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Meeting Report

XIth International Symposium on Respiratory Viral Infections

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Introduction

The X1th International Symposium on Respiratory Viral Infections (ISRVI) was held in Bangkok, Thailand on 19–22 February 2009. This annual meeting convenes noted public health specialists, vaccinologists, clinicians, virologists, and pharmacologists to enable interdisciplinary discussion regarding all aspects of respiratory virus research. Program topics included virus evolution, transmission, pathogenesis, epidemiology and surveillance strategies, and advances in antiviral and vaccine design for several respiratory viruses of concern, including influenza, rhinovirus, and adenovirus, among others. Here, we provide a topical overview covering areas of ongoing focus and research in the field of respiratory viral infections, with particular attention given to the advances and contributions presented by conference participants. The emergence of common themes and goals presented by subject matter experts during the symposium exemplifies the need for continued collaboration across disciplines and illustrates the potential for progress regarding the understanding, management, and prevention of respiratory viral infections.

Transmission of respiratory viruses

The transfer of virus from an infected individual to a susceptible recipient is essential for the spread of respiratory viral pathogens. However, there are many potential modes of respiratory virus transmission, and despite decades of study, the relative contribution of different routes of transmission remains unclear. A keynote address by Robert Couch of the Baylor College of Medicine, and subsequent presentation by Arnold Monto of the University of Michigan, addressed the need to more accurately define and understand the transmission of respiratory viruses. This knowledge is especially important given the implications of this data for the development of control policies in the event of an outbreak or pandemic of a recognized or novel respiratory virus.

In general, respiratory viruses may be spread by contact (either by direct physical contact or indirectly by the transfer of virus from fomites or contaminated environmental surfaces), large droplets generated by the respiratory tract ($\geq$5 μm in diameter) that do not remain suspended in the air and thus require close proximity, or by airborne transmission (virus contained in droplet nuclei $\leq$5 μm in diameter).[1] Aerosol spread of virus in humans has been demonstrated experimentally with numerous viruses including coxsackie A21, rhinovirus, and influenza virus; hand contamination with subsequent self inoculation into the eye or nose has also been documented.[2,3] While transmission has been documented to occur by aerosol, droplet, and contact routes, a consensus regarding the principle mode of virus spread has not been reached and may vary with setting, temperature, humidity, population and virus strain.[1,4] It is important to consider that social contact patterns in populations vary by geographic region, as discussed in a presentation by Annette Fox from Oxford University, and an accurate understanding of the location, frequency, and duration of social contacts in a specific area is critical to create a reliable model of virus transmission at the population level.

Elucidating the predominant mode of transmission of individual respiratory viruses will rely on a more comprehensive understanding of many biological parameters, including the location and kinetics of virus replication in the respiratory tract, the physical size of expelled particles during coughing and sneezing, and the survival of particles once released in the environment. Among multiple studies discussed at this symposium representing the breadth of

research ongoing in this area, a review of pre-clinical research papers presented by Malik Peiris of the University of Hong Kong discussed publications utilizing the ferret and guinea pig models to study influenza virus strain-specific differences which may impact efficient transmission.[5,6] Further research in this area will allow for a greater understanding of the transmission of influenza and other respiratory viruses.

Despite uncertainty regarding the dominant modes of transmission of respiratory viruses, it is reasonable to predict that implementing methods to reduce viral shedding into the environment by an infected host will decrease the likelihood that a contact will become infected. The transmission of respiratory viruses can be interrupted or reduced following the use of relatively low-cost non-pharmaceutical interventions, like patient isolation, hand hygiene, and use of face masks.[7] Several randomized controlled trials have explored the potential effectiveness and compliance of these methods in different populations. In a community-based, randomized trial in Hong Kong, Benjamin Cowling and colleagues of the University of Hong Kong found a reduction of RT-PCR-confirmed influenza in household contacts following the combined use of hand hygiene and face masks when implemented within 36 hours of index case symptom onset.[8,9] Arnold Monto described initial findings from the first year of the M-FLU study, in which the use of disposable face masks and hand sanitizer in a young adult collegiate population reduced overall influenza-like illnesses (by 35–51%) in university residence halls during the influenza season.[10] In discussing the Bangkok HITS study, which is evaluating the effectiveness of these same non-pharmaceutical interventions in Bangkok metropolitan area households which include a child with laboratory-confirmed influenza, Piyarat Suntarattiwong underscored difficulties in achieving high compliance with these intervention methods among study participants, especially among children. A more statistically rigorous assessment of the relative contribution of these interventions in reducing virus transmission among household contacts will necessitate larger study populations, but encouraging results from available studies demonstrates the potential use of non-pharmaceutical interventions in the event of an outbreak or pandemic, when adequate supplies of influenza vaccines and antivirals may not be readily available.

Influenza virus pathogenesis

Influenza viruses are responsible for both seasonal epidemics and infrequent global pandemics of varying severity. The extensive diversity of influenza viruses makes it difficult to predict which viruses or virus subtypes possess the greatest pandemic threat. It has been widely believed that, in order to cause a pandemic, a virus must bear a hemagglutinin (HA) subtype to which the human population is immunologically naïve, be able to infect humans, and transmit efficiently between people. However, shortly after the XIth ISRVI was held, the emergence of the 2009 pandemic virus, a swine-origin quadruple reassortant that was of the same subtype (H1N1) as viruses circulating in humans since 1977, once again highlighted the unpredictability of influenza, as well as the potential for rapid worldwide spread of novel influenza viruses. Continuing sporadic human cases of lethal avian H5N1 infections have emphasized the importance of understanding the receptor binding preference of avian influenza viruses to identify which avian viruses or virus subtypes might acquire mutations that allow for greater binding to α2–6 linked sialic acids (SA), the linkage abundantly expressed in the human upper respiratory tract and the preferred receptor for human influenza viruses. James Paulson of the Scripps Research Institute discussed use of glycan microarrays (which possess a diverse range of glycan structures coupled to a single chip) as a tool to monitor the receptor specificity of circulating influenza viruses. In contrast to avian H5N1 viruses which have maintained a classical avian α2–3 linked SA binding preference with few exceptions,[11] H9N2 viruses isolated from humans in 1999, 2003, and 2009 were found to possess enhanced binding to α2–6 linked SA, as did an H7N2 virus isolated from a human in 2003.[12,13] These findings indicate that avian influenza viruses of other subtypes besides H5N1 have pandemic potential and should be monitored carefully.

Surface glycoproteins like the HA are not the only viral proteins which influence virus pathogenicity. The influenza virus accessory protein PB1-F2, first discovered in 2001, is a pro-apoptotic protein encoded by an alternate reading frame in the PB1 gene.[14] A point mutation N66S in this protein is present in many highly virulent influenza viruses and correlates with increased virus pathogenicity in the mouse model, although the mechanism responsible for this heightened virulence is unclear.[15] Gina Conenello of the Mount Sinai School of Medicine demonstrated that an excessive cellular infiltrate in the lungs and concomitant increase in cytokines and chemokines contributes to the heightened pathogenicity of viruses bearing the N66S point mutation in mice.[114] In addition to virus-induced pathology, secondary bacterial pneumonia can result in excess morbidity and mortality in humans. The PB1-F2 protein augments the frequency and severity of secondary bacterial infection following viral infection in the mouse model.[16] Using a mouse model of secondary bacterial pneumonia following influenza virus infection, Jon McCullers of St. Jude Children's Research Hospital demonstrated that treatment of mice with the protein synthesis inhibitor clindamycin resulted in increased survival.[17] Further understanding of viral-bacterial co-pathogenesis remains an important area of

study given the contribution of secondary bacterial infections to severe illness and mortality in influenza.

While viral determinants greatly impact the overall pathogenicity of a particular virus, it is crucial not to overlook the contribution of potentially deleterious host responses during influenza. Studies utilizing mice deficient in selected components of the innate immune response show that host responses following infection significantly impact the virulence of influenza viruses.[18] Research assessing the role of innate immune responses and their contribution to lung injury in viral pneumonia was reviewed by Joseph Mizgard of Boston University. Infection of mice deficient in both TNF and IL-1 signaling with a highly pathogenic H5N1 virus resulted in diminished cytokine induction in the lungs, diminished airway inflammation, and delayed mortality compared with wild-type controls.[19] It is important to note that while influenza virus primarily manifests as a respiratory disease in humans, studies in the mouse model have demonstrated avian influenza viruses are additionally capable of using the eye as a portal of entry to mount a productive and lethal infection, as presented by Jessica Belser of the US Centers for Disease Control and Prevention (CDC).[20] Collectively, these studies emphasize that both virus and host responses contribute to the overall pathogenesis following influenza virus infection.

Influenza virus animal–human interface

Wild aquatic birds are the natural reservoir of all influenza A viruses.[21,22] While all subtypes of influenza have been identified in avian species, to date only viruses within the H5 and H7 subtype have been associated with severe disease and mortality in avian populations, most frequently occurring following introduction into domestic land-based birds.[23] Interspecies transmission of avian influenza viruses from wild bird reservoirs into domestic poultry and sporadic transmissions to mammals represent a continuing public health threat.[24] Albert Osterhaus of Erasmus MC in The Netherlands provided an overview of H5N1 viruses in non-avian species, including descriptions of the pathology and disease profile observed following lethal H5N1 infection among tigers and leopards in Thailand.[25,26] The severe pathology observed in domestic cats following the consumption of H5N1-virus infected chickens, and subsequent transmission of this virus to naïve animals, underscores the importance of continued studies to identify the determinants of zoonotic virus transmission.[27,28] Gregory Gray from the University of Iowa, now at the University of Florida, presented additional work on swine, equine, and canine influenza viruses, revealing increased rates of seroconversion to animal influenza viruses among individuals with occupational exposure to these species, demonstrating the risk of virus transmission from these hosts to humans.[29,30] This research illuminates the importance of including individuals who face occupational exposure to potentially infected animals, such as swine and poultry workers, in surveillance and immunization programs.[31]

The infection of poultry or swine with avian influenza viruses may facilitate the adaptation of these viruses for human infection. As discussed by Ruben Donis of the US CDC, H5N1 viruses isolated from infected poultry in Vietnam from 2008 to 2009 display extensive genetic and antigenic divergence compared with previously identified strains, indicating that rapid evolution of these viruses has been occurring.[32] Pigs have long been thought of as a potential ''mixing vessel'' for influenza viruses, acting as an intermediate host for avian influenza viruses to adapt to humans, either by reassortment of human and avian influenza viruses, or by continued adaptation of an avian virus to replicate in an environment which more closely resembles the human respiratory tract. Yoshihiro Kawaoka of the University of Wisconsin-Madison described research demonstrating that H5N1 viruses isolated from swine in Indonesia are less virulent in mice compared with avian isolates, an observation suggesting that virus replication in swine has altered the phenotype of these viruses.[33] Exposure of humans to virus-infected poultry and/or swine poses clear health risks, necessitating both the continued active surveillance of influenza virus in these mammalian and avian populations as well as in persons exposed to potentially infected animals.

Vaccination of poultry would offer one avenue to reduce occupational and accidental exposure of avian influenza viruses to humans, however as discussed by David Swayne of the US Department of Agriculture, the effectiveness of this approach to date has been limited. Vaccination policies vary by country and are influenced by the veterinary infrastructure, economic status, and production and export sector regulations specific to each location. Furthermore, many of the challenges that face vaccine effectiveness of avian influenza vaccines for humans similarly apply to poultry vaccination, including the lack of a universal vaccine, poor immunogenicity of unadjuvanted vaccines, and antigenic drift of circulating strains.[34] Antigenic cartography, a method which allows for the quantification and visualization of antigenic evolution using a heterologous panel of viruses and/or vaccine strains from a particular subtype and ferret antisera, is a useful tool to visualize these potential antigenic differences between virus and candidate vaccine strains.[35] Derek Smith of the University of Cambridge further demonstrated the application of this method for assessing both veterinary and human vaccines and its utility in visualizing in new ways the titer and breadth of the immune response post-vaccination.

International epidemiology and surveillance of influenza

The emergence of avian influenza H5N1 in humans has provided an impetus for increased global surveillance of both influenza-like illness and acute respiratory illness (ARI). Representatives from several Southeast Asian countries described national surveillance and epidemiologic efforts. Zhou Lei from the Chinese Center for Disease Control and Prevention presented national ARI surveillance data which highlighted the distinct geographic patterns of influenza activity in China, demonstrating seasonal winter peaks in Northern China and year-round influenza activity with sub-tropical/tropical patterns in Southern China. In addition to surveillance for seasonal influenza viruses, the pneumonia of unknown origin surveillance program has contributed to the early detection of HPAI H5N1, SARS, and clusters of severe pneumonia cases in China. Sirenda Vong from Institut Pasteur du Cambodia discussed a multi-tiered system for ARI surveillance, which includes community-based active surveillance for febrile illness including dengue and a severe acute respiratory surveillance system to identify the viral and bacterial etiology of acute respiratory illnesses. Kumnuan Ungchusak from the Thailand Ministry of Health discussed Thailand's population-based active surveillance for pneumonia, which revealed a high burden of pneumonia in children under the age of 5 years with respiratory syncytial virus (RSV) the most commonly associated viral pathogen.[36] The avian influenza and severe pneumonia surveillance programs have not identified new cases of H5N1 in Thailand in 2007–2009. Khuntirat Benjawan of the Armed Forces Research Institute of Medical Science, Thailand presented results from a prospective cohort study of 1600 adults to study avian influenza transmission in Cambodia and Thailand. Individuals self-reporting any prior poultry exposure were more frequently found to be seropositive to H5N1 virus by microneutralization assay compared with individuals without such exposure. In the neighboring country of Laos, ARI surveillance has contributed to the detection of H5N1 virus and control of H5N1 virus infections. As presented by Phengta Vongphrachanh of the National Center for Laboratory and Epidemiology Vientiane, poultry outbreaks of H5N1 have been contained by the culling of flocks identified through their surveillance program; no human cases have been detected since 2007.

W. Abdullah Brooks from the International Centre for Diarrheal Diseases Research-Bangladesh discussed the national influenza-like illness (ILI) and population-based pneumonia surveillance programs implemented in Bangladesh. Children under the age of 2 years old are at the greatest risk for developing influenza-related pneumonia, most frequently associated with H3N2 virus infection, but other respiratory viruses including RSV, metapneumovirus, parainfluenza virus, and adenoviruses contribute to lower respiratory tract disease in this population. A prospective cohort study in Nicaragua, as presented by Aubree Gordon of the University of California, Berkeley, found that one quarter of all nasopharyngeal swabs collected due to ILI are influenza virus positive in children aged 2–12.[37]

Other presentations highlighted several considerations related to achieving a full understanding of the epidemiology of influenza and other respiratory virus infections. Nancy Cox from the US CDC discussed differences in sensitivity and specificity among diagnostic test platforms for the detection of respiratory pathogens, parameters which impact the numbers of positive cases detected. An update regarding the ongoing development of novel diagnostic tests for human use, in particular tests which can differentiate between seasonal and avian influenza strains, was provided by Roxanne Shively and Mike Perdue of the Office of the Biomedical Advanced Research & Development Authority (BARDA) in the US Department of Health and Human Services. Susan Chiu of the University of Hong Kong further discussed difficulties in determining the appropriate model for disease burden estimates for respiratory virus infections. Several factors can mask the accurate quantification of influenza activity in a region, including less predictable seasonality in tropical and sub-tropical areas, co-circulation of other respiratory viruses like RSV, lack of timely and intensive virologic diagnosis, and difficulties in capturing a defined representative population.[38] The application of models which capture these variables, in addition to reliable and accurate data provided by diagnostic tests, are essential for informed clinical and public health decision making.[39]

Development of H5N1 vaccines

With over 500 cases since 2003 and a mortality rate of approximately 60%, H5N1 viruses continue to pose a significant health threat.[40] During the past decade much effort has been devoted towards the development of effective H5 vaccines for human use, and considerable technical and conceptual advances have been made, including expanded surveillance of avian influenza virus isolates, the development of reverse genetics systems for seed virus strain generation, expanded assay and reagent availability, and studies of novel vaccine targets, adjuvants, and delivery approaches.[41,42] Wendy Keitel of the Baylor College of Medicine discussed many studies highlighting both the strengths and the limitations of available vaccine candidates. While inactivated seasonal vaccines contain 15 μg of non-adjuvanted HA from each subtype to elicit a protective response in the majority of healthy adult recipients, the HA from avian H5 viruses has demonstrated reduced immunogenicity, even when administered at quantities as

high as 90 μg or administered with the adjuvant aluminum hydroxide (alum).[43–45] However, several proprietary oil-in-water adjuvant preparations (MF59, AS03, AF03) have demonstrated enhanced immunogenicity resulting in antigen-sparing and cross-clade immune responses.[46–48] Prasert Auewarakul of the Faculty of Medicine Siriraj Hospital in Thailand presented the results of a phase 3 study that evaluated the use of AS03-adjuvanted inactivated, split H5N1 vaccine and found that following two doses, >90% of vaccinees in a Thai population demonstrated at least four-fold rises in neutralizing antibody titer to both homologous and heterologous H5N1 viruses following two doses.[49] These results were similar to previously published studies, demonstrating a significant enhancement of neutralizing antibody responses with oil-in-water adjuvanted vaccine compared with non-adjuvanted preparations.[50]

A central obstacle for the design of an H5N1 virus vaccine is the uncertainty of cross-reactivity between a potential pandemic H5N1 strain and the strain chosen in advance for vaccine preparation. Inca Kusters of Sanofi Pasteur discussed pre-clinical studies in mice evaluating an oil-in-water adjuvanted (AF03), low-dose (0·3 μg of HA) H5N1 vaccine candidate previously shown to elicit cross-clade neutralizing antibodies in young adults following two doses.[47] Otfried Kistner, representing Baxter vaccines, presented data from clinical trials using a whole-virus, non-adjuvanted H5N1 vaccine generated in Vero cells. Two doses of this vaccine elicit neutralizing antibodies which cross-react with heterologous strains of H5N1 virus representing other clades.[51] Vaccines which incorporate conserved T cell epitopes may also offer an avenue to achieve a pandemic vaccine effective against heterosubtypic strains of virus.[52] John Oxford of Retroscreen Virology Ltd. in the United Kingdom presented data which analyzed the frequencies and magnitude of responses to immunodominant proteins (nucleoprotein and matrix) following influenza virus infection, information which contributes towards understanding and implementing the potential use of vaccine formulations which induce cytotoxic T lymphocyte responses.[53]

With the development of new vaccine preparations, adjuvants, and delivery methods, it will be increasingly important to accurately assess the effectiveness of these influenza vaccines in susceptible populations. David Shay of the US CDC discussed the unique challenges of measuring vaccine effectiveness, including the need to choose the most appropriate outcome measure and the importance of controlling for confounding factors based on the sample population in observational studies. The US CDC recommends the use of laboratory-confirmed outcomes in measuring effectiveness when possible, including the effectiveness of vaccine against both common outcomes and more serious, rarer complications.[54]

Influenza virus antivirals and therapeutics

While vaccines offer the best protection against influenza virus infection, there is a 6–8-month timeframe necessary to manufacture an antigenically well-matched vaccine towards a specific virus strain,[55] vaccine availability and uptake are limited in many countries, and some persons develop illness despite immunization. As such, antiviral drugs which are efficacious against a broad range of virus subtypes serve as a first line of defense before a well-matched vaccine is produced and complementary intervention thereafter. Two classes of antiviral drugs are currently licensed for human use: M2 ion channel inhibitors (amantadine and rimantadine) and neuraminidase (NA) inhibitors (oseltamivir and zanamivir). However, as a high frequency of seasonal influenza viruses have developed resistance to M2 inhibitors or for H1N1 to oseltamivir, the development of novel antiviral and therapeutic approaches for the treatment and prevention of influenza virus infection remains a high priority.[56]

The 2008–2009 circulating oseltamivir-resistant seasonal H1N1 and M2 inhibitor-resistant H3N2 viruses exhibited efficient transmission and caused illness comparable to susceptible, wild-type virus. Such findings indicate that the associated resistance mutations (His275Tyr in the N1 and Ser31Asn in M2, respectively) have not reduced viral fitness. Two presentations, by Frederick Hayden of the University of Virginia School of Medicine and Angie Lackenby of the Health Protection Agency in London, provided a more detailed characterization of oseltamivir-resistant seasonal influenza H1N1 viruses. These resistant viruses displayed no reduction in viral fitness or person-to-person transmissibility, and in fact replaced oseltamivir-susceptible H1N1 viruses in most parts of the world.[57] The rapid global dissemination of oseltamivir-resistant seasonal influenza H1N1 viruses worldwide did not appear to be linked to drug selection pressure.[58,59] Similarly, the rise of M2 inhibitor-resistance of H3N2 viruses in the United States and other countries has not been associated with increased use of this antiviral,[60] although its initial appearance in China could have been linked to amantadine use. As presented by Martha Nelson of the National Institutes of Health, the resistance of H3N2 viruses to the adamantines appeared to be the cumulative effect of several evolutionary steps, including repeated introductions of viruses with the Ser31-Asn mutation in the M2 gene, intra-subtypic genomic reassortment, and rapid global dissemination of resistant viruses. These presentations highlight the need both for continued surveillance of circulating viruses resistant to existing antiviral drugs, and the development of antivirals with novel mechanisms of action.

In addition to the currently licensed antivirals, several new NA inhibitors or formulations are currently undergoing

clinical trials, and several were recently approved in 2010, including peramivir (Japan, South Korea) and laninamivir (Japan). W. James Alexander of BioCryst Pharmaceuticals presented an update of peramivir, an intravenously administered NA inhibitor. When given as a single dose, IV peramivir was more effective than placebo in reducing the time to alleviation of symptoms and was comparably effective as a 5-day course of oseltamivir in outpatient adults with uncomplicated influenza; two phase 3 studies are currently in progress in hospitalized adult patients.[61] CS-8958, the pro-drug of laninamivir, is a long-acting, topically applied NA inhibitor that has shown activity *in vitro,* including against most oseltamivir-resistant strains, and in murine models.[62] As presented by Makato Yamashita of Daiichi Sankyo, comparative studies in the mouse model have shown similar to significantly higher survival rates of mice when treated once with CS-8958 compared with either multi-day treatments of topical zanamivir or oseltamivir following lethal PR/8 virus challenge. Initial clinical trials of this NA inhibitor found that single inhalations were similarly efficacious to a 5-day regimen of oseltamivir in children with influenza virus infection[63]; other phase 3 clinical trials evaluating a single inhalation dose are anticipated.

Researchers are also looking beyond NA inhibitors to develop novel antiviral approaches. Tsutmo Sakurai of Toyama Chemical presented pharmacologic data from clinical trials with T-705 (favipiravir), a compound which selectively inhibits the influenza virus RNA-dependent RNA polymerase.[64] Phase 1 trials demonstrated the tolerability of single or multiple oral doses of T-705, and phase 3 studies are ongoing in Japan. A plant extract rich in polymeric phenols, CYSTUS052, has demonstrated antiviral activity both *in vitro* and in a mouse model as Oliver Planz of Friedrich-Loeffler-Institut in Germany presented, with a phase 1 clinical trial of this compound currently underway.[65]

It is critical to consider antiviral treatments that work in tandem with host responses to resolve viral infection. As such, the development of treatment regimes which possess immunomodulatory activity, or which target signal transduction pathways exploited by influenza viruses, may offer advantages to currently available antivirals. Ralf Altmeyer of CombinatoRx Singapore presented data evaluating two independent classes of anti-inflammatory drugs, selective serotonin reuptake inhibitors (SSRI) and phosphodiesterase type-4 inhibitors (PDE-4i), and found that, administered in combination with NA inhibitors, mice challenged with a lethal dose of influenza virus demonstrated enhanced survival compared with mice which received the NA inhibitor alone. Furthermore, Stephan Ludwig of the Institute for Molecular Virology in Germany demonstrated that SC75741, which inhibits viral replication and NF-κB dependent cytokine expression *in vitro* and *in vivo*, improved survival of H5N1 virus-challenged mice compared with a placebo. By targeting cellular components and not viral factors, this approach may further reduce the likelihood of generating antiviral resistant viruses.[66]

Clinical disease and drug therapy of H5N1

While human infection with highly pathogenic avian influenza (HPAI) H5N1 viruses remains rare despite widespread exposure to infected poultry, human cases are frequently severe with an approximate fatality rate of 60%.[67] A greater understanding of clinical features, pathological findings, and possible genetic risk factors in severe H5N1 human cases is important for optimizing prevention and treatment strategies. Clinical data from hospitalized patients with H5N1 viruses from Vietnam between 2004 and 2007 were presented by Peter Horby of the Oxford University Clinical Research Unit, Hanoi. Retrospective chart review revealed that fatal cases were younger than surviving cases (median age, 18 years versus 30 years, respectively). Diarrhea, systemic inflammatory response syndrome, and mucosal bleeding were more common at presentation in fatal cases, with neutropenia and elevated transaminases highly predictive of death. In regards to treatment, oseltamivir showed benefit but the addition of corticosteroids resulted in an increased risk of death.[68]

Acute kidney injury has also been reported frequently among severe human cases of H5N1 infection and needs to be considered in oseltamivir dose selection as this drug is renally excreted. As discussed by Bob Taylor from Oxford University Clinical Research Unit, HCMC studies evaluating oseltamivir pharmacokinetics in Vietnamese patients with severe influenza virus infection found that oral absorption of extemporaneous oseltamivir administered via nasogastric tube was good but that reduced renal function led to high serum concentrations of oseltamivir carboxylate.[69] Further studies are needed to delineate optimal dosing regimens in various forms of renal replacement therapy and to investigate drug concentrations at sites of infection including bronchalveolar lavage and pleural effusion samples.

Menno de Jong of the University of Amsterdam highlighted numerous papers that described the clinical presentation of HPAI H5N1 infection and its susceptibility to oseltamivir.[67,70] It is clear that the timing of antiviral treatment initiation post-symptom onset influences the effectiveness of antiviral treatment. In one epidemiological study of HPAI H5N1 virus infection in Indonesia, initiation of treatment within 2 days of symptom presentation was associated with a lower mortality than initiation at 5–6 days post-symptom onset.[71] Greater emphasis on rapid diagnosis would allow for earlier initiation of treatment

with oseltamivir.[67] Furthermore, different clades of H5N1 viruses exhibit variable sensitivities to antiviral drugs. Whereas clade 1 and most clade 2.1 viruses are resistant to M2 inhibitors, most clade 2.2 isolates are susceptible so that combination antiviral therapy with oseltamivir and amantadine or rimantadine would be appropriate in such cases.[72,73] Other therapies for treatment of HPAI H5N1 viruses including parental NAIs, neutralizing antibody preparations, the sialidase DAS181, and the polymerase inhibitor favipiravir, as well as combinations evaluating synergistic antiviral potency, are under investigation in preclinical models.

Other respiratory viral infections

Frederick Hayden from the University of Virginia and Tawee Chotpitaysunondh of Queen Sirikit National Institute of Child Health, Thailand commented on selected publications in adult and pediatric respiratory viral research, respectively. Morens *et al.* demonstrated that secondary bacterial infections were a major contributor to mortality observed during the 1918 influenza pandemic.[74] Viral–bacterial co-infections also contributed to mortality during the pandemics of 1957 and 1968 despite the availability of antibiotics, demonstrating a need to consider management and prevention of bacterial infections during pandemic planning. While the greater availability of influenza vaccines and antiviral therapies would suggest that we are better prepared, changes in the health status of susceptible populations should be considered. For example, the prevalence of asthma in western populations has increased dramatically in recent years. The mechanisms for this epidemiologic finding remain to be explained but it is clear that respiratory viruses, especially rhinoviruses, are the major infectious cause of asthma exacerbations and that an enlarging asthma population puts more persons at risk for severe illness. One study detected persistent rhinovirus in the lower respiratory tract of a majority of individuals tested with asymptomatic asthma,[75] a potentially key observation that needs to be confirmed. The spread of respiratory viral infections has been further influenced by the increasing popularity of international travel and tourism. One study from Spain found amongst returned travelers with respiratory symptoms, 56% were positive for a viral respiratory pathogen, most commonly influenza.[76] Demonstrating the rapid dissemination of a respiratory pathogen by this route of transit, worldwide air-traffic patterns accurately predicted the spread of the 2009 H1N1 pandemic influenza virus.[77]

A survey of pediatric literature highlighted clinical epidemiology studies on RSV, human bocavirus (HBoV), and rhinovirus. RSV is associated with substantial morbidity in children, as evidenced by a population based surveillance study in the U.S. which detected RSV in 20% of hospitalized children under the age of 5.[78] HBoV has also been associated with respiratory tract disease in children.[79] It is important to keep in mind that co-infection with several viral pathogens, serial viral infections, or secondary infection with bacterial agents such as *Streptococcus pneumoniae* are also detected in pediatric patients exhibiting respiratory illness.[80–82]

Respiratory virus infections in the immunocompromised host

The immunocompromised host represents a challenging population for the treatment and prevention of influenza and other respiratory viruses. Diagnosis and management need to encompass diverse subpopulations of immunocompromised patients including transplant recipients, HIV-infected persons, and pregnant women. One population especially at risk for respiratory viral infections is lung transplant recipients, due to use of immunosuppressive drugs, impaired mucociliary clearance, abnormal lymphatic drainage, and direct exposure of the graft to airborne viruses. Laurent Kaiser of Université De Genève studied lung transplant recipients and other immunocompromised patients to determine the incidence of respiratory viral infections. Respiratory viruses, most frequently coronaviruses and rhinoviruses, were associated with respiratory symptoms in transplant recipients, notably among patients exhibiting a poor response to antibiotic therapy.[83]

Michael Ison of Northwestern University discussed the clinical epidemiology, management and prevention of influenza in organ transplant recipients. The prevalence of influenza is estimated to be 1–3% among hemapoietic stem cell transplant recipients and 1–12% among solid organ transplant recipients. Immunocompromised hosts often present with atypical symptoms (only a third of patients present with fevers), shed virus for a prolonged period of time, and are at increased risk of co-infections. Vaccination, including ring immunization of close contacts, and antiviral prophylaxis have been used to prevent influenza virus infection in these patients, however poor immune responses to vaccination, especially during the first 12 months after transplantation, decreases the efficacy of this approach depending on the degree of immunosuppression.[84,85] Dr. Ison also presented recent data from clinical trials demonstrating that prolonged prophylaxis with oral oseltamivir or inhaled zanamivir is well-tolerated and can reduce the frequency of viral culture-proven or RT-PCR-positive influenza detection in transplant patients; guidelines are being developed on the optimal management of influenza in transplant populations.[86]

Children with HIV disease represent an under-recognized immunocompromised population at risk for respiratory infections. Vaccination with live attenuated influenza vaccine (LAIV) or trivalent inactivated vaccine (TIV) represents one approach to protect this population from infection with influenza, as both vaccines possess good safety profiles in HIV-infected children.[87] However, the magnitude and breadth of the resulting antibody response in this population following vaccination is not fully known. Myron Levin from The Children's Hospital in Denver, Colorado showed that HIV-infected children with CD4% ranging from 15 to ≥25 at the time of the study possessed similar serum hemagglutinin inhibition and neutralizing antibody responses as in HIV-uninfected children following vaccination, indicating that these serologic responses are not affected. To better understand the role of cell-mediated immunity, Adriana Weinberg from the University of Colorado compared ELISPOT responses to LAIV and TIV in HIV-infected children with respect to their CD4 T-cells, CD8 T-cells, and HIV viral loads.[88] This study found paradoxical decreases in influenza-specific CD8 T-cell mediated immune responses following TIV, whereas LAIV preserved such responses in HIV-infected children. No difference was noted in CD4 and HIV viral load after immunization in comparison to baseline.

During seasonal epidemics, the very young and very old are at increased risk of influenza-related morbidity and mortality, as influenza vaccination is either not recommended (in young infants) or less efficacious (among the elderly) in these populations. While influenza vaccination is not currently advised for infants under the age of 6 months, vaccination of pregnant women, a practice recommended by the WHO, is one means to protect young infants. Mark Steinhoff from Cincinnati Children's Hospital and Medical Center presented evidence that maternal influenza vaccination improved fetal growth, reflected in higher birth weights, in addition to demonstrating virological and clinical effectiveness against influenza illness in both mothers and infants.[89]

With regard to the elderly, the reduced efficacy of vaccination in this population necessitates further efforts to improve immunogenicity through the use of adjuvants or increasing the quantity of HA in vaccine preparations.[90,91] Robert Booy from the University of Sydney discussed findings from a large scale, cluster-randomized, controlled trial of antiviral treatment across multiple aged care facilities in Sydney, Australia. No serious adverse effects were associated with the use of oseltamivir in this population, and oseltamivir use (given as treatment only or with prophylaxis) was associated with reduced incidence of influenza in these facilities. Strikingly, while vaccine coverage of residents ranged from 59 to 100%, only 33% of staff at the aged care facilities was vaccinated; efforts to improve vaccination coverage among staff as a means to minimize the risk of nosocomial infections are essential.[92]

Rhinovirus and other respiratory viruses

Human rhinoviruses are single-stranded, positive-sense RNA viruses which are a major cause of upper and lower respiratory infections worldwide. As there are significant phenotypic variations among the 99 known serotypes and many non-cultivable genotypes of this virus, a greater understanding of the molecular and evolutionary characteristics of rhinoviruses is needed for future vaccine and antiviral development. Sebastian Johnston of Imperial College London highlighted a publication by Palmenberg *et al.* which analyzed the complete genome sequencing of all known rhinovirus serotypes[93] and revealed both common and species-specific RNA sequence and structural elements. In addition to rhinoviruses, enteroviruses also belong to the Picornaviridae family and are important contributing causes of respiratory infections, especially in children.

Caroline Tapparel of Université De Genève, Switzerland, discussed the development and application of real-time PCR assays to identify genomic features which support phenotypic differences between rhinoviruses and enteroviruses and to further identify new variants among circulating strains.[94] This work established phylogenetic analyses of circulating group C viruses compared to reference strains and demonstrated that rhinoviruses evolve by recombination in their natural host.[95] Beyond causing disease in their own right, virus infection can serve as a risk factor for serious illnesses. For example, RSV and rhinovirus are the two most prevalent viral agents identified in infants presenting with bronchiolitis. In a study presented by Gláucia Paranhos-Baccalà of the Emerging Pathogens Laboratory, Fondation Mérieux in France, infants co-infected with both viruses were significantly more likely to require admission into an intensive care unit when hospitalized.[96] There is an association between early childhood infection with these respiratory viruses and an increased risk of recurrent wheezing and likely subsequent asthma development.[97] Recently developed mouse models of rhinovirus infection and exacerbation of allergic airway inflammation have provided the opportunity to more closely study the interplay between respiratory infection and allergy.[98] Ross Walton of Imperial College London described studies performed in an OVA T-cell receptor (TCR) transgenic mouse model which revealed that infection with rhinovirus leads to both enhanced recruitment of allergen-specific T cells into the airway and augmented cytokine production in the lung. Understanding the role of viral infection in airway disease is crucial to identify therapeutic targets to mitigate these adverse effects which can persist long after acute infection.

Investigational vaccines and therapeutics for several respiratory viruses are under study. Infection with human metapneumovirus (HMPV) can result in acute respiratory disease, most frequently in pediatric, elderly, and immunocompromised populations. While no licensed vaccine currently exists for HMPV, Sander Herfst of Erasmus MC presented ongoing studies in hamster and non-human primate models evaluating the efficacy of cold-passaged, temperature-sensitive HMPV strains as vaccine candidates.[99,100] Palivizumab, a monoclonal antibody directed against RSV, is an FDA-approved treatment which inhibits viral entry into host cells by targeting the conserved RSV F glycoprotein; other drug treatments are currently under investigation.[101] John DeVincenzo of the University of Tennessee discussed the application of RNA interference (RNAi) as a therapy for several respiratory viral infections, including influenza and RSV.[102] As respiratory viruses generally infect epithelial cells in the respiratory tract, a topical administration (e.g. by aerosol) with a therapy such as RNAi could theoretically reach infected target cells. A recent randomized study in adults experimentally infected with RSV demonstrated a 38% decrease in infections among volunteers who received prophylaxis and treatment with an RNAi based therapy administered by nasal spray compared with subjects receiving a placebo.[103] Given the paucity of effective vaccine and antiviral treatments for many respiratory viral infections, future work evaluating the effectiveness of these and other preventative approaches is needed.

Adenoviruses

Adenoviruses present a wide spectrum of clinical disease in infected individuals, including respiratory symptoms, gastroenteritis, and conjunctivitis. Most people are infected during childhood with asymptomatic or mild illness; severe cases are most frequently observed among young children and immunocompromised hosts. Among the over 50 recognized serotypes of adenovirus (Ad), Ad type 7 (Ad7), along with types 3, 4, and 21, are most frequently associated with severe disease.[104] However, outbreaks of Ad type 14 (Ad14), a subtype not previously associated with severe disease, have occurred since 2006, resulting in substantial morbidity and mortality. Larry Anderson of the US CDC presented clinical and epidemiological findings from several recent outbreaks of Ad14, including a community-based outbreak of severe respiratory disease in Oregon and a cluster of severe respiratory illness at U.S. Air Force training base in Texas.[105,106] To ascertain levels of preexisting immunity to Ad14 and other circulating adenovirus serotypes, David Metzgar of the Naval Health Research Center presented results from seroprevalence studies among young adults in the United States over the past decade. These studies revealed that antibody to Ad14 was not detected in the tested population prior to 2006, suggesting that a lack of preexisting immunity may have contributed to the severe disease observed in some settings, and that Ad14 does not appear to be continuously circulating in the population and severe illness and death is generally uncommon.

The recent emergence of Ad14 highlights the need to improve methods of serotype specific detection and treatment of infected individuals with all serotypes. Cicely Washington of the Walter Reed Army Institute of Research discussed ongoing development of Luminex xMAP technology to detect the five most common Ad serotypes (3, 4, 7, 14, and 21) in a single assay well, which would facilitate rapid serotyping of samples and could ultimately be expanded to include detection of a greater array of serotypes. Unfortunately, no antiviral drugs for the treatment of adenovirus infections are approved for use in humans.[107] In a presentation given by Michael Ison on behalf of Karl Hostetler of the University of California, San Diego, current research exploring novel therapies for adenovirus infections were discussed. To overcome the limitations of *in vitro* antiviral testing, the development of an *in vivo* model of severe systemic disease following infection with Ad type 5 in immunosuppressed Syrian hamsters has allowed improved pre-clinical evaluation of antiviral drugs for protection from Ad pathogenesis.[108] Use of this model demonstrated efficacy of the antiviral drug hexadecyloxypropyl-cidofovir (CMX001) in preventing adenovirus-induced mortality following prophylactic or therapeutic oral administration. Clinical trials of this drug are currently ongoing.

Conclusions

The emergence of the 2009 A(H1N1) influenza virus and subsequent declaration by the World Health Organization (WHO) on June 11, 2009 of the first pandemic in over 40 years provides a timely example of the importance of studying respiratory viral infections.[109] At the time of this symposium, as presented by Sylvie Briand of the WHO, most countries had already developed pandemic preparedness plans, with many countries conducting pandemic exercises and some implementing changes/revisions in their plans since their initial creation. This level of awareness and groundwork is essential not only for an influenza pandemic, but also contributes to the appropriate actions and response to outbreaks of other respiratory viral infections. The rapid mobilization of efforts in the early stages of the A(H1N1) outbreak was remarkable, and many of the conference participants played key roles in this work. Antigenic and genetic analysis of circulating A(H1N1) viruses occurred very quickly,[110] and studies on the dominant modes of influenza virus transmissibility were directed towards this new virus.[111,112] The topics of vaccine production and antiviral use discussed at this symposium proved

especially relevant in the context of the pharmaceutical responses to the pandemic.[113] The emergence of respiratory viral infections which pose a threat to public health has and will continue to occur; the recent international response to A(H1N1) offers a positive outlook for future responses to other respiratory viral infections. Moreover, the continuing high impact of respiratory viral infections throughout the world, especially in children in the developing world in whom ARI is the leading cause of mortality, require continued commitment to improved understanding, prevention, and management of these infections.

Key messages from XI ISRVI

- Transmission of respiratory viruses
  - Spread of respiratory viruses can occur by multiple modes (including direct contact, respiratory droplets, and short- and possible long-distance aerosols) and can be influenced by both biological parameters (e.g., symptom profiles, humidity, temperature) and social behaviors
  - Non-pharmaceutical interventions aim to limit infectious virus contamination of the environment by an infected host or to reduce risk of exposure in susceptible populations
- Influenza virus pathogenesis
  - Identification of viral determinants of pathogenicity can contribute towards understanding the pandemic potential and virulence of a given virus strain or subtype
- Influenza virus animal–human interface
  - Interspecies transmission of avian influenza viruses from wild bird reservoirs into domestic poultry, and sporadically to other species, represents a continuing public health threat
  - Poultry vaccination is one approach to reduce occupational and accidental exposure of avian influenza viruses to humans, but faces numerous biological and socioeconomic hurdles to effective implementation
- International epidemiology and surveillance of influenza
  - Global surveillance of respiratory viral pathogens is essential to understand geographic-, seasonal-, and population-specific rates of infection and to identify predominant viruses causing acute respiratory illness
- Development of H5N1 vaccines
  - The use of oil-in-water adjuvants in H5N1 vaccine candidate formulations can result in increased immunogenicity, antigen-sparing, and cross-clade immune responses
- Influenza virus antivirals and therapeutics
  - High proportions of seasonal influenza viruses have developed resistance to one or more currently licensed antiviral drugs
  - Novel antiviral/therapeutic approaches in development include intravenous and long-lasting, inhaled neuraminidase inhibitors, as well as formulations which target other viral factors and/or host cellular components
- Clinical disease and drug therapy of H5N1
  - A greater understanding of clinical features, pathological findings, and possible genetic risk factors in severe H5N1 human cases is important for optimizing prevention and treatment strategies
- Other respiratory viral infections
  - The incidence of viral and/or bacterial co-infections, increasing prevalence of asthma, and frequency of international travel and tourism complicates the ability to contain and treat numerous respiratory viral infections worldwide
- Respiratory viral infections in the immunocompromised host
  - Immunocompromised hosts represent a challenging population to treat and prevent respiratory viral infections, notably organ transplant recipients who may present with atypical symptoms and are at increased risk of severe illness and co-infections
  - Children with HIV disease, pregnant women, and the elderly represent additional immunocompromised populations which require further attention with regard to optimizing vaccination and antiviral recommendations
- Rhinoviruses and other respiratory viruses
  - Important phenotypic variations with respect to lower respiratory tract illness may exist among serotypes and genotypes, such that a greater understanding of the molecular and evolutionary characteristics of rhinoviruses is needed for future vaccine and antiviral development
  - Investigational vaccines and therapeutics for several respiratory viruses, such as HMPV and RSV, are under study
- Adenoviruses
  - The recent emergence of severe Ad14 infections highlights the need to improve methods of adenovirus serotype-specific detection and treatment of infected individuals with all serotypes

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