative viruses from each lineage for the development of live attenuated vaccine (LAIV) candidate reassortants. The master donor virus (MDV) for Russian LAIV, A/Leningrad/134/17/57 (H2N2) (Len/17), is antigenically close to the wt H2N2 viruses and selection of reassortant progeny with antiserum after classical co-infection with two similar strains is challenging. Alternative approaches would include: (i) the use of a new MDV with HA and NA subtypes other than H2N2; and (ii) transfer of six internal genes of Len/17 from any seasonal 6:2 LAIV vaccine reassortant from a subtype other than H2N2. The A/Puerto Rico/8/59/1 (H1N1) (59/1) virus, a cold-adapted variant of A/Puerto Rico/8/34 (PR8) virus, is an alternative MDV since it has temperature sensitive (ts), cold-adapted (ca) and attenuated (att) phenotype. However, MDV has been less favored due to its resistance to adamantane antiviral drugs. Here we describe the engineering of an adamantane-sensitive 59/1 virus to be used as MDV.

Materials and Methods

Wild-type H2N2 strains A/Tokyo/3/67 and A/California/1/66 were obtained from CDC repository. MDV 59/1 and 6:2 reassortant virus A/17/New Caledonia/99/145 (H1N1) (17/NC), carrying six internal genes of Len/17 and HA and NA of A/New Caledonia/10/99 (H1N1) were obtained from IEM. A set of eight dual-promoter plasmids carrying all gene segments of PR8 was provided by CDC. Standard reverse genetics methods were used to introduce 59/1-specific mutations into six plasmids carrying internal genes of PR8 and rescue mutant virus in 293T/MDCK cells. H2N2 vaccine reassortant viruses were generated by classical reassortment of wt H2N2 virus with either new MDV or 17/NC virus in eggs using hyperimmune rat sera and cloning by limited dilutions. Vaccine candidates were fully sequenced using 3130xl Genetic Analyzer (Applied Biosystems). Ts and ca phenotypes of vaccine strains were determined by virus titration in eggs at different temperatures. Virus titers were calculated by Reed and Muench method and expressed as $\log_{10}EID_{50}$/ml.

Results

Six internal genes of PR8 were mutated to achieve consensus sequence of MDV 59/1. In addition, the two mutations (T27V and N31S) to restore adamantane sensitivity were inserted into the M2 protein. A reverse genetics virus named A/PR8/59/M2 (H1N1) (59/M2) was generated from from the six internal genes of 59/1 and HA and NA genes of wild-type PR8. Ts and ca phenotypes of 59/M2 strain were similar to original 59/1 MDV with titer reduction values of 6.7 $\log_{10}$ at 39°C compared to 33°C and 2.3 $\log_{10}$ at 26°C compared to 33°C. Two 6:2 reassortant vaccine candidates A/59/M2/Tokyo/67/22111 (H2N2) and A/59/M2/California/66/2212 (H2N2) were prepared in eggs and shown to have the same ts and ca phenotype as 59/M2 strain. We failed to generate 6:2 reassortants between wt H2N2 viruses and Len/17 MDV by classical method, so the vaccine strain 17/NC was used for reassortment as a source of internal genes of Len/17. The resulting reassortants, A/17/Tokyo/67/912 (H2N2) and A/17/California/66/4412 (H2N2), inherited six genes from Len/17 and HA and NA from the wild-type virus. Len/17-based LAIV reassortants were characterized as ts and ca with $\log_{10}$ titer reduction values comparable with Len/17 MDV.

Conclusion

We generated four A(H2N2) LAIV candidate reassortants based on two different MDV which can be evaluated in preclinical models with a view to entering clinical trials.

SPA6 - VACCINE SAFETY

A602P

Maternal outcomes in U.S. pregnant women <18 years of age receiving live attenuated influenza vaccine

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Background
Live attenuated influenza vaccine (LAIV) is approved for use in the European Union for eligible individuals 2–17 years of age; LAIV has been approved in the United States for use in this population for several years. LAIV is not recommended for use during pregnancy; however, rare administration during pregnancy can occur.

Objective
To estimate the rate of LAIV use in U.S. pregnant women 12–17 years of age and describe maternal outcomes after vaccination with LAIV

Methods
Data from a health insurance claims database in the United States that covers approximately 50 million individuals were analyzed over 6 influenza seasons from 2003 through 2009. Any woman 12–17 years of age with claims indicating vaccination with LAIV during pregnancy and the delivery of a child were included in the analysis. Primary diagnoses for emergency department (ED) visits and hospitalizations occurring within 42 days of vaccination were tabulated. Cohort characteristics were summarized using descriptive statistics.

Results
Over the entire study period, 14 women ≤17 years of age were identified as having received LAIV during pregnancy. Less than 0.05% of all pregnancies among women 12–17 years of age indicated vaccination with LAIV. Subjects were 14–17 years of age (mean, 16 years). Two subjects had underlying asthma while another had underlying type 1 diabetes. Most subjects were vaccinated in the first trimester (n=8), followed by the second (n=4) and third (n=2). Two pregnancies resulted in preterm deliveries (each at 35 weeks gestational age); all others had full-term deliveries. One subject was seen in the ED within 42 days of vaccination for a primary diagnosis of limb pain (day 29 postvaccination). No other ED visits or hospitalizations apart from delivery were seen.

Conclusions
In the U.S., administration of LAIV to pregnant women ≤17 years has been rare. In this limited cohort, there was no evidence of significant maternal adverse outcomes after vaccination with LAIV.
Sponsored by MedImmune, LLC.

SPA6 - VACCINE SAFETY

A603P

A post-licensure evaluation of the safety of live attenuated influenza vaccine in U.S. children 5–17 years of age

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Background

Live attenuated influenza vaccine (LAIV) was initially approved in the U.S. for eligible children >5 years of age in June 2003. A post-licensure commitment was made to describe LAIV safety among 40,000 pediatric recipients.

Objective

Evaluate the post-licensure safety of LAIV among individuals 5–17 years of age

Methods

Eligible children received LAIV as part of routine care from October 2003 through March 2008. Using the Kaiser Permanente healthcare database, rates of medically attended events (MAEs) in LAIV recipients were compared with rates in multiple nonrandomized controls: a self-control, matched unvaccinated controls, and matched trivalent inactivated influenza vaccine (TIV) recipients. Matching was based on age, gender, and prior healthcare use. Subjects with high-risk medical conditions were excluded. MAEs were identified in the clinic, emergency department, and hospital. All MAEs through 42 days postvaccination and all hospitalizations and deaths through 6 months postvaccination were analyzed. In the prespecified analysis, statistical significance was assigned without multiplicity adjustment.

Results

43,702 subjects 5–17 years of age were vaccinated with 53,369 doses of LAIV and matched with a similar number of TIV-vaccinated and unvaccinated children; all groups had similar demographics. Approximately 9500 MAE incidence rate comparisons were performed, 341 of which yielded statistically significant differences: 191 were higher and 150 were lower in LAIV recipients relative to controls. No asthma/wheezing MAEs were statistically increased in LAIV recipients; in 30 comparisons, asthma/wheezing MAEs were decreased after LAIV. No anaphylaxis events occurred within 3 days postvaccination. Only 1 MAE was associated with a significant increase among LAIV recipients relative to all three control groups; breast lump/cyst in subjects 9–17 years of age (n=7). Serious adverse events (SAEs) within 42 days postvaccination occurred in 64 subjects. Two SAEs were considered possibly related: Bell palsy and nonspecific paroxysmal spell. Three deaths occurred within 180 days postvaccination; all were considered unrelated to LAIV.

Conclusions

The results of this post-licensure evaluation of LAIV safety in U.S. children 5–17 years of age are consistent with pre-approval clinical studies and Vaccine Adverse Event Reporting System (VAERS) reports in the years after LAIV approval, both of which demonstrated no significant increase in asthma/wheezing events or other adverse outcomes among eligible children who received LAIV.

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SPA6 - VACCINE SAFETY

B604P

Safety and efficacy of live attenuated influenza vaccine in young children with asthma or a history of wheezing

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Background

Wheezing disorders and asthma are common in pediatrics and place these children at an elevated risk of complications due to influenza illness. In the European Union (EU), an Ann Arbor strain live attenuated influenza vaccine (LAIV) is approved for eligible children 2 through 17 years of age, including those with mild to moderate asthma. Although LAIV safety and efficacy among individuals with asthma have been described in children 6 through 17 years of age, there are limited data for children 2 through 5 years of age. Two previous randomized, controlled studies were conducted among children 6 to 71 months of age, but safety and efficacy were not specifically analyzed among the subgroups of children with asthma or a history of wheezing.

Objective

To evaluate the safety and efficacy of LAIV relative to trivalent inactivated influenza vaccine (TIV) among children 2 through 5 years of age with asthma or a history of wheezing

Methods

Based on the differences between the studies in data collection, inclusion/exclusion criteria, geography, and influenza season, the 2 study populations were analyzed separately. The primary analysis endpoints were wheezing events through 42 days postvaccination in LAIV versus TIV recipients for 4 groups: all children with a history of wheeze or asthma, children with wheezing within the previous 12 months, children without wheezing in the previous 12 months, and children with a diagnosis of asthma. Secondary outcomes included rates of solicited reactogenicity events through 10 days postvaccination, adverse events (AEs) through 28 days postvaccination, all-cause hospitalization through 180 days postvaccination, and the relative efficacy of LAIV compared with TIV against culture-confirmed influenza illness. AEs were summarized by system organ class and preferred term using MedDRA; AEs due to lower respiratory illness and wheezing were also summarized.

Results

The post hoc analysis population included 1940 children 24–71 months of age with asthma or a history of wheeze; 679 children had a diagnosis of asthma. Within each study, LAIV and TIV recipients were well matched for age, gender, race, and wheezing/asthma history. In the two studies, there were no statistically significant increases in wheezing among children receiving LAIV compared with TIV overall, or in any subgroup; rates (LAIV vs TIV for studies 1 and 2, respectively) for the overall population were 9.3% vs 10.3% and 18.5% vs 19.8% for wheezing, and 7.2% vs 8.6% and 13.5% vs 14.4% for medically-attended/documented wheezing. There were no statistically significant differences between LAIV and TIV in the rates of lower respiratory illness (9.4% vs 10.6% and 7.6% vs 9.5%) and hospitalization (2.4% vs 1.9% and 4.7% vs 3.3%). Postvaccination adverse events were similar between LAIV and TIV recipients with the exceptions of increased upper respiratory symptoms and irritability among LAIV recipients. There were 47% and 40% fewer cases of influenza among LAIV recipients in studies 1 and 2, respectively, consistent with the results observed in the overall study populations.

Conclusions

The results of the current analysis and previous studies support the safety and efficacy of LAIV among children 2 to 17 years of age in the EU with a history of prior wheezing illness or mild to moderate asthma. There are currently insufficient data to support LAIV use among individuals with severe asthma (e.g., those requiring oral corticosteroids) or with wheezing within the 7 days before vaccination.

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SPA6 - VACCINE SAFETY

A605P

Preclinical studies of live cold–adapted reassortant H5 and H7 influenza vaccines in a ferret challenge model

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Introduction

In the past few years the focus has mainly been placed on potentially pandemic influenza viruses which may acquire mutations facilitating their transmission to humans and subsequent human–to–human spread. More recently, the emergence of new H1N1 2009 strain which resulted from the reassortment of three viruses has produced a pandemic worldwide spreading in a matter of weeks. Continuing transmission of avian influenza viruses from birds to humans illustrates the urgent need for an efficacious, cross–protective vaccines to prevent their spread among humans besides the necessity to restrict H1N1 pandemic. In this study five potential pandemic live attenuated vaccine strains type H5 and H7 were investigated for their safety, immunogenicity and protection in ferrets – the best mammalian model for the study of human influenza.

Aim

In vivo characterization of type A influenza H5 and H7 reassortants as candidates for live attenuated vaccines against avian influenza viruses with pandemic potential.

Methods

Viruses. Reassortant vaccine strains containing either HA or HA and NA genes of low or highly pathogenic H5 and H7 avian influenza viruses and the rest 6 or 7 genes of A/Leningrad/134/17/57 (H2N2), master donor virus for Russian live attenuated reassortant influenza vaccine – A/17/Vietnam/1203/04 (H5N1), A/17/Vietnam/04/65107 (H5N2), A/17/turkey/Turkey/05/133 (H5N2), A/17/duck/Pottsdam/86/92 (H5N2), A/17/mallard/Netherlands/00/95 (H7N3). Animals. Ferrets 6–12 months of age received 2 doses 25–28 days apart of the 10^{7-8} EID_{50} of vaccine strains intranasally or were mock (PBS) vaccinated. Serum and nasal wash samples were collected and were frozen at –80°C until antibody assays were performed. Ferrets were challenged intranasally approximately six to seven weeks post–boost with a lethal dose of appropriate homologous or heterologous wild type virus. Clinical signs, body temperature and weight measurements were determined daily. The presence of viral antigen was determined by immunohistochemistry on formalin fixed tissues in paraffin and stained for influenza A nucleoprotein. Virus replication kinetic and neurovirulence were examined after vaccinations and challenge.

Results

Challenge viruses were fatal to intranasally inoculated ferrets of mock–vaccinated groups. After homologous or heterologous challenge with lethal virus, mock–vaccinated controls displayed signs of neurological dysfunction, showed severe symptoms of infection including virus shedding, significant weight loss (approximately ≥ 20% of their original body weight), temperature elevation, which peaked one to two days post–challenge (maximum temperature increased compared to average baseline temperature was 1.5°C to 2°C), caused severe macroscopy lung lesions etc. In contrast, ferrets vaccinated with live attenuated vaccines did not exhibit overt clinical signs of infection and survived the full experimental period. Vaccinated groups didn't show any significant loss of weight after the challenge. All vaccinated animals didn't shed challenge virus or shed it in low titers. Two doses of H5 or H7 live attenuated vaccines induced significantly high levels of virus specific antibodies agains the appropriate homologous strains, as well as heterologous strains. Additionally, vaccination provided complete protection against lethal homologous virus challenge and a significant reduction in disease severity following heterologous virus challenge.

Conclusion

We demonstrated that H5 and H7 live attenuated vaccines induced comprehensive cross–protection against disease outcomes and upper respiratory tract replication of a lethal virus. The protection against lethal challenge in ferrets was achieved by two vaccinations with homologous or heterologous live attenuated vaccines.

These results suggest that H5 and H7 live attenuated reassortant vaccines based on the A/Leningrad/134/17/57 master donor virus are a promissing public health tool for pandemic preparedness.

This research was supported by PATH and WHO.

SPA6 - VACCINE SAFETY

A606P

Similar safety and immunogenicity of the seasonal 2010-2011 vaccine in subjects who previously had or had not received an A/H1N1 pandemic vaccine

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Aim

Safety and immune response to the 2010-2011 NH seasonal trivalent influenza vaccine (TIV, Vaxigrip®) was assessed in 180 subjects who received two injections of the A/H1N1 pandemic influenza vaccine with or without adjuvant approximately 13 months earlier and in 103 subjects who did not receive the A/H1N1 pandemic influenza vaccine.

Materials and Methods

Anti-hemagglutinin (HA) antibody titers were measured in serum samples drawn at day 0 (before vaccination) and 21 days after one vaccine injection using the hemagglutination inhibition (HAI) assay method. The occurrence of adverse events was reported up to 21 days after vaccination.

Results

Prior to vaccination with TIV, high levels of HA antibody to the A/H1N1 strain in subjects who received the two injections of the A/H1N1 pandemic influenza vaccine were still present, showing good persistence of the immune response up to 13 months, regardless of the formulation. More than 80% of adult subjects and 50% of elderly subjects had seroprotective levels of HA antibody against the A/H1N1 strain (i.e., ≥40 (1/dil)).

Vaccination with the trivalent influenza vaccine 13 months after the A/H1N1 pandemic influenza vaccine injection induced a strong immune response against the A/H1N1 and the A/H3N2 strains. More than 80% of subjects reached the seroprotection threshold 21 days after TIV injection in all age groups, regardless of history of previous vaccination with the A/H1N1 pandemic influenza vaccine in all age groups. A weaker immune response was observed for the B strain, particularly in elderly subjects. The weaker immune response observed against the B strain can possibly be explained by a lower sensitivity of the HAI assay for the B influenza strain.

In adults, the three EMA immunogenicity criteria (e.g., seroprotection rate, GMT ratios and seroconversion rate) were reached against all three strains. In the elderly subjects, the three EMA immunogenicity criteria were met in all groups for the two A strains. For the B strain, all criteria were met in subjects who received only the TIV, one criterion was met in subjects who received the A/H1N1 pandemic influenza vaccine without adjuvant and no criteria were met in subjects who received the A/H1N1 pandemic influenza vaccine with adjuvant. However, post-vaccination GMTs were similar across the 3 groups.

The safety profile of the TIV was similar regardless of the history of previous vaccination with the A/H1N1 pandemic influenza vaccine.

Conclusions

The results of this study show that previous vaccination with a pandemic A/H1N1 influenza vaccine with or without adjuvant had no impact on the immune response and the safety of the 2010-2011 NH seasonal trivalent Influenza vaccine (TIV, Vaxigrip®) in adults and elderly.

SPA6 - VACCINE SAFETY

A607P

Acceptance of an intradermal influenza vaccine in routine clinical practice in the Czech Republic and Turkey

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Aims

Intanza®/IDflu® 9 µg and Intanza®/IDflu® 15 µg (sanofi pasteur) are split-virion trivalent influenza vaccines delivered by intradermal (ID) injection using the BD Soluvia™ microinjection system. Intanza® 9 µg is designed to provide protective immunity against seasonal influenza in adults aged 18-59 years and Intanza® 15 µg is designed to provide enhanced immune responses against seasonal influenza in adults 60 years and older. Intanza® 9 µg and 15 µg became available for vaccination of adults aged 18 to 59 years in the Northern Hemisphere beginning with the 2010-2011 influenza season. The aim of this survey study was to assess the acceptability of ID vaccination with Intanza® 9 µg and Intanza® 15 µg in routine clinical practice by patients and their prescribers in the Czech Republic, while in Turkey, Intanza® 9 µg vaccinees, their vaccinators, and prescribers were surveyed.

Methods

Vaccinees: In the Czech Republic, 1013 healthy adult vaccinees aged 18–59 years (Intanza® 9 µg) or 60 years or older (Intanza® 15 µg) were enrolled. In Turkey, 249 vaccinees were surveyed. The vaccinees' age range expected in Turkey was 18 to 59 years as only 9 µg ID vaccine was available. However, 5.6% of the patients responding to the questionnaire were 60 or older in this country. All vaccinees surveyed had elected to received either Intanza® 9µg or Intanza® 15 µg (Czech Republic) or Intanza® 9 µg (Turkey) when offered a choice between Intanza® and standard intramuscular (IM) influenza vaccine (Vaxigrip®, sanofi pasteur).

Vaccine prescribers/vaccinators: In the Czech Republic, 28 vaccine prescribers of whom 85.7% represented general practitioners also responded to a questionnaire measuring their opinions on influenza vaccination and acceptance of the ID vaccination. In Turkey, 18 vaccinators and 15 prescribers (mainly specialists) participated in this survey.

Results

Czech Republic

Vaccinees: Of the 1013 vaccinees in the Czech Republic, 96.1% reported they were 'satisfied' or 'very satisfied' with Intanza® 9 µg or Intanza® 15 µg vaccine. The main reasons for satisfaction were the injection was considered minimally painful and that the vaccine was administered quickly. Most vaccinees (92.9%) reported that they preferred ID over IM vaccination. When surveyed on the day of immunization, 92.8% reported a preference for ID vaccination for the following influenza season. 87.5% reported a preference for ID vs. IM vaccination 8 days post vaccination. Survey responses were similar across age and dosage groups.

Prescribers: Of the 28 vaccine prescribers who responded to the questionnaire, 92.9% reported being 'satisfied' or 'very satisfied' with Intanza® and 71.4% reported a preference for ID over IM vaccination.

Turkey

Vaccinees: In Turkey, most of the vaccinees had felt at risk for contracting influenza (90.3%; 224). In all, 27.0% reported that they were vaccinated every year, while 51.6% reported that they had not been vaccinated in the past. 96% of vaccinees reported been 'satisfied' or 'very satisfied' with Intanza® 9µg ID influenza vaccination with a minimal pain seen as the main reason for satisfaction. 94.2% of vaccinees who had previously been vaccinated declared a preference for ID vaccine.

Vaccinators: Of the 18 vaccinators surveyed, 94.4% reported being 'satisfied' or 'very satisfied' with Intanza® 9 µg. All preferred ID over IM vaccination.
Prescribers: Of the 15 prescribers who responded to the survey, 5 reported to be 'very satisfied' with Intanza® with 10 reporting being 'satisfied'. All preferred ID over IM vaccination.

Conclusions

ID vaccination against seasonal influenza using Intanza® 9 µg and Intanza® 15 µg is well accepted both by vaccinees and prescribers. Therefore, Intanza® 9 µg and Intanza® 15 µg may have the additional benefit of increasing vaccination rates in adults against seasonal influenza.

Conflict of interest

Employee:: Francoise Weber sanofi pasteur Serdar Altinel sanofi pasteur Radka Sichova sanofi pasteur; Commercially-sponsored research:: Roman Prymula sanofi pasteur Gaye Usluer sanofi pasteur;

SPA6 - VACCINE SAFETY

A608P

Twelve Month Follow-Up Safety Data After a Two-Dose Priming Schedule with MF59®-Adjuvanted H1N1 2009 Pandemic Influenza Vaccine in Paediatric, Adult and Elderly Subjects

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Aims

Controlled clinical trials have demonstrated MF59®-adjuvanted, monovalent A/H1N1 pandemic vaccine to be highly immunogenic and well tolerated. Further to previous safety analyses covering periods up to three weeks post-vaccination, we now report the results of twelve month follow-up periods to fully assess the safety profiles of various investigational vaccine formulations.

Methods

Data were obtained from four separate trials including subjects aged from 6 to <36 months-old (Group A, 1 trial), 3 to <9 years-old (Group B, 2 trials), 9 to 17 years-old (Group C, 1 trial), 18 to 64 years-old (Group D, 2 trials), and elderly subjects ≥ 65 years of age (Group E, 1 trial). All trials investigated levels of immunogenicity and safety profiles following two primary vaccine doses administered three weeks apart. Subjects included in the long-term safety follow-up from Groups A (N = 627), B (N = 655), D (N = 679) and E (N = 678) were randomly assigned to one of four immunization groups (1:1:1:1) to receive vaccine either containing 3.75 mg of A/California/7/2009 strain H1N1 influenza antigen with half the standard dose of MF59 adjuvant (3.75-50%), 7.5 mg antigen with half a dose of MF59 (7.5-50%), or non-adjuvanted formulations containing 7.5 mg (7.5-0%) or 15 mg (15-0%) of antigen. Subjects in the safety follow-up from Group C (N = 137) were assigned (~1:2) to receive vaccine containing 7.5 mg antigen with a full dose of MF59 (7.5-100%) or the 15-0% formulation. Selected adverse events (AEs), including serious adverse events (SAE), were recorded over the twelve month safety periods (Day 43 to Day 387).

Results

In **Group A** (6 to <36 mo), across the four vaccination groups, SAEs were experienced by 1-6% of subjects. The onset of a new chronic disease occurred in 3-4% of subjects, and 68-72% of subjects required the non-routine attention of a health care provider. None of these events were considered to be related to vaccination. No AEs occurred which resulted in the withdrawal of infants from the study. In **Group B** (3-9 yr), 0-4% of subjects experienced a SAE, the onset of a new chronic disease occurred in 1-4% of children, and 54-60% required the attention of a health care provider. None of these events were vaccine-related. No study withdrawals due to AE occurred in this group. No adolescents in Group C (9-17 yr) receiving the 15-0% vaccine formulation experienced an adverse event during the twelve month observation period; the attention of a health care provider was required by 26% of vaccinees. Four percent of **Group C** subjects receiving the 7.5-100% formulation experienced a non-vaccine-related SAE, no incidences of new chronic diseases or study withdrawals occurred, and 18% of subjects required the attention of a health care provider. In **Group D** (18-64 yr), 2-3% and 4-7% of subjects experienced a SAE or developed a new chronic disease, respectively; none of these events were considered to be vaccine-related. The attention of a health care provider was required by 44-49% of subjects, and no AEs resulting in study withdrawal occurred. **In Group E** (≥ 65 yr), 4-10% of elderly subjects experienced a SAE, 77-82% required the attention of a health care provider, and 6-10% developed a new chronic disease over the twelve month follow-up period; none of these events were related to vaccination. One subject receiving the 7.5-0% vaccine, and one subject receiving the 7.5-50% vaccine were withdrawn from the study because of non-vaccine-related AEs.

Conclusions

These safety data demonstrate that all of the investigational MF59-adjuvated A/H1N1 vaccine formulations studied were well tolerated and showed robust safety profiles in individuals from six months to over sixty-five years of age.**Trial registration:** www.clinicaltrials.gov: NCT00973349; NCT00972816; NCT00973700; NCT00996307

SPA6 - VACCINE SAFETY

A609P

On-field acceptability of a new intradermal trivalent, inactivated, seasonal influenza vaccine in Liguria, Italy

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Aims

A new intradermal (ID), trivalent, inactivated, seasonal influenza vaccine (Intanza®, Sanofi Pasteur, France) has been available in Italy for the season 2010/2011. An "on field" randomised study was carried out in elderly people (i) to evaluate its acceptability and (ii) to compare it with that of a trivalent subunit virosomal formulation (Inflexal V®, Berna,Switzerland), given using the intramuscular (IM) route.

Methods

Between November and December 2010, 500 subjects aged ≥ 60 years, vaccinated at the outpatient clinics of the Department of Health Sciences, San Martino Hospital, University of Genoa and of the Local Public Health Unit of Genoa (ASL 3 Genovese), Italy, were enrolled into the study: a previously validated and self-administered questionnaire - VAPI© (Vaccinees' Perception of Injection) - was given to each subject, to be completed and returned to investigators 21 days following vaccination. The questionnaire, according to recommended standard procedures, was made-up of twenty-one questions concerning the acceptability of injection site reactions (ISRs), in terms of anxiety and bothers associated with the administration, the effects of ISRs, partucularly pain, on the ability to perform everyday activities, focusing on sleeping and arm movement, the general satisfaction with the injection system and, finally, the willingness to be revaccinated. The questions were answered using a 5-point verbal rating scale (1= most favorable, 5= most unfavorable response), and mean scores were calculated for each item investigated in the two treatment groups.

Results

Four-hundred and eighty-one fully completed questionnaires were returned (96.2%) and evaluated: 255 and 226 were filled by subjects vaccinated with ID-vaccine and IM-vaccine, respectively. The mean age of the study population was 75.3 years (SD= 8.1) and the male-female ratio was ~ 1:1.

Overall, subjects immunized expressed a very favourable opinion concerning the acceptability of both influenza vaccines, with the most positive answers ranging between percentages of 76.5% and 94.8%. Answers to questions investigating the role of bothers and effects of the ISRs on the change of the ability of the individual in performing routine activities resulted generally favourable in both groups: these items, even those related to pain, were considered "totally acceptable" in nearly 90% of the subjects, and more than 80% of the individuals resulted "very satisfied" with the injection system. Ninety-five percent of the subjects declared not to be feared about the idea of immunisation, and nearly 100% of the individuals reported a positive willingness to be re-vaccinated against influenza during the following season. With respect to the comparison between treatment groups, only few differences were observed. The majority of subjects reported that there was "no bother at all" due to ISRs, but, as expected, "little" bother due to redness and itching occurred more frequently in the ID-group than in the IM-group, with values of 18.4% vs 6.2% ($p<0.001$) and of 14.9% vs 7.1% ($p=0.006$), respectively. Another significant difference resulted for a "moderate" bother due to induration, described in 2.8% of the individuals immunised with the ID-vaccine vs 0% of those vaccinated by IM route ($p=0.029$).

Conclusion

The study showed a very good acceptability of both the vaccines under survey in the elderly. As expected, since the ID injection is close to the skin surface, an higher bother due to redness, itching and induration was observed in the ID-group, but it was in a few proportion of subjects and its clinical relevance was not meaningful.

SPA6 - VACCINE SAFETY

A610P - Vaccine safety

Adverse effects of influenza vaccine between 2009/2010 and 2010/2011 in the Valencian Community

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Background

Monitoring vaccine safety is a complex and shared responsibility. It can be carried out in many ways; one of them is to reporting from the individual cases of adverse reactions, caused by the vaccination. Health care workers of the Valencian Community (VC) report suspected adverse effects following immunization (AEFIs) through the Immunization Information System (SIV). The adverse effect of the immunization is reported in the vaccine records of each individual in the SIV. The VC carries out a vaccination campaign of seasonal influenza every year. In 2009-2010, it also was included the vaccination against AH_1N_1 influenza virus.The aim of the study is to describe suspected adverse effects of influenza vaccine in health care workers, and over 6 months of age with risk of severe complications caused by influenza in 2009-2010 and 2010-2011 seasons in the Valencian Community.

Methods

A descriptive analysis was done, including the cases of influenza vaccine adverse effects reported through the SIV. We calculated the rates by sex , by age groups, by risk groups and by type of reaction to vaccination (local or general), and by influenza season. The study period was from October 1st 2009 to February 28th 2011. We used SPSS 14.0 for the statistical analysis.

Results

A total of 1,708,428 doses of influenza vaccines were administrated and recorded in the SIV between October 1st 2009 and February 28th 2011. During that time 463 possible AEFIs from 257 reports (rate 15,04 X 10^5) were received. 310 of the total of reported AEFIs were systemic symptoms, and 153 local symptoms.

Table 1. Reporting rate (per 1000 persons vaccinated) by age, by sex and by risk group, by influenza season.

			Healthcare workers	> 6 month of age (risk factors)
Influenza season	Age	Sex	Rate (95% CI)	Rate (95% CI)
2009 / 10	< 15 yr.	M	0,000	0,057 (-0,055 - 0,170)
		F	0,000	0,000
		TOTAL	0,000	0,034 (-0,033-0,100)
	15-60 yr.	M	0,177 (-0,170 - 0,525)	0,040 (-0,015 - 0,094)
		F	0,142 (-0,055 - 0,340)	0,043 (-0,016 - 0,101)
		TOTAL	0,152 (-0,020 - 0,325)	0,041 (-0,001 - 0,081)
	> 60 yr.	M	0,000	0,004 (-0,004 - 0,012)
		F	0,000	0,017 (0,002 - 0,031)
		TOTAL	0,000	0,011 (0,002 - 0,020)
	TOTAL	M	0,177 (-0,170 - 0,525)	0,013 (0,000 - 0,026)
		F	0,142 (-0,055 - 0,340)	0,019 (0,005 - 0,034)
		TOTAL	0,135 (-0,018 - 0,2888)	0,016 (0,001 - 0,026)
AH1N1 09/10	< 15 yr.	M	0,000	2,396 (1,223 - 3,569)
		F	0,000	3,193 (1,523 - 4,864)
		TOTAL	0,000	2,712 (1,743 - 3,681)
	15-60 yr.	M	6,021 (3,619 - 8,423)	0,759 (0,426 - 1,091)
		F	10,377 (8,009 - 12,745)	1,386 (0,906 - 1,866)
		TOTAL	8,801 (7,058 - 10,545)	1,052 (0,766 - 1,337)
	> 60 yr.	M	6,803 (0,159 - 13,447)	0,128 (0,049 - 0,208)
		F	12,500 (2,561 -22,439)	0,203 (0,097 - 0,309)
		TOTAL	9,363 (3,587 - 15,139)	0,163 (0,098 - 0,229)
	TOTAL	M	6,122 (3,861 - 8,382)	0,415 (0,295 - 0,535)
		F	10,512 (8,206 - 12,818)	0,622 (0,465 - 0,779)
		TOTAL	8,851 (7,181 - 10,521)	0,366 (0,284 - 0,449)
2010 / 11	< 15 yr.	M	0,000	0,106 (-0,101 -0,313)
		F	0,000	0,305 (-0,118 - 0,728)
		TOTAL	0,000	0,187 (0,025 - 0,399)
	15-60 yr.	M	0,236 (-0,227 - 0,700)	0,024 (-0,023 - 0,072)
		F	0,736 (0,191 - 1,281)	0,027 (-0,026 - 0,079)
		TOTAL	0,582 (0,179 - 0,986)	0,025 (-0,010 - 0,061)
	> 60 yr.	M	0,993 (-0,952 - 2,938)	0,013 (-0,002 - 0,028)
		F	1,933 (-0,252 - 4,118)	0,022 (0,004 - 0,039)
		TOTAL	1,563 (0,032 - 3,094)	0,018 (0,006 - 0,030)
	TOTAL	M	0,382 (-0,147 - 0,911)	0,018 (0,002 - 0,034)
		F	0,904 (0,344 - 1,464)	0,028 (0,010 - 0,046)
		TOTAL	0,736 (0,320 - 1,153)	0,023 (0,011 - 0,036)

The most frequent report of suspected adverse reaction was fever (22.25%), followed by pain local site (15.98%).

Conclusions

The rate of AEFIs caused by pandemic influenza is higher than seasonal influenza 2009-2010 and 2010-2011. The risk group with highest AEFIs rate is in health care workers. And the group of people over 60 years of age has the lowest AEFIs

YOUNG SCIENTIST

SPB6 - MATHEMATICAL MODELLING

B601P

Structured meta-population dynamics of human influenza A H3N2 virus

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In temperate regions, populations of seasonal influenza virus experience strong annual bottlenecks that pose a considerable extinction risk. It has been suggested that a possible influenza source population, perhaps located in tropical Southeast or East Asia, seeds annual temperate epidemics. Here we investigate migration of influenza A H3N2 virus by analysis of virus samples obtained between 2003 and 2006 from Australia, Japan, New York, New Zealand, Southeast Asia and newly sequenced viruses from Hong Kong. Bayesian phylogeographic analysis using discrete temporal and spatial characters revealed migration between all urban centers tested, with no evidence that East or Southeast Asia maintains a viral population that seeds epidemics in other regions over this time period. Rather, multiple divergent lineages often seed annual influenza epidemics, and each region can function as a potential source population. Persistent lineages that seed subsequent epidemics emerge as virus populations migrate through highly connected urban centers. The combined effect of influenza outbreaks in major urban centers with coinciding epidemic peaks and migration between these centers enables the continued circulation and maintenance of seasonal influenza in humans. We therefore propose that the global persistence of H3N2 influenza A virus is due to a migrating meta-population, in which different localities seed seasonal epidemics in temperate regions in a given year. Such complex global migration dynamics may confound control efforts and contribute to the emergence and spread of antigenic variants and drug resistant viruses.

SPB6 - MATHEMATICAL MODELLING

B602P

Validating early estimation of the transmission potential of pandemic influenza (H1N1-2009): Sample size estimation for post-epidemic seroepidemiological studies

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Introduction

Seroepidemiological studies before and after the epidemic wave of influenza (H1N1-2009) are useful for estimating final size with a potential to validate early estimates of the reproduction number, R, in modeling studies. Nevertheless, a glance at the literature shows that various seroepidemiological studies published so far have adopted a binomial sampling process to quantify the uncertainty of the proportion of infected individuals.

Methods

In the present study, we employ the use of an asymptotic distribution of the final epidemic size which is derived from a stochastic epidemic model. This allows for the computation of approximate 95% confidence intervals of the proportion of individuals in a population infected during an epidemic, is proposed since infection events are not independent. More importantly, this approach allows the comparison of observed final sizes against model studies based predictions (R=1.15, 1.40 and 1.90) while yielding simple formulae for determining acceptable sample sizes for future seroepidemiological studies.

Results

Eleven published seroepidemiological studies of H1N1-2009, which took place after observing the peak incidence in a number of countries, are used in the testing of the methodology. Observed seropositive proportions in six studies appear to be significantly smaller than those predicted from R=1.40; four of the six studies sampled serum less than one month after the reported peak incidence. Comparisons of observed final sizes against R=1.15 provide evidence that all eleven studies do not significantly deviate from the prediction with R=1.15 while comparisons with R=1.90 suggest that the final sizes in nine studies would be overestimated.

Conclusions

Sample sizes of published seroepidemiological studies were too small to assess the validity of model predictions except when R=1.90 was used. We recommend the use of the proposed approach in determining the sample size of post-epidemic seroepidemiological studies, calculating the 95% confidence interval of observed final size, and conducting relevant hypothesis testing instead of the use of methods that rely on a binomial proportion,

SPB6 - MATHEMATICAL MODELLING

B603P

Attributable deaths due to influenza in Austria - a comperative study of seasonal (from 1999/00 through to 2008/09) and pandemic (2009) influenza.

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Background

Influenza epidemics occur nearly every winter, leading to an increase in hospitalizations and deaths. Up to now the overall impact of attributable deaths due to seasonal and pandemic influenza viruses in Austria has not been investigated in detail.

Objectives

To determine and compare the number of attributable deaths due to seasonal and pandemic influenza A(H1N1) 2009 in Austria in different age groups adjusted for the confounding effect of co-circulating Respiratory Syncytial Virus (RSV) that may also cause excess mortality in the very young and in the elderly.

Methods

A poisson model, relating age and daily deaths to week of influenza season using national mortality and viral surveillance data, was used to estimate the contribution of influenza associated deaths in Austria for ten consecutive seasonal influenza waves and for the pandemic influenza A(H1N1) 2009 season in Austria.

Results

Using this mathematical model, we calculated an average of 316 influenza associated deaths per seasonal influenza epidemic occurring from 1999/00 to 2008/09 in the area of Vienna, Austria. Based on this data the total number of deaths due to seasonal influenza for the whole country of Austria is estimated to be about 1300 influenza associated deaths per season. For the pandemic influenza season 2009/10 we calculated 241 deaths attributable to influenza A(H1N1) 2009 virus in the area of Vienna with an estimate of about 1000 deaths for the whole country of Austria. Comparing the mortality data calculated for seasonal and pandemic influenza viruses in different age groups revealed a statistically significant increase in mortality for pandemic A(H1N1) 2009 influenza virus in the age groups of 0-34 years and a significant decrease in mortality in the age groups above 45 years.

Conclusions

Our data adjusted for circulating RSV further confirm that, mortality associated with seasonal influenza disproportionately affects persons above 45 years, whereas the largest impact of pandemic influenza virus on mortality could be observed in those below the age of 34 years.

SPB6 - MATHEMATICAL MODELLING

B604P

Characterizing the epidemiology of the 2009 influenza A/H1N1pandemic in Mexico

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Introduction

Mexico's local and national authorities initiated an intense public health response during the early stages of the H1N1pdm influenza pandemic in 2009. Here we analyze the epidemiological patterns of the pandemic during April-December 2009 in Mexico and evaluate the impact of non-medical interventions, school cycles, and demographic factors on influenza transmission.

Material & Methods

We used influenza surveillance data compiled by the Mexican Institute for Social Security, representing 40% of the population, to study patterns in influenza-like illness (ILI) hospitalizations, deaths, and case fatality rate by pandemic wave and geographical region. We also estimated the reproduction number (R) based on the growth rate of daily cases, and used a transmission model to evaluate the effectiveness of mitigation strategies initiated during the spring pandemic wave.

Results

A total of 117,626 ILI cases were identified during April-December 2009, of which 30.6% were tested for influenza, and 23.3% were H1N1pdm-positive. A three-wave pandemic profile was identified, with an initial wave in April-May (Mexico City area), a second wave in June-July (southeastern states), and a geographically-widespread third wave in August-December. The median age of laboratory confirmed ILI cases was ~18 years overall and increased to ~31 years during autumn ($p<0.0001$). The case-fatality ratio among ILI cases was 1.2% overall, and highest (5.5%) among people over 60 years. The regional R estimates were 1.8-2.1, 1.6-1.9 and 1.2-1.3 for the spring, summer and fall waves, respectively. We estimate that the 18-day period of mandatory school closures and other social distancing measures implemented in the greater Mexico City area was associated with a 29-37% reduction in influenza transmission in Spring 2009. In addition, an increase in R was observed in late May and early June in the southeast states, after mandatory school suspension resumed and before summer vacation started. State-specific fall pandemic waves began 2-5 weeks after school re-opened for the fall term, coinciding with an age shift in influenza cases.

Conclusions

We have documented three spatially heterogeneous waves of the 2009 H1N1 pandemic virus in Mexico, which were characterized by a relatively young age distribution of cases. Our study highlights the importance of school cycles on the transmission dynamics of pandemic influenza and suggests that school closure and other mitigation measures can be useful to mitigate future influenza pandemics.

YOUNG SCIENTIST

SPB6 - MATHEMATICAL MODELLING

B605P

Impact of the 2009 influenza pandemic on pneumococcal pneumonia hospitalizations in the US

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Background

Experimental and epidemiological studies have established that infection with influenza virus increases the risk for developing pneumococcal disease. However, both pathogens display strong winter seasonality in temperate locations, and in a typical year, it is difficult to quantify the independent effect of influenza on pneumococcal disease. The recent A/H1N1pdm influenza pandemic peaked in the spring and fall of 2009 in the US, allowing for the observation of this synergistic relationship in the absence of typical seasonal cofactors.

Methods

Using weekly age- and cause-specific hospitalizations from the State Inpatient Databases of the Healthcare Cost and Utilization Project, we used regression models to quantify the increase in pneumococcal disease incidence above a seasonal baseline during the 2009 pandemic period. Additionally, we compared the magnitude of the excess pneumococcal pneumonia hospitalization rates in 2009 with influenza-attributable incidence estimates from recent years and evaluated variations between states in the effect of influenza on pneumococcal disease.

Results

We found a significant increase in pneumococcal pneumonia hospitalizations in the period from late August-mid December 2009, which corresponded to the timing of thepandemic. The 5-19 year old population, which has a low baseline level of pneumococcal disease, had the largest relative increase in hospitalizations, with a 1.65-fold increase over baseline (95% CI 1.42-1.89). In absolute terms, the largest increase in incidence was observed among the 40-64 year old population with 1.45 excess hospitalizations/100000 (95% CI: 1.18-1.71). No increase in pneumococcal pneumonia was observed among the 65-plus population. The effect of influenza also varied between states according to the timing of the fall influenza pandemic wave.

Conclusions

The 2009 influenza pandemic had a significant impact on the incidence of pneumococcal disease hospitalizations, with the magnitude of this effect varying between age groups and states, mirroring observed variations in influenza activity.

SPB6 - MATHEMATICAL MODELLING

B606P

Pretopological modelling of influenza epidemics by multi-agent system and geographic information system

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Aims

In this paper, we are proposing a mathematical model that allows us to efficiently model social networks more subtly than small world model. For example, our model considers that relationships between people are not symmetrical. So this modelling is a necessary step if we want to simulate in an appropriate manner an epidemic phenomenon in which individual's behaviours play a major role. Hence, only considering the virus strength is not sufficient to explain how epidemics spread out: events enabling various contacts between people are essential in epidemics outbreaks. These contacts are made through various relationships between people and according to complex modalities. As a lot of papers deal with the spreading of influenza's epidemics, in this work, we focus on providing a decision-making tool in order to help a decision-maker take appropriate decisions so as to preserve the minimal economic functions for the society's survival, in the case of a sanitary crisis situation such as the influenza A\H1N1.

Methods

As the society takes a central role in our model, we base our approach on the concept of social networks. Social networks are mainly modelled by random graphs with some assumptions that are not too realistic. So, we introduced a new mathematical model: the stochastic pretopology that extends random graphs while suppressing their assumptions. Stochastic pretopology is in fact a coupling of two mathematical theories: extended topology and random sets. At the same time, stochastic pretopology provides a useful mathematical framework to develop simulation modelling based on the multi-agent system paradigm as detailed hereafter.

That mathematical model is implemented through a multi-agent system approach. We opted for this approach because we wish to set up a model of decision-making support. Multi agent systems reveal the best tool to model complex systems and to allow us to simulate them. Furthermore, we can define an explicit and dynamic representation of people's behaviour. Multi-agent system also enables us to take into account simultaneously the way individual behaves, interactions between people and the emergence of a general behaviour from individuals. This approach is coupled with a geographical information system to integrate the spatial aspect in the model. By exploiting epidemiological data gathered by Regional Groups of Observation of the Flu (GROG) and socio-demographic features from the National Institute for Statistics and Economic Studies (INSEE), we can perform simulation and then test the robustness of the model.

Results

To run simulation, we differentiate three types of agents because the impact of their infection on economic dynamics is not the same: the "simple" people, the medical staff and people who have a critical job regarding society functioning such as decision-maker, policemen, nuclear power plant employees,... We also integrate into the model four types of relations: professionals, household, transport and leisure activities. By mean of stochastic pretopology, throughout the day, we can follow step by step the evolution of the health status of the different type of agents. Having such information, a decision-maker can take the most adequate decisions such as, for example, to close a highway to protect the workers of a nuclear power plant.

Discussion/Conclusion

The choice of stochastic pretopology instead of small-world model is guided by the fact that stochastic pretopology enables simultaneously considering several types of relationships, giving a way to be more close to the real world in the modelling process. Another advantage of stochastic pretopology is to be able to give conclusion about groups of people, not only on people. Its third interest is how it is adapted as a conceptual background for multi-agent systems.

SPB6 - MATHEMATICAL MODELLING

B607P

Determinants and predictability of the spatio-temporal dynamics of the 2009 h1n1 pandemic in europe

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Background

Introduction Influenza pandemics of last century were characterized by successive waves and differences in impact and timing between different regions, for reasons not clearly understood. The 2009 H1N1pdm influenza has spread very rapidly across the globe, but still in a rather heterogeneous way, beyond expected timing differences between Northern and Southern hemisphere. A notable pattern occurred in Europe, with UK exhibiting a first wave of transmission in the early summer, followed by a second one in the autumn, while all other European countries had only a limited transmission before the summer and a single wave in autumn/winter. Moreover, a clear West to East pattern of spread was observed, similar to that sometimes seen for seasonal flu. Here we analyze which factors are most responsible for the observed geographical differences in Europe, and to which extent the pattern was predictable on the basis of the first available data on the spread of H1N1pdm in Mexico, US and UK.

Material & methods

To such aim, we developed an individual-based stochastic simulation model for Europe. Specifically, the simulation is a spatially-explicit discrete-time SEIR model with force of infection decreasing with the geographical distance which explicitly models transmission in households, schools and workplaces. Country-specific socio-demographic data were used to parameterise the distribution of individuals in households, schools and workplaces. Infection spread between countries is modelled through cross-border diffusion and long-distance travel, making use of European air and railway transportation data.

The model was parameterised using data about H1N1pdm collected by the beginning of June 2009. We did not fit the model to the observed pattern of spread (which is possible only after the pandemic); rather, we used parameter values estimated from the first published analyses and examined the extent to which the model predicted spread agrees with the pattern of spread seen in the Europe in the summer and autumn of 2009.

Results

The model predicts almost inevitably two waves in UK and a single wave in autumn/winter in the rest of Europe as a consequence of timing of H1N1pdm spread, fluxes of travels from US and Mexico, and timing of school vacations, with timing of the peaks of the two waves strongly determined by the dates of summer and autumn school holidays. The model provides a description of pandemic spread through Europe, depending also on inter-European mobility patterns and socio-demographic structure of the different European populations, which is in broad agreement with observed peak weeks in the different countries. As a consequence of marked differences in the socio-demographic structure of European countries, the model predicts substantial variation in cumulative infection attack rate across Europe, with predicted age dependent attack rates broadly agreeing with available serological data.

Conclusions

Results suggest that the observed heterogeneity can be partly explained by the characteristics of the European populations: marked differences in school calendars, mobility patterns and socio-demographic structures, also in consequence of age-dependent susceptibility to infection, strongly affected the pattern of spread of the 2009 pandemic. Moreover, it emerges that it would have been possible to obtain before the summer a broad-brush prediction of pandemic spread, hardly achievable with simpler models or pre-pandemic parameterisation. Results support the use of models accounting for the structure of complex modern societies for giving insight to policy makers. We believe that the work presented here supports the use of this type of modelling for assessing in real time the likely effects of future flu pandemics and for evaluating mitigation measures.

YOUNG SCIENTIST

SPB6 - MATHEMATICAL MODELLING

B608P

Estimation of mortality burden associated with 2009 pandemic H1N1 in Hong Kong

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Despite great efforts have been made for intensive laboratory tests for suspected cases with influenza infections during the pandemic in 2009, it remains difficult to obtain the true burden of 2009 pandemic influenza (pH1N1) because laboratory tests could be done in a small proportion of patients with severe influenza like symptoms. Some fatal cases might have died from secondary bacteria infections and exacerbation of their preexisting conditions, so they could have been neglected by the surveillance system if they showed only mild respiratory symptoms at the onset of infections. In this study, we aimed to develop a modeling strategy to estimate the excess mortality burden associated with pH1N1 and to compare the estimates between the pandemic and inter-pandemic periods. We adopted a "Poisson prediction" modeling approach, by which a Poisson model containing an influenza virus proxy variable and other seasonal covariates was first fitted to the de-trended mortality data of 1998-2008 (inter-pandemic period) in Hong Kong. The excess mortality of pH1N1 was calculated by deducting the baseline mortality predicted from this model from the observed mortality during the pandemic period of July-December, 2009. The similar modeling process was also applied to the inter-pandemic period of1998-2008 to estimate excess mortality associated with seasonal influenza. Our results suggested that there were 127 all-cause excess deaths attributable to pH1N1 infections, of which 115 deaths were coded as cardiovascular and respiratory diseases, and 22 as pneumonia and influenza. Although the attack rate was higher in children and young adults, the higher excess mortality rate associated with pH1N1 was found in the old people aged 65 years or over. The annual estimates of excess mortality associated with influenza were comparable between the pandemic year 2009 and inter-pandemic years 1998-2008. Our estimates of excess mortality for children and younger adults matched well with the reported numbers of laboratory confirmed pH1N1 fatal cases, but the estimates for the older population were more than two times higher. In conclusion, the elders still had a higher mortality risk associated with influenza during the pandemic period but the mortality burden of this group was likely underreported in the surveillance system.

SPB6 - MATHEMATICAL MODELLING

B609P

Uncoordinated behavioral changes during 2009 h1n1 influenza pandemic

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Introduction.

As most European countries, Italy has experienced one single pandemic wave during fall-winter 2009 and no substantial activity has been detected during the summer. The pandemic has mainly spread starting since the reopening of schools in mid-September until mid-December. By analyzing the Influenza-Like Illness (ILI) incidence, as reported to the national surveillance system, the hypothesis appears plausible that spontaneous behavioral changes have contributed to change the timing of spread and the transmissibility potential. In fact, after an initial period characterized by a slow exponential increase in the weekly ILI incidence, a sudden and sharp increase of the growth rate was observed by mid-October. Over the whole period schools remained open and only moderate mitigation measures were enacted. However, during the initial phases of the epidemic the Italian population has been exposed to a massive information campaign on the risks of an emerging influenza pandemic, which can have contributed to alter the perceived risk triggering moderate behavioral responses that would have affected the initial spread of the infection.

Material and methods.

In order to validate this hypothesis a mathematical model is proposed. The transmission process is based on a Susceptible-Infective-Recovered (SIR) model where individuals can reduce the number of potentially infectious contacts (e.g. by avoiding crowded environments, limiting travels or increasing hand washing) in response to the perceived risk of infection. The diffusion of responsiveness in the population is explicitly modeled as an imitation process based on the idea that individuals change behavior as they become aware that their payoff can increase by adopting a responsive behavior when the risk of infection is large.

Results.

A classical SIR model is not able to catch two distinct exponential growth rates in the incidence dynamics unless by considering a time-dependent transmission rate and it does not provide any motivation underlying sudden changes in the transmissibility potential. On the contrary, the proposed model perfectly fits observed data providing an initial overestimation of the perceived risk as a plausible explanation of the mechanisms responsible for the observed pattern. Such phenomenon is compliant with the high perceived risk of infection at the beginning of the pandemic highlighted by specific survey studies and supported by the analysis of temporal pattern of drug purchases during the 2009. In fact, when the pandemic arrived in Europe (end of April) antiviral drug purchases immediately increased reaching a peak at the end of July, despite no substantial ILI activity has been detected in Italy during the summer. During fall, the number of sold antivirals complies with the observed ILI temporal dynamics, while until mid-October an excess of purchase can be observed, possibly caused by the information campaign about the use of antivirals for treating H1N1 infections.

Discussion.

As a matter of fact, if human behavioral changes are not taken into account, estimates of the growth rate based on the observations during the early phases of the epidemic may lead to underrate the impact of the epidemic. On the other hand, predictions based on robust available estimates of the reproductive number may lead to overestimate the growth rate of the epidemic during its early phases leading to predict a faster spread than the actual one.

This study represents a first step for the estimation of the quantitative effects of spontaneous behavioral changes on the spread of an epidemic, potentially useful for planning public health control strategies and better estimating the burden for health care centers over time.

SPB6 - MATHEMATICAL MODELLING

B610P

A new approach to characterising infectious disease transmission dynamics from surveillance data: application to the 2009-10 A/H1N1 pandemic in Italy

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Introduction

Syndromic and virological data are routinely collected by many countries and are often the only information available in real time. The analysis of surveillance data poses many statistical challenges that have not been fully addressed. For instance, the fraction of cases that seek healthcare and are thus detected is often unknown; the size of the population that is monitored by primary-care based surveillance tends to change over time and only a fraction of syndromic cases who are detected by the surveillance system have really been infected by the aetiological agent of interest (e.g. H1N1 virus, in the past 2009-2010 influenza pandemic), with other cases being due too other pathogens. These problems are usually either ignored or corrected by scaling the epidemic curve with multiplicative factors, something which is expected to bias the variance of the estimates. Here we present a general framework to tackle these issues and analyze syndromic and virological data by taking stochasticity in the surveillance system explicitly into account.

Methods

We couple a deterministic age-structured SEIR (Susceptible-Exposed-Infectious-Removed) model of transmission dynamics with a statistical description of how the surveillance data is generated. Estimation of epidemiological parameters such as the reproduction number R and age-dependent reporting rates and susceptibility is then performed via Bayesian Markov Chain Monte-Carlo (MCMC) sampling. The approach is applied to surveillance data (i.e. Influenza-Like-Illness and virological data) collected in Italy during the 2009-2010 A/H1N1 influenza pandemic.

Results

We estimate that the reproduction number R was initially into the range 1.3-1.4, that case detection in children was about 3 times higher than in adults and that school-age children experienced the highest infection rate overall. We also estimate a drop in susceptibility to H1N1 beyond school-years. In terms of both estimated peak-incidence and overall attack rate (final size of the epidemic), the 5-14 years age-class was about 5 times more affected than the 65+ years old age-group and about twice more than the 15-64 years age-class; the 0-4 years age-class was about 3 times more affected than the 65+ years old age-group and the overall estimated attack rate was about 30%.

Conclusion

In this work we propose a general and rigorous statistical framework which explicitly takes into account the way surveillance data are generated and allows to estimate the incidence of H1N1 cases at the national population level without scaling the epidemic curve with some multiplicative factor. We show that a simple model like the one we used appears adequate for an overall description of the epidemic course and the age distribution of the cases. The general modelling framework proposed in this work can be applied to a variety of different infections detected by surveillance system in many countries and is potentially a powerful tool to be used in the future to provide policy makers with important information in real time.

YOUNG SCIENTIST

SPB6 - MATHEMATICAL MODELLING

B611P

Predicting Excess Mortality due to Influenza Epidemics using the Extreme Value Theory

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Introduction

Influenza viruses are responsible for annual epidemics, causing more than 500,000 deaths worldwide. A crucial question for resource planning in public health would be to predict the mortality burden of unusual epidemics, such as pandemics.

Modelling extreme values is the appropriate statistical method to attempt such a forecast and it has not yet been applied to epidemic data. It is based on the extreme value theory which is applied in many different areas such as environmental sciences for predicting extreme events such as inondations or disaster. We applied this statistical methodology to influenza excesses mortality estimated for the US over the period 1957-2003 and we provided predictions of maximal influenza excess mortality for periods of up to a century.

Material and ethods:

Monthly death rates were calculated from influenza and total mortality for 34 seasons (1969 to 2008) in the US and were standardized using the age distributions of the 2008 US population as reference. A Serfling cyclical regression approach was applied to these data to identify epidemic months and to estimate seasonal excesses mortality rates during influenza epidemic months. Influenza virus dominant subtypes were taking into account during the study period.

We collected the US excess mortality estimated between 1957 and 1969 from literature.

We estimated the maximum excess mortality rate which may be exceeding with a 95% probability over a given time period using the extreme value theory. We assumed that the maximum of seasonal excess mortality followed a Generalized Extreme Value (GEV) distribution. Parameters of this distribution were estimated by the maximum-likelihood. We then computed the return level i.e. the expected maximal excess mortality rate from the fitted distribution for given time interval of ten to one hundred years, termed return period.

A primary analysis was performed from the 1957-2003 excess mortality rates.

We performed a secondary analysis excluding excess from the 1957-58 and 1968-69 pandemics seasons during which the A/H2N2/HK and A/H3N2/HK pandemic strains circulated respectively and were associated with a particularly high mortality compared to inter-pandemics seasons.

Results

In all analyses, the model fit (quantile-quantile plot) for the US suggested that the determined GEV law correctly described the excesses' behavior of the distribution tail.

The maxima excess mortality estimated was 20.1 per 100,000 during the 1957 pandemic and 14 per 100,000 during the 1968 pandemic.

In the primary analysis, predicted maxima over the next 10 years was 16.5 per 100,000 (95%CI: 14.1-20.7), and over the next 100 years, predicted maxima was 24.7 per 100,000 (95% CI: 20.2-40.6).

The two maxima excesses mortality estimated during inter-pandemics periods was 25 per 100,000 (1963) and 15 per 100,000 (1978).In the secondary analysis, predicted maxima for inter-pandemic periods were similar to those observed with the 1957-1969 excess mortality for instance ten years (predicted maxima=15.8, %CI: 13.5-20.0), 50 years (predicted maxima=21.6, %CI: 18.1-32.7) and 100 years (predicted maxima=23.8, %CI: 19.6-39.2).

Conclusions

We predicted the excess mortality attributable to influenza during the most severe epidemic expected for the next decades. These results showed that an inter-pandemic season could have a mortality impact as high as a pandemic season. However, it is important to conclude with caution since patient care evolved between 1969 and 2008, possibly impacting the rates of mortality. We believe that extreme value methods can be applied to other topics in epidemiology or in other areas of public health where prediction of the maximal burden of a disease could be achieved on the basis of longer records.

SPB6 - MATHEMATICAL MODELLING

B612P

Model selection in time series studies of influenza-associated mortality

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Background

Underreporting of influenza cases is not unusual in clinical practice, because only a few of them are confirmed by laboratory tests. As a result, estimation of disease burden due to influenza relies on modeling approaches. An approach with Poisson regression model has been increasingly popular in recent studies. In this model, clear seasonality of influenza is not a prerequisite and several confounders can be properly adjusted for by smoothing functions. However, it is unclear as to what extent the confounding can be adjusted for. Here we conducted a simulation study with the aim to determine the selection criteria for Poisson models, which could lead to least biases in the estimations.

Methods

We generated 500 mortality datasets from a Poisson model with a known coefficient of influenza proxy variable of weekly specimens positive for influenza, and with fixed degrees of freedom for natural cubic spline smoothing functions of seasonal confounders including temperature, humidity, air pollution and seasonal trend of mortality data. The true coefficient for influenza variable was derived from a previously developed model fitted to the empirical mortality datasets of Hong Kong from 1998 to 2008. We compared the performance of three selected criteria commonly used in the time series studies: quasi Akaike information criterion (QAIC), partial autocorrelation function of residuals up to the lag time of five weeks (PACF), and generalized cross-validation (GCV). Sensitivity analyses were also conducted by generating mortality datasets from the models with different degrees of freedom for smoothing functions of time and seasonal trends.

Results

The GCV performed better than the QAIC and PACF as a criterion providing the smaller bias and root mean squared error (RMSE) for the estimation of influenza coefficients. The better performance was consistently observed while selecting the models fitted to the mortality datasets simulated from the models with various degrees of freedom for adjustment of seasonal trend confounders. The QAIC and PACF criteria gave dramatically large bias and RMSE, particularly when the data were simulated from the model with higher degrees of freedom.

Conclusions

Our study suggests that the GCV criterion is preferred in selecting the best fitted Poisson models for mortality data while assessing the influenza associated mortality burden. These findings shall help refinement of the statistical modeling approach for future disease burden studies.

YOUNG SCIENTIST

SPB6 - MATHEMATICAL MODELLING

B613P

Modeling of influenza seasonality in Italy

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Introduction

Mathematical modeling plays important roles in understanding the causal and contributing factors of influenza seasonality. Historically, influenza seasonality was proposed to be connected to the seasonal variations of solar radiation (by Hope-Simpson). More recently, temperature and humidity have been found to strongly modulate the survival and transmission of influenza virus. In 2009, a non-linear model with environmental and socio-behavioral variables was developed by Zhou et al to explain and project influenza activity. We tried to use the model to simulate the seasonality of the flu in Italy.

Method:

Data of influenza like illness (ILI rate) in Italy has been weekly collected within a sentinel surveillance system to monitor the timing and severity of seasonal influenza. We analyzed ILI surveillance data from 1999/2000 to 2010/2011 season using the statistical software proposed by Zhou et al to identify the potential explanatory factors for influenza seasonality in the country. The screened factors were then fed into the non-linear model to simulate the seasonal variation of the flu. Analysis of variance (ANOVA) was performed to measure the goodness of the modeling. We also considered population susceptibility to circulating viruses and school closures in the model.

Results

The monthly mean ILI rate was found to correlate to the dewpoint temperature (T_d) (r = - 0.73, P = 0.007), monthly sunshine hours (S_h) (r = - 0.71, *P* = 0.009), and monthly number of days with precipitation (R_n) (r = + 0.51, P = 0.09) in the previous month. A linear regression of monthly ILI rate on the three variables gave a R-square of 0.86 (*P* < 0.001), meaning 86% of the seasonal variation of ILI rate in Italy could potentially be explained by these environmental variables. When school closures and long public holidays (S_o) were taken into account, R-square increased to 0.88 (*P* = 0.002). Using the non-linear model containing previous month ILI rate (ILI_0), we obtained stronger correlations (r = - 0.92, *P* < 0.0001; r = - 0.89, *P* = 0.0001; r = + 0.76, *P* = 0.004; respectively). R-square of the non-linear regression of monthly ILI rate on the environmental variables came up to 0.93 (*P* < 0.0001). When school closure and a seasonal variation of population's susceptibility (VPS) to influenza virus were incorporated into the model, a R-square of 0.98 (*P* = <0.0001) was achieved. The model for seasonal influenza activity in Italy can be formulated as:

$$Incidence*1000 = [(Incidence*1000)_0 + 50] \times \exp(-0.902-0.278Td+0.014Sh-0.075Rn+3.057S0-7.396VSP) + 0.2$$

During the 12 seasons analyzed the A/H3N2 virus has been the dominant strain. Subtype B have sometimes co-circulated with virus A or become dominating in the spring. In the past 12 years (except pandemic 2009), maximum ILI rate was recorded in the 5th week of 2005 after a sharp drop of dewpoint temperature and days of overcast and precipitation in week 3 and 4. Weekly ILI rate was significantly reduced during the months with long public holidays and school breaks. Immunity to circulating viruses in the population seems to begin to decline 8-9 months after a ILI peak.

Conclusion

The strong associations found between influenza activity and the environmental variables are in agreement with findings from other studies. The high consistency between model output and the actual ILI surveillance data indicates that the non-linear model is applicable for the explanation and projection of seasonal influenza activity in Italy and strongly suggests that environmental variables, school closure, transmission dynamics with background prevalence, and variation of population susceptibility against the viruses are the four crucial determinants for influenza seasonality. Modeling results suggest that timely vaccination, social distancing measures, comfortable dewpoint temperature are beneficial strategies for the prevention and control of the flu. No conflict of interest

YOUNG SCIENTIST

SPB6 - MATHEMATICAL MODELLING

B614P

The antigenic evolution of influenza

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Introduction

Human influenza viruses are known to be in rapid antigenic flux, manifesting in the sequential replacement of dominant antigenic types during inter-pandemic periods. Among swine and birds however, the antigenic evolution of influenza is usually considered to be more sedate despite the rapid genetic evolution of the virus as a whole. This is generally attributed to host life expectancy: that pigs and birds simply don't live long enough to exert significant antigenic selection pressure through herd immunity.

Materials and Methods

Whilst there are several competing hypotheses that seek to explain the antigenic evolution of influenza within a single host species, none of these have yet been used to explicitly consider the impact of lifespan on virus evolution. Here we present a simple model to explore the relationship between host lifespan and viral evolution in a finite antigenic space, paying careful attention to (and resolving) the conceptual paradox of antigenic drift. We also briefly touch on the effects of transmission between hosts of different lifespan within this model in an effort to capture the impact of cross-species transmission of influenza.

Results

Our model predicts that antigenic evolution of the influenza virus should occur for a wide range of host life expectancy – wide enough to expect antigenic evolution of swine and avian influenza in their natural hosts. Importantly however, the infectious period of the virus and close epidemiological linkage between different hosts both have significant effects on the mode and tempo of antigenic evolution.

Conclusion

Together, these results can explain conflicting reports of antigenic evolution of influenza in different hosts as a function of both host life-expectancy and its role in the wider influenza ecology. We illustrate these principles with examples from avian influenza.

SPB6 - MATHEMATICAL MODELLING

B615P

Estimation of Mortality and morbidity attributable to influenza by influenza virus types and subtypes in Korea

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Introduction

Influenza infections cause substantial mortality and morbidity every year in Korea. But the numbers of deaths or hospitalizations associated with influenza are difficult to estimate directly. The aims of this study was to estimate the numbers of deaths and hospitalizations attributable to influenza by age groups, virus types and subtypes in Korea

Materials & Methods

We used the national influenza viral surveillance data (Korean Centers for Disease Control), national causes of deaths statistics (National Statistics Office) and national health insurance data (Korean Health Insurance Review and Assessment) from 2005-2006 to 2008-2009 influenza season in this analysis. The Korean national health insurance database is representative of the disease and health care of Korean population. The Poisson regression model was applied to estimate the numbers of deaths and the numbers of hospitalizations by age groups, by death categories (pneumonia, underlying respiratory diseases, underlying cardiovascular diseases), and by virus types and subtypes.

Results

The annual mean influenza-associated mortality rates in persons under 65 years and over 65 were 3.4 and 40.7 per 100,000 person-years respectively during these 3 seasons. Influenza virus was associated with 3.0% of underlying pneumonia and influenza deaths, 1.8% of underlying respiratory and cardiovascular deaths and 1.0% of all causes deaths in Korea. Of total 1,366 influenza-associated deaths in age under 65 years, 905 (66.3%) were attributable to influenza virus A. But in persons over 65 years, this proportion was 33.5% (1,922 of 5,743), and the rest were attributable to influenza virus B. The annual mean influenza-associated hospitalization rates for underlying pneumonia, for underlying respiratory diseases and for underlying cardiovascular diseases were 2005.3, 680.8 and 712.8 per 100,000 person-years respectively in persons over 65 years. These rates in age under 65 were 602.4, 157.5 and 57.3 per 100,000 person-years respectively. 14.1% of underlying pneumonia hospitalizations and 10.0% of underlying respiratory and circulatory hospitalizations were attributable to influenza viruses in these seasons in Korea. The influenza-associated hospitalization rates in the persons under 65 years were also more sensitive to influenza virus A epidemics then influenza B. But in case of over 65 years, these rates were more sensitive to influenza virus B epidemics relatively.

Conclusion

The Poisson regression model is useful to estimate the mortality and morbidity associated with influenza. The elderly persons are more seriously affected by influenza in mortality and morbidity in general. And the circulating influenza virus types disproportionately affect the mortality and hospitalization rates according to age group.

SPB6 - MATHEMATICAL MODELLING

B616P

What is driving the seasonal epidemic of influenza in French Guiana?

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Introduction.

Data on seasonal influenza in tropical regions remain scarce compared with that in temperate region. Our objectives in this study were to describe the pattern of the seasonal epidemic of influenza in French Guiana, a tropical region of South America and to analyze the role of climatic factors on the epidemiology of influenza transmission.

Material and Methods

Weekly Influenza-like illnesses (ILI) were recorded from the sentinel GP network whereas isolation and identification of influenza virus from 2006 to 2010 were performed at the Pasteur Institute laboratory of virology. Climatic parameters (rainfall, temperature and relative humidity) were obtained from the regional office of Météo France. Times series analysis (ARIMA models) and dynamic regression models were used to investigate relationship between ILI incidence and climatic parameters. The pandemic period was excluded from the analysis.

Results.

From January 2006 to June 2010, a total of 586 viruses were isolated from the sentinel surveillance.The univariate analysis of the ILI incidence showed a seasonal autoregressive variation with a mean of 81 ILI/100,000 inhabitants. In bivariate analysis, we observed that including rainfall or specific humidity as input series result in models with better performance than the univariate one where the ILI incidence series depend only on its past values and error signal. Using multivariate dynamic regression analysis, we estimated that an increase of 1 mm of precipitation induced in an average delay of 1 week, an increase of 0.33% in ILI incidence ($p < 0.01$); while an increase of 1kg/kg for specific humidity resulted with an average delay of 3 weeks, in a significant decrease of 11% in the ILI incidence rate.

Conclusion.

Rainfall and specific humidity played significant roles on the transmission of seasonal influenza in French Guiana

SPB6 - MATHEMATICAL MODELLING

B617P

Measuring total and infectious viral concentration in order to more accurately estimate within-host model parameters

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Introduction

For in vivo studies of influenza dynamics where within-host data is measured and fit with a mathematical model, infectivity assays (such as 50% tissue culture infectious doses (TCID50) or plaque forming units (PFU)) are often used to estimate the infectious virion concentration over time. Less frequently, measurements of the total (infectious and non-infectious) virion concentration (obtained using methods such as polymerase chain reaction (PCR) or hemagglutination assays) have been used as an alternative to infectivity assays. However, in vivo measurement of the total virion concentration has never been used in conjunction with measurements of the infectious virion concentration in order to better inform model fitting to data and within-host parameter estimation. We investigated the degree to which simultaneous measurement of both infectious (via TCID50) and total (via PCR) virion concentration allows within-host model parameters to be determined with greater consistency and reduced uncertainty.

Materials and Methods

The dataset used in this work consists of viral load data taken from 10 ferrets that were intranasally infected with a strain of AH1N1. We fit two different models to these viral load data. The first model is a target cell-limited model which has no latent (eclipse) phase for infected cells, and has been used frequently in the within-host literature as a "benchmark" model. This model is fit solely to measurements of infectious virion concentration (TCID50 assays). We also develop a new model which includes compartments for both the infectious and total virion concentrations. The model assumes that infectious virions become non-infectious at a rate which we fix to a value determined previously from an in vitro study. This model is then fit to corresponding measurements of infectious and total virion concentrations (TCID50 and PCR assays). Global optimisation is performed for both models using a genetic algorithm, and likelihood confidence regions are calculated in order to examine the uncertainty of the parameter estimates.

Results

Firstly we note that the ratio of total (PCR) to infectious (TCID50) virion concentration is not constant over time, which reflects the fact that each of these measurements are probing different aspects of the underlying biological dynamics.
Secondly, the confidence regions for the parameter estimates obtained using our new model are tighter than those obtained using the benchmark model. This result is consistent across all of the the 10 ferrets' data, and indicates that our model is more identifiable than the other model. Thirdly, the confidence regions obtained using our new model were also often located in different regions in parameter space compared with those of the benchmark model, meaning that our model made different predictions for the values of some of the within-host model parameters. Lastly, the best-fit parameter estimates obtained using our new model were sometimes different than those obtained using the other model, as well as having less variability between the different ferrets.

Conclusions

The fact that the ratio of total (PCR) to infectious (TCID50) virion concentration is time-dependent indicates that the biological processes underlying infection can be probed more comprehensively by including both measurements within our analysis, when compared with just including one of them alone. Indeed, we found that measuring both infectious and total virion concentrations simultaneously, when fit with an appropriate model, allows within-host model parameters to be determined with reduced uncertainty and greater consistency between subjects. This technique thus has the potential to more accurately estimate the underlying biological processes of influenza infection.

SPB6 - MATHEMATICAL MODELLING

B618P

Human respiratory tissue tropisms determine influenza virus reproductive fitness

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Introduction.

The location of influenza virus infection along the respiratory tract is recognized as a key determinant of the virus ability to spread between humans. Location of infection along the respiratory tract also at least partly determines the severity of the resulting disease, with deeper infection causing more severe disease. Transmissibility and pathogenicity both affect the fitness of influenza virus in human populations, yet the impact of tissue tropism on the virus reproductive fitness is poorly understood. Here, we aim at determining the location of infection along the lower respiratory tract that maximizes influenza virus reproductive fitness at the population level.

Material & methods.

We developed a spatially-structured mathematical model of influenza virus infection in the human lower respiratory tract (trachea to alveoli) to determine the tropisms associated with influenza virus maximal reproductive fitness (R). Scores of viral production and damage were used to estimate the transmission rate, recovery rate and disease-induced mortality rate of influenza viruses with variable receptor binding affinity and tropism patterns along the human lower respiratory tract.

Results.

The optimal location of infection of influenza viruses with human-like receptor binding affinity in the lower respiratory tract depended on the level of pre-existing immunity of the hosts. The infectivity for bronchiolar epithelial cells that maximized R was consistently higher for human influenza viruses circulating in a naïve population (e.g., pandemic influenza viruses) or following substantial antigenic drift (e.g., following cluster jumps), than for human influenza viruses circulating in a partially immune population (e.g., non-drift variants of seasonal influenza viruses). On the other hand, the infectivity for tracheal and bronchial epithelial cells that maximized R was consistently higher for human influenza viruses circulating in a partially immune population. Therefore, the optimal location of infection which maximized the ability of human influenza viruses to spread at the population level was deeper down the respiratory tract for viruses circulating in populations with no or little pre-existing immunity.

Preferred tropism of influenza viruses with avian-like or mixed receptor binding affinity for epithelial cells of the deeper regions of the lower respiratory tract strongly impaired R. Yet, viruses with reduced infectivity for these cells had R values above unity, thus potentially spreading in human populations. Therefore, the optimal location of infection which maximized the ability of zoonotic influenza viruses to spread in human populations was higher up the respiratory tract than that of pandemic human influenza viruses. However, a switch from avian-like to human-like receptor binding affinity substantially increased the fitness of avian influenza viruses, leading to optimal location of infection deeper down the respiratory tract following such a switch.

Conclusions.

The model presents a novel synthesis of within-host and population level dynamics of influenza virus that clarifies the reciprocal impacts between influenza virus tissue tropism in individual hosts and the virus ability to spread at the population level. The model suggests that pre-existing herd immunity drives influenza virus tissue tropism towards the upper regions of the respiratory tract, while antigenic drift or antigenic shift drives it towards the lower regions of the respiratory tract. The model thus proposes an evolutionary basis for the deeper tissue tropism and higher pathogenicity of pandemic influenza viruses and of drift variants of seasonal influenza viruses, and for the lower pathogenicity of non-drift variants of seasonal influenza viruses in humans. In addition, the model proposes evolutionary mechanisms for the adaptation of zoonotic influenza viruses to efficient human-to-human transmission, including an evolutionary basis for the increased transmissibility and pathogenicity of pandemic viruses of avian origin during subsequent waves of the pandemic in human populations.

SPB6 - MATHEMATICAL MODELLING

B619P

Estimating infection attack rates and severity in real-time during an influenza pandemic

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Introduction

In an emerging influenza pandemic, severity estimate is an urgent public health priority. As many influenza infections are subclinical, serologic surveillance is needed to allow reliable real-time estimates of infection attack rates (IAR) and severity such as the case-hospitalization probability (CHP).

Methods

We tested 14,766 sera collected during the first wave of the 2009 pandemic in Hong Kong using viral microneutralization. We estimated IAR and CHP from the serial cross-sectional serologic data and hospitalization data using a convolution-based method without modeling transmission dynamics.

Results

Had our serologic data been available weekly in real-time, we would have obtained reliable estimates 1 week after, 1-2 weeks before and 3 weeks after epidemic peak for 5-14 yo, 15-29 yo and 30-59 yo. The ratio of IAR to pre-existing seroprevalence, which decreased with age, was a major determinant for the timeliness of reliable estimates. Had we begun serologic surveillance 3 weeks after community transmission was confirmed with 150, 350 and 500 specimens per week for 5-14 yo, 15-19 yo and 20-29 yo, we would have obtained reliable estimates for them 4 weeks before the peak. For 30-59 yo, even 800 specimens per week would not have generated reliable estimates until the peak because their ratio of IAR to pre-existing seroprevalence was low. The performance of serial cross-sectional sero-surveillance would substantially deteriorate if test specificity was not near 100% or if pre-existing seroprevalence was not near 0. These potential limitations could be mitigated by choosing a higher titer cutoff for seropositivity. Using simulations of future pandemics, we estimated that serial cross-sectional sero-surveillance with 300 specimens per week would yield reliable estimates as soon as the true IAR reached around 6%.

Conclusions

Serial cross-sectional serologic data together with clinical surveillance data can allow reliable real-time estimates of IAR and severity in an emerging pandemic. Serologic monitoring should be considered in pandemic surveillance.

YOUNG SCIENTIST

SPB6 - MATHEMATICAL MODELLING

B620P

Epidemiology of influenza strains: competition, prediction, and associated mortality

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Introduction

The epidemic sizes of influenza A/H3N2, A/H1N1 and B infections vary from year to year in the United States. We use publicly available US CDC influenza surveillance data between 1997 and 2009 to study the temporal dynamics of influenza over this period. Additionally, we relate the incidence proxy for the three influenza strains above that we've defined to weekly excess mortality data for various causes

Materials and Methods

Regional outpatient surveillance data on influenza-like-illness (ILI) and virologic surveillance data were combined to define a weekly proxy for the incidence of each strain in the US. For each strain, Spearman rank association between the cumulative incidence proxy (CIP) for the whole season for that strain and the early CIP of the other two strains (the complementary CIP) was examined. A method is introduced to predict a particular strain's CIP for the whole season by following the incidence of each strain from the start of the season until either the CIP of the chosen strain or its complementary CIP exceed certain thresholds. Weekly mortality data underlying each of the several causes is regressed linearly in terms of the influenza strain incidence proxies (shifted forward by an appropriate number of weeks specific to each cause), the temporal trend and the annual baseline (modeled by periodic cubic splines).

Results

All strains exhibited a negative association between their cumulative incidence proxy (CIP) for the whole season (from calendar week 40 of each year to calendar week 20 of the next year) and the CIP of the other two strains (the complementary CIP) from the start of the season up to calendar week 2 (or 3, 4, or 5) of the next year. The prediction method yielded accurate predictions which generally occurred within a few weeks of the peak of incidence of the chosen strain, sometimes after that peak. For the largest seasons in the data, which were dominated by A/H3N2, prediction of A/H3N2 incidence always occurred at least several weeks in advance of the peak. Strong correlation was observed between the weekly mortality data and the model fit, with the prominent role played by the influenza A/H3N2 contribution. Baselines for non-influenza associated mortality for various causes were estimated. For Pneumonia and Influenza (P&I) mortality, a notable change in the baseline mortality coinciding with the introduction of the pneumococcal conjugate vaccine in the US was observed.

Conclusions

Early circulation of one influenza strain is associated with a reduced total incidence of the other strains, consistent with the presence of interference between subtypes. Routine ILI and virologic surveillance data can be combined using this method to predict the relative size of each influenza strain's epidemic by following the change in incidence of a given strain in the context of the incidence of co-circulating strains. Incidence proxies for the major influenza strains can be related to the weekly mortality data for various causes and the corresponding baselines for non-influenza associated mortality can be estimated in the process.

SPA7 - LATE BREAKERS

A701P

Structure based design of anti-influenza molecules targeting the cap-snatching activity of the viral polymerase

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Introduction.

Influenza virus polymerase transcribes and replicates the viral RNA genome within the context of a ribonucleoprotein complex that has been hitherto remarkably intractable to high resolution structural analysis. As a result many aspects of the detailed mechanism of action of the polymerase remain obscure, despite years of study. However in the last few years, crystal structures of independent domains covering roughly half of the heterotrimeric polymerase have been determined (Towards an atomic resolution understanding of the influenza virus replication machinery. Ruigrok RW, Crépin T, Hart DJ, Cusack S. Curr Opin Struct Biol. 2010 Feb;20(1):104-13.. These results will be reviewed with a particular focus on the mRNA cap-binding and endonuclease domains, critical for the unique cap-snatching mechanism of influenza viral mRNA transcription. The structural results have given new impetus to structure-based anti-influenza drug design targeting the polymerase.

Material & methods.

Domains from influenza virus polymerase subunits have been expressed, crystallized and their atomic structures determined by X-ray crystallography. High-throughput screening, small molecule synthesis and structure determination of polymerase-inhibitor complexes has been used to iteratively optimize inhibitors.

Results.

Crystal structures will be presented of model compounds bound to the influenza polymerase cap-binding and endonuclease domains and discussed in relation to structure-based drug deisgn.

Conclusions.

Crystal structures of the PB2 cap-binding domain and PA endonuclease domain reveal new insights into the mechanism of transcription by cap-snatching by influenza polymerase. Co-crystal structures with inhibitors show the mode of binding to each active site and will allow structure based optimization of inhibitor potency.

SPA7 - LATE BREAKERS

A702P

A microarray-based approach for serological surveillance of avian influenza

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Influenza A viruses are associated with frequent seasonal epidemics and occasional pandemics. In the past century, the exposure of the naïve human population to the novel subtypes H1N1, H2N2 and H3N2 resulted in three major pandemics claiming many lives. In 2009, a swine-originating subtype H1N1 emerged and caused the first pandemic of the 21st century. Phylogenetic analysis revealed that all pandemic-causing strains obtained parts of their genetic material from an animal source. To date, 16 subtypes of influenza A viruses are known to asymptomatically circulate in avian reservoirs, mainly shore- and waterbirds, and have repeatedly been reported to cross the species barrier from birds to mammals including humans. In particular, since 1997 the circulation of highly pathogenic avian influenza virus (HPAI) strains of the subtype H5N1 has raised concerns of becoming the cause of the next pandemic. HPAI H5N1 infections in humans have resulted in a high fatality rate of more than 60%. In addition, also other subtypes have been reported to infect humans and thus might be of importance for public health. Therefore, serological surveillance of animal reservoirs and measuring exposure in humans - especially in influenza hot-spot areas - is important. However, current standard serological surveillance tools, the hemagglutination inhibition- and virus microneutralization assay, either fail to detect antibodies against avian influenza viruses in humans or are too laborious and costly for widespread use. Furthermore, standardization of these assays has been challenging. In this study, we developed a protein-microarray comprising commercially available HA1 subunits of 13 hemagglutinin-types. The antigens were spotted on nitrocellulose-coated slides using a Piezorray non-contact spotter. Following spotting, the slides were dried overnight and serum samples were subsequently analyzed according to a standardized protocol. Validation using rabbit, human and chicken sera showed that this novel screening technique allows simultaneous detection of subtype-specific antibodies against different human and animal influenza hemagglutinin-types, whilst requiring only little amounts of serum. In conclusion, antigen-microarrays might constitute a promising, rapid serological surveillance tool.

SPA7 - LATE BREAKERS

A703P

A prospective randomised trial of a 10-day oseltamivir treatment regimen in patients with pandemic A(H1N1) 2009 influenza

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Aim:

During the early stages of the 2009–10 influenza pandemic, information on the pathogenicity of pandemic A(H1N1) 2009 virus and the likely response of the virus to antiviral therapies was limited. This study aimed to compare the efficacy of treatment with an extended (10-day) oseltamivir regimen and the standard 5-day regimen in children and adults with pandemic A(H1N1) 2009 in New Zealand, and to monitor emergence of resistant viral strains.

Methods:

The study used a prospective, unblinded, randomised design and recruited patients during the Southern hemisphere 2010 influenza season. Eligible patients were those aged ≥5 years who presented within 48 hours of the onset of influenza symptoms (fever ≥37.8°C at screening or self-reported fever in the 24 hours before screening, and at least one respiratory symptom), and were influenza-positive by rapid test. All participants received oseltamivir for either 5 or 10 days; the dosages were 75mg b.i.d. in adults and 30–75mg b.i.d. in children according to body weight. Nasopharyngeal swabs were taken on Days 0 (baseline), 5 and 10, and tested for the presence of influenza virus by RT-PCR and culture. All PCR positive samples were tested for the H275Y resistance mutation, while cultured isolates were phenotypically tested (NAStar® Kit, Applied Biosystems) and in cases with reduced sensitivity, resistance mutations were identified by sequencing. Eight influenza signs and symptoms were assessed on Days 0, 5 and 10 and adverse events recorded on Days 5 and 10. The primary endpoints were the proportion of patients who shed virus at Days 5 and 10 and the proportion infected with resistant viruses.

Results:

A total of 97 patients (mean ± SD age: 19.2 ± 14.3 years) were randomised to treatment, 48 at standard duration (including 21 children <14 years old) and 49 at double duration (including 23 children <14 years old). The groups had similar baseline demographics and clinical and virological characteristics. The proportion of patients reporting at least one symptom fell from baseline in both groups at Day 10, but not at Day 5 (Table). The decline in the proportion of patients shedding virus (RT-PCR positive for pandemic [H1N1] 2009 virus) was similar in the two groups at Days 5 and 10, with the majority being RT-PCR negative by Day 10 (Table). Viruses with reduced susceptibility to oseltamivir (IC50: 111–134nM) were detected in three cases on Day 5 (all children); the associated mutation was H275Y in two children and unknown in the third. In all three cases, viral cultures on Day 10 were negative for influenza A. Adverse events were reported by 18 patients (19%) in the standard duration group and 17 patients (18%) in the double duration group. One serious adverse event (abdominal pain) was reported by a patient in the double duration group.

Conclusions:

The rate of reduction of viral shedding and the rate of symptom resolution in patients receiving extended-duration oseltamivir treatment was similar to those treated for the standard duration. The incidence of oseltamivir-resistant viruses was low.

	Standard duration (n=48)			Double duration (n=49)		
	Baseline	Day 5	Day 10	Baseline	Day 5	Day 10
RT-PCR positive for pandemic (H1N1) 2009, n (%)	48 (100)	30 (62.5)	8 (16.7)	49 (100)	38 (77.6)	6 (12.2)
Reporting at least one symptom, n (%)	48 (100)	45 (93.8)	37 (77.1)	49 (100)	49 (100)	41 (83.7)

SPA7 - LATE BREAKERS

A704P

Paediatric Influenza Vaccine Outcome Trial (PIVOT) – Respiratory Virus Infection Outcomes

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Introduction

Despite high annual burdens of influenza in young children, few governments recommend influenza vaccination for them.

Aims

Identify health and quality of life (QoL) impacts of influenza illness and vaccination in young children.

Methods

Observational cohort study of influenza vaccinated (pandemic monovalent or trivalent 2010) and unvaccinated children aged 6-36 months at study enrolment followed over the 2010 Sydney influenza season. Parents reported when their children developed influenza-like-illness episodes (ILIs, definition: body temperature ≥37.8C/feverishness plus ≥1 respiratory symptom) and they collected swabs (for multiplex respiratory virus polymerase chain reactions) from the children's noses and/or throats. Health and QoL impacts were assessed for each child and family by telephone interviews before the influenza season (baseline) and 2 weeks after onset of each ILI (along with a non-ILI comparison group).

Results

Of 381 children (208 males) from 358 families followed over 13 weeks from 30 July 2010 (at which date subjects were aged 0.92-3.41 years), 88 fulfilled Australian criteria for "fully vaccinated against influenza A/California/7/2009 (H1N1)" (pH1N1); 55 partially and 238 were unvaccinated. 124 ILIs occurred in 105 children (12 had two ILIs each, 3 had 3). 117 ILIs were swabbed; nose, throat or both. 175 viruses were identified from 103 ILIs: 5 pH1N1, 39 adenovirus, 39 rhinovirus, 22 parainfluenza 3, 12 human metapneumovirus, 14 coronavirus (9 HKU-1, 5 NL63), 3 respiratory syncytial virus, 11 bocavirus, 29 polyomavirus (7 KI, 22 WU), and 1 parainfluenza 2. 36 ILIs yielded 2 viruses each, 10 yielded 3 viruses, 4 yielded 4, 1 yielded 5. Adenovirus infections were more common in females. Polyomavirus infections were more common in children aged <2 years than in older children.

In addition to fever and respiratory symptoms, the 124 ILIs were associated with: loss of appetite (26), otitis media (17), vomiting (17) diarrhea (7), conjunctivitis (9), wheezing (8). Managing ILIs required 134 GP visits (for 70 of the ILI episodes), 106 pharmacy visits (for 64 ILIs), 5 emergency department visits and 3 specialist visits but no hospitalisations; 52 ILIs were treated with antibiotics, 73 were treated with analgesic/antipyretics. The median duration of ILIs was 8 days (16 [13%] lasted >28 days). Children's ILIs significantly reduced parents' QoL compared to baseline ($p<0.001$) and non-ILI controls ($p<0.001$) with greatest score reductions across the questionnaires' social and emotional domains increasing with duration and perceived severity of the child's ILI. Compared to non-influenza ILI cases, the parents' QoL physical component score was lower for the five influenza cases.

Conclusion

Study enrolment was impeded by the April 2010 national suspension of influenza vaccination for children aged <5 years when one manufacturer's influenza vaccine caused high rates of febrile convulsions in young children. Sydney's 2010 influenza season was mild compared to other years. Few influenza infections occurred in study subjects. Other respiratory viruses caused ILI in 26% of subjects. When the child's illness was severe or persistent the ILIs had significant negative impacts upon parents' QoL.

SPA7 - LATE BREAKERS

A705P

Evaluation of influenza antiviral susceptibility testing in Europe: results from the first influenza antiviral susceptibility quality assessment exercise

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On behalf of the members of the Quality and Training and Antiviral task groups and the Community Network of Reference Laboratories for Human Influenza in Europe.

Background

Virological surveillance for influenza in European Union (EU) countries is undertaken by the Community Network of Reference Laboratories for Human Influenza in Europe (CNRL) as part of the European Influenza Surveillance Network (EISN), formerly the European Influenza Surveillance Scheme (EISS). EISN has been coordinated by the European Centre for Disease Prevention and Control (ECDC) since 2008. Virological surveillance activities are supported by CNRL Coordination Group and CNRL Task Groups composed of experts from member laboratories. The Antiviral Task Group and the Quality and Training Task Groups organised an influenza antiviral susceptibility testing quality assessment exercise during the winter influenza season 2010/11. To our knowledge this was the first antiviral susceptibility quality assessment exercise for influenza undertaken anywhere in the world. The objectives of the exercise were to provide participants with an independent mechanism to check the performance of the influenza antiviral susceptibility testing methods that were currently performed in their laboratories. Assessment of laboratory competency and ability to perform antiviral susceptibility testing is important now that there is widespread clinical use of antiviral therapy for influenza. Furthermore the exercise would provide an insight into the performance of the different techniques used for influenza antiviral susceptibility in European laboratories. The outcomes can be used by CNRL to determine training priorities and produce guidelines on the harmonisation of interpretation of antiviral data and reporting of results.

Methods

Twenty different laboratories from 16 European countries participated in the quality assessment exercise during December 2010 and January 2011. Most participating laboratories were WHO recognised National influenza Centres (NICs) and also affiliated to CNRL. Each laboratory received a panel of 10 coded samples containing recent influenza A viruses, including H1N1 2009, previous seasonal H1N1 and H3N2 subtypes, and influenza B viruses. Viruses contained substitutions known to confer resistance to antiviral drugs oseltamivir, zanamivir or the adamantanes. Sensitive control viruses for each type/subtype were also included. Participants tested the viruses using the genotypic and phenotypic methods for antiviral susceptibility testing that were currently implemented in their laboratories. Reporting of results into a web-based portal hosed by Quality Control for Molecular Diagnostics (QCMD) was made within a defined timeframe.

Results

All 20 participating laboratories performed genotypic testing to identify mutations by SNP PCR or sequencing in H1N1 2009 influenza virus and 55-75% tested all influenza subtypes. Participants correctly identified the presence or absence of substitutions in the viral neuraminidase (NA) gene (47-100%) or M2 gene (69-93%) associated with resistance to the neuraminidase inhibitor (NI) drugs oseltamivir and zanamivir or amantadine respectively. 12/20 laboratories also reported results of phenotypic testing (IC50 assay) to determine susceptibility to oseltamivir and zanamivir. Variation in the range of IC50 values reported for each sample was observed with IC50 values generated by chemiluminescence assay lower than those generated by fluorescence assay. For both genotypic and phenotypic testing participants were asked to provide an interpretation of the result in terms of sensitivity, reduced susceptibility or resistance to the respective antiviral drugs. Variation in interpretations (50-100% matching consensus) was seen between laboratories and was associated with the method of phenotypic analysis.

Conclusion

The results of the first influenza antiviral susceptibility testing quality assessment exercise will be used to promote harmonization of antiviral susceptibility testing, data interpretation and reporting in the European network through implementation of recommendations for improvement and training activities. Recommendations arising from the experience of designing, producing and analysing the first influenza antiviral susceptibility testing quality assessment exercise will be used to inform future quality assessment programmes.

SPA7 - LATE BREAKERS

A706P

Cost effectiveness model comparing vaccination of older adults (>=65 years) with Fluad® vs. non-adjuvanted influenza vaccines in the United Kingdom

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Introduction

Due to the natural aging process of the immune system, influenza causes significant disease burden among adults older than 65 years of age. Fluad containing MF59® adjuvant can provide greater protection for these people than conventional influenza vaccines. The aim of this poster is to assess the potential cost-effectiveness of vaccinating older adults with Fluad, compared to conventional influenza vaccines in the United Kingdom.

Methods

A dynamic, compartmental SIR (Susceptible-Infected-Recovered) model was developed to simulate the spread of the disease and to estimate the associated costs and outcomes for three vaccination strategies in a single influenza season in the UK. Non-clinical parameters were taken from UK specific data sources; vaccine efficacy is derived from international reviews. Figures supporting Fluad's incremental clinical benefit were utilized from a recent, large-scale, observational, population-based cohort study in Northern Italy.

Results

A vaccination strategy that replaces non-adjuvanted vaccines with Fluad in older adults (≥ 65 years) could have the potential to prevent up to 1,500 hospitalizations and 800 deaths per year in the UK. Assuming a 23% Fluad risk-reduction in influenza cases, then 300,000 influenza cases, 4,400 hospitalizations and 1,500 deaths per year could be prevented. Excluding vaccination cost, Fluad could potentially generate cost-savings of £5.5 million to £15.6 million per influenza season in older adults and an overall health improvement of 5,200 to 13,300 quality-adjusted life years (QALYs) gained. Fluad is estimated to be cost-effective up to a price level of £21/dose (Willingness-to-pay threshold: £20,000 per QALY gained).

Conclusions

Vaccinating older adults with Fluad MF59 was estimated to have the potential to reduce the clinical and economic burden of influenza in the United Kingdom. The modeling results support the current clinical evidence of the MF59-adjuvanted influenza vaccine, Fluad as a more effective influenza vaccine for adults aged ≥65 years as compared to non-adjuvanted seasonal influenza vaccine.

SPA7 - LATE BREAKERS

A707P

A serological correlate of protection demonstrated in an efficacy study using a Vero cell derived split seasonal influenza vaccine

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Introduction

Conventional methodologies used to manufacture influenza vaccines have a number of disadvantages. Public health authorities have been recommending for decades that alternative cell culture systems be developed that allow production of influenza vaccine using robust, well characterized manufacturing systems. The ideal substrate to produce biologicals has been defined as a continuous cell line which can be fully characterized with respect to absence of tumorogenicity and freedom from adventitious agents. We have developed a Vero cell culture platform which has been optimized for the development of a wide variety of candidate vaccines against emerging virus diseases and particularly influenza viruses. Both seasonal and pandemic Vero cell derived influenza vaccines (VCIV) have been demonstrated to be well tolerated and immunogenic in a number of clinical studies.Generally, safety and immunogenicity studies are sufficient for the licensure of seasonal influenza vaccines. The induction of hemagglutinin (HA)-specific antibodies, as determined by the hemagglutination-inhibition (HI) assay, is a well established correlate of laboratory-confirmed efficacy and clinical effectiveness of seasonal influenza vaccines. However, it remains to be confirmed that correlates of immunity established for egg-derived vaccines apply equally to VCIV.

Materials & Methods

In the present study, culture-confirmed influenza infection (CCII) and antigenic typing were used to provide a stringent assessment of the ability of a novel inactivated Vero cell-derived influenza vaccine (VCIV) to prevent seasonal influenza infection in healthy adults. This double-blind, placebo-controlled phase III study was conducted in season 2008/2009. Subjects were randomized in equal numbers to receive VCIV or placebo, and a total of 7,243 subjects were vaccinated. Subjects who developed symptoms of influenza infection from 21 days after the date of vaccination until end of influenza season were instructed to return within 48 hours of symptom-onset to the study site for a nasopharyngeal swab for the purpose of determining culture-confirmed influenza infection. Efficacy and immunogenicity data were then analyzed to investigate whether hemagglutination inhibition (HI) titer can be used as a correlate of VCIV-induced protection from seasonal influenza infection.

Results

In this study overall protective efficacy was 78.5% for antigenically-matched CCII and 71.5% for all laboratory confirmed infections (Table 1).

Table 1
Efficacy of Vero cell-derived vaccine in CCII

	Culture-confirmed influenza infection antigenically matched to vaccine strains			Influenza infection (culture-confirmed or PR-PCR identified), regardless of antigenic match		
	VCIV (n=3619)	Placebo (n=3617)	Efficacy (95% CI)	VCIV (n=3619)	Placebo (n=3617)	Efficacy (95% CI)
A/H1N1	11 (0.3)	52 (1.4)	79.0% (59.7 to 89.0)	14 (0.4)	56 (1.5)	75.2% (55.4 to 86.2)
A/H3N2	2 (0.1)	4 (0.1)	50.0 (-173.0 to 90.8)	2 (0.1)	4 (0.1)	50.0% (-173.0 to 90.8)
B	0	4 (0.1)	100% (4.1 to 100.0)	8 (0.2)	20 (0.6)	60.1% (9.5 to 82.4)
Total	13 (0.4)	60 (1.7)	78.5% (60.8 to 88.2)	23* (0.6)	80 (2.2)	71.5% (54.7 to 82.1)

Data presented as n (%), unless otherwise indicated. * one subject was infected with two strains.

Analysis of the Youden's index (sensitivity + specificity –1), which was calculated for the different reciprocal HIA titer cut-off values, suggested that a reciprocal HI titer of =15 provides a reliable correlate of VCIV-induced protection and there was no additional benefit with titers >30. Furthermore an analysis of efficacy over time showed that there was no decrease in efficacy throughout the full influenza season. In addition, all CPMP and FDA criteria for immunogenicity thresholds i.e. seroprotection, seroconversion and GMFI were met for all three vaccine strains.

Conclusions

These data confirm previous study results that this novel VCIV is immunogenic and well tolerated, and clinical efficacy has been proven. Our results further indicate that the existing correlates of protection established for egg-derived seasonal influenza vaccines also apply to cell culture-derived seasonal influenza vaccines.

SPA7 - LATE BREAKERS

A708P

An intradermal influenza vaccine elicits cross-reactive antibody responses against heterologous a(h3n2) influenza viruses

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Background

The Intradermal (ID) route of administration has been demonstrated to induce higher immune responses to influenza vaccination in the elderly, but little is known about the ability of an ID vaccine to induce cross-reactive antibodies against heterologous circulating viruses. Hence, we evaluated antibody responses elicited by an ID or an intramuscular (IM) trivalent inactivated influenza vaccine administered in the fall of 2006 against H3N2 viruses that differed antigenically from the vaccine strain including circulating field isolates.

Methods

The study population included 50 subjects 60 years or older who were randomly assigned to receive a single dose of Intanza® 15 µg intradermal or standard IM Vaxigrip® split-virion trivalent inactivated influenza vaccine containing A/Wisconsin/67/05(H3N2) as the H3N2 component. Each vaccine contained 15 µg of hemagglutinin per strain. Serum samples were collected before and 21 days after vaccination. In this descriptive study, antibody responses were measured against six A/H3N2 strains: the homologous vaccine reassortant strain A/Wisconsin/67/05, the A/Brisbane/10/07 reassortant vaccine strain, and four H3N2 field isolates: Genoa/62/05, Genoa/3/07, Genoa/02/07, and Genoa/3/06. The test viruses were antigenically closely related to A/California/7/04(H3N2), A/Nepal/921/06(H3N2), and A/Brisbane/10/07(H3N2). Antibody responses were assessed using hemagglutination inhibition (HI) and neutralization (NT) assays.

Results

With the HI assay, at least one Committee for Medicinal Products for Human Use (CHMP) immunogenicity criteria for vaccine approval in the elderly was met by both vaccines against all of the viruses tested. Seroprotection and seroconversion criteria were met for all six strains by the ID vaccine, while criteria for seroprotection against circulating Nepal/921/06-like and Brisbane/10/07-like viruses and seroconversion against A/Brisbane/10/07-like strains were not met by the IM vaccine. Neutralizing antibody post-immunization titers against five of six of the A/H3N2 strains tested were significantly higher with the ID vaccine compared to IM.

Conclusion

The ability of an ID vaccine to enhance the immune response and to elicit a stronger immune response against homologous influenza strains is consistent with other findings reported during the last years in subjects >60 years. When the immune response was evaluated against heterologous circulating H3N2 viruses, the immunogenicity profile of IM and ID vaccines differed markedly with the ID vaccine able to induce higher titers and to confer broader immunity.

SPA7 - LATE BREAKERS

A709P

Conformation Stability of the HA of the H1N1 pandemic vaccine strain A/California/7/2009 X-179A propagated in different substrates

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Introduction

Growth characteristics of the influenza viruses recommended for vaccine production is a key element in successful vaccine supply. At the same time, the quality of the viral hemagglutinin (HA), the major antigenic component of influenza vaccines, should be also taken into consideration. The reassortant virus A/California/7/2009 X-179A (X-179A) with improved growth properties (Fulvini et al., PLoS One 2011; 6(6):e20823), was widely used as a vaccine strain since H1N1 2009 pandemic. In this study the conformation status of the viral hemagglutinin (HA) of X-179A produced in different substrates was analyzed.

Materials and Methods

-179A was propagated in chicken embryonated eggs (eggs), monolayer culture of MDCK cells (ATCC, CCL-34), and suspension culture of the genetically modified MDCK*siat7e* cells (Chu et al., PNAS 2009; 106:14802-7). The produced virus particles were purified through sucrose gradient, and the virion-associated HA was analyzed for pH conformation stability, HA1/HA2 activation cleavage status, and HA-trimerization status. pH-conformation stability was evaluated by the exposure of the purified virus preparation to the pH ranging from 4.5 to 7.2. Treated virus samples were then analyzed for hemagglutination activity (with 0.5% suspension of chicken erythrocytes), and evaluated for their resistance to trypsin digestion. HA1/HA2 cleavage status was determined by polyacrylamide gel electrophoresis/Western blot analysis (PAGE/WB, using anti-HA1 polyclonal antibodies). The HA oligomerization status was evaluated in the molecular cross-linking experiments followed by PAGE/WB visualization.

Results

Viral HA produced in eggs was found to be proteoliticly activated by HA1/HA2 cleavage, and represented predominantly as trimers. The conformation change associated with the exposure to the low pH, when the viral HA loses its hemagglutination activity and becomes sensitive to trypsin digestion, was observed at pH 5.6. Virus preparations produced in MDCK cell monolayer culture were characterized by partial proteolytic activation of HA molecules, with relatively similar amounts of HA1 (cleaved form) and HA0 (uncleaved form) observed in PAGE/WB. Though virions contained predominantly trimerized HA molecules, a significant portion of the HA was also found in a form of dimers. Low-pH conformational change was observed at the pH 5.6. The HA of virus preparations produced in suspension culture of MDCK*siat7e* cells were represented only as uncleaved HA0 molecules, predominantly in a form of dimers rather than trimers. Although the hemagglutination activity of HA remains unaltered at the pH range from 7.2 to 5.7, the viral HA was not resistant to trypsin digestion even at neutral pH (pH 7.2). The combined data indicate that the folding and oligomerization of the virion-incorporated HA molecules can be significantly affected depending on the specifics of the host cell synthesis pathways.

Conclusion

This study shows that the properties of HA of X-179A can vary significantly depending on the substrate used for virus replication. The differences in HA characteristics can be attributed to the differences in post-translation modifications, including specifics of glycosylation pathways. On the other hand, such a significant variations of the HA properties, produced in different substrates, also indicate an intrinsic instability of the HA of the X-179A strain. Whether these findings are strain specific or can be extended to other H1N1 2009-pandemic strains is still unclear, but the consideration of the described HA characteristics would serve as an additional quality control in the selection of virus strains and substrates for successful application in vaccine development.

YOUNG SCIENTIST

SPA7 - LATE BREAKERS

A710P

When is the next pandemic going to occur?- Association of influenza pandemics with sunspot and the Southern Oscillation

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Introduction

Theoretically, the occurrence of an influenza pandemic is unpredictable, for nobody knows when a novel strain will appear from genetic re-assortments. However, this belief is being challenged with more and more findings on the association between influenza activity and environmental elements. A nice pioneering study by Viboud et al. shows that the impact of influenza epidemics depend on both the circulating subtype and the magnitude of El Nino Southern Oscillation (ENSO). Mazzarella et al. hypothesized a possible role of El Nino in the occurrence of influenza pandemics. Alternatively, Yeung used Sunspot Number (SSN) to detect pandemics, achieving a sensitivity of 85.7% and a specificity of 51.2%. Nevertheless, further endeavors are required for hypothesis tests, higher specificity, and biological explanations for the association. This study improves the specificity/sensitivity of pandemic detection through stringent criteria of epidemiology significance.

Method

Comprehensive reviews on pandemics and occurrence hypotheses were conducted. Yearly mean sunspot numbers (YMSSN) (1701 ~ 2010) was obtained from NOAA, USA. Monthly Southern Oscillation Index (SOI) (1876 ~ 2011) was retrieved from Bureau of Meteorology, Australia. Solar factor (SF) was calculated by amplifying the absolute deviation of YMSSN from the mean SSN with the constant e (2.718). Years in SF peaks (SF >100) and next years after the peaks were extracted. These years were further filtered by choosing those years with (1) positive mean SOI in the previous year and negative mean SOI in the SF peak year and next year; or (2) a mean SOI greater than +10 in the previous year.

Results

From 1876 to 2010, five (and only five) years were found to meet the criteria: 1888/89, 1918, 1957, 1968 and 2009. Historical pandemics exactly began in these years (and peaked in or after the years). Under a relaxed criterion (SF > 95), 1976 (swine flu) was also detected to be a pandemic year. The sensitivity of using the loose criterion to detect pandemics is 100% and the specificity is 99.2%. When the previous year of a SF peak (SF > 100) was examined, it was found that major epidemics occurred in the years with a positive mean SOI in the previous year and a negative mean SOI in the year and next year. These include 1877 (plague), 1887 and 1900 (yellow fever), 1911 (plague), 1932 (influenza epidemic), and 1946 (plague & relapsing fever).

DiscussionDiscussion

All nine influenza pandemics since 1800 and 10/12 pandemics since 1700 appeared in the SSN extreme years or±one year, rendering a ratio (83.3% ~ 100%) that is much higher than it would be (54.2%) if pandemics were fully randomly distributed. Meanwhile, since SOI was recorded all the pandemics (1889-90, 1918-19, 1957-58, 1968-69, and 2009-10) have occurred during El Nino. These associations have been brought to our attention, for no evidence has verified that these are purely due to coincidence. However, controversies exist because not all SSN extremes or El Nino years cause pandemics. The criteria in this study ease the argument by combing SSN and ENSO effects and improving detection sensitivity/specificity. The results indicate that pandemics occurred in a particular solar-climatic background: extreme sunspot activities and strong/persistent La Nina followed by medium/strong El Nino. The reason for this phenomenon warrants further investigation. A hypothesis is that the anomalous magnetic field during sunspot extremes affects the polarity of virus and the pathways/timing of birds' migration. The strong/presistent La Nina and El Nino as well as their transition lead to climate anomolies with borad impacts on viruses, vectors, herd immunity, and socio-behaviors. A significant outbreak of influenza epidemic is anticipated in 2012-2013 if current La Nina persists and sunspot number peaks in 2012.

SPA7 - LATE BREAKERS

A711P

Combination of carrageenan and a neuraminidase inhibitor as superior treatment for influenza A

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Introduction

The 2009 flu pandemic and the appearance of neuraminidase-inhibitor resistant H1N1 influenza strains highlight the need for treatment alternatives. One such option is the creation of a protective physical barrier in the nasal cavity. Consequently, we tested a novel combination of carrageenans together with the neuraminidase inhibitor Zanamivir in *in vitro* and *in vivo* infection experiments. This combination showed a high protection level against novel H1N1 influenza.

Material and Methods

For infection experiments Influenza A PR8/34 and novel H1N1 HH/09 viruses were used.
Plaque reduction assays were performed with Madine Darbey Canine Kidney cells (MDCK). *In vivo* infection experiments were performed using C57BL/6 mice infected intranasally, following FELASA guidelines, and survival was monitored over a 14 day period. Animals were monitored and treated twice daily. The treatment consisted of a preparation containing carrageenans together with Zanamivir.

Results

Treatment of mice infected with a lethal dose of influenza A PR8/34 H1N1 or novel H1N1/09 virus resulted in a strong protection of infected animals.

Conclusions

Novel treatment options for influenza are desperately needed. Based on these encouraging results in animals we suggest testing a nasal spray containing carrageenans in combinbation with neuraminidase inhibitors in a clinical trial for prevention or treatment of influenza A in humans.

SPA7 - LATE BREAKERS

A712P

Prognostic factors for mortality from influenza A (H1N1) new viral subtype, case-control study, State of São Paulo, Brazil, 2009.

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Introduction

On April 12, 2009, Mexico was identified an outbreak of acute respiratory illness characterized by high attack rate (28.5%), with later identification of positive samples for a new viral subtype of influenza A (H1N1). In the same period, the CDC reported two cases of febrile respiratory illness in California with similar laboratory diagnosis. On April 24, WHO warned countries members of the network of global alert and response, and on June 11 raised the alert level to the global pandemic phase 6. In the World, were laboratory confirmed cases of influenza A/H1N1 in over 214 countries, including at least 18,449 deaths. In Brazil, the mitigation phase, 44,544 were confirmed cases associated with Severe Acute Respiratory Syndrome (SARS). In the State of São Paulo, during the period, 6643 were confirmed cases of influenza A/H1N1 associated with SARS and 586 deaths with a mortality rate of 1.4 per 100,000 population.Objective: Identify predictors of mortality from influenza A (H1N1) new viral subtype.

Methods

Case-Control Study developed in the regional Greater Sao Paulo and Campinas, State of Sao Paulo, Brazil. The cases were confirmed deaths by influenza A/H1N1 in the period from June 28 to August 29, 2009. For each case, two controls were randomly matched by epidemiological week of admission, patients with SARS, with laboratory confirmation and progressing to cure. For cases and controls were elaborated two structured questionnaires, home and hospital with social, clinical and laboratory data. Logistic regression was used to determine the independent risk factor(s) for mortality.

Results

During the period, there were 193 cases and 386 randomly selected controls. The analysis of questionnaires hospital (193 cases and 385 controls) and household (161 cases and 336 controls) showed the following crudes odds ratios associated: diabetes mellitus Odds Ratio (OR) = 7.65 (95% Confidence Interval (CI) 3.51 to 16.65), neoplasms OR = 3.77 (95% CI 1.46 to 9.78), thyroid disease OR = 3.13 (95% CI 1.17 to 8.39), obesity (adults with 20 years and more) OR = 1,80 (95% CI 1.03 to 3.17), other diseases OR = 2.17 (95% CI .35 to 3.47), cardiovascular OR = 2.95 (95% CI 1.95 to 4.47) and as a protective factor against the use of oseltamivir OR = 0.65 (95% CI 0.44 - 0, 96) and pneumococcal vaccine OR= 0,16 (95% CI 0,06-0,45). Logistic regression analysis was performed considering the variables with p <0.15 control and confusion, with the following variables: age, sex, influenza vaccine, pneumococcal vaccine, caridiovascular diseases, diabetes, thyroid diseases and neoplasm. The fit of the model was made by the Hosmer-Lemeshow test and the following variables were significant in the final model using the Wald test: diabetes OR = 4.59 (95% CI 1.8 to 11.5), neoplasms OR 3.23 (95% CI 0,99 -10.43), pneumococcal vaccine OR = 0.25 (95% CI 0.08 - 0.75) and influenza vaccine OR = 0.23 (95% CI 0.08 - 0.66).

Conclusions

Pandemic influenza A/H1N1 represented major impact on morbidity and mortality in the state. The study showed as predictors of mortality, the presence of some comorbidities, especially diabetes mellitus, neoplasms, obesity. Use of oseltamivir, pneumococcal vaccine and influenza vaccine were protective factors. It is important to remember that there were differences in the vaccine prior information between cases and controls, recall bias, especially the history of the pneumococcal vaccine, which can take information bias.

SPA7 - LATE BREAKERS

A713P

Adaptation of 2009 pandemic virus to MDCK cells leads to antigenicity change and increased neuraminidase activity

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The 2009 H1N1 pandemic influenza strains have been shown to replicate inefficiently in eggs. Serial passages of these wild type viruses into embryonic eggs result in egg-adapted mutations which promote viral growth but also lead to loss of their lethality in mice. However, it is unclear whether cell adaption of these pandemic viruses would result in similar phenomena. Hence, we have serially passaged these wild type viruses into MDCK cells at 1:1000 dilution. Despite the original isolates grow poorly in cells at the beginning, these wild type pandemic strains quickly adapt into new host environment and replicate efficiently in MDCK cells after 2-3 passages. Unlike egg-adapted viruses, these cell-adapted viruses still reserve their pathogenicity in mice. However, we have observed that serial passages of these pandemic wild type viruses into MDCK cells lead to quick loss of the original antigenicity. Compared to egg grown wild type A/Mexico/4108/2009 (egg passage 4) which has an HAI titer of 1280 by standard ferret anti-A/California/07/2009, by cell passage 3 the HAI titer drops 2-fold and by cell passage 7 the HAI titer drops to 80. Full-length gene sequencing reveals that cell-adapted A/Mexico/4108/2009 has acquired two amino acid changes in the HA gene by cell passage 7. One is in the global head of HA from N to D at aa 173 and the other one is in the stem region from S to N at aa 306. In addition to antigenicity change, the NA gene of cell-adapted A/Mexico/4108/2009 has also acquired a mutation at aa 72 from T to P. Accompanied by this change, cell-adapted A/Mexico/4108/2009 also shows increased NA activity than the egg-adapted virus. To understand whether these genetic instabilities are unique to viruses of swine origin, we performed similar cell adaptation in A/New Jersey/8/1976 which caused a regional outbreak in Fort Dix military base, New Jersey 30 years ago. Unlike the 2009 pandemic viruses, A/New Jersey/8/1976 shows neither antigenicity loss nor NA activity change after cell adaptation. The observed genetic instabilities suggest that the 2009 pandemic viruses were at transition stage from swine origin to human virus when the first pandemic of 21st century took place.

SPA7 - LATE BREAKERS

A714P

Oral replicating Adenovirus serotype 4 vector vaccine for avian influenza: initial safety and efficacy results and next steps

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Introduction

We have developed a novel, oral avian influenza vaccine. The vaccine is a replication competent, vector vaccine based on adenovirus serotype 4 (Ad4) expressing the H5N1 influenza hemagglutinin (HA) 1194/ Vietnam (HA-Vtn), administered as an enteric-coated capsule. Cellular immunological and antibody results of the Phase 1 study of the Ad4-H5-Vtn vaccine suggest the need for a novel efficacy study to interpret and fully understand the results. The immunological findings and proposed study design of the Phase 2 efficacy study are described.

Materials & Methods

The Ad4-H5-Vtn vaccine was administered as enteric-coated capsules in a randomized, double-blind, placebo-controlled (vaccine:placebo=3:1), ascending dosage study. We enrolled 166 adult volunteers and all (87) of their household contacts (HHCs), in 5 cohorts, 107, 108, 109, 1010, and 1011 viral particles, dosed at 0, 56, 112 days. Three to 12 months after last Ad4-H5-Vtn/placebo immunization, vaccinees/ placebo recipients received a single parenteral booster immunization with inactivated H5N1 vaccine. Response to H5N1 was evaluated by hemaggluination inhibition (HAI), neutralizing and HA-ELISA antibody, and by HA-ELISPOT responses. All vaccinees and HHCs were evaluated for virological safety and evidence of transmission.

Results

There was no serious or dose limiting toxicity nor transmission to HHCs. There was no increase in AEs, reactogenicity, or clinical laboratory abnormalities as dosage or number of doses increased; vaccine vs. placebo comparison did not suggest a pattern of vaccine-related toxicity. 30% had pre-existing immunity to Ad4 (neutralizing antibody ≥1:6). Vaccine "take" (Ad4 seroconversion and/or Ad4-H5-Vtn shedding) was >80% in the 4 highest dosages among Ad4 seronegatives and ranged from 30% to 75% in Ad4 seropositives. Most of the vaccine takes had occurred following the first immunization. HAI seroconversion ranged from 0 to 19% (10% in placebo), but there was evidence of priming; cellular immune response to HA ranged from 52% to 74%. However, following a single boost with H5N1 inactivated vaccine, HAI seroconversion rates among Ad4-H5-Vtn recipients ranged from 61% to 80%; overall, 72% of vaccinees compared to 37% of placebo recipients seroconverted (p=0.0073). 68% of Ad4-H5-Vtn and 16% of placebo recipients had post-boost HAI titers ≥1:40 (p=0.00013). At the higher dosages, post-boost response rates were not diminished despite pre-existing immunity to the Ad4 vector.

Conclusions

The oral, replicating Ad4-H5 vector induced a potent HA-specific cellular response, and primed for a robust antibody (HAI) response following heterologous boost with the H5 HA protein. But, whether the cellular response, HAI levels ≥ 1:40, or both together will protect against H5N1 infection is unknown. Therefore, the components of the cellular immune and antibody responses will be evaluated in an H5N1 human challenge study. Volunteers will be immunized by different vaccine regimens on Day 0 and Day 30, and challenged by nasal installation of an attenuated H5N1 strain at Day 60; and then will be followed for clinical, immunological and microbiological evidence of H5N1 infection. In order to determine which vaccine regimens and which components of the immune response provide the best protection, the vaccine regimens will be (1) a single oral priming regimen (Ad4-Vtn-H5 at Day 0), (2) a heterologous prime boost regimen (oral Ad4-Vtn-H5 at Day 0 and parenteral inactivated H5N1 at Day 30), (3) "standard" parenteral regimen (inactivated H5N1 at Days 0 and 30), and (4) placebo (oral and parenteral placebos at Days 0 and 30). Thus, the efficacy of a single oral dose (predominantly cellular immune response), heterologous prime boost (cellular immune and antibody responses), or standard two dose parenteral regimen (predominantly antibody response) will be compared.

SPA7 - LATE BREAKERS

A715P

Aciclovir in Influenza, A Novel Experience

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Background

Influenza (flu), is an infectious disease caused by RNA viruses of the family Orthomyxoviridea. This is one of the most contagious and morbid viral condition worldwide. It has a big social and economical burden. Flu could be fatal in some instances. The two classes of antiviral drugs used against influenza are neuraminidase inhibitors and M2 protein inhibitors. No study we found that worked on aciclovir in flu.

Methods

We prescribed aciclovir 400 mg/ 6hrs orally along with acetaminophen 500 mg/ 6hrs for 5 days for 15 cases of clinically diagnosed with flu with constellation of high grade fever, myalgia, headache of less than three day duration in epidemic period of flu in 3 sequential years in Yazd, central Iran from 2009-2011.

Results

mean range of ages was 32 +/_ 6 years. Six patients had history of recent flu in their family. 12 patients had rapid clinical improvement within 48 hrs that was expressed dramatic by them. Remaining three, improved rather slowly within five days. Temperature, myalgia and headache dropped to normal condition rapidly. All of them returned to work after three days of treatment.

Conclusion

Unlike previous data, It seems that aciclovir could have a powerful theraputic effect of non-herpes related infections notably flu. Further RCTs should potentiate results of this trial.

SPB7 - IMMUNOLOGY

B701P

How dendritic cells contribute to the maintenance of tertiary lymphoid structures in lung tissue during influenza virus infection

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Tertiary lymphoid organs (TLO) are organized aggregates of B and T cells formed in post-embryonic life in response to chronic immune responses to infectious agents or self-antigens. Although CD11c$^+$ dendritic cells are consistently found in regions of TLO, their contribution to TLO organization has not been studied in detail. Here, we found that CD11c$^+$DCs are essential for the maintenance of inducible bronchus-associated lymphoid tissue (iBALT), a form of TLO induced in the lungs following influenza virus infection. Elimination of DCs after the virus had been cleared from the lung resulted in iBALT disintegration and reduction in germinal center (GC) reactions. This local disruption led to significantly reduced numbers of class switched plasma cells in both lung and bone marrow, and reduction in protective antiviral serum immunoglobulins. Mechanistically, DC isolated from the lungs of mice with iBALT did not present viral antigens to T cells, but were a source of lymphotoxin-ß and homeostatic chemokines (CXCL-12, and -13, CCL-19, and-21) known to contribute to TLO organization. Like depletion of DCs, blockade of LTß receptor signalling following virus clearance led to disintegration of iBALT and GC reactions. Together our data reveal a previously unappreciated function of lung DCs in iBALT homeostasis and humoral immunity to influenza virus.

SPB7 - IMMUNOLOGY

B702P

Generation of protective CTLs against the influenza A virus using single matrix epitope

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Introduction

Influenza viruses continue to pose a severe threat worldwide, causing thousands of deaths and enormous socio-economic loss. The major problem in fighting influenza virus is the high genetic variability which allows the virus to infect new host species and quickly overcome protective immunity. Vaccination with viral epitope, conserved in all the types and sub-types of influenza A virus, may efficiently counter the virus infection.

Materials & Methods

The study was carried out in which the protein transduction domain (PTD) of Tat protein of human immunodeficiency virus (HIV) was fused to the epitopic segment of the matrix gene of influenza A virus as the PTD domain is known to deliver the peptides < 10 kDa into the eukaryotic cells without the assistance of any external agent. The ligated oligo was cloned in pSecTag2 vector and expressed in MDCK cell line. The fusion protein was isolated, purified and expressed on antigen presenting cells (APCs) to generate immune response against the virus infected cells.

Results

The sensitized dendritic cells (DCs), when co-incubated with the cultured naive T cells, generated the cytotoxic T lymphocytes (CTLs) against the virus infected cells. The marked reduction in viral plaque count was observed in lungs of Balb/c mice when the intra-peritoneal (i.p.) injection of sensitized DCs was given and followed by intranasal instillation of influenza A virus strain [A/PR/8/34 (H1N1)]. The real time RT PCR assay also showed a significant decrease in viral titer as compared to the mock infected mouse. Cytokine analysis of the BAL fluid collected from the mice showed the enhanced expression of cytokines viz. IFN-ϒ, IL-12, IL-10 and IL-4 as compared to control.

Conclusion

Thus, the protective effect was observed with a single carrier protein coupled to the desired peptide for priming the APCs and thus activating the host immune system against the pathogenic virus.

SPB7 - IMMUNOLOGY

B703P

Application of a novel trogocytosis-based method of influenza-specific T-cells detection after LAIV vaccination

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Introduction.

Effective influenza immunity depends on induction of immunological memory B- and T-cells. Recently a new approach to determine T-cells specificity based on trogocytosis detection - TRAP assay (T-cell recognition of antigen presenting cells by protein capture) has been developed (Daubeuf S. at al., 2006). TRAP assay has been previously used for studies where T-cells specific to herpes virus, lymphocytic choriomeningitis virus and non-infectious antigens were determined. In our study, we modified the TRAP assay which measures antigen-activated cells involved in trogocytosis to determine influenza-specific memory T cells in peripheral blood of young adults vaccinated with live attenuated influenza vaccine (LAIV).

Materials&Methods.

Peripheral blood virus-specific memory T cells and HAI antibodies were studied in 20 volunteers (18-20 years old) vaccinated with LAIV reassortant strain A/17/Solomon Islands/06/9 (H1N1) and in 10 volunteers (18-20 years old) inoculated with placebo. Venous blood collection was performed prior and 21 days after vaccination. Peripheral blood mononuclear cells (PBMC) isolation, serum separation and HAI (haemagglutination ingibition) assay was performed by standard procedures. TRAP assay was based on previous protocols (Daubeuf S. at al., 2006) with modification. Briefly, PBMCs were divided into “effectors” and “targets”. Half the targets were loaded with virus by exposure to 1.5 MOI of purified A (H1N1) influenza virus vaccine strain for 1 h and incubated overnight at 37°C in 5% CO2 followed by labeling of the target cells. “Target” cells surface was biotinylated using EZ-Link Sulfo-NHS-LC-Biotin at the final concentration of 1 mg/ml: cells were incubated for 10 min at 25°?, 10 min with equal volume of fetal bovine serum at 4°?, and washed three times with RPMI-10. “Effectors” cells were labeled with 0.05 μM CFSE by standard procedure. The “effectors” cells were mixed with the loaded or control “targets” at a ratio 1:1 and after pelleting co-cultured for 1 h at 37°C in 5% CO2 to allow the trogocytosis. The mixture was treated with cold PBS with 2,5 mM EDTA, washed and stained with the fluorescently labeled human anti-CD4 antibodies and fluorescently labeled strepavadin (BD Biosciences, USA). The samples were analyzed by flow cytometry using a COULTER EPICS ALTRA.

Results.

By the TRAP assay data, unvaccinated volunteers 18-20 years old had marked variation in baseline levels of trogocytosis-reactive virus-specific CD4+ T-cells (range from 0.01 to 1.12%, mean 0.09%). Vaccination with LAIV increased levels of virus-specific CD4+ T cells in peripheral blood persons (rise up to 3.4 times, mean level 0.34%, $p<0,01$). Raised levels of these cells were shown not only in persons with four-fold seroconversion of HAI antibodies (HAI+) but in persons without these changes (HAI-) after vaccination. Thus, mean ratio of levels of virus-specific CD4+ T cells (ratio of individual post- to prevaccination levels) was 34.0, 14.8 and 1.9 times in HAI+ group, HAI- group and placebo group respectively. Also, an inverse dependence between preexisting levels of virus-specific $CD4^+$ T-cells before vaccination and their magnification after vaccination was shown in this study (correlation coefficient –0.70, $p<0.01$). Individual increases were considered significant if virus-specific CD4+ T-cells exceeded maximal value in placebo group in 10.0 times and more. Using these criteria significant increase of virus-specific CD4+ T-cells was shown in HAI+ group (70%) and in HAI- vaccinated volunteers (40%).

Conclusion.

It was shown that TRAP assay can be utilized to virus-specific $CD4^+$ memory T-cells estimation in a LAIV vaccination studies as alternative to intracellular cytokine staining assay.

SPB7 - IMMUNOLOGY

B704P

Performance of the Hemagglutination Inhibition Assay Using Native, Ether-treated, or Triton X-100 split B Influenza Antigen Preparations

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Aims

Influenza Hemagglutination Inhibition (HI) is one of the reference methods for measuring the receptor binding antibodies to the surface protein hemagglutinin (HA). Lower HI antibody titers against influenza B strains have been observed and reported by various laboratories in serum samples from both clinical trial subjects and subjects following influenza infection. To enhance and maximize the influenza B HI assay detection capability without compromising assay specificity, performance of the HI assay was evaluated using various preparations of influenza B antigen.

Methods

Pre (Day 0)- and post (Day 21)-vaccination human serum samples from adult and elderly volunteers immunized with 2009/2010 (containing Victoria linage of B/Brisbane/60/2008, n = 64) or 2008/2009 (containing Yamagata lineage of B/Florida/04/2006, n = 15) trivalent inactivated influenza vaccines were tested with sanofi pasteur validated influenza B HI assay using native (whole virion), ether-treated, or triton-split antigens. The validated HI assay using turkey red blood cells was adapted from the WHO/CDC HI assay protocol with minor modifications. The serum-virus incubation temperature used in the assay previously had been evaluated and optimized. Seroprotection (sp) rate (≥1:40), seroconversion (sc) rate (4-fold rise), geometric mean titer (GMT), and GMT ratio (GMTR) between pre- and post-vaccination samples were compared using various antigen preparations.

Results

Data analyses demonstrated that the lowest HI GMTs, sp and sc rates, and GMTRs were obtained when native (whole virion) B HA antigen was used in the HI assay. Relatively higher HI titers were observed in both pre- and post-vaccination samples when triton-split B antigen was used, resulting in a high sp rate, but neither the sc rate nor the GMTR was significantly enhanced. Nevertheless, ether-treated B HA antigen increased the post-vaccination titers while the pre-vaccination titers remained low. Use of the ether-treated antigen resulted in relatively higher sp rates, sc rates, and the GMTRs especially in serum samples from elderly persons. The HI GMTs and GMTRs, with 95% CI, of B/Brisbane/60/2008 are illustrated in the following table; similar observations were seen with the immunogenicity evaluation of the B/Florida/04/2006 strain.

HI GMTs and GMTRs of B/Brisbane/60/2008 Using Different Antigen Preparations

	B/Brisbane/60/2008		
	18-59 YO		
	GMT, Pre-bleed	GMT, Post-bleed	GMTR
Native (whole) B HI	16.8 (12.6, 22.3)	87.2 (64.0, 118.8)	5.19 (3.44, 7.84)
Tx-100 split B HI	84.2 (57.9, 122.4)	732.7 (546.4, 982.5)	8.71 (5.46, 13.89)
Ether-treated B HI	36.1 (24.7, 52.9)	321.6 (230.9, 447.9)	8.90 (5.43, 14.60)
	> 60 YO		
	GMT, Pre-bleed	GMT, Post-bleed	GMTR
Native (whole) B HI	20.8 (15.3, 28.2)	48.3 (36.5, 63.9)	2.33 (1.55, 3.50)
Tx-100 split B HI	135.3 (98.4, 186)	360.5 (283.6, 458.3)	2.67 (1.80, 3.94)
Ether-treated B HI	49.9 (35.7, 69.7)	148.4 (115, 191.4)	2.98 (1.97, 4.49)

Conclusions

The performance of the Influenza B HI assay in detecting strain-specific anti-HA antibodies can be enhanced by using either triton X-100 split B or ether-treated B antigen. Use of ether-treated B antigen in the HI assay can further provide a greater GMTR enabling better characterization of anti-HA antibody response against influenza B viruses following vaccination with either B/Brisbane/60/2008 (Victoria lineage) and B/Florida/04/2006 (Yamagata lineage). Importantly, the specificity of this enhanced HI assay remained unaltered, upon confirmation using a competitive inhibition HI assay.

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Composition, structure and fusogenic properties determine the capacity of classical, non-adjuvanted influenza vaccines to induce cross-protective immunity

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Introduction

Recent outbreaks of highly pathogenic influenza strains and the unpredictable emergence of new pandemic strains demand the introduction of cross-protective influenza vaccines. Cross-protection against influenza could be induced by eliciting antibody responses targeting conserved viral epitopes, such as conserved hemagglutinin (HA) epitopes or epitopes of conserved proteins (nucleoprotein, matrix protein). Additionally, a cross-protective vaccine could induce T cell responses, particularly cytotoxic T cell (CTL) responses against conserved proteins. Here, we compared the capacity of non-adjuvanted, seasonal influenza vaccines – subunit, split, fusogenic and non-fusogenic whole inactivated virus (WIV) – to induce cross-protection against heterologous challenge in mice.

Materials and Methods

C57Bl/6 mice were subcutaneously vaccinated with H5N1 vaccine (subunit, split or WIV) and challenged with a heterologous influenza strain (PR/8). In a separate group of mice, CD8+ T cells were depleted by injecting specific depletion antibody before challenge. Survival, body weight change, lung virus titers, antibody titers and systemic and local influenza-specific CTL numbers together with granzyme B were measured to assess the protection and immunity.

Results

WIV, but not subunit and split vaccine platforms, protected mice against heterologous challenge by inducing rapid clearance of the virus from their lungs. Cross-protection induced by WIV was mainly mediated by specific CD8+ T cells, i.e. CTLs, as WIV-immunized mice depleted of CD8+ T cells were not protected against challenge. Next, we focused on the role of the HA-mediated fusion activity of WIV in the induction of cross-protection. We compared the cross-protective capacity of WIV inactivated by formaldehyde, which abrogates fusion activity, to that of WIV inactivated by ß-propiolactone, which preserves membrane fusion activity. All the mice immunized with WIV survived the heterologous challenge. In the group vaccinated with fusion-inactive WIV 5 out of 12 mice developed severe symptoms. In contrast, none of the mice vaccinated with fusion-active WIV developed severe symptoms. Moreover, we observed lower lung viral loads, a higher influx of influenza-specific CTLs and a higher production of granzyme B in the lungs of mice vaccinated with fusion-active WIV compared to mice vaccinated with fusion-inactive WIV.

Conclusions

In summary, WIV, but not subunit or split vaccine, induced cross-protective immunity, which was mainly CTL-dependent. WIV produced by methods that preserve its membrane fusion activity has the highest capacity to induce influenza-specific CTL responses, and is therefore a promising candidate for induction of cross-protective immunity.

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B706P

Isolation of 279 single-domain antibody fragments that specifically recognize the 9 neuraminidase and 16 haemagglutinin subtypes of influenza A virus

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Antigenic differences are used to classify influenza A viruses into 9 neuraminidase (N1-N9) and 16 haemagglutinin (H1-H16) subtypes. The H1-H3 and N1-N2 subtypes occur only in human influenza viruses whereas all subtypes are found in avian viruses. Subtyping of influenza viruses is most easily performed by sequence analysis of the NA and HA genes. However, serological analysis of the influenza subtype that caused a prior infection in birds requires ELISA assays that specifically recognize the NA and HA subtypes. For use in such assays we isolated 279 single-domain antibody fragments (VHHs) against all NA and HA subtypes. VHHs are the N-terminal antigen binding domains derived from llama heavy-chain antibodies that naturally lack a light chain. They have particular advantages for biotechnological applications such a high stability, high microbial production level and high amenability to phage display selection.

VHHs were selected by immunization of four llamas with mixtures of sucrose density gradient purified influenza viruses that represent all subtypes and subsequent phage display selection of specifically binding VHHs using, preferably, recombinant HA and NA molecules of all subtypes. Recombinant HA was derived from commercial suppliers (Abcam, Sinobiological) whereas recombinant NA was expressed in HEK293 cells. Isolated VHHs were characterized by sequence analysis. Unique VHHs were expressed by secretory yeast expression. Sandwich ELISAs for detection of influenza antigen were developed by coating with unlabelled VHH, subsequent incubation with influenza antigen, and detection of bound antigen using biotinylated VHH and streptavidin peroxidase conjugate.

By phage display selection we isolated 172 VHHs against the nine NA subtypes, comprising at least 10 clones for each N subtype. We similarly isolated 107 VHHs binding the 16 HA subtypes. Only 1 or 2 VHHs were selected that bound either H2, H7, H14, H15 or H16, whereas more diverse sets of VHHs were obtained for the other HA subtypes. The influenza subtype specificity of the isolated VHHs was characterized by binding to different influenza strains in ELISA. This allowed us to identify for each NA subtype several VHHs that specifically bound the NA subtype. The analysis of the subtype specificity of the HA binding VHHs is ongoing.

We currently develop sandwich ELISAs for the various NA and HA subtypes, with a focus on the 9 NA subtypes. A competition ELISA for the specific detection of a chicken polyclonal antibody response against N2 subtype was successfully developed, whereas ELISAs against the other 8 NA subtypes are in various stages of development.

These ELISAs could allow future subtyping of the serological response in birds against the various HA and NA types for use in epidemiological studies. They could also allow the identification of prior infection with influenza strains that have potential for spread to humans, which until now are only of the H5, H7 or H9 subtype. Finally, the availability of VHHs against any subtype could be usefull research reagents in various assays, especially for the more rare subtypes against which monoclonal antibodies are not yet publicly available.

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B707P

Innate immunity as driving force of nucleotide bias in the influenza virus genome

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The innate immune system provides the critical first line of defense against virus infections. It is becoming increasingly clear that host cells recognize viral genomes as non-self via pattern recognition receptors. Such detection of invading nucleic acids results in a signaling cascade leading to the induction of inflammatory cytokines and type I interferon, and ultimately priming of adaptive immune responses. Viral genomes can be recognized by their structure, modifications and/or sequence. The 5' triphosphate ends of influenza virus RNA are recognized by the RIG-I receptor. It has been hypothesized that besides this sequence-independent recognition, the influenza virus genome can also be recognized in a sequence-dependent manner.
Clues about which sequence motifs in the influenza virus genome might be recognized by the innate immune system could be drawn from computational studies examining non-random patterns in the frequency of sequence motifs (nucleotide bias) in the influenza viral genome. One of these studies reported that CpG motifs (cytosine-phosphate-guanine) are underrepresented in the influenza virus genome. It has been suggested that this has been caused by the evolution of influenza viruses escaping from recognition of CpG motifs by the innate immune system. However, it is still unknown whether influenza virus CpG-RNA is indeed being recognized by the innate immune system and whether this innate immunity is the driving force of the observed nucleotide bias. Other factors like selection on the protein level, a bias in the total number of cytosines and guanines, a codon usage bias or RNA structure/stability may also explain the CpG nucleotide bias. Furthermore, it is unknown whether besides the sequence, also the secondary structure in which the motif is present plays a role in the recognition.

We aim to elucidate the relation between innate immunity and the CpG nucleotide bias in the influenza virus genome, by the following key objectives:
1) Can other factors than innate immunity explain the nucleotide bias present in the influenza virus genome? Does the underrepresentation of CpG motifs remain when corrected for this?
2) In which secondary structures of the influenza genome are CpG motifs underrepresented?
3) Are CpG motifs in viral RNA recognized by the innate immune system?

We investigated whether other factors than innate immunity could be responsible for the underrepresentation of CpG motifs in the influenza virus genome by computational analyses on thousands of influenza virus sequences. We found that natural selection on the protein level, a bias in the total number of cytosines and guanines or a bias in codon usage cannot explain the low level of CpG motifs in influenza virus RNA. In other computational analyses we have tested whether the presence of CpG motifs is related to the predicted secondary structure of the genome of influenza viruses. We distinguished between predicted double-stranded ('stems') and single-stranded ('loops') parts of the folded influenza virus RNA segments and found that CpG motifs are especially underrepresented in stems when compared to the GpC motifs (guanine–phosphate–cytosine). This indicates that CpG motifs in the influenza virus RNA genome may be specifically recognized by the immune system when they are present in double-stranded stem structures. We are currently testing the immunogenicity of CpG motifs in viral RNA by immunological assays.

Thus, so far we can conclude that innate immune recognition may be a driving force in the CpG nucleotide bias in the influenza virus genome. By further investigating this, we will shed more light on the interplay between the genome of influenza viruses and human innate immunity. This knowledge may be ultimately used for immunotherapy and vaccine development.

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B708P

Application of a new vaccine principle to combat infectious salmon anemia virus (isav) – a piscine orthomyxovirus.

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Infectious salmon anemia virus (ISAV), a piscine orthomyxovirus, is a viral pathogen of farmed Atlantic salmon. Over the past 25 years this pathogen has caused major disease outbreaks in the Northern hemisphere (Norway, Scotland and Canada), and has recently spread to the Southern hemisphere (Chile). The virus causes a multisystemic disease leading to severe anemia and death, and no effective vaccines are available. The severe economic losses in the fish farming industry due to this viral disease make ISAV one of the most costly emerging pathogens in this industry.

The virus belongs, together with the influenza viruses, to the *Orthomyxoviridae* family. Accordingly, these viruses share several important features such as morphology, replication strategy and interactions with their respective hosts, including how to enter and exit cells.

To determine the immune correlates of protection against ISAV and to develop efficient vaccines we use several different approaches; a reverse genetics system has been adapted for the rescue of recombinant virus from cloned cDNA [1] and a yeast two-hybrid approach is being used to study virus-host interactions.

Recently, we have started to explore the possibility of using a targeted DNA fusion vaccine principle (Vaccibodies) [2-6] to analyze immune responses to ISAV antigens and as a putative vaccine approach. The efficiency of DNA vaccines targeting antigen presenting cells (APCs) caused by enhanced presentation has been demonstrated [2-6], and very promising results have been achieved by applying this principle to influenza (G. Grødeland et al., unpublished). Due to the many similarities between influenza and ISAV, we will explore the comparative aspect of this research. By targeting ISAV-antigens to MHCII molecules on salmon APCs viral peptides will be presented to the immune system, mounting strong and efficient responses, without the need for potentially harmful adjuvants.

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B709P

Protective haemagglutination-inhibiting antibody titre against infection with pandemic influenza a/h1n1

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Introduction

Haemagglutination-inhibiting (HI) antibody titre is frequently used to assess protection against influenza infection. HI titre is the main criteria for influenza vaccines evaluation by the European Medicines Agency (EMA) or the US Food and Drug Administration (FDA). However, the association between an HI titre and a level of protection has rarely been assessed. Hobson et al. in a seminal paper published in 1972 reported a titre of 1:18-1:36 to be 50% protective against experimental challenge (J Hyg. 1972;70(4):767-777). More recently, Coudeville *et al* (BMC Med Res Methodol. 2010;10:18) reported a 50% protective titre of 1:17 against natural or challenge infection, irrespective of the type of viral strain (A or B). We explored the relationship between the HI antibody titre and the risk of infection with the pandemic influenza A/H1N1 virus.

Material & Methods

We used serological data from 2 cohort studies conducted in the general population: 1321 subjects with paired sera (pre and post-pandemic sera) from the CoPanFlu-Réunion cohort and 1200 subjects with post-pandemic sera from the CoPanFlu-France cohort.
We developed a Bayesian model for estimating protection against influenza infection using serological titres, individual characteristics of subjects and epidemiological data from surveillance networks. This model estimated the level of protection in relation to the HI titer, the antibody kinetics following an infection, the posterior distributions for individual pre-pandemic and post-pandemic titres and the dates of infection.

Results

Preliminary estimates show that a pre-pandemic HI titre of 1:30 [95% confidence interval: 1:22; 1:42] was 50% protective of infection with the pandemic H1N1 virus. This finding is in line with the criteria used by the EMA and the FDA (1:40) and with the studies referenced above. The modeled antibody kinetics followed a biexponential function, with a quick increasing post-exposure phase from the predicted baseline titre and a slow decreasing phase until the predicted final titre.

Conclusions

This model built from serological data allows us to make predictions of infection rates, pre and post-pandemic protection levels and antibody kinetics following infection.

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B710P

Seroprevalence against pandemic influenza A(H1N1) 2009 after the first season of circulation in Italy

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Introduction

Studies on the extent of infection with pandemic A (H1N1) 2009 virus are crucial for better understanding the proportion of subjects who are still susceptible to the virus. A previous study, conducted in Italy on 587 serum samples collected before the circulation of the 2009 pandemic virus, showed that the proportion of subjects with protective levels (HI titres ≥ 40) of antibodies cross-reacting with the pandemic virus was about 6%, 12%, and 22% in individuals aged < 55, 56-65 and >65 yrs, respectively. To assess seroprotective antibody levels after the first season of pandemic 2009 virus circulation, we carried out a survey of antibody levels against the pandemic virus in further 1439 serum samples, representative of the Italian population by age and gender.

MATERIALS AND Methods

Left over serum samples were collected in 7 diagnostic labs, located in different Italian regions, between September and November 2010 before the start of the 2010/2011 influenza season, and were included in this study. About 200 samples were collected for each of the following age groups: <1, 1-4, 5-14, 15-44, 45-64, 65-74, >75 yrs. Sera were provided anonymously and without additional clinical detail (e.g., testing indication or vaccination history).

The antibody titres against the pandemic A(H1N1) 2009 virus (A/California/7/2009 strain) were measured by the haemagglutination inhibition (HI) assay, supplemented by the microneutralization (MN) assay. Geometric mean titres (GMT) and the proportion of people with seroprotective antibody levels were assessed. A HI titre of 10 or more were considered as seropositive.

Results

When considering the cut-off value for protection required by EMA criteria (HI titre ≥40), the lowest proportion of subjects with protective antibody titres was found, as expected, in children aged < 1 yr (1%). In the other age groups, the proportion of protected individuals was higher among people less than 15 yrs of age (37% in 1-4 yrs group; 58% in 5-14 yrs group), when compared with those subjects belonging to the 15-44, 45-64, 65-74, >75 yrs age groups, where the values of seroprotection against the pandemic virus were respectively 17%, 7%, 12% and 20%. When using a minimum level of detection of 1:10 in HI, the proportion of subjects with positive sera increased in each of the 7 age groups showing the following values, respectively: 3% (<1), 43% (1-4), 72% (5-14), 28% (15-44), 22% (45-64), 25% (65-74), 44% (>75). Overall, the results we obtained using HI and MN assays were well correlated. With regard to the GMTs of the above age groups, the values found were respectively 5.2, 18.6, 29.5, 8.8, 6.8, 8.1 and 11.1.

Conclusions

The results obtained in this study show that, after the first season of circulation of the pandemic influenza A (H1N1) 2009 virus in the community, the highest increase in seropositivity rates to the new virus, when compared to the results observed in sera collected before the pandemic, were found among young children and young adults (1-14 yrs of age), in agreement with higher attack rates of pandemic (H1N1) influenza in these groups observed during the pandemic season. On the contrary, the lowest increase in the rate of seroprotection after the 2009 pandemic was found among subjects aged 45–74 yrs, whereas no substantial changes were observed among the elderly aged >75.

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B711P

Evaluation of influenza A H1N1 (2009) sero-susceptibility in children in scotland post pandemic.

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Introduction

There is evidence that the serological attack rate (SAR) in children during the Influenza A H1N1 (2009) pandemic was higher than indicated attack rates or virological testing, and that it varied across different regions in England. As the pandemic epidemiology in Scotland differed from the rest of the UK, we undertook a cohort study looking at Influenza A H1N1 2009 sero-epidemiology in children to provide a more definitive picture on the level of H1N1 (2009) susceptibility in children to inform influenza disease public health planning.

The primary objective of this study was to estimate the proportion of children (under 15 years) in Scotland sero-positive for Influenza A H1N1 (2009) antibodies. Secondary objectives of this study were to identify susceptible age groups within this cohort to inform the 2010/11 influenza programme, and to investigate whether there were any regional differences in the level of susceptibility within this cohort.

Method

Residual blood samples collected for routine clinical diagnosis (April to October 2010) in three Paediatric Hospitals in Scotland (Aberdeen, Glasgow and Edinburgh) were analysed in this study. A total of 1580 samples (age and sex representative) were collected and tested anonymously using a micro-neutralisation assay (1:40 dilution) to determine sero-positivity levels. Binomial analysis and logistic regression were used to determine whether there were any differences in the levels of sero-positivity due to age, gender, and region.

Results

A total of 1580 samples were collected, of which 1482 met the study criteria and were included in the analysis (98 samples were excluded as outside age range/inadequate volume).

A key finding of this study was that the rate of sero-positivity in those aged under 1 year was significantly lower when compared to children aged 1 year and above ($p<0.05$, table 1). Individuals aged 5-9 years had the highest level of sero-positivity followed by the 10-15 years, with the 1-4 years age group slightly lower than both.

There was a significant difference in the sero-prevalence rate in the samples collected in the Aberdeen hospital compared to the Glasgow and Edinburgh hospitals ($p=0.045$). This appeared to be primarily due to the lower sero-prevalence rate in the under 1 year olds compared to the other two sites ($p<0.05$, table 2).

There was no evidence of any differences in the levels of sero-positivity by age group over 1 years of age ($p=0.17$), region ($p=0.07$) and gender ($p=0.15$) in this study.

Table 1: Sero-positivity rate broken down by age

Age (years)	Number of samples tested	Number positive for Influenza A H1N1 (2009)	Sero-positivity rate	95% CI, Lower limit	95% CI, Upper limit
<1	268	49	18.3	14.1	23.4
1-4	381	159	41.7	36. 9	46.7
5-9	412	198	48.1	43.3	52.9
10-15	421	196	46.6	41.8	51.3

Table 2: Sero-positivity rate in infants aged less than1 year broken down by region

Region	Number of samples tested	Number positive for H1N1 (2009)	Sero-positivity rate	95% CI, Lower limit	95% CI, Upper limit
Edinburgh	95	23	24.2	16.7	33.7
Aberdeen	48	3	6.3	2.1	16.8
Glasgow	125	23	18.4	12.6	26.1

Conclusion

This study suggests a significant proportion of children under the age of 15 years were at risk of developing Influenza A H1N1 2009 at the start of the 2010/11 season, and in particular those aged under 1 year were at highest risk as the sero-positivity rate was lowest. There is a public health need to ensure that all pregnant women are immunised against Influenza A H1N1 (2009) to protect themselves, and indirectly to protect children under 1 year of age. The results of this study informed the influenza 2010/11 season and will inform future influenza seasons..

YOUNG SCIENTIST

SPB7 - IMMUNOLOGY

B712P

Hetero-subtypic antibody responses against group 1 influenza a viruses are predominantly induced by pandemic influenza vaccination

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Hetero-subtypic antibodies (Ab) that bind to conserved regions of the influenza A hemagglutinin (HA) protein exist as part of a sub-dominant response to either infection or vaccination and cross-react with group 1 influenza A viruses. To estimate the frequency and potency of heterosubtypic Ab responses towards both group 1 and 2 influenza A viruses, sera from 114 health care workers vaccinated at San Raffaele Scientific Institute in Milano, against pandemic H1N1 influenza in 2009 were tested for the presence of Ab capable of displacing the binding of the murine monoclonal Ab (mAb) C179 to H5 or of the human mAb FI6 to H7, respectively. Both mAbs recognize an epitope located in the stem region of the HA molecule.

Prior to vaccination, the proportion of individuals with serum Ab that cross-reacted on H5 was remarkably high (97%). However, a significant increase of H5 binding displacement titer 80 (BD_{80}) was observed 28 days after vaccination (reciprocal BD_{80} before vaccination of 170 vs. 417 after vaccination, $p<0.0001$). In addition, the BD_{80} values correlated with the microneutralization (MN) titers of a lentiviral pseudotype expressing the same H5 but not with the titers of hemagglutination inhibition Ab to the vaccine strain. The pre and post-vaccination MN_{80} titers were 235 and 590, respectively, ($p<0.0001$). In contrast, the proportion of individuals with serum Ab that cross-reacted for H7 binding was low (27%) and the H7 titers were lower than those of H5 (47 vs. 170, $p<0.0001$). No increase of H7 BD_{80} titers was observed following vaccination (BD80 of pre and post-vaccination titers of 47 vs. 70, respectively, $p=0.24$) except for 2 individuals; for these reasons, the MN responses were not tested.

In summary, pandemic 2009 vaccine can boost the titers of a preexisting hetero-subtypic Ab response against group 1 but rarely against group 2 influenza viruses. The protective role of these responses against infection and disease of seasonal or pandemic strains remains to be determined.

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B713P

Population immunity to influenza viruses in Norway in August 2010: good correspondence between pre-seasonal immunity and subsequent virus circulation

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Aims

The main objective of this study was to assess the prevalence of antibodies to the pandemic A(H1N1) 2009 influenza virus as well as to contemporary seasonal influenza viruses in the Norwegian population. The sera included in the study were collected in August 2010, approximately ten months after the main peak of the 2009 pandemic wave and after an extensive pandemic H1N1 (2009) virus vaccination campaign in October to December of 2009.

In addition, the study aimed to analyse how the observed immunity in August 2010 corresponded to the extent of circulation of the various influenza viruses during the subsequent 2010/11 influenza season.

Methods

A panel of age- and geographically representative anonymised residual sera (n = 2062) was collected in August 2010 from hospital laboratories in all regions of Norway. Serum antibody titres were determined using the influenza hemagglutination test (HI). Viral antigens used were the pandemic H1N1(2009) influenza virus A/California/07/2009, in addition to the following antigens: A/Brisbane59/2007(H1N1), A/Brisbane/10/2007(H3N2), A/Perth/16/2009(H3N2), B/Brisbane/60/2008 (Victoria lineage), and B/Florida/04/2006 (Yamagata lineage). Serum HI titres of ≥ 40 and ≥80 were considered protective to influenza A and influenza B viruses, respectively.

Results

In August 2010 the prevalence of protective antibodies to pandemic and seasonal influenza A viruses was high in most age groups even though there was no circulation of these viruses after the main wave of pandemic influenza H1N1 (2009) during Oct-Dec of 2009. H1N1/California (26%), H1N1/Brisbane (15%), H3N2/Brisbane (33%) and H3N2/Perth (23%). However, the antibody prevalence to the pandemic A(H1N1) 2009 virus had decreased compared to the observed very high prevalence seen in January 2010 (26% versus 45%, all ages), which is a reduction of 42%. Seroprevalence to pandemic H1N1 (2009) was highest in the age groups 5-14 and 15-24 year-olds with 39% and 43%, respectively.

For influenza B viruses, prevalence of protective antibody was 10% to B/Brisbane/60/2008 (Victoria lineage), and 25% to B/Florida/4/2006 (Yamagata lineage). For the Yamagata lineage virus the highest antibody prevalence was seen among adolescents and young adults, but also among the elderly, particularly those above 80 years. In contrast, the antibody prevalence to the Victoria lineage virus was particularly low among children, adolescents and young adults (those below 30 years), 7% and 6%, respectively.

Discussion/Conclusion

During the last four-to-five years the immunity to the influenza B Victoria lineage viruses in the Norwegian population has been low.

During the subsequent 2010/11 influenza season, and contrasting to the pattern observed elsewhere in Europe, B/Brisbane/60/2008-like Victoria-lineage viruses predominated in Norway, and the outbreak was comparatively strong and peaked unusually early for an influenza B outbreak. Large outbreaks of influenza B viruses at this time of the year have been very uncommon in Norway, even when looking as far back as the 1980s.

The observed weak population immunity to B/Victoria-lineage virus may have provided 'an open door' for the Brisbane/60-like viruses. Conversely, substantial prevalence of antibody against the other antigens corresponded well to the limited circulation observed for these viruses, including the H1N1(2009) virus. The 2010 influenza serosurvey thus was predictive for the following influenza season in Norway.

SPB7 - IMMUNOLOGY

B714P

T cell responses contribute to protection from influenza

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Until now, serology has been the golden standard to assess influenza vaccine efficacy. Several studies have reported, however, that cellular responses are also associated with protection, especially in high risks groups such as the elderly and infants. In this study, we examined the role of T cell responses in protection from laboratory confirmed infection in healthy adults (18-60 years) who were vaccinated with the trivalent inactivated (TIV) seasonal influenza split-unit vaccine of 2006/2007. Blood samples were taken at three time-points: before vaccination, 4 weeks after vaccination (i.e. before the influenza season) and at the end of the influenza season. Peripheral blood mononuclear cells (PBMC) were isolated and cryopreserved. In addition, hemagglutination inhibiting (HI) titers were determined. During the influenza season, 5 individuals were identified with clinical influenza A infection (4x A/Wisconsin/ 67/2005 H3N2, 1x A/New Caledonia/20/99 H1N1). All infected individuals had low HI responses. To exclude any protection mediated through HI antibodies, seasonal influenza-specific T cell responses of sick individuals were compared with non-infected individuals who had similar low HI responses (HI≤40). Strikingly, sick individuals showed significantly lower CD4+-IL-2+ T cell responses for the influenza strain (A/Wisconsin/ 67/2005) as compared to the non-infected controls. In contrast, degranulation and IFN gamma-production by CD8+ T cells were not different between both groups. Therefore we conclude that in addition to HI responses, CD4+-IL-2+ T cell responses are associated with protection against influenza.

YOUNG SCIENTIST

SPB7 - IMMUNOLOGY

B715P

Broadly cross-reactive antibodies dominate the human B cell response against 2009 pandemic H1N1 influenza virus infection

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The 2009 pandemic H1N1 influenza pandemic demonstrated the global health threat of reassortant influenza strains. Here we have analysed the plasmablast and monoclonal antibody responses induced by pandemic H1N1 infection in humans. Unlike antibodies elicited by annual influenza vaccinations, most neutralizing antibodies induced by pandemic H1N1 infection were broadly cross-reactive against epitopes in the hemagglutinin (HA) stalk and head domain of multiple influenza strains. The antibodies were from cells that had undergone extensive affinity maturation. Based on these observations, we postulate that the plasmablasts producing these broadly neutralizing antibodies were predominantly derived from activated memory B cells specific for epitopes conserved in several influenza strains. Consequently, most neutralizing antibodies were broadly reactive against divergent H1N1 and H5N1 influenza strains. This suggests that a pan-influenza vaccine may be possible, given the right immunogen. Antibodies generated potently protected and rescued mice from lethal challenge with pandemic H1N1 or antigenically distinct influenza strains, making them excellent therapeutic candidates.

SPB7 - IMMUNOLOGY

B716P

T Cell Response in Highly Pathogenic Human Influenza A (H5N1): Insights from a Convalescent Cohort in Vietnam

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Antibodies are critical to the clearance of influenza virus infections and to that effect current vaccines are antibody-based. Even in H5N1 influenza, convalescent plasma and manufactured antibodies are reported to be beneficial. But, with negligible antibody-mediated immunity to H5-HA reported in most human populations due to antigenic variation (shift/drift), a H5N1 pandemic would be catastrophic. H5N1 is a highly virulent strain of Influenza A virus producing severe disease, at c.60% fatality, an aggressive clinical course and cytokine storm resembling 1918 H1N1, which remains incapable of sustained human-to-human transmission, but may in future evolve such capability by mutation or reassortment.

While the antibody response is not detected for days, T cell responses can be mounted within several hours and early viral suppression promotes survival. Evidence in mice and humans suggest influenza-specific CD8 cells play a critical role in viral clearance, and that memory CD4 cells maintain this CD8 response. The purported protective potential/capacity of T cells is not to prevent, but rather, control or limit an infection. However, caveats remain in understanding the nature, dynamics & kinetics of a protective cellular response in influenza including whether virus-specific CD8 T cells actually protect.

We investigated memory T cell response associated with a good clinical outcome in our cohort of convalescent H5N1 subjects -mapped H5N1 epitopes and quantified H5N1-specific and cross reactive T cells ex vivo with synthetic overlapping peptides spanning the full proteome of influenza A/Viet Nam/CL26/2005 (H5N1). We provide a picture of functional immunity with antigen-specific CD4 and CD8 T cells to a broad range of viral proteins including H5-HA, varying immunodominance and frequencies, most prominently NP & M. The study will next assess T cell quality, kinetics, ability to control infection in vitro; and may contribute insight into T cell vaccine strategies.

No conflict of interest

SCIENCE POLICY INTERFACE

I102P - SPI

U.S. Pediatric Influenza Vaccine Utilization from 2006 to 2011

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Background:
The recommendations for pediatric influenza vaccination use in the United States have expanded significantly, adding all children 6–23 months of age in 2004, all children 24–59 months of age in 2006, and all children 5–18 years of age in 2008. Three vaccine types are available for use in eligible U.S. children: preservative-containing inactivated influenza vaccine, preservative-free inactivated influenza vaccine, and the nasal spray live attenuated influenza vaccine. Limited data are available regarding influenza vaccine utilization by U.S. pediatric providers.
Objectives: To describe U.S. pediatric influenza vaccination during the previous 5 influenza seasons

Methods
Electronic healthcare reimbursement claims data representing more than 60% of all medical claims from the U.S. outpatient setting were analyzed. Data were available regarding the date of service, provider type (pediatricians, family physicians, and other), age of the vaccine recipient, and the type of vaccine used. Weekly counts of influenza vaccinations given to children 6 months through 18 years of age between August 1 and March 31 for the 2006–2007 through 2010–2011 seasons were projected to yield national estimates for all children with private insurance. Vaccinations provided through the federal Vaccines for Children program and those provided in other settings where no insurance claim was generated, such as schools, were not captured.

Results
From 2006–2007 through 2010–2011, total seasonal vaccinations increased 78%. Among children 6–23 months of age, use of preservative-free injectable vaccine increased each year, from 38% in 2006–2007 to 64% of 2010–2011 vaccinations. Among children 2–18 years of age, use of the intranasal vaccine increased each year, from 8% in 2006–2007 to 37% of 2010–2011 vaccinations. Among children 6–23 months of age, use of preservative-free injectable vaccine increased from 39% to 65% of pediatrician vaccinations and from 28% to 43% of family practitioner vaccinations. For children 2–18 years of age, use of intranasal vaccine increased from 9% to 41% of pediatrician vaccinations and 3% to 11% of family practitioner vaccinations.

Conclusions
Consistent with national recommendations, U.S. pediatric influenza vaccination has increased significantly in recent years. From 2006–2007 to 2010–2011, use of preservative-free TIV and LAIV increased significantly in children 6–23 months and 2–18 years of age, respectively, with the highest use of each among pediatricians.

Sponsored by MedImmune, LLC.

SCIENCE POLICY INTERFACE

SCIENCE POLICY INTERFACE

I103P – SPI

"Hardly a house without ill people" the spanish flu in Styria, Austria

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Introduction

The Spanish flu of 1918/19 was the most devastating influenza pandemic in human history, which caused about 50 million deaths.
For the Austrian historiography the research of the Spanish Flu is an empty sheet of paper. The silent and lonely dying because of the „ordinary" flu could easily be overseen in the 20th century, which is full of human tragedy.

The author Thomas Hörzer tried to fill this gap with his diploma thesis at the end of his academic studies of history at the University of Graz, Austria. The data presented in that paper are, to our knowledge, the first scientifically investigated data for Austria.
Mortality data in three communities - Geistthal, Gratkorn (rural areas) and Graz (the capital city) - in Styria, a county in the south of Austria, were analysed.

The dimension of the pandemic in Styria

The number of deaths in Graz because of flu exploded in the 4th quartile of 1918 from 89 (in the 4th quartile 1917) to 1.020. The number of stillbirth doubled in the same time period. The mortality rate was 7.2.

In the small communities Geistthal and Gratkorn the „flu-autumn" reached its maximum in November 1918, which means a time shifting of about one month later than in the city of Graz. There is a clear trend observable that the farer a place from the main roads had been located, the later appeared the flu-maximum.

The influenza mortality rate in Gratkorn was 8.2, in Geistthal 27.4. On the average (without 1918) about 30 people per year died in Geistthal and about 70 in Gratkorn. During the pandemic mortality increased in Gratkorn about 69% and in Geistthal about 86%. In Geistthal, more people died because of influenza than soldiers in the first world war.

However, the estimated number of unreported cases is most probably much higher.

We do not have an explantion for the higher mortality rates in rural areas now, but this will be an issue of further investigations.

Reactions of the public authorities

The reactions were cautious and late, the dimensions of the disease were minimized, actually a kind of disinterest was present.
The Austrian government was not able to combat the flu in a coordinated action and the crisis management was done by subordinated authorities. Therefore several actions were done at different time points.

Examples for recommendations were the closing of schools, cinemas and theatres or the isolation of ill people, if „*in a place an increased occurrence of influenza cases is observed*". But there was no precise description of what „an increased occurrence" meant, therefore the range of discretion for the authorities was wide.

Disinterest and influenza - an Austrian phenomenon also in the 21st century

Ignorance and disinterest regarding prevention and control of influenza are still dominating in Austria. This fact is also reflected by the low vaccination rate of about 10%, which is among the lowest in the world. Although Austria has very good recommendations (e.g. vaccination for everybody over 50 years or for all children), the implementation is absolutely lousy.

Much work has to be done to increase awareness among the population but also among the health care workers and the medical system.

SCIENCE POLICY INTERFACE

I104P - SPI

Vaccination against 2009 influenza A (H1N1) among pregnant women in the Netherlands

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Introduction

At the end of 2009, the Dutch government advised pregnant women (second or third trimester) to get vaccinated against 2009 influenza A (H1N1). For future decisions on vaccination during pregnancy it is important to get insight into the participation of pregnant women and the reasons why they get vaccinated or not.

Material & methods

In April 2010, the RIVM invited 14,529 pregnant women by letter to participate in an internet survey on vaccination against 2009 influenza A (H1N1) and vaccination during pregnancy in general; 3,067 (21.1%) women participated. Differences in background characteristics between unvaccinated and vaccinated women were tested by using Pearson's χ^2 or Fisher's exact test. To determine which of the survey statements on (vaccination against) 2009 influenza A (H1N1) had the greatest impact on vaccination status, a standard prediction analysis was carried out with the randomForest software.

Results

Of the total group of respondents 58.1% received two vaccinations against 2009 influenza A (H1N1); 5.2% received only one vaccination. Note that women with higher educational level were overrepresented (60%). Vaccinated women responded more positively to statements regarding protection of the vaccine, less negatively to statements regarding harmfulness of the vaccine, and expected a higher risk of (harmful effects of) 2009 influenza than unvaccinated women. Furthermore, vaccinated women agreed more on statements that the advice of the government and health care professionals played an important role in their decision. The prediction analysis predicted a women's vaccination status about 91.3% of the time with a sensitivity of 92.6% and a specificity of 89.1% and showed that the following three statements had the greatest predictive value for the 2009 influenza A (H1N1) vaccination status:

1. By getting vaccinated I expected to protect my child after birth as well
2. The advice of the government played an important role in my decision
3. I was afraid that the vaccine would be harmful for my unborn child

Conclusions

A considerable part of pregnant women in the Netherlands was vaccinated against 2009 influenza A (H1N1). The protection of their child (after birth), the advice of the government and the possible harmful effects of the vaccine for their unborn child had the greatest predictive value for their vaccination status. This information should be used in case any future vaccination will be introduced during pregnancy.

SCIENCE POLICY INTERFACE

SCIENCE POLICY INTERFACE

I105P - SPI

Influenza vaccine uptake among health care workers in general hospitals: a cross-sectional management study from the netherlands

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Aims

To quantify the association between beliefs of administrators of general hospitals (GH) in the Netherlands and influenza vaccine uptake among health care workers (HCW).

Methods

All Dutch GH were approached to participate in this management questionnaire study by the end of 2010. The following organisational items were assessed: beliefs about the vaccine's effectiveness, whether the hospital had a written policy on influenza vaccination and how the hospital informed their staff about influenza vaccination. The questionnaire also included items on mandatory vaccination and costs of the vaccination campaign. The primary outcome was the overall influenza vaccination rate of HCW in the hospitals.

Results

In all, 79 of 81 GH were eligible for participation in the questionnaire study. Of these, 42 questionnaires were returned (response rate 53.2%). The average influenza vaccination rate among all HCW was 17.7% (95%CI = 14.63 – 20.75, range from 0.5% to 45.4%). GH in which the administrators agreed with organisational statements (see table 1) did not reach substantial higher vaccination rates among their HCW than those GH that disagreed. Analysis of the influenza campaign costs showed a significant, though not substantial, higher vaccination rate above the cut-off of €1250 (24.0% versus 15.0%).

Discussion/conclusion

Our results support findings from behavioural studies that failed to show large effects of voluntary vaccination programs in health care.

Table 1: Effect of predictors on mean influenza vaccination rate of Health Care Workers in General Hospitals (n=42)

Predictor	HCW vaccination rate n (%)		Mean difference
	Yes	No	(95% CI)
HCW are personally informed about influenza vaccination	24 (18.9)	14 (15.6)	3.36 (-2.97 – 9.70)
Agreement with the effect of vaccination on mortality of patients	27 (19.0)	6 (16.7)	2.24 (-6.50 – 10.98)
Agreement of management with the statement that they are responsible for offering the vaccine to HCW	32 (18.8)	3 (10.0)	8.78 (-2.75 – 20.32)
Believing that an intervention program to stimulate vaccination has a positive effect on vaccination rate	18 (16.5)	8 (17.3)	-0.85 (-8.15 – 6.46)
Hospitals willing to implement an intervention program	20 (17.4)	5 (12.7)	4.70 (-2.66 – 12.06)
Hospitals willing to implement mandatory vaccination	3 (18.0)	30 (17.5)	0.51 (-11.49 – 12.51)
Believing that mandatory vaccination will reduce costs	11 (16.7)	13 (15.6)	1.08 (-5.23 – 7.38)
Believing that the vaccine against influenza is effective	27 (18.7)	5 (14.2)	4.48 (-5.20 – 14.16)

SCIENCE POLICY INTERFACE

SCIENCE POLICY INTERFACE

I106P - SPI

The impact of official recommendations, reimbursement policies, web-scale communications and national development status on influenza vaccine provision

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Introduction

Although the World Health Organization (WHO) and 40% of countries around the world recommend seasonal influenza vaccination for those at risk, no systematic worldwide data are available to assess the level of vaccine provision and impact of local immunization policies. To address this, the International Federation of Pharmaceutical Manufacturers and Associations Influenza Vaccine Supply task force (IFPMA IVS) collected national supply data from its member companies, which distribute the majority of the world's influenza vaccines. In addition, information was collected from a sub-group of countries on local immunization recommendations, reimbursement policies, communications and national development status, to analyse the influence they may exert on vaccination levels.

Methods

The study collected supply data from 157 WHO member states from 2004-2009, and immunization policy information from a sub-group of 26 countries taken from each region of the world. Population, gross national income and national development status were taken from the United Nations Statistics and Population Divisions. The study introduced the concept of a hurdle rate to assess the level of vaccine provision (set at 15.9% of the population, based on WHO recommendations for immunization of the elderly). This was correlated to local vaccination policies and national development status across the 26 sub-group countries. The correlations were based on the anticipated effect of these different factors, providing an impact factor for each.

Results

The results show that global vaccine supply increased by 72% to 449 million doses during the six year study period. Despite this, no country provided sufficient vaccine for half of its inhabitants, and only 20% of the 157 countries met the study's modest hurdle rate. On a global scale, vaccine provision did not correspond to national wealth, and nearly 30% of the countries that achieved the study hurdle rate were classed as 'less developed' by the UN, while 20% that supplied below the hurdle were classed as 'more developed'.

In the 26 country sub-group, half supplied vaccines above the hurdle rate, and approximately half (54%) were classified as 'more developed'. Almost every country (92%) recommended immunization for the elderly and those with chronic illnesses. In 65% of countries, reimbursement was available for vaccination of these two groups, and 74% undertook wide-scale communication activities. When compared across all 26 countries, the level of vaccine provision did not correlate well with official immunization recommendations (positive:negative correlation = 1.3:1) or national development status (2.7:1). Reimbursement policies (4.5:1) and communication initiatives (5.3:1) correlated more strongly. Similar analyses were applied to healthcare worker (HCW) vaccination policies in the sub-group countries. Of these, 88% recommended HCW immunization and 61% provided reimbursement. Neither policy correlated with overall vaccine provision (1.1:1 and 1.3:1 respectively).

Conclusions

The study provides a unique insight into global seasonal influenza vaccine supply, and the influence local policies may have on usage. The results show that vaccine provision remains below recommended levels, and simply recommending immunization is insufficient to drive high levels of coverage. For instance, although US vaccination recommendations covered 85% of the population in 2009, only 36% of the country's inhabitants were actually vaccinated.

Similarly, national development status did not correlate closely to coverage, and recommending vaccination for HCWs and supporting this financially did not appear to encourage vaccine uptake in the broader population. In contrast, reimbursement and communication policies that have a direct impact on patients appeared to have by far the strongest influence on local vaccine provision.

The current post-pandemic period provides health authorities with an opportunity to reprioritize seasonal influenza vaccination. These results offer important policy insights that can be used to inform local immunization plans, and thereby help protect populations against the threat of influenza.

SCIENCE POLICY INTERFACE

SCIENCE POLICY INTERFACE

I108P - SPI

How can we manage decision making in the de-escalation or escalation of response during pandemics of influenza? the pandemic influenza severity matrix as a management tool

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Introduction

A criticism of the 2009 Influenza A (H1N1) pandemic was that most pandemic plans locked countries into a predetermined management, predicated on the expectation of a severe outcome akin to that expected should Influenza A (H5N1) have developed a pandemic potential. Once embarked on this pandemic response, many countries then had difficulty "reigning-in" their response once it was recognised that in the main the features of the pandemic virus were mainly mild with only relatively small numbers of cases requiring hospitalisation or resulting in death. In response to this finding, ECDC have proposed that one possible way of dealing with pandemics in the future would be to develop a risk matrix that could inform a more flexible response.

Method:

The authors propose a pandemic severity risk matrix that may allow policy makers flexibility in escalating or de-escalating their response predicated on contrasting the observed severity with that witnessed across multiple prior influenza seasons. The proposed risk matrix considers three groups of criteria - indices – under the banner of illness severity, effectiveness of response measures and the impact on health and society. Each of the indices are related to each other but score independently. The results are presented diagrammatically using a cube as a mechanism to present summary information on the risk assessment. The overall risk assessment and its three component indices are illustrated using the following traffic light system. Each country examines their own data and colour codes the indices in accordance with their knowledge base - available colours = Red, Amber, Green, or Grey. The colour coding is then predicated on how the new pandemic infection compares in its risk assessment with that of prior experience of seasonal influenza. Red = exceptionally high value beyond that seen in a normal influenza season; Amber = up to a level of activity seen in a worse than normal flu season; Green = up to the normal level seen in previous flu seasons; Grey = no data available. In the absence of data for the new influenza under consideration, the international view may be a consensus statement on the basis of expert opinion until an evidence base is established. Precautionary principle may be applied in the absence of other data to give overall view. A number of illustrative scenarios have been developed demonstrating which sets of indices might generate a Red, Amber or Green pandemic severity risk assessment. Regular re-examination of parameters within the three indices, perhaps on a weekly basis, allows the pandemic severity matrix to be revisited allowing scope for escalation and de-escalation of the threat assessment as new information emerges.

Next steps:

Airing this concept in an international academic forum allows intellectual discussion on the merits and de-merits of the concept shaping the final shape of the tool to be designed. In the interim a number of EU partners are being recruited as volunteers to examine the practicality of this model by considering its utility grounded against their prior seasonal influenza data. The first task of this group will be the generation of the standard protocol that each country should use in interpreting their data. Preliminary performance of the pandemic severity matrix will then be assessed before decision is made on its likely utility or further testing undertaken.

SCIENCE POLICY INTERFACE

SCIENCE POLICY INTERFACE

I109P - SPI

The SneezeSafe Programme: A Novel Education Approach for the Community Mitigation of Influenza and other Respiratory Virus Infections

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Introduction

Non-pharmaceutical interventions are crucial for the control or mitigation of influenza and may be the only interventions available to a large proportion of the world's population in future pandemics. Infection control measures exist for healthcare workers which can reduce their exposure to respiratory infections. These include the use of masks and respirators; however their usefulness as a community intervention remains controversial. Indeed community studies to define their role will be difficult because of the differing modes of transmission of respiratory viruses. Further, the relative contribution of the different modes is likely to vary in different settings.

Methods

A novel strategy developed in New Zealand (NZ) to limit the spread of respiratory disease in the community is the Kleenex® SneezeSafe programme. Developed and funded by Kimberly-Clark NZ, it has been designed to help schools educate children about the importance of covering coughs and sneezes, in addition to hand-washing. It was first offered to pre-schools in 2005 with a view to good respiratory hygiene practices in young children being taken back into homes and communities. In 2011 the SneezeSafe programme is available to all teachers in primary and intermediate schools and public health nurses throughout New Zealand. The SneezeSafe lesson has been designed to engage children in fun activities which help them learn good respiratory hygiene practices. The lesson's content responds to the New Zealand health curriculum and its messaging has the support of the Ministry of Health.The principles are simple and teach a child to:

1. TRAP a cough or sneeze with a tissue. If caught short without a tissue, trap with the inside of the elbow or with cupped hands covering the nose and mouth;
2. BIN the tissue after use;
3. WASH hands after sneezing into them.

SneezeSafe teaching resources include a 30-minute lesson plan, classroom posters, fun science facts and stickers. Practical teaching aids include a spray bottle to allow the simulation of a sneeze with water and the distribution of small and large droplets across the room, and glitter to demonstrate the transfer of pathogens during hand-to-hand contact and the need for hand washing. The programme's teaching resources are made available on an interactive website www.sneezesafe.co.nz. The on-line 'virtual sneeze' and fun character ACHOO the Flu Bug have added to the interactive aspect of this programme in classrooms.

Outcomes:

Surveys have been undertaken to identify the respiratory hygiene habits of children, their parents and others in the community to help shape the programme's key educational messages. A 2011 study is planned to gather teachers' views and assess the programme's effectiveness. The SneezeSafe programme is an exciting health education initiative which has succeeded through an informal 'public private partnership', linking private business with the education and health sectors. In 2011, a partnership with the Counties Manukau District Health Board has seen the SneezeSafe programme become a component of their new Infection Control kits. Another measure of the success of the SneezeSafe programme has been its international adoption, with similar programmes now established in Australia, the United Kingdom and Poland.

Conclusion

The SneezeSafe programme has been designed to be fun for children and an innovative approach to respiratory hygiene education for teachers and nurses. Through effective public relations the programme has taken positive messaging about the importance of good respiratory hygiene into homes and communities and brought the topic of respiratory hygiene to the attention of health, education and mainstream media nationally.

SCIENCE POLICY INTERFACE

SCIENCE POLICY INTERFACE

I110P - SPI

FLURESP : a new European Commission project assessing cost-effectiveness of European influenza human pandemic response strategies

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Background :

The constant threat of emerging subtipes influenza viruses with pandemic potential imposes to European countries to prepare efficient responses adapted to pandemic planning. Most of the European countries had pandemic preparedness plans in place when the Pandemic H1N1 (2009) strain emerged in april 2009. These plans need to be revised to take into account the lessons learned form the 2009 pandemic. The objective of the FLURESP project is to assess performance and socio-economical impact of response strategies in order to improve European public authorities ability to better respond to various categories of threats thru better preparedness plannings.

Method :

The FLURESP consortium is a multidisciplinary team composed by European experts in influenza and public health response to influenza outbreaks, Health Economics, Public Health, Computarized Sciences, Legal, Epidemiology and Statistics. The objective of the FLURESP project is support decision making process regarding the revision of national pandemic preparedness plans. The activities carried out by FLURESP will help to define main pandemic scenarios at the European level, describe and cluster response strategies and assess these response strategies in the frame of multi-criteria and cost-effectiveness analyses, taking into account lessons from the 2009 pandemic situation in Europe.

Expected Results :

The first phase will be dedicated to the description of possible pandemic scenarios by criteria, such as severity or spread types. The second phase will focus on listing potential response strategies per flu pandemic scenario. During the third phase, Multi-criteria analyses will be carried out on each response strategy in order to assess their performance and efficiency. The fourth phase will perform cost-effectiveness assessments of the response strategies, which will be ranked by level of cost-effectiveness. The fifth phase will propose guidelines and recommendations for policy decision makers.

Conclusion :

If flu pandemic scenarios and main related responses are well documented and investigated, they have never been assessed and ranked using both multi-criteria and cost-effectiveness approaches. The integrated approach of Decision Making proposed by the FLURESP consortium would constitute a premiere at the European and global level, which would support European member states to select the most appropriate and efficient public response to various scenarios of human pandemic. The outcome of the FLURESP project will provide an extensive assessment of Influenza pandemic response strategies at European level, based on cost-effective analysis of the possible response strategies for each pandemic scenario.

SCIENCE POLICY INTERFACE

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I111P - SPI

Healthcare resource use in the elderly due to seasonal influenza, a UK study

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Objective:

Seasonal influenza incurs a substantial clinical and financial burden. Meanwhile, the specifics of the burden of influenza in the elderly are lacking. A study was conducted to evaluate the extent of healthcare resource utilisation and direct healthcare costs in the elderly in the UK.

Methods

A retrospective database analyses was done focusing on 65+ years who had been diagnosed with either confirmed influenza or who presented with influenza-like-illness (ILI) during a influenza season between October 2008 and March 2009. Subjects of the general practice (GP) database were selected using the defined clinical terms (READ codes). Subjects admitted to hospital were selected with a primary or secondary diagnosis of pneumonia and/or influenza. Unit costs were obtained from National Schedule of Reference Cost, the Doctors Laboratory, and British National Formulary. Analyses were carried out using SAS statistical software and multivariate regression models were used to investigate the gender and age relationship. Number of GP visits and average cost per GP visit by-age group, and gender were measured. The average for medications, laboratory tests, and radiology tests per visit were estimated. The average length of hospital stay (LOS), costs of inpatient stay, and of emergency room visits were estimated by-age group and gender.

Results

A total of 3,157 subjects were taken from the GP database, 56% were female. The mean age was 73.6 (min=65, max=86) years and 70% of the patients were from England, 23.4% from Scotland and the rest from Wales. All subject had one GP visit within 15 days of the initial visit. The average cost of a GP visit was £35.71(min=33.79, max=36.69) with males having a significant higher average cost than females, i.e. £38.8 and £33.28 ($p<0.0001$), respectively. The mean cost for a GP visits was similar across age groups. The majorities of resources were office visit (61.6%), representing the most expensive resource, followed by laboratory tests (36%), radiology (1.8%) and medication (0.5%). The average cost per GP visit was higher in males for laboratory assessments, and radiology tests. The hospital database included 82 patients of whom-28 (34.1%) were females. The overall average age was 72.2 (min=65, max=84) years with female slightly older than males. The average LOS per subject was 9.2 days (min=5, max=15). The LOS was similar in both genders and had a trend toward longer LOS in older subjects. Primary diagnosis of congestive heart failure had the highest length of stay of 11.8 days (min=10, max=13). The average cost of inpatient stay was £1717.7 (1614.7, 1820.6). The average total cost per hospital stay including emergency room visit was £1785.2 (1678.4, 1891.9). There is a non-significant trend for an increasing average total cost with increasing age. Eight subjects had a primary diagnosis of pneumonia and influenza with an average LOS of approximately 8 days (6.5, 10.1) and an average total cost of £1592.8 (1389.7, 1795.8). Subjects with other primary diagnoses had an average of £1806 (1690.4, 1921.5). The acute hospital database was limited by the small number of subjects assembled.

Conclusion

The clinical and financial burden of influenza appears to be substantial as revealed by the associated costs in GP visits and hospital settings. Applying the outcomes of this study to the elderly incidence data reported in Pitman et al (2007), the costs associated with elderly GP visits would be between 2 and 6 million Pounds, and for hospitalisations between 18 and 39 million Pounds. Therefore, the total seasonal influenza healthcare costs in the elderly would range from 20 to 45 million Pounds. Hospitalization cost would account for 88% of total seasonal influenza cost and represented an economic burden in health care system.

SCIENCE POLICY INTERFACE

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I113P - SPI

Bringing innovation into practice: influenza battles in Thai society

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Introduction

Thailand has a high burden of seasonal influenza disease and experienced pandemic influenza since the summer 2009. While non-pharmaceutical intervention procedures such as hand hygiene and the use of face mask could play important role in reducing the transmission of influenza in households, schools, workplaces, and other public places, pharmaceutical intervention including antiviral drug and vaccine also play significant role in preventing individuals from getting influenza. It is therefore important to better understand knowledge, attitude and practices (KAP) of hand hygiene and the use of face mask, antiviral drug and influenza vaccine among Thai people. The findings will be greatly useful for public health strategies regarding influenza in Thailand and in the global community.

Methods

This KAP study is a qualitative study that employs focus group discussion and in-depth interview as data collection techniques and Content Analysis as methodological framework for data analysis. Ten households in Bangkok, Thailand where their family members became diagnosed of influenza were selected for KAP study. The total of ten focus group discussions (one per household) and 30 in-depth interviews (three persons per one household) were conducted.

Results

In Thai society, while health promotion is a socialized view, conceptions of flu among Thai people are subject to traditional perceptions of illness. While Thai people generally have good knowledge and positive attitude about hand hygiene and the use of face mask against influenza transmission, they do not practically follow these non-pharmaceutical intervention procedures to protect themselves against influenza transmission. Instead, they describe other prevention methods localized to their everyday life, and they look forward to universal protection methods such as antiviral drug and influenza vaccine.

Conclusions

Since preferred influenza prevention methods among Thai people are localized to everyday life in Thai society, it is crucial that Thailand's national influenza vaccination program take the fundamentals of community-based programming into its design and implementation. The research findings about knowledge, attitude and practices regarding seasonal and pandemic influenza among Thai people, in particular, can be constructed into a new social innovation and stakeholders in Thailand can utilize it to inform the country's public health policy regarding influenza prevention. It is known scientifically how influenza is spread, yet a variety of environmental and socio-economic factors that encourage/prevent influenza transmission in Thailand has not yet been brought into policy discussions.

SCIENCE POLICY INTERFACE

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I114P – SPI

Evaluation of pandemic preparedness activities and the response to the pandemic (H1N1) 2009 – key lessons learned in Germany

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Introduction.

It is not possible to predict when a pandemic will occur and what course and impact a future pandemic will have. This emphasizes the importance of developing and updating national pandemic preparedness plans. Following the pandemic (H1N1) 2009, countries worldwide carried out evaluations of their pandemic preparedness activities and their response to the pandemic. The Review Committee of the World Health Organization concluded in its final report: "The world is ill-prepared to respond to a severe influenza pandemic or to any similarly global, sustained and threatening public-health emergency". In Germany, evaluations were performed by different institutions of the public health sector at the national, regional and local level. We reviewed international and national reports and articles regarding the evaluation of national pandemic preparedness activities and the response to the pandemic (H1N1) 2009 in Germany in order to identify key lessons learned.Methods. A comprehensive literature research has been performed using PubMed and Scopus. Additionally, reports of international institutions such as WHO-Euro, the European Commission and the Global Health Security Initiative and national institutions such as Health Authorities of the national, regional and local level were gathered. We reviewed all documents and grouped all topics discussed under the following thematic subheadings: vaccination, surveillance, communication, antivirals, diagnostics, infection protection measures, flexibility of public health measures, resources of the public health authorities and ethics. A matrix was created, specifying the key lessons learned and their frequency of occurrence in the evaluation reports. Results. In total, six international and ten national documents concerning the evaluation of pandemic preparedness activities and the response to the pandemic (H1N1) 2009 in Germany were reviewed. In both the international and national evaluations, vaccination, surveillance and communication were the most frequently stated areas that could be improved in Germany. In detail, key findings regarding vaccination issues were: optimize concepts for logistics, distribution and cost coverage, define a clearer role of medical and public health professionals in immunisation, aim for more flexible contracts and joint vaccine procurement. Influenza surveillance could benefit from strengthening the instruments for timely monitoring of severe disease (hospital and mortality surveillance) and of vaccination coverage. Risk and crisis communication have been identified as important areas for the planning process e.g. enhanced involvement of medical associations and the response to the pandemic e.g. improvement of communication channels to medical professionals and more transparent and clear content of communication messages. National regional and local health authorities additionally emphasized the need for sufficient resources in public health services.Conclusions. It is of utmost importance that countries thoroughly evaluate their pandemic preparedness activities on different levels and their response to the pandemic. In our study, we have identified key lessons learned that need to be taken into account in the revision process of the national pandemic preparedness plan. Our methodological approach for summarizing topics that should be more emphasized in future pandemic planning may allow comparison with other countries. On the basis of these findings, preparedness for future influenza pandemics could be improved.

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I115P - Should we prevent and treat seasonal influenza and how?

Differential use of antivirals during the pandemic (H1N1) 2009 in Germany

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Introduction.

In 2009, the pandemic (pdm) influenza A(H1N1) virus emerged, causing morbidity and mortality during the pandemic (H1N1) 2009 and the following influenza seasons. The World Health Organization recommends early treatment with antiviral drugs for patients who present with severe influenza illness or who are at increased risk of severe disease. However, little is known about the use of antivirals during the pandemic (H1N1) 2009 in Germany. We aimed to determine the proportion of notified cases with A(H1N1)pdm infection that have been treated with antiviral drugs during the pandemic (H1N1) 2009 in Germany and to identify factors that were associated with the use of antivirals. Method. We analysed cases with laboratory confirmed A(H1N1)pdm infection notified to national health authorities between week 29/2009 and 17/2010 according to a special legal ordinance in Germany. Analysed surveillance data included information on demographic characteristics, underlying medical conditions (diabetes mellitus, impairment of cardiovascular system, impairment of respiratory system, obesity, immunosuppression and other conditions), treatment with antivirals, pneumonia and death. For the purpose of analysis pneumonia and death were grouped as an indicator for severe disease. We investigated factors associated with antiviral treatment using multivariable logistic regression to estimate odds ratios (OR) and 95% confidence intervals (CI). Results. In total, 170,856 cases with laboratory confirmed A(H1N1)pdm infection were notified during the study period. The proportion of cases with antiviral treatment increased with age: 14% (903/6,701) of 1 to 4 year-old cases, 15% (2,817/18,301) of 5 to 9 year-old cases and 17% (4,603/26,684) of 10 to 14 year-old cases were treated with antivirals, whereas 25% (14,134/ 57,146) of 15 to 49 year-old cases and 28% (2,087/ 7,553) of cases over 50 years old received antivirals. Multivariable analysis including information on age group, gender, underlying medical conditions and severe disease revealed that the chance for being treated with antivirals was lower for cases 1 to 4 years old compared to cases aged 15 to 49 years (OR=0.45; 95%CI 0.42 - 0.49; p<0.001). This finding also applies to cases 5 to 9 years old (OR=0.55; 95%CI 0.52 - 0.57; p<0.001) and to cases 10 to 14 years old (OR=0.63; 95%CI 0.61 - 0.66; p<0.001). There was no significant difference for cases over 50 years of age (p=1.000).In addition, the odds of antiviral treatment was slightly lower for female patients (OR=0.90; 95%CI 0.87 - 0.93; p<0.001). The chance for being treated with antivirals was higher in patients with one or more underlying medical condition(s) than in patients without the condition(s) (OR=2.7; 95%CI 2.6 - 2.8; p<0.001). Similarly, patients with severe disease were more likely to be treated with antivirals than patients without severe disease (OR=1.6; 95%CI 1.4 - 1.9; p<0.001). Conclusion. Our analyses of national surveillance data confirm the use of antivirals in patients with underlying medical conditions or severe disease in line with international recommendations. The results show that children and female patients with A(H1N1)pdm infection were treated less often with antivirals than adults and male patients, independent of underlying medical conditions and severity of disease. The factors leading to this patient management remain to be investigated. These findings stress the need to improve prevention and treatment strategies in this age group in order to prevent morbidity and mortality.

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I116P – SPI

Preparedness and response plans of korea for next pandemic influenza

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Since we had experienced recent pandemic outbreaks of A/H1N1 in 2009, it caused more national and global concerns over the potential for a new influenza pandemic. Although it is impossible to predict when the next pandemic will occur, it is not too much for us to emphasize its importance of appropriate planning for efficient use of resources and minimizing loss of social security and function against influenza. Korea Centers for Disease Control and Prevention (KCDC) has been a national hub for infectious disease control including A/H1NI contol and A/H5N1, SARS prevention. Based on our experiences from 2009 pandemic, KCDC totally revised and upgraded our national guideline for next pandemic considering two factors; not only level of community spread of inflenaza but also its severity. Response plans for intervention are in both ways - non-pharmacological intervention (NPI) and pharmacological intervention (PI).First, Non-pharmacological intervention (NPI) - Designated hospitals including negative pressure isolation room construction - School Dismiss and social distancing plan - Risk communication plan Pharmacological intervention (PI) - Antiviral and vaccine stockpile - Antiviral distribution plan in pandemic - Vaccination plan in pandemic

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