Workshop report

Current research on respiratory viral infections: XIIth International Symposium

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Introduction

The XIIth International Symposium on Respiratory Viral Infections was convened by The Macrae Group (New York, NY, USA) in Taipei, Taiwan on 11–14 March 2010. This annual symposium provides a forum to discuss recent advances in respiratory virus research in an interdisciplinary fashion. The current report summarizes presentations during this symposium that ranged from basic virology and pathogenesis to epidemiology, vaccines, antivirals and management strategies. The 2010 symposium provided a detailed examination of lessons learned from the 2009 influenza pandemic with a common theme, emphasized by many speakers, being the importance of international collaboration and cooperation in responding to new infectious disease threats.

Pandemic 2009 H1N1

First cases of 2009 pandemic influenza: history of detection and lessons learned (Alexander Klimov, Influenza Division, US Centers for Disease Control and Prevention, Atlanta, GA, USA)

The rapid detection of the first cases of pandemic H1N1 (pH1N1) is a tribute to pandemic preparedness. Since 1997, when first cases of human infections with highly pathogenic avian influenza (HPAI) A(H5N1) viruses were documented, there was a need for sensitive, specific tests for novel influenza viruses. In September 2008 (6 months before the start of the pandemic) the US Food and Drug Administration (FDA) approved the US Centers for Disease Control and Prevention (CDC) 5 target reverse transcription (RT)-PCR assay, which is capable of detecting influenza A, influenza B, A(H1), A(H3) and A(H5, Asian lineage) viruses. However, because of sporadic zoonotic infections with other influenza viruses (for example, avian H7, and swine H3 and H1), other assays were developed. Consequently, when the first case sample (collected from a 10-year-old boy in San Diego, CA, USA) yielded an unsubtypeable influenza A, the CDC rapidly determined that it was positive for swine-H1 HA and for swine-NP. Complete genome sequencing and analysis of samples from the two first cases showed that pH1N1 was a triple reassortant influenza virus of swine origin with a unique genome composition. On 20 April 2009, the new sequences were posted on the Global
Initiative on Sharing Avian Influenza Data website and the Morbidity and Mortality Weekly Report described the first two cases.

On 25 April 2009 the first case of pH1N1 was recognized in Canada, and antigenic analysis showed that the pH1N1 viruses were antigenically distinct from seasonal viruses, the 1976 swine influenza virus, and recent triple reassortant swine viruses. Only 73% similarity was observed when the haemagglutinin (HA) amino acid composition was compared to seasonal influenza virus. On 28 April 2009 the CDC RT-PCR assay to detect the pH1N1 virus was approved by the FDA under an Emergency Use Authorization. As of February 2010, 2,479 kits had been distributed to 320 international labs in 140 countries and 156 domestic labs. On 23 May 2009 the vaccine strain, A/California/7/2009, was sent to manufacturers.

Dr Klimov stated that some valuable lessons were learned. There was unprecedented international cooperation, openness and free sharing of materials like reagents and virus isolates. However, there was some delay with the diagnosis of the first cases in Mexico, and gaps remain in human influenza surveillance in Asia, Africa and South America and in surveillance at the animal–human interface, particularly in swine. Early in the epidemic, there was a lack of standardized guidance on prioritization of testing that resulted in an overwhelming demand. Better point-of-care diagnostic tests for influenza are needed, as are development of new drugs and rapid tests for drug resistance. Comprehensive surveillance has to be continued since more virulent virus variants may appear.

Epidemiology of 2009 H1N1 infections in Mexico (Celia M Alpuche Aranda, Instituto de Diagnostico y Referencia Epidemiologicos, Delegacion Miguel Hidalgo, Mexico)

Prior to April 2009, increases in influenza-like illness (ILI) had been seen in Mexico with peaks occurring in November 2008 and February–March 2009, above levels seen in the two preceding years. In mid-March 2009, outbreaks of suspected influenza were reported in Mexico with 31 cases confirmed in Tlaxcala. By the end of that month, unconfirmed cases had been reported from La Gloria, Perote and Veracruz. On 12 April 2009, Oaxaca State reported the death of a 39-year-old diabetic woman who had experienced a rapidly progressive pneumonia. Coronavirus (CoV) was initially detected in samples from this patient by an independent diagnostic laboratory, but a government laboratory subsequently did not detect CoV. Retrospective testing of the same sample 10 days later in Canada revealed the presence of pH1N1 viral RNA. By mid-April, Veracruz notified national authorities of an outbreak of 616 suspectedILI cases with an estimated attack rate of 30%. Only 3 out of 60 samples were reported to be positive for influenza. The outbreak was reported under the International Health Regulations as probable seasonal influenza. However, unofficial reports described atypical pneumonia cases in young adults, including fatalities, and these events triggered a national public health investigation and an emergency response. Active surveillance identified cases of severe pneumonia in young adults admitted to hospitals in Mexico City, cases in which influenza A was detected but could not be subtyped. External analysis by government laboratories in the US and Canada revealed that a significant proportion of submitted samples contained the novel pH1N1 virus, with results being released to the Mexican authorities on 23 April 2009. At this point, a new declaration was issued and a national pandemic influenza response was triggered [1].

In an attempt to improve surveillance, existing algorithms were reviewed and weaknesses identified. Sentinel surveillance was changed to active surveillance across clinical units and daily reporting of deaths due to ILI/severe acute respiratory infection was introduced. Guidelines on case definitions, laboratory diagnosis, clinical management and public health aspects were updated. The CDC RT-PCR for influenza was introduced along with rapid funding from the federal government for the purchase of new instruments, and a new, internet-based national informatics system was introduced. Logistical and biosafety considerations meant that high-throughput processing of initial samples was challenging. Following improvements in epidemiological surveillance and laboratory diagnostics, 40 centres had the capability to run a total of 5,000 samples per day. Retrospective analysis of stored samples revealed the presence of pH1N1 in Mexico as early as February 2009. By 10 March 2010, 71,393 cases of confirmed pH1N1 had been reported, including 1,108 deaths. Similar to other parts of the world, young adults were being disproportionately affected and pH1N1 became the predominant circulating strain during 2009.

Dr Aranda stated that various lessons have been learnt in Mexico following the emergence of the 2009 pandemic. Although no plan is perfect, preparedness is essential for an optimal response. Surveillance requires epidemiological intelligence, and early warning systems are essential for known and unknown pathogens. Protocols for managing unexpected events need to be flexible and adaptable. Honest and transparent communication is essential, and international collaboration is a key component of an effective response campaign. Although <15% of Mexican cases required hospitalization, significant demands were placed upon critical care facilities and increases in overall ILI during the pandemic meant that primary care was also placed under great pressure. Communicating risk is
complicated and influenced by multiple factors, but messages from opinion leaders can have great impact during a pandemic. Efforts to mitigate severe cases and death will need to be ongoing as long as the pH1N1 continues to circulate in its current form.

China's perspective on planning for and responding to the H1N1 pandemic (Yu Wang, Chinese Center for Disease Control and Prevention, Beijing, People's Republic of China)

As of 21 February 2010, there were 127,167 confirmed cases, 7,485 severe cases and 789 reported deaths due to pH1N1 in mainland China. In October–November 2009, pH1N1 virus accounted for approximately 90% of all ILI that were influenza-positive in a network of sentinel hospitals. A cross-sectional study initiated in January 2010 in 11 provinces found that the overall seroprevalence for pH1N1 averaged 29.5% in unvaccinated people, compared to a rate in self-reported immunized people of 69.9%, suggesting that approximately 80% of the vaccinated population was protected. After the pandemic started, adjustments kept being made to coping strategies according to the epidemic situation. When it first became clear that the virus was spreading quickly, the government responded immediately to form a multi-sector Emergency Committee led by the Ministry of Health with the involvement of 38 government departments and agencies. This first phase from 25 April to 10 July 2009 focused on limiting domestic transmission caused by imported cases. This containment stage consisted of border entry screening, case isolation in designated hospitals and contact quarantine, expanding the surveillance system and intensifying laboratory diagnosis capacity. As of 23 August, 56 million travellers were screened and 17,909 febrile persons identified, of whom 757 (4.2%) had confirmed pH1N1 infection. As of 10 August, >9,900 close contacts had been quarantined, either at home or at designated hotels, among whom 531 (5.5%) had confirmed pH1N1 infection. The influenza laboratory network and the ILI sentinel hospital surveillance system was expanded from 197 hospitals (and 63 laboratories) before May 2009 to 556 hospitals (and 411 laboratories) after June 2009. After 10 July, domestic clusters of pH1N1 cases showed that domestic transmission had started. Because the containment strategy was resource-intensive and costly, the policy was changed to emphasize reducing the pandemic impact through control and management of school outbreaks, acceleration of vaccine development and mass immunization, distribution of antiviral drugs, and promotion of health education and public communication.

After receiving the WHO-recommended vaccine strains, companies were encouraged to work together to enhance limited production capacities. A large-scale, multisectoral clinical trial was coordinated by China CDC to test 10 candidate vaccines in 12,691 volunteers of four different age groups. This was the first clinical pH1N1 vaccine trial completed in the world, and the results showed that one dose of non-adjuvanted split-virion vaccine containing 7.5 µg HA was immunogenic in those aged 12 years and above, whereas children younger than 12 years might require two 7.5 µg doses [2]. In September 2009, China became the first nation to start mass vaccinating high priority groups for pH1N1. As of 6 March 2010, 101 million doses had been distributed and 81 million people were vaccinated. Priority was given to healthcare workers and other essential public service providers, people with underlying medical conditions, primary and middle school students and children 6 months to 3 years of age. Oseltamivir production was increased by two domestic manufacturers for national stockpile and deployment, particularly for treatment of severe cases and those with underlying medical conditions.

Dr Wang stated that among the multiple lessons learned, determining when and how to take action while facing the uncertainty of the epidemic was critical to minimizing both health and social impacts of the event. The Ministry of Health and China CDC reacted swiftly and communicated in a timely manner, including international sharing of data on pH1N1 virological, epidemiological and clinical features and on vaccine trials. Open and honest mass communication played an essential role in coping with the pandemic. However, challenges like timely access to vaccine and antiviral drugs in remote areas and shortcomings in surveillance and research remain.

Taipei's perspective on planning for and responding to the H1N1 pandemic (Steve Hsu-Sung Kuo, Taiwan CDC, Chinese Taipei, Taiwan)

The severe acute respiratory syndrome (SARS) outbreak in Taiwan in 2003 showed that an infectious disease outbreak can be a national security issue and that panic due to misinformation may be more dangerous than the virus itself. In response the major system changes implemented in Taipei were the formation of a centralized Central Epidemic Control Center, establishment of a national stockpile of antivirals and work on the manufacturing of influenza vaccines. A nationwide in-school influenza vaccination (NISIV), an epidemic intelligence centre (EIC) and a national influenza centre expanded the public communication capacities.

In response to pH1N1 pandemic, Taiwan activated its Central Epidemic Control Center supported by the National Security Council and the 25 local epidemic command centres, and started border controls on 29 April 2009. The Real-time Outbreak and Disease Surveillance system covers approximately 99% of the population. The first domestic case with unknown
source occurred on 2 July and on 20 October 2009 the first case of oseltamivir-resistant pH1N1 was identified. The virological surveillance was done by a network consisting of the national influenza centre and 10 laboratories in all major medical centres around the island. School children were seen as both a risk group as well as ‘superspreaders’, and sick children were asked to stay home unless they needed to seek medical care; classes were suspended on the basis of a 3-2-5 protocol: within 3 days, if 2 or more cases, then 5 days of class suspension. The national antiviral stockpile was increased from 10% to 25% coverage of the population, 2,920 local clinics were established for distribution. The vaccination programme was launched on 1 November 2009 and Taiwan started the NISIV, which ultimately immunized 75% of all middle school students. The overall vaccination coverage rate was approximately 25% of the population, with the highest percentage (83%) in the healthcare workers. A national immunization day was held on 12 December 2009, when 2.4% of the entire population received the vaccine. Unfortunately, 10 days later the son of a doctor died 1 month after vaccination with the locally made vaccine, and the father suspected a causal link. This increased the number of reported adverse events after vaccination and triggered a wave of concern about vaccine safety that adversely affected government credibility and brought the vaccine programme to a halt. Subsequently, conclusive evidence showed that the death was not related to the vaccine.

Based on reviewing and modelling the data, Taiwan had approximately 41 deaths, 269 intensive care unit hospitalizations and 1.2 million people infected. The case fatality rate was estimated at 5 in 100,000. During the peak phase of the pandemic, 60% of ILI-cases were positive for pH1N1, >70 patients were admitted due to pH1N1 per week and 20% of patients visited emergency rooms. However, the normal winter peak of pneumonia- and influenza-related deaths were not observed. In summary, Dr Kuo stated that the lessons learned from the SARS outbreak contributed significantly to Taiwan’s handling of the 2009 pandemic. In particular, further evaluation of vaccine effectiveness (VE) and safety are needed, as rebuilding public trust with a better communication strategy is an important task.

Perspective on planning for and responding to the H1N1 pandemic (Hitoshi Oshitani, Tohoku University Graduate School of Medicine, Sendai, Japan)

Japan revised its pandemic action plan and guidelines in February 2009, just before the pandemic started. The objectives of the pandemic response in Japan were to mitigate social and medical impact by reducing transmission. Because the original plan was based on a severe pandemic scenario caused by HPAI H5N1 virus, very stringent measures were implemented in May–June 2009. The very first indigenous cases were identified on 16 May and subsequent investigations found >370 cases, mainly in high school students. Extensive contact tracing failed to identify the index case, and adult cases without any epidemiological link to a school or outbreak at a school were identified. The government decided to close all schools in affected areas for 1 week, as well as isolate and treat confirmed cases and quarantine possible contacts. These measures appeared to contain the outbreaks. By mid-June 2009 clusters were identified in other places, most of which appeared to be contained after similar measures, but in September 2009, after schools reopened following the summer holidays, the number of cases increased, particularly after October 2009 and peaked in November. During the winter holidays a decrease in cases was observed, followed by a small increase again after the schools reopened after the New Year holidays, observations suggesting a potentially important role for school closures on transmission.

Japan noted some unique epidemiological characteristics of pH1N1 influenza, particularly a very low case fatality rate nationally (196 confirmed deaths with an estimated number of 20.6 million cases, or about 1 in 100,000). High proportions of cases and hospitalizations were found in the group of children aged between 5 and 14 years; 79% of hospitalized cases were under the age of 15 years. The case fatality rates were very low in children, and only 0.4% were pregnant women who were hospitalized, with no severe cases. In contrast with other countries, very few adult cases were hospitalized, although the number of deaths and mortality per age group increased for those aged over 40 years. During seasonal influenza in Japan the ILI peak is usually around January or February, and all age groups show the peak at the same time. When looking at pH1N1, a sudden increase in the number of cases in school-aged children 5–9 and 10–14 years old occurred in October–November 2009, but the number of cases in children <5 years old was much lower and the increase was slower, compared with seasonal influenza. In adults, no clear, sharp peak was observed.

The cause of this different epidemiologic pattern is unclear, but the control measures implemented in Japan were different from previous seasons. For example, in Japan approximately 10,000 classes were suspended for seasonal influenza, but >160,000 schools implemented school closures for pH1N1. Recommendations on hand washing, coughing etiquette and self-isolation of sick individuals at home were emphasized. Vaccines were only available at the end of October 2009, and were initially targeted to the healthcare workers and people with risk factors. By the end of 2009 approximately 17.3 million doses of vaccines have been distributed.
Much like in seasonal influenza in Japan, most pH1N1 patients received antiviral treatment. The clinicians in Japan believe that the low case fatality rate was due to the extensive treatment with antivirals, although many fatal cases also received antiviral treatment, sometimes on the day of onset or 1 day afterwards, indicating that antiviral treatment did not prevent death in some cases. Dr Oshitani stated that transmission in households and the community may have been reduced by non-pharmaceutical measures, post-exposure prophylaxis with antivirals and perhaps behavioural changes.

Pandemic planning: lessons learned from the H1N1 pandemic: viruses, vaccines and vision for the future (Nancy Cox, Influenza Division, National Center for Immunization and Respiratory Diseases, US Centers for Disease Control and Prevention, Atlanta, GA, USA) The global community was better prepared for the 2009 pandemic than for the spread of avian H5N1 in 2003–2005, but various challenges remain when it comes to global preparedness for epidemic/pandemic influenza. The idea that the next pandemic was going to be severe shaped expectations and plans. The emergence was expected in Asia and not North America, with the origin from avian species and not swine. There are many challenges in risk assessment of influenza viruses with regard to their pandemic potential when they are detected in animals or humans. Consequently, public education that a pandemic does not always mean a severe event (or follow a specific pattern), and flexibility in estimating severity early on, are key. Many aspects of the pH1N1 response went well, as there was excellent sharing of viruses and information in turn accelerated vaccine and diagnostics development. In the US, funding was available for stockpiling antivirals, and some investments were made in vaccine production in developing countries. Diagnostic preparedness due to the swine influenza infections in humans allowed rapid production of the CDC RT-PCR test that recognized the various types of influenza, and the number of public health laboratories with reagents, equipment and trained staff was increased. Enhanced syndromic outpatient and hospital-based surveillance helped to close the gaps in surveillance nationally and globally. The US CDC developed an Influenza Reagent Resource, which is a contract for the rapid manufacture and distribution of influenza diagnostic reagents.

While the US CDC shipped seed viruses to vaccine manufacturers at the end of May 2009, poor seed virus growth and the lack of initially required biosafety level 3 containment levels facilities delayed production. The first vaccine lots were shipped in early October, which was already during the second wave of the epidemic in the US. Release 26 weeks earlier would have been needed to distribute vaccine in a timely fashion and would have most likely made large differences in both disease reduction and in vaccine acceptance in the US. Therefore, faster influenza vaccine development and availability are needed, including better growing vaccine seed strains and faster methods for producing potency reagents. Of note, early in the pandemic, the world was reliant on sheep from one particular regulator in the UK which was able to induce high enough titres. The whole paradigm for potency testing of influenza vaccines may need to change.

To further reduce delays, improved vaccine production through development of better growing seed viruses, identification of molecular determinants for high virus yield in cells, quantification of antigen using new platforms and streamlining production of potency testing reagents are needed. Vaccine manufacturers could incorporate HPLC into standard practice for vaccines, as the Chinese saved considerable time using this technique to quantify the vaccine antigen for use in clinical trials. The US CDC continues to work on mass spectrometry isotope dilution methods to quantify the HA and neuraminidase content in vaccines. In conclusion, Dr Cox emphasized that the rapid detection of new influenza viruses should be linked to sustainable multi-use respiratory disease surveillance platforms for timely identification of novel virus emergence globally. Also the availability of vaccines could be improved by streamlining the methods for measuring vaccination antigen content and improvements in vaccine capacity domestically and globally. The ultimate goal should be the availability of vaccines prior to major outbreaks.

Influenza virus contamination of common household surfaces during the 2009 influenza A(H1N1) pandemic in Bangkok, Thailand – implications for contact transmission (James Mark Simmerman, Influenza Division, International Emerging Infections Program, Thailand MOPH-US Centers for Disease Control and Prevention Collaboration, Nonthaburi, Thailand) Influenza is thought to be spread by contact, droplet and airborne transmission, but the relative importance of these routes is uncertain and likely varies depending on the setting and possibly the virus strain. Viruses can be cultured from experimentally infected surfaces for hours, while immediate reductions of influenza virus have been observed on experimentally inoculated hands. Dr Simmerman and colleagues investigated the importance in transmission of indirect contact via contaminated hands and surfaces. A total of 90 Bangkok households with an influenza-positive child were included during the July–August 2009 time period. The intervention arm included hand washing education and supplies, while the control arm received advice on nutrition, exercise and smoking cessation. At the day-3 visit, six common household surfaces were swabbed:
the bathroom door knob, refrigerator door handle, television remote control, light switch in main room, a frequently used children’s toy and a mobile phone, as well as fingertips of the dominant hand of the pediatric index case and any other household member reporting influenza symptoms. In 16 of the 90 households, ≥1 surface (most frequently the TV remote) was positive for viral RNA, more often in households with index cases <8 years of age. Overall, 15 of 90 index cases had positive fingertips. No viruses were cultured from surface swabs, but one virus was cultured from a positive finger swab. While participants in the intervention arm washed their hands more often than the control arm, no differences were observed in secondary influenza infections.

Young Investigator Award: household transmission of pandemic and seasonal influenza A in 2009 (Benjamin J Cowling, School of Public Health, The University of Hong Kong, Pokfulam, Hong Kong SAR, People’s Republic of China)

Few data exist concerning the transmissibility of pH1N1, compared with seasonal influenza. To address this, the investigators assessed the patterns in viral shedding, course of illness, antibody responses and transmissibility associated with seasonal and pH1N1 influenza infections. ‘Index case’ outpatients were recruited when they met the following criteria: acute influenza infections. ‘Index case’ outpatients were recruited when they met the following criteria: acute respiratory infection (ARI) within 48 h of onset and living with ≥2 other people, none of whom have had ARI the preceding 2 weeks. For those with a positive QuickVue rapid antigen test (Quidel Corporation, San Diego, CA, USA) for influenza A or B, home follow-up visits were conducted by trained nurses to collect nose and throat swabs from all household members at initial visit and after 3 and 6 days, regardless of the presence of illness. Patients were asked to keep symptom diaries and record body temperature, and blood for serology/immunology was drawn on days 0, 6 and 21–30.

Of 348 index cases, 148 had influenza confirmed by QuickVue; three cases had influenza B but the remainder had influenza A. RT-PCR-confirmed secondary attack rates were similar among household contacts of index cases with pH1N1 (8% [95% CI 3, 15]) and seasonal influenza (9% [95% CI 5, 14]). Patterns in viral shedding and course of illness were also reported to be similar. Index cases infected with seasonal H1N1 showed cross-reactive antibody responses to pH1N1 and vice-versa. Convalescent antibody titres by viral neutralization tended to be lower for index cases with pH1N1 who received oseltamivir treatment compared with those who did not. Dr Cowling concluded that pH1N1 was similar to seasonal influenza A infections in outpatients in terms of viral shedding, clinical illness and transmissibility in the household setting [3].

Clinical aspects of pandemic H1N1 and human infection with highly pathogenic H5N1 (Tim Uyeki, US Centers for Disease Control and Prevention, Atlanta, GA, USA)

Dr Uyeki compared and contrasted severe cases of pH1N1 and highly pathogenic H5N1 virus infections. The pH1N1 pandemic has been considered mild overall, while in H5N1 virus infection, the clinical spectrum has been characterized by cases with severe disease and high mortality. Severe disease in pH1N1 has predominantly been reported in persons with underlying high-risk conditions, while H5N1 disease has occurred in previously healthy children and young adults. The true case fatality ratio for H5N1 virus infection is not clear, but is likely to be quite high. As of March 2010, 59% of 488 confirmed cases reported to WHO had fatal outcomes. Risk factors for H5N1 remain direct or close contact with sick or dead poultry, and visiting live poultry markets. The risk factors for severe pH1N1 are very similar to risk factors for seasonal influenza and include underlying chronic conditions, but also pregnancy and obesity. However, approximately 25–30% of pH1N1 patients with severe disease have no known risk factors.

Severe pH1N1 disease is similar clinically to H5N1 illness. In the early stages, ILI symptoms are typically present, but as disease progresses, increasing productive cough, dyspnoea and shortness of breath develop. Progressive viral pneumonia leading to acute respiratory distress syndrome is the major cause of death in both. Sepsis syndrome, renal insufficiency and multi-organ failure appear to occur more often in H5N1 than pH1N1 disease. In 26–41% of fatal cases of pH1N1, bacterial coinfections are found, while in H5N1 infections, bacterial coinfection is rarely reported at time of hospitalization [4,5]. Although viral RNA has been reported in blood in some severe cases, extra-pulmonary pH1N1 virus replication has not been conclusively demonstrated, whereas H5N1 virus has been isolated from cerebrospinal fluid, serum, throat and rectal swab samples in some patients.

Concerning antiviral treatment, early oseltamivir administration appears to be associated with survival in both severe pH1N1 and H5N1 patients, although some patients have progressed to fatal outcomes despite treatment within 48 h. Oseltamivir resistance has been documented during treatment of both pH1N1 and H5N1 illness, and H5N1 virus strains have different in vitro antiviral susceptibilities of possible clinical significance. Intravenous peramivir was authorized in the US for treatment of hospitalized pH1N1 patients, but its effectiveness in complicated pH1N1 illness is uncertain; intravenous zanamivir is available for compassionate use, especially in severe illness when oseltamivir resistance is suspected. Controlled studies of both agents are in progress.
Epidemiology and impact of respiratory viral infections

Enterovirus 71 and hand, foot and mouth disease (Jing Zhang, Chinese Center for Disease Control and Prevention, Beijing, People’s Republic of China)

Enterovirus (EV)71 is the primary causative agent of hand, foot and mouth disease (HFMD) in many countries. In the Asia-Pacific region, large numbers of cases have occurred during outbreaks, often associated with severe illness and sometimes fatality. An outbreak in Taiwan in 1998 recorded 129,106 cases, of which 405 were severe and 78 fatal. Several genotypes of the virus are known to be circulating in the Asia-Pacific region. The surveillance system in mainland China consists of laboratory detection (RT-PCR and virus isolation), case notification and cluster case/outbreak investigation. Since 2008, HFMD has been a notifiable disease in China and approximately 1 million cases have been reported. Children <5 years of age are the group predominantly affected (93% of all cases in 2009). The peak age for infection and fatality appears to be 2 years of age, although severe disease has been seen in adults up to 23 years old. Fatal cases have a median duration of illness of 3 days (range 0–35 days) with 88% of cases dying within 5 days of symptom onset. Estimates suggest that HFMD results in hospitalization in 16% of those infected and causes 2.6 deaths per 10,000 reported cases.

In suspected mild cases of HFMD, EV71 was only detected in samples from 41% of patients in 2009, with Coxsackie A16 virus and other enteric viruses being detected in the remainder. The proportions due to EV71 increased to 81% and 93% for severe and fatal cases, respectively. Risk factors for developing HFMD include age <5 years, poor personal hygiene, contact with known cases or those symptomatically infected, and contamination of the immediate environment. Patients may remain infectious for more than 2 weeks following clinical recovery, and cross-protective immunity against different serotypes is limited. During outbreaks, children who attend schools and kindergartens appear to be infected earlier than those who stay at home. For every symptomatic child with confirmed EV71 infections, there are an estimated 1.6 asymptptomatically infected ones. The economic cost is estimated to be USD 75–150 million per year. EV71 infection is an important public health problem, and vaccine development is therefore a priority area of research.

Disease burden of respiratory viruses: Taipei perspective (Chien-Jen Chen, Academia Sinica, Taipei, Taiwan)

Taiwan has established influenza surveillance systems, including sentinel ILI reporting from 530 physicians (one sentinel practice per 43,000 people), a virological laboratory network and a notifiable disease system for H5N1 and influenza with severe complications. Mortality data between 1981 and 2005, along with monthly virological data between March 1999 and December 2003 have been analysed. Seasonal influenza epidemics are estimated to cause 2,500–4,500 deaths in Taiwan each year, with >70% of deaths occurring among persons aged 65 years or older. Seasonal influenza-related mortality rates generally remained stable during the years studied, although higher excess influenza deaths were noted in 2003–2004 and 2004–2005, when influenza H3N2 and influenza B viruses were reported to be the dominant circulating viruses, respectively. In general, the population-adjusted mortality rates seen in Taiwan are comparable with those reported by the US, tropical Singapore and subtropical Hong Kong.

Disease burden of respiratory viruses: Mexican perspective (Ethel Palacios Zavala, Emergency Preparedness for International Health Emergencies, Ministry of Health, Mexico City, Mexico)

Using data from the national epidemiological surveillance systems and a national network of public health laboratories in Mexico, efforts were made to assess the burden of 163 different diseases for each of the 32 states of Mexico. In 2006, 15 million person-years were estimated to be lost to disability, of which 46% were associated with premature death. Importantly, respiratory disease was the 10th cause of years of life lost among highly marginalized populations. Acute respiratory disease was identified as the primary cause of healthy years lost to disability and premature death in the 0–4-year age group, and lower respiratory tract infection was identified as a cause of years lost to disability and premature death in the elderly.

Following the emergence of the 2009 H1N1 pandemic, the various surveillance systems were reviewed, with greater emphasis being placed on the incorporation of rapid laboratory detection. Emphasis was placed upon establishing a surveillance system with enhanced analytical capabilities, with continued laboratory capacity building and the development of unknown pathogen diagnostics. The 2009 pandemic in Mexico increased influenza and pneumonia mortality in all age groups compared to data collected over the preceding 10 years, with a 40% increase seen in those aged 15–64 years. From August 2009 to March 2010, 44% of 857 samples tested positive for ≥1 respiratory viruses. Peak detections of most viruses generally occurred in the 0–4-year age group, with a few exceptions such as CoVs and parainfluenza-2 and -4. Dr Zavala summarized that the 2009 pandemic necessitated the review of strategic planning and methods of data collection and analysis in Mexico and other countries.
Disease burden of respiratory viruses: Vietnamese perspective (Rogier van Doorn, National Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam)

Vietnam is a rapidly developing country with a childhood pneumonia incidence rate of 0.31–0.40 episodes per child year, similar to India and several African countries. Among 309 children attending the Hospital for Tropical Diseases (Ho Chi Minh City, Vietnam) with ARI between November 2004 and January 2008, 70 children required critical care, two children died and 89% made a complete recovery. Samples were analysed for a range of respiratory viruses using molecular and antigen detection methods. Seasonal peaks in human metapneumovirus (hMPV) and respiratory syncytial virus (RSV) were detected, most commonly between July and September, and coinfections were detected in 62 patients. RSV, influenza and bocavirus were the most frequently identified viruses. Combined nose and throat swabs had similar diagnostic yields to nasopharyngeal aspirate (NPA) overall (78–79%), with certain sampling methods appearing to be more beneficial for specific viruses. For example, diagnostic yields for influenza were 53%, 45% and 75% for nasal swabs, throat swabs and NPAs, respectively, but for EV were 50%, 85% and 48%, respectively. Antibiotic resistance is also a problem in Vietnam within the context of ARIs; over-the-counter antibiotic availability is a major contributory factor. A study of 500 patients given antibiotics for ARI in 2009–2010 found selection of cephalosporin-resistant Enterobacteriaceae in faeces at follow-up.

From 27 April to 24 July 2009, approximately 760,000 passengers who entered Ho Chi Minh City on international flights were screened at the airport by a body temperature scan and symptom questionnaire. Over one-half of these arrivals were from endemic countries. Approximately 0.15% of the incoming passengers were intercepted, 200 of whom tested positive for pH1N1 by RT-PCR and an additional 121 out of 169 non-travellers tested positive after self-reporting or contact tracing. ILI was noted in 61% of patients and no patients experienced pneumonia or severe outcomes [6].

Global Emerging Infections Surveillance and Response System (Kevin L Russell, Armed Forces Health Surveillance Center, Silver Springs, MD, USA)

The US Department of Defence (DoD) global surveillance system includes electronic health records for 1.3 million military personnel undertaking active duty and a global, laboratory-based surveillance system. Particular emphasis is placed upon the surveillance of respiratory tract illnesses, including influenza and respiratory viruses in vulnerable populations, for example military recruits and personnel confined to ships. The Global Emerging Infections Surveillance and Response System, operating across eight different countries, also facilitates programmes in training, communication and maintenance of standards. The role of this syndromic surveillance system in global health was highlighted by the early detection of pH1N1 cases by military physicians in California in April 2009, representing four of the first five recognized cases in the world. Overall, >40,000 samples were collected and analysed in 2009. Although pH1N1 predominated during 2009, regional differences in influenza strain cocirculation were observed in different continents.

Research efforts are not confined to military personnel, and DoD surveillance efforts are based on rapid sharing of results, as occurred during the 2009 pandemic. Prospective studies of avian influenza transmission in Asia are in progress, with the aim to determine transmission rates and risk factors. Other collaborations include work to identify risk factors for zoonotic influenza transmission to man in Romania, Nigeria and Mongolia. Furthermore, work includes influenza phylogenetic analysis, susceptibility studies and vaccine safety evaluation, thus supporting the work of the US CDC. The role of military-based systems in detecting outbreaks can be complex, particularly when outbreaks are detected in countries other than the US, but non-reporting and non-transparency can be detrimental to global health and should be avoided where possible [7].

Nosocomial respiratory viral infections (Allison McGeer, Mt Sinai Hospital, University of Toronto, Toronto, ON, Canada)

Attempts have been made to improve our understanding of the epidemiology of nosocomial respiratory virus infections (RVIs). The incidence of RVIs may be higher in hospitals than that in the community and there may be opportunities to interrupt or prevent nosocomial transmission. In Canada, RSV is the most frequently detected nosocomial RVI pathogen, but influenza, parainfluenza and adenovirus also contribute. Annual nosocomial rates of RSV infection for children <3 years of age have ranged from 10,600 to 35,600 per 100,000 population [8,9]. Nosocomial influenza has been shown to be associated with an average case fatality rate of >7% and significant additional healthcare expenditure in the US. Preliminary results assessing hospital-acquired pH1N1 in Toronto indicated that 36 (3.9%) hospitalized cases were thought to be nosocomially acquired infections; 7 of these patients required critical care and 5 died.

Nosocomial RVIs can occur in outbreaks and occupational disease can also occur in hospital staff. Transmission has been shown to occur between patients, between healthcare workers and between healthcare workers and patients. However, several challenges for controlling nosocomial RVIs exist. Most adult patients with fever and respiratory symptoms do not have a communicable disease, some infected...
patients have no fever and staff often continues to work during an ARI. Several potential interventions to interrupt transmission were proposed, including increasing humidity in winter, improving hand hygiene, and the use of personal protective equipment and isolation facilities.

Influenza pathogenesis

New insights from studies on influenza (Yoshi Kawaoka, University of Wisconsin, Madison, WI, USA and University of Tokyo, Tokyo, Japan)

Professor Kawaoka reviewed new insights into the characterization and options for control of pH1N1 virus. Using a porcine infection model, titres of virus at different sites of the respiratory tract were similar for pH1N1 influenza (A/California/04/09) and classical swine influenza virus (A/swine/Hokkaido/81 [H1N1]). Infected pigs also demonstrated replication of pH1N1 in the lungs but without significant histopathological lesions. Such studies help to explain the efficient replication in multiple outbreaks of pH1N1 seen in pig populations worldwide. In a murine model, greater loss of body weight was seen following similar inocula of A/California/04/09 compared with seasonal H1N1 virus. Lethality was seen with pH1N1 but not with seasonal H1N1 infection, although differences in lethality occurred among different strains of pH1N1, with three out of five strains causing infection that resulted in death. In ferrets, pH1N1 showed enhanced replication in trachea and lung compared with seasonal H1N1 influenza, similar to the results reported by others [10]. Unlike other groups, virus replication was not found outside of the respiratory tract in ferrets, specifically there was no evidence of pH1N1 replication in the intestines, although previous work has demonstrated replication of classical swine influenza viruses in ferret intestine [11]. In non-human primate models, greater replication of pH1N1 was seen in both the upper and lower respiratory tracts, compared with infection with seasonal H1N1 virus. Influenza infiltrates and fluid-filled alveoli developed following infection with pH1N1 with replication in both type I and type II pneumocytes, and influenza antigen was much more readily identifiable in pH1N1 infection. Such studies in non-human primates may improve understanding of viral pneumonitis due to pH1N1 in humans.

Dr Kawaoka emphasized the importance of the North American avian virus-derived PB2 gene in pH1N1 virus; PB2-590S and PB2-591R mutations appear to contribute to its efficient replication in humans [12]. Based on the three dimensional structure of PB2, mutational studies were undertaken to assess the effect of different PB2 mutations on replication and transmissibility of pH1N1 virus. Three mutant Cal04 H1N1 viruses were created: PB2-627K, PB2-701N and PB2-591Q. When ferrets were then coinfected with wild-type virus and a single mutant at a 1:1 ratio, transmission studies found that isolation of wild-type Cal04 virus clearly predominated over Cal04 PB2-627K and Cal04 PB2-701N in both inoculated ferrets and the contact animals, whereas Cal04 PB2-591Q was found in greater proportions of isolates from inoculated animals and appeared to transmit more efficiently than wild-type virus to some, but not all, contact animals.

With regard to antiviral options for the control of pH1N1 influenza, the existing neuraminidase inhibitors and selected, novel antiviral agents show efficacy in murine models. Replication of pH1N1 in the presence of oseltamivir and zanamivir in mice revealed decreases in viral titres in the lung compared with no treatment, and the investigational topical application may also be applied neuraminidase inhibitor laninamivir (CS-8958) and the investigational oral RNA polymerase inhibitor favipiravir (T-705) did well as well [10]. In animal H5N1 models, both favipiravir and laninamivir demonstrated dose-dependent improvements in survival rates, and a single dose of laninamivir improved survival when given as prophylaxis 7 days before infection, again in a dose-dependent manner.

Can systems and computational virology help us understand deadly influenza viruses and develop better drugs and vaccines? (Michael Katze, Washington National Primate Research Center, University of Washington, Seattle, WA, USA)

The Katze laboratory studies highly pathogenic viruses and those with pandemic potential in humans. In addition to the characteristics of the infecting viruses, host factors are important in determining outcomes of infections with these pathogens and a structured approach to understanding the process of infection is required. Systems biology methods incorporate techniques like genome-wide association studies, network analysis, RNA interference screens, evaluation of different arms of the host immune response, computational modelling and ‘omics’ disciplines, such as metabolomics. Using a computational infrastructure, for example, enables the study the global impact of virus infection on host gene expression, the discovery of cellular regulatory pathways targeted by viruses and the identification of novel targets for antiviral therapy. Starting with an experimental model of virus infection, from which virology and ‘omics’ data are generated, these data are then integrated into more refined models incorporating key genes and pathways in an iterative process involving biologists and modellers working together.

The hypothesis of the Katze laboratory is that highly pathogenic respiratory viruses use both unique and common strategies to remodel the host cell to enhance virus replication, regulate disease severity
and promote virus transmission. Key host immune events occurring in response to infection happen very quickly; as such, early innate immune signalling is a key area of investigation. Examples of systems biology approaches to understanding the mechanisms of severe influenza infection include genomic analyses of host immune and cell death responses induced by 1918 influenza virus infection in mice [13,14]. Recombinant 1918 virus was shown to cause early and sustained expression of genes associated with specific immune cell populations. A group of cellular microRNAs have also been identified that are associated with virulence of the 1918 virus, along with an enrichment of inversely-correlated targets of these microRNAs [15]. Bioinformatics analysis suggests an association of the inversely-correlated targets with certain key biological functions.

Swine-origin pH1N1 viruses cause significant pathology, but different strains appear to be more pathogenic than others in infected primates. For example, Mexico4487 pH1N1 obtained from a fatal human case generated higher clinical and pathology scores, compared with pH1N1 strains sourced from mild and moderate cases, and all of these viruses demonstrated more efficient replication and greater pathogenicity than seasonal H1N1 influenza in this model. Mexico4487 was also found to replicate for longer in the lower respiratory tract, which correlated with prolonged and more severe pneumonia. Gene expression patterns in the lung at day 1 post-infection also distinguished the individual viral isolates, with pH1N1 viruses inducing a more robust proinflammatory response in the lungs compared with the seasonal isolate [16]. Murine studies also suggest that there is a species-specific response to different strains of influenza; furthermore, differing responses in weight loss, lung pathology and viral titres in various founder strains of mice infected with influenza suggest that predetermined host genetic response is important. Such results indicate that one should exercise caution when comparing different animal models and how results from such models relate to human infection. Expression quantitative trait loci analysis combined with microarrays will become a powerful tool to identify novel host susceptibility loci that confer resistance to or protection from infection. Increasing complexity in studies of disease pathogenesis is inevitable, as understanding not only the functions of proteins, but also the roles of RNAs, including non-coding RNAs generated in response to infection, will be required.

Tropism and host responses of the 2009 pandemic H1N1 influenza virus in ex vivo and in vitro cultures of human conjunctiva and respiratory tract (Renee WY Chan, The University of Hong Kong Li Ka Shing Faculty of Medicine, Queen Mary Hospital, Pokfulam, Hong Kong SAR, People's Republic of China)

Dr Chan presented results comparing tissue tropism and replication kinetics in different regions of the respiratory tract and the innate immune dysregulation effects of pH1N1 and seasonal H1N1 viruses in ex vivo organ culture models and in vitro cell models. Comparable viral replication was observed in human nasopharyngeal, bronchial and lung biopsies ex vivo and no differences were observed in vitro between the viruses at 37°C. Greater replication competence of pH1N1 was observed in bronchial epithelium at 33°C, which may contribute to a slight increase in virulence. As other influenza variants are able to infect human conjunctiva, the ability of pH1N1 to infect conjunctival tissue was investigated. At 24 h after infection of ex vivo human conjunctival cultures, replication of pH1N1 was observed for H7N7 and H5N1 viruses, but not for the seasonal H1N1 and H3N2 variants. Lectin staining of the human conjunctival tissues confirmed that abundantly more sialic acid α2,3 and less α2,6 receptors are expressed; sialidase treatment showed that the pH1N1 virus replication is sialic-acid-dependant. Investigation of the cytokine responses showed that the pH1N1 virus does not differ from seasonal influenza virus in their intrinsic capacity for cytokine dysregulation. Also the global gene expression profile was broadly similar between pH1N1 and the seasonal H1N1. Both viruses were able to trigger interferon (IFN)-related genes, including type III IFNs and Mx1, but seasonal H1N1 virus down-regulates many more host genes than pH1N1 virus [17].

Overview of influenza pathogenesis and transmission in the ferret model: comparing the 2009 H1N1 virus to H5N1, 1918 and seasonal H1N1 viruses (Terrence Tumpey, US Centers for Disease Control and Prevention, Atlanta, GA, USA)

Ferret models have the benefit of natural susceptibility to human influenza viruses, a clinical course that is similar to that in humans and a distribution of sialic acid receptors in the respiratory tract that is also similar to that found in humans. Controlled transmission studies using set distancing of caged ferrets to assess respiratory droplet transmission between inoculated and contact ferrets have revealed differences in transmission patterns between different H1N1 viruses. For example, A/Texas/36/91 transmitted effectively to contact animals, whereas an avian H1N1, A/duck/NY/96, did not. Similar differences in transmission were also seen with human and avian H2N2 strains. To address which genes from the 1918 H1N1 virus confer efficient transmission, 1918 HA and PB2 were inserted into Dk/NY/96; this conferred efficient transmission in this model. Furthermore, viruses that
preferentially bind to α2,6 receptors show more efficient respiratory transmission than those viruses with a preference for α2,3 receptors [18]. These findings suggest that human adaptation of the HA and PB2 proteins of avian H1N1 influenza viruses are required to generate viruses that are readily transmissible between people. Comparing human 1918 (SC18) and avian H5N1 (VN/1203) viruses, the 1918 strain was much more transmissible and was associated with greater lethality and more significant weight loss. Although H5N1 replicates well in ferrets, it does not transmit effectively.

Infection with three different isolates of pH1N1 obtained from humans with uncomplicated, severe or fatal infection, and a seasonal H1N1 (A/Brisbane/59/07) show varying impacts in ferrets. The main characteristics of pH1N1 infection were significant weight loss, high levels of virus in nasal turbinates and lung tissue and detection of infectious virus in the intestinal tract. Moderate to severe alveolitis was seen with A/Mexico/4482/09, and unlike seasonal H1N1, high amounts of NP antigen-staining were seen in alveolar pneumocytes. A/Mexico/4482/09 antigen was also detected in the glandular epithelial cells of the upper airways and also in bronchiolar epithelial cells. Only infection with A/Mexico/4482/09 resulted in death, although all three pH1N1 isolates caused greater weight loss and detectable virus lung titres in the lungs of infected animals compared with infection with seasonal H1N1. However, transmission of different strains of pH1N1 was less efficient and more sporadic than with seasonal strains. Importantly, pH1N1 viruses transmit relatively well by direct contact but not by droplet transmission, which has implications for animal studies of transmission. Viruses from zoonotic cases of triple reassortant swine influenza virus [19] also appear to transmit efficiently by contact but not by respiratory droplets in the ferret model.

Pathogenesis of pandemic H1N1 in humans (Nelson Lee, Chinese University of Hong Kong, Sah Tin NT, Hong Kong SAR, People’s Republic of China)

Key features of pH1N1 illness described in published series indicate that the case fatality is <1% overall and the mortality rate among hospitalized patients is 9–15%. Of those requiring hospitalization, 10–34% require critical care following the development of severe pneumonia, acute respiratory distress syndrome and multi-organ failure. Critical illness has been associated with mortality rates of 17–41%. The clinical course can become rapidly progressive within 1–2 days, resulting in refractory hypoxaemia and the requirement of intubation and mechanical ventilation. Acute respiratory distress syndrome and multi-organ dysfunction occurs in many cases of H5N1 infection, in some cases of pH1N1 influenza, and less frequently with seasonal influenza strains [20–30].

Dr Lee described a prospective, observational study of hospitalized patients with laboratory-confirmed pH1N1 in Hong Kong, over a 16-week period during the first wave of infection in the city. Three categories of illness were defined: severe pneumonia for those with radiographic pneumonia and a requirement for supplemental oxygen to maintain oxygen saturations >95%, mild pneumonia for those without hypoxaemia (oxygen saturations >95% on room air) and no pneumonia. Of 66 adult patients, 39% had comorbidities and the mean age was 43 years. The vast majority had fever, 75% had cough, but less than one-third had coryza. Comorbidities were more common in those with severe pneumonia; 20% of the total cohort required admission to intensive care and 3% died. All patients received antiviral therapy, 77% received antibiotics and bacterial coinfection was reported to have been detected in 18% overall and in 32% of those with severe pneumonia. Elevations in creatine kinase, c-reactive protein and lactate dehydrogenase were common, with greater increases above normal range occurring in the severe pneumonia group. Moderate lymphopaenia was common in all patients. Multivariate logistic regression analysis identified the following factors to be associated with severe pH1N1 pneumonia: age >35 years, delayed presentation >2 days from onset and without antiviral treatment, dyspnoea and absence of rhinorrhoea at presentation.

The main radiographic features were airspace consolidation and ground-glass opacity. At presentation, one, two and three to four lung zones were involved in 47%, 37% and 17% of cases, respectively. Lower zones were generally more frequently involved than the upper zones. Severe pneumonia was shown to be significantly associated with higher NPA viral RNA concentration at presentation. Viral RNA was also detected in peripheral blood in 13%, in urine in 13% and in faeces in 28% of patients; most of these patients had severe pneumonia and/or comorbidities, although extra-respiratory virus was detected in a small number of previously healthy patients with mild pH1N1 illness. Compared with seasonal influenza cases, pH1N1 was associated with higher viral RNA concentrations at presentation.

The median duration of detectable viral RNA following initiation of oseltamivir therapy in nasopharyngeal flocked swab samples was significantly longer in patients with severe pneumonia compared with those with mild disease (6 days and 2 days, respectively). Median duration of viral RNA detection in tracheal aspirates was 11 days following commencement of oseltamivir, and viral RNA load was substantially higher in the lower respiratory
tract samples compared with upper respiratory tract samples. Viral rebound (viral RNA concentration increasing after stopping oseltamivir) was detected in a small number of patients, but no H275Y mutations were found. The persistence of viral RNA in the lower respiratory tract when upper respiratory tract samples were negative has important implications for the choice of anatomical sampling, particularly in those patients with lower respiratory tract involvement.

A variety of soluble immune mediators were measured in plasma. After adjusting for confounders, elevated interleukin (IL)-6 was independently associated with severe pneumonia and significant elevations of chemokine (C-C motif) ligand (CCL)2 (monocyte chemotactic protein [MCP]-1) and IL-8 were also found. Furthermore, the plasma concentrations of IL-6, IL-8, MCP-1 and soluble tumour necrosis factor (TNF)-α receptor 1 were found to correlate with the radiographic extent of pneumonia. Most patients had undetectable or very low plasma levels of TNF-α, IFN-γ, IL-12p70 and IL-10. Other published trials described Th1/Th17 hypercytokinaemia as an early host response signature in severe pH1N1 and in a retrospective cohort analysis high levels of IL-6 and MCP-1 at presentation were seen in severe cases with associated delayed viral clearance in upper respiratory tract samples [31,32].

Dr Lee highlighted the proinflammatory activity of IL-8, the neutrophil chemotactic properties of CXCL-8 and the monocyte/granulocyte recruiting/activating potential of CCL2, to support their role in pneumonia pathogenesis. He proposed that severe viral pneumonia may result from a combination of high-level, uncontrolled viral replication alongside an abnormal innate immune response. A lack of pre-existing immunity and/or presence of comorbid conditions may influence viral kinetics and patient outcome. The duration of detectable viral RNA described and the phenomenon of viral rebound, particularly in those with the most severe disease, raises the question of whether a more sustained antiviral regimen is warranted and whether methods of administration to achieve higher levels of antiviral in the lungs need to be pursued. In the absence of conclusive data, the role of immunomodulatory agents remains controversial [33,34].

Strain-specific function of influenza A virus PB1-F2 [Shin-Ru Shih, Research Center for Emerging Viral Infections and Graduate Institute of Medical Biotechnology, Chang Gung University, Tao-Yuan, Taipei, Taiwan]

The functions of PB1-F2 derived from different human and avian influenza strains have been investigated using a combination of techniques: localization studies, sequence analysis, expression of proteins of FLAG-tagged PB1-F2, RNA transcription analysis, and measurement of induction of apoptosis, viral growth and viral polymerase activity. Different cellular localizations of PB1-F2 proteins exhibited by both H5N1 and H7N7 viruses reveal that these PB1-F2 proteins have different cellular functions compared with the PB1-F2 of PR8 virus. Similar to the PB1-F2 of the other strains, the putative pH1N1 virus PB1-F2 can increase viral ribonucleoprotein activity. The plaque size and growth rate of the viruses differed greatly with and without pH1N1 PB1-F2; plaques were more than double the size in virus without PB1-F2. The H5N1 PB1-F2 does not appear to have apoptosis-enhancing ability, perhaps due to its intracellular localization. Overall, the distinct differences in cellular localization and functions of PB1-F2 may help explain the differing replication abilities and virulence seen among different strains of influenza [35].

General virology of respiratory viruses and diagnostics

Virology of parechoviruses (Katja C Wolthers, Academic Medical Centre, Amsterdam, the Netherlands)

Human parechoviruses (HPeVs), formerly in the genus Enterovirus, have been reclassified into their own
genus within the family Picornaviridae. This is divided into 11 genera with >200 serotypes. These small, non-enveloped, symmetrical, single-stranded positive-sense RNA viruses usually have four capsid proteins – three outer ones including VP1, VP2 and VP3, and VP4 inside the capsid – but the parechoviruses do not have VP4. The genome consists of a single open reading frame, of which the 5′-untranslated region is highly conserved, whereas the VP1 region to which neutralizing antibodies are directed is highly variable. Classification of the EVs, formerly done on the basis of serotyping by neutralization with antibody pools (five serogroups), is now done by sequencing of the capsid-encoding region, usually the VP1 region. Like human EVs, HPeVs are assumed to be transmitted faeco-orally and replicate in mucosal lymphoid tissue of the oropharynx and the gut, then spreading through the blood to their different target organs.

HPeVs were first recovered from children in cell culture, causing the same cytopathic effects that are observed with EVs. Mainly children are infected (adult seroprevalence is approximately 95%) and infection is associated with gastrointestinal and respiratory symptoms, as well as sepsis-like illness in neonates. It was not possible to detect HPeVs with EV-specific molecular probes. For example, echovirus 22 was reclassified as HPeV1 and echo 23 virus as HPeV2a. Discovery of new HPeVs increased the number to 14 by 2010. Various 5′-untranslated region real-time PCR assays have been published, and surveillance studies found that the prevalence of HPeV infections is similar to that of EVs, with HPeV1 being the most prevalent.

HPeV infections have been associated with respiratory symptoms. In a study performed in children from Canada, 50% of the children with HPeV1 infection had bronchiolitis [36]. In 59 children followed from birth, HPeV1 infection was associated with acute otitis media [37]. A third study of children from 6 months to 5 years found HPeV in 1.2% (27/2,220) of respiratory samples [38]. Only type 1 and type 6 were detected, with a peak in the number of infections in July and August. However, the relationship of HPeVs with respiratory symptoms has been questioned, since many individuals positive for HPeVs have had coinfections with other viruses, much more so than for other respiratory viruses except bocaviruses. In a general screening from 1,644 respiratory samples from Dutch patients <18 years of age in 2007–2009, the prevalence was 1.7% for HPeVs and 3.1% for EVs. No seasonal peak was observed for infection with HPeV, and a high number of coinfections was observed in patients with either HPeV (68% of 28 patients) or EV (94% of 52 patients) infections, which was much higher compared with RSV (35%) and influenza A (19%).

In a prospective study of 102 hospitalized patients <7 years old with suspected respiratory infection and an aged-matched, hospitalized control group of 51 without respiratory symptoms, NPAs detected similar percentages positive for HPeV and EV in cases and controls. However, the cycle threshold values, as a measure for viral load, showed a clear trend for higher HPeV loads in the cases. This pattern was also found for human rhinovirus (HRV) infections but not for EVs. Dr Wolthers summarized that HPeVs are widespread in the population with type 1 being the most prevalent and associated with mostly mild respiratory symptoms. Type 3 infects primarily young infants and appears to be associated with central nervous system symptoms and neonatal sepsis. Therefore, HPeV detection should be included in viral diagnostics, but the importance of HPeVs in causing respiratory and other syndromes requires further study.

Virology of coronavirus (Kathryn Holmes, University of Colorado School of Medicine, Denver, CO, USA) CoVs are large, enveloped, positive-sense RNA viruses with a helical nucleocapsid and the largest of all RNA genomes. Of the total genome, 2/3 codes for the polymerase protein which is formed as a polyprotein and subsequently processed by several viral proteases. These proteases are targets for antiviral drugs. The gene order of the structural proteins is conserved among the various groups of CoVs, but there are several accessory proteins that are different in position and length across virus groups.

Different groups of CoVs exist, and phylogenetic analyses increased the number of CoV groups from 3 in 2002 to almost 8 different groups in 2009, highlighting the tremendous biodiversity in these viruses.

The coronavirus responsible for SARS is part of group II. Due to increased surveillance after the SARS epidemic in 2003, new human CoVs (NL63 and HKU-1) have been identified [39,40].

After the SARS outbreak, SARS-like CoVs were also identified by two different groups simultaneously in five species of bats, CoVs have now been identified in bats from all over the world, but SARS-like CoVs have been detected only in Africa and South-East Asia to date. Bioinformatic analysis suggests that all CoVs might originate from bats. In any case there is an enormous reservoir of CoVs in nature with sporadic transmission from one host to another.

In cross-species transmission, CoVs sometimes swap the entire S gene from an unknown host or sometimes a small piece of S (usually the receptor binding domain or receptor binding motif), which changes the host range. Mutations occur in the S gene as the virus adapts to its new host and changes the binding specificity to receptor homologues and allow for antigenic drift over time. Interestingly, the receptor binding domain of SARS has absolutely no recognized homology to any other CoV group.
and it is unknown where it came from. Surprisingly, the group 1 CoV NL63 and the group 2 SARS-CoV use the same human receptor, angiotensin converting enzyme 2, but the receptor binding motifs have completely different structures. NL63 has three loops and SARS has 1 cradle-like structure. Therefore, the spike region is composed of modular domains that can be swapped with others, changing receptor specificity and host range. Using reverse genetics, introduction of the SARS receptor binding domain into a bat CoV virus enables production of infectious virus.

Human CoVs contribute to the estimated 17% of all global deaths in children <5 years due to ARIs. One study in 2009 examining 1,854 respiratory samples from children found that 5% were positive for one of the human CoVs (229e, OC43 or NL63). Of all respiratory infections in children from 6 to 23 months, 8% were positive for CoVs compared with 12% for hMPV, and in hospitalized children in Denver the frequency of coronavirus detection was 5%.

CoV research has been very difficult in the past because these viruses do not grow easily in cell culture, although this has been circumvented due to the increased usage of human differentiated airway epithelial cell culture systems and the use of reverse genetics. Antiviral drugs are currently being developed that can inhibit the receptor binding, viral replicase or other RNA enzymes, virus-encoded proteases, the conformational changes in the spike protein, and virus assembly. Also, currently, many vaccines are being produced that target the viral S protein.

Identification of human rhinovirus strains associated with severe respiratory illness (Wai Ming Lee, School of Medicine and Public Health, University of Wisconsin, Madison, WI, USA)

HRV infection is the most common RVIs in humans, causing >50% of common colds. They also have a wide range of clinical outcomes, namely the common cold, exacerbation of severe lower airway illness (asthma, chronic obstructive pulmonary disease [COPD] and cystic fibrosis) and bronchiolitis/pneumonia hospitalization of young children. An estimated 20–30% of infections are asymptomatic. There are many different serotypes (100) and many newly discovered HRV group C (HRV-C) strains, but it is unclear whether there are HRV strains that cause more or less severe illnesses. Using a large cohort called the Childhood Origins of Asthma (COAST) project, now in its 11th year, 1,645 nasal samples from 285 1-year-olds were collected at scheduled visits (months 2, 4, 6, 9 and 12) and during ARI sick visits. At each visit, the severity of illness was assessed by a clinician using a numerical respiratory symptom scorecard and was categorized into one of the 3 groups (none, mild and moderate–severe). All samples were analysed by a high throughput, multiplex RT-PCR detection system that detects all common respiratory viruses and for HRV positives, by a molecular typing assay to determine the serotypes/genotypes.

HRV was detected in 72.2% of virus-positive samples and was the most common virus in each illness severity category. The next common virus (RSV) was present in only 10.4% of the virus positive samples. HRV infection occurred year-round and showed no seasonality, regardless of illness severity. HRV group A (HRV-A) and HRV-C were both common in infants and together accounted for 94% of all HRV infections. Also, they both induced a similar rate of moderate-to-severe illness (approximately 12% of detections) that was significantly higher than HRV group B (HRV-B; approximately 1–2%). Analysis of individual strains shows the least pathogenic serotype was HRV-B R52 (approximately 1%), and the most pathogenic was the HRV-C W12 strain (approximately 21%). These findings highlight the importance of studying differences among HRVs in infection rates and virulence.

Rhinovirus-induced chemokines and role in recruitment of allergen-specific Th2 cells (Nathan Bartlett, National Heart and Lung Institute, Imperial College London, London, UK)

HRV infections cause most cases of the common cold but also frequently trigger exacerbations of chronic respiratory diseases, such as asthma, that are associated with significant morbidity, mortality and healthcare-related costs. Recruitment of Th2 cells is believed to be important in the pathogenesis of asthma and is thought to be regulated by the chemokines CCL17 and CCL22 binding to CCR4 on Th2 cells. It is hypothesized that RV infection induces expression of such chemokines, resulting in recruitment of Th2 cells to the airways. In Balb/c mice infected with HRV-1B, the BAL levels of CCL17 peaked at day 1 post-infection and of CCL22 at day 2, compared to control mice dosed with ultraviolet-inactivated HRV-1B. Chemokine gene expression in lung tissue was also significantly increased; CCL22 staining revealed increased expression in blood vessels and in bronchi, whereas CCL17 was only found in the bronchi.

In infected IFN receptor-α (IFNRA) knock-out mice, the expression of the CCL17 and CCL22 remained intact, but that of Th1 chemokines CXCL11 (I-TAC) and CCL5 (RANTES) was completely absent, indicating a role for IFN signalling on expression of these chemokines. To functionally investigate HRV-induced CCL17 expression, ovalbumin-specific Th2 polarized cells were administered to mice, followed by a single intraperitoneal dose of anti-CCL17 antibody given 24 h prior to infection. Significant inhibition of HRV-induced recruitment of lymphocytes in BAL was seen.
in antibody-treated, infected mice, and lung allergen-specific CD4+ T-cells were reduced by 60%. Suppression of IL-4 production by mediastinal lymph node cells and lung cells was also seen with anti-CCL17 treatment. These findings indicate that HRV infection can increase expression in the lung of chemokines involved in Th2 cell recruitment, implicating the CCR4 receptor/CCR4 ligands as potential targets for therapeutic intervention in HRV-induced asthma exacerbations.

Clinical aspects of measles (Diane Griffin, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA)

Measles is a negative-sense, single-stranded RNA paramyxovirus in the morbillivirus genus. The virus particle includes two surface glycoproteins (HA and fusion [F] protein) that are important for attachment, entry and fusion. The HA protein mediates attachment to the target cells including epithelial and endothelial cells, monocytes/macrophages and lymphocytes. The first identified cellular receptor was CD46 but its distribution does not account for the tissue tropism of the virus. Wild-type viruses use CD150/signaling lymphocytic activation molecule as receptors present primarily on activated immune cells, including lymphocytes and dendritic cells. The receptor used for infection of epithelial cells and endothelial cells remains unknown.

Measles infection is initiated in the respiratory tract and consequently spreads systemically through the lymphatic system to many other organs. A substantial amount of viral replication and spread occurs before presentation of clinical disease 10–14 days after infection. It is not clear which cells are infected first. In vitro studies have shown that ciliated respiratory epithelial cells can be infected, but are most susceptible to infection from the basolateral surface rather than the apical. Alternatively, alveolar macrophages or dendritic cells may take up measles virus and transport the virus to lymphoid tissue where most early replication occurs.

The immune response to measles poses a paradox in that the adaptive immune response is effective in clearing virus and establishing lifelong protection from re-infection, but this is accompanied by immunological abnormalities that increase susceptibility to other infections and autoimmune disease. The potential mechanisms of measles-induced immunosuppression include lymphopaenia, virus-induced and/or bystander lymphocyte cell death, and type 2 cytokine skewing with suppression of IL-12 production. The lymphopaenia includes a decrease in both CD4+ and CD8+ lymphocytes during the acute phase of measles [41]. Suppression of lymphoproliferation may be due to measles virus-induced cell cycle arrest [42]. Cytokines produced during the acute phases of measles have a type 1 phenotype with activated CD4+ and CD8+ T-cells producing IL-2 and IFN-γ, but CD4+ T-cells remain activated and later produce IL-10, IL-4 and IL-13. These Th2 cytokines alter macrophage activation, reduce the Th1 immune responses and increase susceptibility to other infections. The importance of CD8+ T-cells for virus clearance has been demonstrated experimentally; monkeys depleted of CD8+ T-cells prior to infection have higher viraemia and slower clearance of infectious virus. In both children and monkeys, virus cannot be cultured from any site after the rash has cleared. However, RT-PCR analysis of nasopharyngeal washings, urine and peripheral blood mononuclear cells (PBMCs) show that measles virus RNA is still detectable in up to 50% of children with measles 1 month after discharge from the hospital and in 35% at 3 months [43,44]. In monkeys, measles virus RNA is detectable in PBMCs 4–6 months after infection. Using FACS analysis of monkey PBMCs, Dr Griffin showed preliminary results that during the acute phase of infection, virus is present in CD4+ T-cells but the majority of virus is in B-cells. At day 25 the RNA is primarily in B-cells, and at day 90 viral RNA can be found in monocytes and B-cells. During this period, T-cell responses are multiphasic confirming the importance of the slow clearance of RNA for ongoing stimulation and maturation of the virus-specific immune response and for prolonged immune suppression.

Comparing influenza and RSV viral and disease dynamics in experimentally infected adults predicts clinical effectiveness of RSV antivirals. (John DeVincenzo, Paediatrics and Molecular Sciences, University of Tennessee Health Sciences Center, Children’s Foundation Research Center, and LeBonheur Children’s Hospital, Memphis, TN, USA)

RSV is the most common cause of respiratory illness hospitalization in infants in the developed world. Of the entire birth cohort, 70% is infected within the first year of life and about 1% of the birth cohort is hospitalized for within the first year of life. RSV causes a significant burden of disease in immunocompromised hosts, those with underlying cardiopulmonary conditions and the elderly. Several RSV antivirals exist with documented in vitro and in vivo effects, but the capability of these experimental antivirals to reduce RSV loads and clinical disease is uncertain in key patient groups. In a study comparing the viral dynamics and clinical manifestations in human experimental infection models of influenza and RSV, healthy volunteers were inoculated intranasally with wild-type GMP-manufactured RSV-A or influenza A/H3N2. Volunteers were observed within a quarantine unit for 7–8 days (influenza) or 11–12 days (RSV) depending on symptoms or positive rapid test. For precaution, all volunteers inoculated with influenza received oseltamivir starting on day 6 post-inoculation.

The incubation period differed and was on average 3.5 days for RSV and around 1 day for influenza.
Infectious RSV load and symptom scores tracked very closely together, whereas in experimental influenza infection the symptom scores continued to rise for 2 days after the viral load fell. The same pattern was observed for nasal mucus production, as experimental RSV infection produces greater amounts of nasal mucus than influenza. Dr DiVincenzo summarized that finding a correlation between disease severity and viral load in this model was encouraging and suggested that an antiviral-mediated reduction of RSV load might be expected to reduce RSV disease.

Bocavirus (Olli Ruuskanen, Turku University, Turku, Finland)

Human bocavirus (HBoV) was identified 5 years ago in Stockholm by systemic molecular virus screening [45]. The new virus was found in 3% of the mucus samples; subsequent studies have shown an average prevalence between 5% and 10% in symptomatic persons. However, frequent concomitant respiratory viruses have been detected with HBoV, raising the issue of the contribution of HBoV to illness. Dr Ruuskanen presented data from a study to determine the aetiology of the common cold in children. In 92% of cases, ≥1 possible causative agent was identified [46]. Within the group of cases with ≥1 positive test, HBoV was found in 14% of cases, and in 80% of these cases HBoV was identified together with other viruses, most commonly HRV. HBoV has been found in approximately 10% of bronchiolitis cases, being the third most common agent detected after RSV and HRV. The prevalence of HBoV was approximately 15% in asthma exacerbations and 12–18% in childhood pneumonia. A study of acute wheezing in 259 children found HBoV in 19% of cases, being the third most common cause after RSV and HRV [47]. Overall, 12 (5%) cases were solely positive for HBoV, 8 cases with bronchiolitis, 3 with recurrent wheezing and 1 with acute asthma. HBoV PCR was positive in 53% of 43 acute serum samples and in 19% of convalescent serum.

Dr Ruuskanen noted the difficulties concerning the actual pathogenicity of HBoV as coinfections are very frequent, low copy number infections are common, difficulties exist in culturing the virus and no animal model exists. HBoV has also been often detected in asymptomatic individuals. Serological tests for HBoV on the acute and convalescent serum samples of the patients included in the acute wheezing study showed that 43% of the samples had evidence of a past infection and 19% had evidence of acute infection. Of 28 cases with high-load HBoV, 96% had serological evidence of acute infection compared with 38% with low HBoV load. Of the 34 patients that were NPA- and serum PCR-positive, 97% had a serodiagnosis and of the 39 cases coinfected with other virus 64% had a serodiagnosis [48]. A prospective study on 109 children collected serum samples every 2 months until they were 2 years of age and every 6 months thereafter. HBoV immunoglobulin (Ig)M and IgG testing found that primary immune responses were uncommon in children <1 year of age, but almost all children had primary infection between 1 and 4 years of age. By age 5 years, all children had been infected by HBoV, much like hMPV or RSV. Preliminary data suggests that that repeated infections occur. Dr Ruuskanen summarized that HBoV is a common cause of systemic respiratory infections in young children but is rare in adults, infections occur usually during winter months, and essentially all children are infected before 5 years of age. High copy numbers are found in NPA and serum, and HBoV should be included in multiplex PCR assays. Infection often occurs with other respiratory viruses and is associated with the common cold, acute wheezing, pneumonia, otitis media and diarrhoea.

GIS H1N1 microarray: accurate, low-cost, high-throughput method for biosurveillance and molecular epidemiology (Christopher W Wong, Genome Institute Singapore, Singapore)

Many relevant clinical questions need to be answered for pH1N1, including whether particular mutations correlate with disease severity or response to treatment. This group developed a resequencing microarray for pH1N1 screening and molecular epidemiology using 120,000 probes. An initial microarray was developed to investigate possible reassortment events between pH1N1 and seasonal H1N1, H3N2 (384,000 probes). Subsequently a smaller resequencing microarray was developed for high resolution mapping of the pH1N1 genome. This consisted of 121,928 oligos designed to provide a 2× coverage of the entire genome and up to 8× coverage for selected regions at 1 nucleotide resolution. If proficient, the entire process can be done in 2 days. In comparison to capillary sequencing, often considered the gold standard, the overall call rate was approximately 99% with an accuracy of the called nucleotides around 100%. The assay was field-tested in Mexico in July 2009, and the Ministry of Health and Ministry of Defence has used it in Singapore for influenza surveillance. The advantages of this technique are that it is easy to use, quick, and costs are <500 USD/virus sample analysed. The limitations of the technique are that it cannot get the first and last 14 bases of any sequence, nor can it deal with homopolymer regions. The technique is also not able to detect possible minority variants in a sample because the strongest signal of each nucleotide is selected for the final sequence.
Current research on respiratory viral infections

Findings from a randomized controlled trial of non-pharmaceutical interventions to reduce household influenza transmission in Bangkok, Thailand (Piyarat Suntarattiwong, Queen Sirikit National Institute of Child Health, Bangkok, Thailand) In an attempt to inform pandemic and seasonal epidemic response guidelines, a block randomized controlled trial of non-pharmaceutical interventions in households was undertaken. Following detection of influenza by a rapid diagnostic in a pediatric index patient presenting with ILI of <48 h duration, each household was randomized to undertake hand washing education or hand washing with additional face mask use. Control households were provided with advice on nutrition, exercise, and smoking cessation only. Nose and throat swabs were collected from patients and household members by nurses at recruitment and at home visits on days 1, 3 and 7. The primary outcome measure was PCR-confirmed influenza in a household contact.

Between April 2008 and August 2009, 442 children and their households (1,147 members) were recruited into the study. Following exclusion of 94 households where there was evidence of influenza in other members on enrolment, 348 households were studied. Of note, 91% of households reported that the index patient slept in the same room as the contacts, and 47% of index patients were children under 6 years of age. Participants in the hand-washing-only arm reported 4.6 hand washing episodes per day compared with 4.9 episodes per day in the hand-washing plus mask-wearing arm and 3.9 times per day in the control arm; wearing face masks was reported for a mean of only 180 minutes per day. At least one secondary infection occurred in 37% of households, and the overall secondary attack rate was 18% in contacts. The odds ratios for a secondary influenza infection were 1.16 (95% CI 0.71, 1.89) in the hand-washing-only arm and 1.38 (95% CI 0.85, 2.25) in the hand-washing and mask-wearing arm, compared with the control group. Increased risk of secondary infection was associated with index case age of 2–5 years, proximity to the index case and younger age of household contacts. In this study setting, modest increases in hand washing alone or with face masking did not provide protection from secondary influenza infection, perhaps because young index cases tended to sleep in the same room as their parents, overwhelming any possible protective effects of the interventions.

Molecular analyses and changes of low pathogenic avian influenza viruses in Taiwan (Chwan-Chuen King, Institute of Epidemiology, College of Public Health, National Taiwan University, Taipei, Taiwan) Sequence analysis was used to compare all eight segments of H5N2 isolates from Taiwan with several avian influenza viruses obtained from around the world and also H5 viruses obtained from nearby Hong Kong and mainland China, via the NCBI Influenza Virus Resource Database. All H5N2 viruses isolated from healthy ducks during 2005 and 2006 were recognized as low pathogenic viruses, because only monobasic amino acid was identified at the HA cleavage site. The receptor binding site also differed significantly from the 1997 Singapore H5N3 virus. Phylogenetic analysis suggested that possible gene reassortment occurred between Eastern Chinese and Taiwanese local viral strains. In part because of the multiple reassortments detected in the H5N2 viruses from Taiwan, continued virological surveillance of avian influenza viruses is recommended, focusing on samples from wild birds and those obtained from live-bird markets.

Influenza vaccine studies

Immunogenicity of one dose of AS03B-adjuvanted H1N1 2009 vaccine in children 6–35 months – preliminary report (Paul Gillard, GlaxoSmithKline Biological, Wavre, Belgium) Dr Gillard presented results of a study evaluating the immunogenicity and reactogenicity/safety of an AS03-adjuvanted vaccine against pH1N1 in young children. In adults the use of AS03, a tocopherol oil-in-water emulsion-based adjuvant system, can significantly enhance immune responses to H5N1 vaccines. Studies with monovalent, non-adjuvanted pH1N1 vaccines indicated that one dose appeared to be sufficient to induce protective immunity in healthy adults. Young children, however, only have had limited exposures to influenza A viruses and are often not immunologically primed, which generally results in inferior immune responses to seasonal influenza vaccines. Two doses of seasonal vaccine are recommended for previously unvaccinated children of 6 months to 8 years of age. In an open-label randomized study, healthy Spanish children aged 6–35 months received two doses (21 days apart) of a split-virion AS03-adjuvanted inactivated A/California/7/2009 (H1N1) vaccine. Participants were stratified into three age strata (6–11 months, 12–23 months and 24–35 months) and received either a half dose of the vaccine (1.9 µg HA and AS03 B [5.93 mg tocopherol], n=104) or the full dose (3.75 µg HA and AS03 A [11.86 mg tocopherol], n=53). At 21 days following the first dose of AS03 B-adjuvanted vaccine (1.9 µg HA), the percentage of children with haemagglutination inhibition (HI) titres of ≥1:40 against the vaccine strain increased from 3.0% to 100% (99% seroconversion rate). The geometric mean titre increased from 6 to 313 and after the second dose increased further to 2008. In both study groups, the HI responses induced in the three age strata were within the same range. The higher dose
AS03$_a$ vaccine (3.75 µg HA) elicits a similar immune response as the half dose AS03$_a$ vaccine. Neutralization data also indicated the production of biologically functional antibodies at titres ≥1:40 in the majority of vaccinees. After the first dose of AS03$_a$-adjuvanted vaccine, the most common adverse events reported were pain at the injection site (35.6%) and irritability (31.7%). Fever (axillary temperature ≥37.5°C) was reported in 20.2%. After the second dose, reactogenicity tended to increase (injection site pain 41.3%, irritability 46.2% and fever ≥37.5°C 67.3%). These reactogenicity rates are substantially higher than reported with non-adjuvanted seasonal vaccine. Dr Gillard concluded that a first dose of AS03$_a$-adjuvanted A/H1N1/2009 vaccine containing 1.9 µg HA in children 6–35 months old was highly immunogenic, potentially affording early onset of protection, and that the overall reactogenicity profile following administration of a single dose of the AS03$_a$-adjuvanted A/H1N1/2009 vaccine was clinically acceptable.

Immunity in adults following vaccination with influenza A/H1N1 2009 vaccine formulated with and without AS03$_a$ adjuvant (Geert Leroux-Roels, Center for Vaccinology, Ghent University and Hospital, Ghent, Belgium)

Dr Leroux-Roels presented results of two studies comparing an AS03$_a$-adjuvanted pH1N1 vaccine with unadjuvanted vaccine. The first study compared the adjuvanted vaccine (3.75 µg antigen per dose) with the unadjuvanted vaccine containing a fourfold higher dose (15 µg antigen per dose), and the second study compared the adjuvanted vaccine with the unadjuvanted vaccine containing the same antigen dose (3.75 µg). All enrolled individuals were given two doses of an egg-derived, split vaccine produced from A/California/4/2009 in the deltoid muscle at 21-day intervals. In both studies, approximately 65 subjects were included in each arm. In the first study 11.2% and 18.2% of subjects already had HI antibody levels exceeding 1:40 at baseline. After administering one vaccine dose, seroprotection rates at 21 days were 94% in the unadjuvanted vaccine compared with 100% in the adjuvanted arm, and the seroconversion factors were 28.7 and 38.1 in the unadjuvanted and adjuvanted arm, respectively. A second dose of adjuvanted vaccine induced a clear boosting effect and a rise in seroconversion factor to 72.9, but no such boosting was seen following a second dose of unadjuvanted vaccine. In the second study administration of low-dose unadjuvanted vaccine resulted in a seroprotection rate of 73.0 whereas the rate was 93.8 following the adjuvanted vaccine, and differences in seroconversion factor following a second dose were again noted (17.8 versus 71.9, respectively). In the first study, an increase in activated CD4$^+$ T-cells was observed after stimulation with either the split vaccine or overlapping HA peptides. The inability to boost with the second dose in the unadjuvanted arm was also observed at the T-cell level, as the frequency of HA-specific CD4$^+$ T-cells was much lower and there was no beneficial effect of a second dose in the unadjuvanted arm.

The combined safety analysis found that in both studies approximately 90% of the 3.75 µg AS03$_a$-adjuvanted vaccine recipients reported pain at the injection site compared with 30–35% of those in the unadjuvanted 15 µg dose of study 1 and 25% in the 3.75 µg unadjuvanted vaccine group in study 2. Redness and swelling were only reported in a small number of recipients of the adjuvanted vaccine with a slight increase after the second dose. More systemic adverse events were reported by participants from the adjuvanted arm compared with the unadjuvanted arm, mainly fatigue, headache and muscle aches. Dr Leroux-Roels summarized that the results showed that a single dose of AS03$_a$-adjuvanted vaccine induced HI responses that exceeded European Medicines Agency Committee for Medicinal Products for Human Use criteria, induced a greater HI response than an unadjuvanted vaccine formulated with the same antigen content, and induced a greater CD4$^+$ T-cell response than an unadjuvanted H1N1 vaccine containing 4× the antigen content. The local reactogenicity was higher in the adjuvanted vaccine than in the non-adjuvanted vaccine and the incidence of general adverse events was slightly higher in the adjuvanted vaccine.

Nationwide in-school influenza vaccination: 3-year experience in Taiwan (Hsu-Sung Kuo, Taiwan Centers for Disease Control, Taipei, Taiwan)

Taiwan has conducted an NISIV programme since 2007. One dose of seasonal vaccine was offered to each student between 2007 and 2009. One and two doses of pandemic vaccine were offered to each student in grades 1–3 and 4–12, respectively, during 2009. For seasonal influenza vaccine, uptake rose from 67% in 2007 to 79% in 2009. After the first 4 weeks of the pandemic vaccine programme, uptake was 70%. Surveillance estimated that 0.63 million children (2.7% of the Taiwanese population) were infected with pH1N1 in 2009 and >50% of those attending outpatient and emergency departments with ILI during the peak period were school-age children. Influenza activity peaked after the start of the NISIV programme and declined for 6 consecutive weeks. The ILI visits of school-age children decreased by 77% and visits by the rest of the population by 28%, during the same 6-week period. The existing seasonal influenza programme facilitated rapid integration of pandemic vaccination in school-age children, and the NISIV programme is thought to have played a key role in mitigating the pandemic in Taiwan, both
by providing individual protection and also providing protection against infection for unvaccinated persons in the community.

New vaccine approaches for influenza vaccines
(Chi-Huey Wong, Academia Sinica, Taipei, Taiwan and The Scripps Research Institute, La Jolla, CA, USA)

New influenza vaccine approaches include designing more effective molecule-based constructs and using better adjuvants. For example, mice immunized using DNA vaccines from consensus HA of H5 viruses were generally well-protected by such vaccines on challenge with a lethal dose of live H5 virus. The sequences of different H1N1 viruses also show a high percentage of sequence identity, and monoglycosylated HA as a vaccine shows cross-protection among the different human H1N1 viruses.

The HA protein is a trimer and most of influenza HAs have 5 to 9 N-linked glycosylation sites that are highly conserved. Host cell receptor binding is affected by the glycans on HA, and the effect of removing these glycans is important to study, because glycosylation also plays a major role in protein folding and structure stabilization and therefore the intact tertiary structure may be key for immunization. Dr Wong presented results in which four HA types were investigated for the effect of HA glycosylation on HA binding. The four HA-types were fully glycosylated HA, neuraminidase-treated HA, high mannose type HA and monoglycosylated HA. The results showed that monoglycosylated HA bound more tightly to the receptor measured by glycan array, suggesting that monoglycosylated HA might be the best for vaccination. Using a glycan array it is possible to determine the contribution of each glycan to the overall binding profile. Removal of all sugars through site-directed mutagenesis showed that position 27 is crucial, as HA binding was abrogated when asparagine was changed to glutamine. In mice immunized with these four different HAs, the monoglycosylated HA elicited better responses measured by neutralization activity, cross-reactive ELISA responses and protection of mice after challenge with virus.

The subtype binding preferences vary across different HAs, such that seasonal H3N2 has a very different binding preference compared with seasonal H1N1 and pH1N1. Although A/Brisbane/59/07 seasonal H1N1 appears to be very similar to pH1N1, the association constants are quite different. A seasonal H1 protein vaccine, adjuvanted with alum, provided serum HI and neutralization titres that were higher for the monoglycosylated forms, compared with the fully glycosylated HA. When mice were challenged with a swine strain, improved survival was observed for the mice vaccinated using the monoglycosylated HA compared with fully glycosylated HA. Cross-protection against heterologous H1N1 viruses was also observed with the monoglycosylated HA. With a consensus H1 HA vaccine, monoglycosylated HA again showed a more protective effect in mice compared with fully or non-glycosylated HA. The three-dimensional HA structure indicated that two glycosylation sites (at positions 142 and 177) were close to the receptor binding surface. In the case of a N177Q mutation, a strong increase in serum HI titres, comparable to the monoglycosylated forms, was observed, indicating that this position is very important for receptor binding and protection.

Recognition of influenza virus by broadly neutralizing antibodies: implications for therapeutic vaccine design (Ian A Wilson, The Scripps Research Institute, La Jolla, CA, USA)

With regard to antibody recognition and antigenicity of influenza viruses, essentially all of the natural variation and escape mutants are located in the HA1 head. Neutralizing antibodies are isolate-specific and normally prevent viral entry by blocking receptor binding and are somewhat restricted by a glycan shield. However, conserved epitopes can be recognized by antibodies to influenza HA. For example, using recombinant HAs and a phage display library, scientists from Crucell, Leiden, the Netherlands, produced a number of antibodies that recognized HAs from H1 and H5 subtypes and, remarkably, all the clones isolated used the V_{H} 1-69 gene [49]. The cross-subtype neutralization profile was restricted in that they inhibited almost all HA subtypes in group 1 influenza viruses, but none from group 2. The crystal structures of the 1918 HA and the H5N1 HA with the antibody CR6261 showed that the antibodies bind to the stalk region, which is substantially different from what has been seen with almost all other HA-directed antibodies that bind in the head region [50]. A second feature is that the antibody binds to the HA with only the heavy chain. The method of neutralization was investigated by testing the protease susceptibility of HA as a function of pH, with and without CR6261. When trypsin was added to the HA trimer alone, it started to degrade below pH 5.5, indicating that the conformational change was occurring at low pH. After addition of the antibody, no degradation was observed, indicating that these antibodies are stabilizing the HA and preventing the pH-induced conformational change. According to analysis of the GenBank database, the epitope is highly conserved (approximately 99% for each residue in HA2) and that it probably correlates with a conserved function, such as membrane fusion, and acts later in the entry process than most antibodies. Dr Wilson stated that this is a fascinating prospect for structure-based vaccine design and strategies can be developed to design immunogens to elicit broadly neutralizing antibodies. Among possible approaches, modifications can be made...
to the HA presenting the epitope of interest and scaffolding proteins can be used for antigen presentation. The crystal structure of the pH1N1 HA showed that like other H1 viruses it has four major antigenic sites (called, Sa, Sb, Ca and Cb) [51]. The antigenic surface is very similar to the 1918 H1N1 but differs from later seasonal and vaccine strains. During the evolution of the antigenic surface of H1 HAs over the years a gradual masking of the surface by a glycan shield can be observed, starting from no glycosylation sites in the 1918 virus up to three glycosylation sites that block recognition by antibodies over the years. The 1918 virus remains the most closely related to the pH1N1 HA, whereas most other H1 viruses up to 2007 differ at approximately 40% of the positions along the HA sequence. Consequently, individuals exposed to a 1918-like virus may have antibodies targeting the Sa site that would then be able to cross-react with the pH1N1 HA. Antibodies from individuals born before 1915 react primarily with 1918 HA and clones isolated shortly thereafter, but show little reactivity with post 1930-HAs up through 2007 [52]. One of the isolated antibodies (2D1) exhibited strong binding to both 1918 and 2009 California-04 HA, but not to H1N1 PR8/34 or HAs of other influenza subtypes [53]. The 2D1 antibody neutralizes both 1918 and pH1N1 in vitro and protects mice from a lethal challenge with either virus in vivo. Dr Wilson summarized that this data suggests that people who were exposed to viruses similar to 1918 in the first part of the 20th century may have cross-protective antibodies and be largely immune to the pH1N1 virus.

Seasonal influenza vaccine and protection against pandemic influenza A (H1N1) virus infection among US military personnel (Matthew C Johns, Armed Forces Health Surveillance Center Maryland, Silver Springs, MD, USA)

The US DoD has a comprehensive monitoring system that allows for ongoing, systematic VE assessments. To provide for a system that gives accurate interim effectiveness outcomes, a population-based surveillance of recruits was started in 2003 and continues at eight training centres. Since it is prospective, the monitoring allows for dynamic assessments of VE. However, military recruits are a highly immunized group, and the VE might be overestimated if many influenza cases occur early (within first 14 days of training). Furthermore, an expanded capacity to evaluate VE among both active duty and beneficiary populations, for illness severity measures, and against influenza viruses circulating in different regions of the world is needed. Using a case-control approach, separate analyses examined 2008–2009 and 2009–2010 seasonal VE against pH1N1-associated clinical end points. Among 1,205 cases matched to 4,810 controls, 2008–2009 seasonal VE, adjusted for age group, sex and prior documented vaccination, was estimated at 45% against pH1N1. Within the different age groups, an unexpected U-shaped relationship was found for VE, such that the point estimate for VE was 50% in the 17–24-year-olds, -6% in those 25–29 years, 9% in those 30–39 years and 55% in the ≥40 years age group. No statistically significant difference was noted between trivalent inactivated vaccine (TIV) and live attenuated influenza vaccine recipients. When pH1N1 hospitalizations were analysed as a marker of severity, VE increased to 62%, indicating that the seasonal vaccination in 2008–2009 gave some protection against pH1N1-associated illness. For the 2009–2010 seasonal vaccine, VE among 1,382 cases matched to 5,520 controls was lower at 22%. Thus, the DoD populations and systems offer an opportunity for rapid assessment of influenza VE in young and middle-aged adults.

Effectiveness of 2008–2009 influenza vaccines in preventing healthcare visits with laboratory-confirmed seasonal and 2009 pandemic influenza A (H1N1) influenza infections (David K Shay, Influenza Division, US Centers for Disease Control and Prevention, Atlanta, GA, USA)

The effectiveness of 2008–2009 seasonal influenza vaccines in preventing healthcare visits with seasonal and pH1N1 influenza infections was investigated in a case-control study conducted from December 2008 until August 2009 in patients seeking care for ARI of <8 days duration. Vaccine recipients were categorized as immunized starting the 14th day after vaccination. Based on testing specimens of nasopharyngeal (>13 years) or nasal swabs (children 6 months to 12 years) by RT-PCR, cases were patients positive for influenza RNA and controls were those who were negative. Among 6,507 participants, 4,221 were from the seasonal influenza period (until 30 April 2009) and 2,286 were from the pH1N1 period (1 May 2009 until 31 August 2009). The adjusted VE (95% CI) for seasonal influenza A among 337 cases and 3,884 controls was 49% (27, 65) for those aged 8 months to 8 years, 37% (5, 58) for 9–49 years, 69% (37, 85) for ≥50 years and 47% (32, 59) overall. For seasonal B influenza, the adjusted VE in a total of 503 cases and 3,718 controls was 17% (-14, 40), 34% (6, 54), 36% (-63, 75) and 28% (10, 43) across these same age cohorts. Dr Shay noted that the data appeared to suggest that the vaccine was not effective in the youngest age group with the caveat that all the influenza B viruses that were characterized were of a lineage that was not present in the vaccine. For the pH1N1 virus the adjusted seasonal VE was -5% (-79, 39), -40% (-129, 14), 29% (-126, 78) and -14% (-58, 18) in the same age groups, respectively. The findings indicated that the immunization with 2008–2009 seasonal influenza vaccines offered...
Key immunological observations from the clinical development of Sanofi Pasteur pandemic influenza A (H1N1) vaccines (Yanee Hutagalung, Sanofi Pasteur, Singapore)

The experience with AF03-adjuvanted vaccine programme for A/H5N1 demonstrated the effectiveness of antigen-sparing formulations. For pH1N1 vaccine the proposed strategy was to develop non-adjuvanted and AF03-adjuvanted split-virion inactivated vaccine candidates in parallel for adult, elderly and paediatric populations. Monovalent vaccines manufactured in Europe were the 3.8 μg HA adjuvanted vaccine (Humenza) and 15 μg HA non-adjuvanted (Panenza). Clinical trials enrolling >10,000 subjects showed that very strong antibody responses were observed after a single injection of AF03-adjuvanted or non-adjuvanted vaccine. After a single injection of non-adjuvanted 7.5 μg HA, approximately 95% of adults had an HI antibody titre ≥1:40 at 21 days after vaccination. Single doses of AF03-adjuvanted 3.8 μg or 7.5 μg HA or 15 μg non-adjuvanted vaccine in adults elicited large increases in geometric mean titre of HI and neutralizing antibodies. A trend toward better antibody responses was observed in the subset of adults with pre-existing antibodies to pH1N1. A second dose increased the antibody responses, especially in children. An enhancing effect of the AF03 adjuvant on immunogenicity was mainly observed in children <3 years. Dr Hutagalung summarized that a single dose of AF03-adjuvanted or non-adjuvanted vaccine elicited high antibody responses in most populations. In children <3 years of age, a single dose of AF03-adjuvanted vaccine induced a sufficient immune response, whereas 2 doses of non-adjuvanted vaccine were required.

Optimal protection by pandemic H1N1 vaccine requires adjuvantation in treatment-naive ferrets (Benoît Baras, GlaxoSmithKline Biologicals, Rixensart, Belgium)

A single vaccination with non-adjuvanted pH1N1 vaccine is sufficient to immunize healthy adults. However, only 50% of infants (6–35 months old) and 75% of children (3–9 years old) were seroprotected with a single non-adjuvanted vaccination [54]. Dr Baras investigated the immunogenicity and protection against a non-lethal pH1N1 challenge induced by an inactivated split candidate vaccine in treatment-naive ferrets. The H1N1/California/7/09 HA dosages tested in this study were 15 μg non-adjuvanted versus 3.75 μg versus 1.9 μg adjuvanted with AS03_A (which contains 11.86 mg α-tocopherol) or AS03_B which contained 5.93 mg α-tocopherol). Ferrets received two immunizations on days 0 and 21 or a single immunization at day 21. On day 49, ferrets were challenged intratracheally with 10^6 50% tissue culture infective dose of pH1N1 A/The Netherlands/602/09, which contains four amino acid differences in comparison to the used vaccine strain. Neutralizing antibodies were observed in all groups immunized with AS03-adjuvanted vaccines with a response rate of 64% after a single dose and 100% after two. The ferrets immunized with AS03-adjuvanted vaccines had lower percentages of affected lung parenchyma, lower lung weights and lower pulmonary virus loads compared with ferrets immunized with non-adjuvanted vaccine at day 4 post-challenge. No virus was detected in the lungs with two doses of AS03-adjuvanted vaccine, but virus was detected in 27% (3/11) of ferrets receiving a single dose. In addition, 24% immunized with AS03-adjuvanted vaccine shed virus from the upper respiratory tract compared with 83% in control groups. Viral replication with the adjuvanted vaccine correlated with the presence of higher humoral immune responses in treatment-naive animals, and the data confirmed an antigen-sparing approach with AS03.

Seasonal and pandemic influenza virus attack rates in a randomized placebo-controlled trial of seasonal influenza vaccine (Sophia Ng, School of Public Health, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, People's Republic of China)

Dr Ng investigated whether influenza vaccination of children could confer indirect benefit to their household members in a developed urban setting. The study design was a cluster-randomized, placebo-controlled, double-blind study in households including ≥1 child aged 6–15 years eligible to receive the seasonal TIV. A pilot study in 2008–2009 consisted of 119 households and the main study from 2009–2010 consisted of 796 households. The households were randomized in a ratio 3:2, and one child in each household received either one dose of TIV or saline. Daily symptom diaries were combined with biweekly telephone calls, and home visits were triggered by ≥2 signs or symptoms of acute respiratory illness. As expected, the proportion with HI antibody titres ≥1:40 after 1 month was significantly higher in the vaccine than control group. In the vaccinated children a trend towards lower infection rate was observed for seasonal H1N1 (P=0.07), but no statistically significant differences in influenza infection rates were observed for household contacts. Dr Ng summarized that there was no substantial antibody response against pH1N1 in children following receipt of trivalent influenza vaccine. Recent infection (within 6–9 months) with seasonal H1N1 virus infection appeared to cross-protect against pH1N1, whereas seasonal vaccination by lowering the risk of seasonal infection might have
In healthy adults, disease usually is mild, but RSV in children and throughout life may be associated with airway reactivity lasting into adulthood. These viruses, and especially RSV, HPIV3 and HMPV, infect essentially everyone worldwide during infancy and early childhood. Approximately 1–3% of primary RSV infections result in hospitalization. In addition to causing exacerbations of asthma, RSV infection early in life may be associated with airway reactivity lasting through childhood, and controversy has been linked to initiation of asthma. These viruses, and especially RSV, also can re-infect symptomatically throughout life. 

Non-influenza vaccines

Status of vaccines for respiratory syncytial virus, parainfluenza virus and human metapneumovirus (Peter Collins, National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA)

RSV is the most important viral agent of paediatric lower respiratory tract disease worldwide, followed by human parainfluenza virus serotype 3 (HPIV3), human metapneumovirus (HMPV) and HPIV1 and 2. These viruses, especially RSV, HPIV3 and HMPV, infect essentially everyone worldwide during infancy and early childhood. Approximately 1–3% of primary RSV infections result in hospitalization. In addition to causing exacerbations of asthma, RSV infection early in life may be associated with airway reactivity lasting through childhood, and controversy has been linked to initiation of asthma. These viruses, and especially RSV, also can re-infect symptomatically throughout life. In healthy adults, disease usually is mild, but RSV in particular is an important cause of morbidity and mortality in immunosuppressed individuals and the elderly. The peak of hospitalization for paediatric RSV disease is 2 months of age. Therefore, vaccination must begin early in life.

RSV vaccine development has been underway since the 1960s, and there has been ongoing research to develop RSV protein vaccines. The most active research to develop paediatric vaccines against RSV, HPIVs and HMPV at the present time involves live-attenuated strains for intranasal immunization. In addition to avoiding possible disease enhancement, this approach has the advantage of inducing local respiratory tract immunity as well as systemic immunity. Also, the topical route of administration is less subject to immunosuppression by maternal antibodies. By contrast, attenuating the replication of these viruses reduces their immunogenicity, and therefore the balance between attenuation and immunogenicity much be carefully evaluated. Live vaccines are presently being developed using reverse genetics, whereby complete infectious virus is produced entirely from transfected plasmids. This provides a method for introducing desired changes into a live virus using a clean source of virus, as DNA is a stable seed. Reverse genetics can be used to develop well-characterized attenuating mutations that can be introduced in desired combinations to adjust the level of attenuation. The present vaccine candidates involve several types of attenuating mutations. More stable attenuating mutations can be developed by stabilizing amino acid substitutions through codon optimization. In addition, it sometimes is possible to delete rather than substitute the codon in question. Stable attenuation can be achieved by deleting entire genes encoding accessory proteins, such as the NS1, NS2, SH and M2-2 coding sequences of RSV. Another strategy involves host range restriction, as RSV, HPIV3 and HMPV have related animal viruses that are attenuated in primates. Genes encoding internal proteins in the human viruses can be replaced with their counterparts from the related animal viruses to combine host range restriction with the surface neutralization antigens of the human virus. Dr Collins noted that the present vaccine candidates involve substitutions and deletions that attenuate by a variety of mechanisms, including effects on viral RNA synthesis, inactivation of viral IFN antagonists, host range restriction and temperature-sensitivity that restricts viral replication in the lower, warmer respiratory tract.

Five recombinant RSV vaccines have been tested clinically, and one promising candidate has been identified. This RSV vaccine has five attenuating elements, namely five cold passage mutations as one attenuating element, deletion of the SH gene as a second, and three independent temperature-sensitive mutations. In 1–2-month-old infants, this vaccine virus replicated to moderate titres and was well tolerated and immunogenic. However, there was evidence of genetic instability evidenced by the finding that some specimens of shed vaccine virus had lost one or another of two temperature-sensitive mutations. These point mutations had not been previously stabilized by codon optimization, and current research is underway to achieve this. Currently this vaccine candidate is being evaluated further in phase I and II studies to investigate efficacy in young infants, which will be a critical proof of principle. Among other RSV vaccine candidates for clinical evaluation, one involves deletion of the M2-2 open reading frame, and a second involves deletion of the NS1 gene. Another approach is the use of a bivalent vector, in which a chimeric virus contains the bovine PIV3 backbone combined with the HPIV3 F and HN genes as well as the RSV F and G genes. Via this approach two needed vaccines could be combined and the poor growth of RSV is overcome. Dr Collins noted that vaccine candidates for all of three viruses are in clinical trials.

Antivirals and therapeutics

A community cluster of oseltamivir-resistant influenza H1N1 2009 cases (Peter Horby, Oxford University Clinical Research Unit, Hanoi, Vietnam)

Although oseltamivir-resistant pH1N1 remains rare and onward transmission is not a widespread
phenomenon, the emergence of resistant pH1N1 remains a significant concern, particularly when one considers the rapid increase in oseltamivir resistance that was seen in seasonal H1N1 influenza prior to 2009 and in the absence of selective drug pressure. Furthermore, the acquisition of the H275Y mutation conferring oseltamivir resistance in seasonal H1N1 was not associated with fitness costs affecting transmissibility or ability to cause significant illness. Therefore, it is important to conduct cluster analysis following any reported cases of oseltamivir-resistant pH1N1 that may be associated with onward transmission.

In July 2009, 10 students socialized together in the same carriage during a 42-h train journey in Vietnam. ILI or contact with known cases of influenza in the weeks prior to the journey did not occur and none of the students knew each other prior to the trip, nor were they taking oseltamivir. Four students developed a febrile illness within 12 h of arriving at their destination and another two students developed febrile illness within 48 h. A person in a different carriage also developed a compatible illness. In samples taken prior to commencing oseltamivir treatment, pH1N1 infection was confirmed by PCR in all seven symptomatic individuals. Neuraminidase inhibitor resistance due to the H275Y mutation was found and confirmed through neuraminidase inhibition assays. Six cases were admitted to hospital on public health grounds and one case was isolated at home. All of the patients recovered uneventfully, but viral RNA was still detectable in day-9 samples taken from the patient infected with the virus demonstrating the highest degree of resistance. The index cases and additional cases were not identified, despite an extensive public health investigation. However, it is likely that six individuals had contact with the index case and were infected with a transmissible, oseltamivir-resistant pH1N1 virus [55].

A review of clinical experience in the treatment of severe 2009 H1N1 influenza with intravenous peramivir under an emergency investigational new drug programme in the United States (Jaime Hernandez, BioCryst Pharmaceutical, Inc., Durham, NC, USA)

The investigational neuraminidase inhibitor peramivir is currently in Phase III trials for the treatment of influenza in hospitalized patients. During the 2009 pandemic, intravenous peramivir was made available in the US under FDA emergency authorization regulations, for the treatment of seriously ill patients infected with pH1N1. Prior to this, peramivir had also been issued to 42 patients as part of a compassionate use programme, 31 of whom had clinical data, including information on outcomes, available. The majority (58%) had no underlying illnesses, although four patients had obstructive lung disease, two diabetes mellitus, three solid organ or stem cell transplants, three history of cancer and three were pregnant or post-partum. The median age was 24 years (range 0.3–77). Clinically, 97% had pneumonitis with respiratory failure, 50% required vasopressor support and 38% had evidence of renal impairment. Treatment with peramivir was delayed by 1 to 15 days from admission.

The case of a 44-year-old female who had recently undergone autologous bone marrow transplant for multiple myeloma was described. Rapidly progressive pneumonitis ensued following admission with febrile neutropaenia and twice daily oral oseltamivir 150 mg was commenced on day 3, with the addition of oral amantadine and ribavirin on day 4 of admission. On day 6, the patient also received intravenous Ig and inhaled ribavirin. Viral RNA was still detectable by PCR in BAL fluid, plasma and stool at day 14, when intravenous peramivir 600 mg once-daily was commenced and oral oseltamivir was continued at a dose of 75 mg twice daily for 10 days. By day 24 of admission, viral RNA was still detectable in BAL fluid but viral culture was subsequently negative. The patient was successfully extubated and discharged home on day 29. Clinical improvement was seen prior to recovery of the blood lymphocyte count.

Overall, 5 of the 42 (12.5%) patients died despite antiviral treatment. Intravenous peramivir was reported to be well tolerated. While treatment with intravenous peramivir may have contributed to recovery in a number of these patients, the uncontrolled nature of the observations preclude definitive conclusions regarding effectiveness.

Oseltamivir treatment in H5N1 patients (Paul KS Chan, Chinese University of Hong Kong, Hong Kong SAR, People’s Republic of China)

Between 2003 and February 2010, 473 cases of H5N1 in humans had been reported across 15 countries, with approximately a 60% case-fatality proportion. A global H5N1 patient registry, funded by Hoffman-La Roche, was developed to describe clinical aspects of human cases of H5N1 including treatment responses and enhanced communication through aggregate data analyses. A retrospective observational study of 308 laboratory-confirmed H5N1 influenza cases from 12 countries was conducted, including detailed case publications from 110 patients in Asia [56]. The median age of infected individuals was 17 years (range 1–75), and the overall crude mortality was 56%. Similar to severe pH1N1 viral pneumonia, symptoms suggestive of lower respiratory tract involvement (cough and dyspnoea) were frequent in those who died. Chest infiltrates and/or acute pneumonia were associated with a crude relative mortality risk of 26.6 (95% CI 1.7, 410.3). Diarrhoea and vomiting were frequently reported, more so in those who died. Neurological involvement
was reported in 68 patients and psychiatric symptoms in 61 patients. Interestingly, rhinorrhea appeared to be more frequent in survivors (77% of survivors versus 23% of fatal cases). Of commonly measured laboratory parameters, raised alanine aminotransferase was found more frequently in those who died. Survival in patients who received oseltamivir was significantly higher across all age groups, compared with those who did not receive antivirals (71% versus 40% in under those <16 years of age, 50% versus 12% in those aged 16–34 years and 67% versus 18% in those >35 years of age, respectively; P<0.001 for all three groups). Survival benefit decreased when treatment was delayed by >2 days from onset of symptoms, although case fatality rates still remained lower in treated patients even when oseltamivir was commenced up to approximately 6 days after illness onset.

The beneficial effect of early delayed antiviral therapy is consistent with earlier observations showing protracted viral replication in such patients. These findings emphasize the importance of timely antiviral treatment in severe influenza but also indicate that some H5N1 patients will progress to fatal outcome despite relatively early oseltamivir therapy. Whether more potent antiviral regimens or other therapies like immunomodulators might improve outcomes remains to be determined.

Inhaled CS-8958: a long-acting neuraminidase inhibitor for the treatment of influenza (Makoto Yamashita, Daiichi Sankyo Co. Ltd, Tokyo, Japan)

CS-8958 (Daiichi Sankyo Co. Ltd, Tokyo, Japan) is a prodrug of the long-acting neuraminidase inhibitor, laninamivir (R-125489). Laninamivir has demonstrated in vitro antiviral activity against influenza A and influenza B viruses, including HPAI H5N1 virus and oseltamivir-resistant strains of seasonal H1N1, H3N2 and HPAI H5N1. Inhaled CS-8958 has been administered as a single, one-off dose for treatment and potentially once-weekly for prophylaxis. The active metabolite, laninamivir, is tightly bound to the neuraminidase and demonstrates long retention in the lungs. In murine studies, single intranasal dosing of CS-8958 demonstrated improved survival at 20 days compared with oseltamivir, in mice infected with A/PR8 virus or HPAI H5N1 virus. Significant decreases in lung viral titre were seen following administration of CS-8958 in mice infected with pH1N1 at days 3 and 6 compared with mice treated with oseltamivir or zanamivir. CS-8958 has been assessed in randomized controlled trials for use in adults and children. The Multinational Asian Clinical Research for Influenza Virus Extermination on Long Acting Neuraminidase Inhibitors (MARVEL) study assessed the efficacy and safety of CS-8958 20 mg and 40 mg compared with oseltamivir phosphate in approximately 1,000 adult patients with influenza A or B infection [57]. Non-inferiority (primary end point was alleviation of influenza illness) to oseltamivir was demonstrated with both doses; 40 mg CS-8958 had superior efficacy compared with 20 mg. Both doses were reported to be well tolerated. The study also compared the same doses of CS-8958 with 2 mg oseltamivir given twice daily for 5 days in 180 paediatric subjects. CS-8958 was reported to have superior efficacy over oseltamivir, particularly in children infected with oseltamivir-resistant seasonal H1N1 virus, and was well tolerated [58]. A new drug approval application has been recently approved in Japan.

Interferon-β development for exacerbations of asthma and chronic obstructive pulmonary disease (Sebastian Johnston, National Heart & Lung Institute, Imperial College London, London, UK)

Acute exacerbations of asthma and COPD remain a major cause of morbidity, mortality and healthcare expenditure. IFN-λ. production has also been shown to be deficient in primary bronchial epithelial (PBE) and BAL cells taken from asthmatics. In vivo, IFN-λ levels have been shown to be directly proportional to HRV viral load in BAL cells and inversely proportional to decrease in forced expiratory volume in the first second (FEV1). In vitro, PBE cells obtained from asthmatic subjects demonstrate significantly lower increases in IFN-β in culture supernatants and higher virus replication following infection with HRV, compared with PBE cells obtained from non-asthmatic subjects. In vivo, IFN-β levels were shown to be lower in COPD patients compared with controls following experimental HRV infection and viral loads were also higher in COPD patients. The Synairgen SG004 trial was a double-blind placebo-controlled single and multiple dose-escalating Phase I study to assess the safety and tolerability of inhaled IFN-β (IFN-β1a) in 40 controlled, moderate asthmatic subjects receiving regular inhaled corticosteroids. Study end points were safety and biomarkers of antiviral activity. Inhaled IFN-β was well tolerated and no effects on standard measures of lung function (percentage predicted FEV1, forced vital capacity and transfer factor of the lung for carbon dioxide) or inflammation (exhaled nitric oxide and sputum eosinophil counts) were seen. To assess lung antiviral biomarker responses to inhaled IFN-β, levels of neopterin, a macrophage-produced pteridine, were measured in sputum and were shown to increase in a dose-dependent manner at 24 h compared with placebo. Additionally, the IFN-β-dependent antiviral genes MxA, 2′-5′-oligoadenylate synthetase (2′-5′ OAS) and IFN inducible protein-10 were shown to be upregulated in lung cells from sputum following administration of inhaled IFN-β. Mid- and top-range
doses administered every 3 days were associated with transient loss of induction during treatment, but this was not seen with once-daily dosing using the top dose. In conclusion, the reported studies have demonstrated successful delivery of investigational inhaled IFN-β to the lungs, resulting activation of antiviral pathways in lung cells from asthmatic patients and no evidence of airway inflammation in vivo. Additionally, data suggest that antiviral response to IFN-β is maintained when once daily, high-dose administration is used, and Phase II trials are in progress.

Novel human monoclonal antibodies in the treatment of influenza (Jaap Goudsmit, Crucell NV, Leiden, the Netherlands)

In an attempt to address the limitations of annual multivalent seasonal influenza vaccination programmes, it is proposed that heterosubtypic neutralizing monoclonal antibodies (mAbs) may have a role. A novel class of mAbs has been developed by creating a phage library using IgM-positive CD27+ memory B-cells obtained from H1N1/H3N2-immune donors. This approach identified a potent antibody (CR6261) that was protective in mice when given before and after lethal H5N1 or H1N1 challenge [49]. In ferret studies, CR6261 mAb prophylaxis prevents clinical symptoms and death and reduces viral load in the lungs, following infection with H5N1 A/Indonesia/5/2005. Additionally, when given 4 h or 24 h post-intratracheal challenge, CR6261 mAb therapy was shown to prevent clinical symptoms and death, and to reduce viral load in the lungs in ferrets infected with H5N1 A/Indonesia/5/2005 [59]. CR6261 has a half-life in ferrets of 1.25 days (95% CI 0.94, 1.86) and 4.8 days in mice. When CR6261 was given 24 h prior to intranasal challenge with H1N1 A/WSN/33, survival was 100% in BALB/c mice given the highest dose (15 mg/kg).

To compare the effect of therapeutic CR6261 with therapeutic oseltamivir, in a separate study, BALB/c mice were infected with H5N1 A/HongKong/156/97 and were given either a single dose of CR6261 or commenced on a 5-day course of oseltamivir, 4 days post challenge. Overall, 100% of CR6261-treated mice survived at 20 days, but only 25% of oseltamivir-treated mice survived at 21 days, with a median survival time of 9 days [60]. Although capable of neutralizing a number of viruses within the group 1 H1 and H9 clades, because the glycan structure in a hydrophobic pocket prevents recognition by VH 1-69 antibody, CR6261 did not neutralize H3, H4 and H7 viruses within group 2. To address this, IgM-positive CD27+ memory B-cells were obtained from H1N1/H3N2 immune donors, immortalized and sorted by FACs and then subclones were selected, with final H3 and H7 clones being identified. mAbs CR8020 and CR8043 were shown to be capable to neutralize multiple phylogenetically distinct H3 strains, as well as H7 and H10 subtypes. In H3N2-challenged mice, prophylactic administration of these antibodies afforded 100% survival at 21 days, prevented weight loss and decreased clinical disease scores compared with control treatment. Survival was also seen following therapeutic administration of CR8020, given at the highest dose to mice 2 days after challenge with H3N2, and prophylactic CR8020 administration protected mice against lethal infection with H7N7.

Competition experiments using A/Wisconsin/67/2007(H3N2) showed that the selected mAbs competed with each other. These antibodies also blocked trypsin cleavage, suggesting that they exert an effect by binding to a region close to the trypsin cleavage site of HA. Escape mutants have been generated, but in the 10 wild-type H3N2 viruses assessed, such mutations were very uncommon; three-dimensional modelling of escape mutants is being undertaken to assess the mechanism. The influence of variability in HA2 region around escape mutants is also being assessed. In closing, Dr Goudsmit stated that mutual exclusivity of HA2 antibodies neutralizing either H1 or H3 clades occurs and cluster 2 mAbs define a second conserved HA epitope key to virus replication, and that such observations have important implications for influenza vaccine development strategies.

Triple-therapy with amantadine, ribavirin and oseltamivir to treat oseltamivir-resistant influenza

A infection in mice (Jack Nguyen, Adamas Pharmaceuticals Inc., Emeryville, CA, USA)

Triple combination antiviral drug (TCAD) therapy for the treatment of influenza may potentially improve efficacy, improve the spectrum of antiviral activity and reduce the emergence of antiviral resistance. The combination of amantadine, ribavirin and oseltamivir incorporates three drugs with three different mechanisms of action. In vitro, TCAD has been shown to display greater synergy against H1N1, H5N1 and H3N2, compared with different pairs of the constituent agents [61]. Although double combinations are additive or modestly synergistic, TCAD has greater synergy than double combinations, and amantadine contributes to the synergy of TCAD against amantadine-resistant (S31N) pH1N1 viruses [62]. In terms of the antiviral effect against pH1N1/09, TCAD decreases the 50% effective concentration 11-fold, compared with oseltamivir monotherapy. Against oseltamivir-resistant (H274Y) seasonal H1N1 viruses, oseltamivir contributes to the synergistic action of TCAD. In studies of BALB/c mice infected with 100 50% lethal dose of H5N1 (A/duck/MN/1525/81), TCAD commenced 24 h post-challenge was associated with significantly improved survival (approximately 90% at 21 days), compared with
double combinations or therapy with single agents and placebo (100% of placebo-treated mice died by day 5). Amantadine/ribavirin and oseltamivir/amantadine combinations both resulted in approximately 60% survival at 21 days. In a similar study using pH1N1/09 challenge, TCAD was associated with >90% survival at 21 days, compared with 60% survival in ribavirin/oseltamivir-treated mice and 20% survival in mice given oseltamivir monotherapy. In vitro resistance studies using serial passage of seasonal H1N1 (A/Hawaii/31/07), at fixed concentrations of drugs, found that treatment with amantadine or combined aman-
tadine/oseltamivir resulted in the breakthrough of amantadine-resistant variants. By contrast, in the pres-
ence of TCAD, 30–40% virus variants had resistance-associated mutations in M2 (V27A, A30T or S31N). In summary, in vitro and animal studies suggest that amantadine/ribavirin/oseltamivir TCAD represents a broad spectrum anti-influenza therapy with the poten-
tial to overcome pre-existing resistance while imposing a high genetic barrier to the emergence of resistant virus strains. An open-label, randomized Phase II study is currently enrolling immunocompromised patients, with the aim to assess safety and compare the efficacy of TCAD against oseltamivir monotherapy [62].

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