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Second isirv antiviral group conference: overview

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Introduction

The 2nd isirv Antiviral Group conference entitled 'Severe Influenza: Burden, Pathogenesis and Management' was organized in conjunction with the National Institute of Hygiene and Epidemiology (NIHE) and held in Hanoi, Vietnam on 29 October–31 October 2012. In the aftermath of the 2009 A(H1N1) pandemic and the continuing threats of avian A(H5N1) infections and other emergent respiratory virus infections such as the Middle East respiratory syndrome coronavirus (MERS-CoV), the purpose of the meeting was to address the many gaps in our understanding of pathogenesis and clinical management of severe influenza and other respiratory virus infections. The meeting was comprised of both invited state-of-the-art lectures, a number of which are the subject of detailed summaries in this supplement, and of oral and poster abstract presentations of new data. The following summary highlights some of the key points made during the meeting. Readers are referred to the nine review articles in the supplement for more comprehensive coverage of particular topics.

The scene was set by a review of conclusions from a previous isirv meeting entitled 'Incidence, Severity and Impact of Seasonal and Pandemic Influenza' in September 2012, and a keynote presentation on Asian perspectives of the epidemiology and impact of severe influenza and severe acute respiratory infection more generally, both of which emphasized the importance of strengthening the various aspects of influenza surveillance. Increased surveillance in various countries in South-East/East Asia continues to provide more information on factors affecting the epidemiology, demographics and seasonality of influenza and other respiratory infections, as well as the role the region plays in the global dynamics of influenza evolution and epidemiology. An overview of the use of influenza vaccines in combatting seasonal and pandemic influenza emphasized the difficulties in implementing effective control strategies and consistent vaccination policies in the Asia–Pacific region (see L. Jennings, this issue).

One of the core functions of the World Health Organization (WHO) is the development and recommendation of guidelines for the management of influenza infection and in particular severe disease. In response to human cases of avian A(H5N1) influenza and the 2009 A(H1N1) influenza pandemic, a series of 'emergency' and 'interim' guidelines were published. More recently, these are being consolidated, incorporating extensive review of existing literature, to produce a comprehensive WHO standard guideline on clinical management of severe influenza disease, in preparation for future emergencies. The broad scope encompasses as follows: treatment for severe influenza, for example viral pneumonia, ARDS, multiple organ failure, septic shock; pharmacological interventions for treatment, including anti-influenza drugs, anti-inflammatory drugs and adjunctive therapies; non-pharmacological clinical interventions, such as mechanical ventilation, oxygen and fluid management; and prevention of severe influenza in patients at higher risk of progression to severe disease. To facilitate the collection of the knowledge needed to address key questions associated with the prevention and treatment for the full spectrum of respiratory viral infections, the WHO is also spearheading a public health research agenda for influenza called the Battle Against Respiratory Viruses (BRaVe) initiative.[1] The associated research agenda has been recently posted on the WHO website (http://www.who.int/influenza/patient_care/clinical/brave/en/).

Pathogenesis of acute respiratory illness

Pathogenesis of severe influenza is affected by variation in the virus, host factors, co-infections with other micro-organisms, and how these interact with one another. Emerging data are shedding light on the complex combination of virus factors (e.g. genetic constellation, virulence, receptor specificity or tropism), host factors (e.g. underlying immunosuppression; pre-existing or cross-immunity; genetic susceptibility) and virus–host interactions [e.g. innate toll-like receptors (TLRs), RLRs, NLRs, inflammasomes, DAMPS, type-I interferons, pro-inflammatory cytokines; adaptive Th1, Th17, B-cell responses] that are involved. The complexities of viral–bacterial co-infections have posed particular challenges to physicians in the management of severe disease.

From observational data, the mortality attributed to seasonal and pandemic influenza and severe acute respiratory syndrome in children <5 years of age is usually significantly lower than in older patients.[2,3] In contrast to seasonal influenza, the 2009 pandemic was associated with lower mortality in the elderly, in part explained by higher prevalence of pre-existing cross-reactive antibodies in older individuals.[4] However, low-avidity serum antibodies directed against influenza may predispose patients to risk of more severe diseases; for example, a higher incidence of low-avidity pulmonary immune complexes with C4d deposition was observed in patients with severe influenza.[5] Presence of pre-existing cell-mediated immunity has also been associated with blunted severity of illness.[6] As with seasonal influenza, pregnant women had a higher rate of hospitalization and death resulting from infection with the 2009 pandemic A (H1N1) virus.[3] However, in contrast to other countries, no maternal mortality among pregnant Japanese women was attributed to the 2009 pandemic, in part because of the aggressive use of antiviral agents for prophylaxis and treatment.[7] Obesity has also recently been recognized to be a risk factor for severe influenza, including increased risk of complications and mortality.[3,8,9]

Two studies reported a shift to older age groups in the severity of influenza due to the A(H1N1)pdm09 virus in post-pandemic seasons. One study of patients in Beijing in 2011–2012 also observed that community-acquired pneumonia was more serious in patients infected with A(H1N1) pdm09 than in patients with seasonal A(H3N2) infection. The other study in England concluded that the increased impact of A(H1N1)pdm09 on critical care in 2010–2011 reflected increased transmission associated with lower temperatures and secondary bacterial infection.

The innate immune system plays a key role in early influenza infection, and cytokine/immune dysregulation is important in disease pathogenesis. In influenza-infected patients, there appears to be differential expression of TLRs on monocytes and dendritic cells, with higher expression of TLR 3, 7, 8 and 9 on dendritic cells and of TLR 8 and 9 on monocytes, and lower levels of TLR 2 and 4 on monocytes. Furthermore, there is a negative correlation between expression of TLR 3, 8 and 9 on monocytes and dendritic cells and virus load. In contradistinction, higher concentrations of pro-inflammatory cytokines, such as interleukin(IL)6, IL8, IL10 and MCP-1, are associated with higher virus loads, worse clinical symptoms, longer length of hospitalization and higher risk of ICU admission for both seasonal and pandemic influenza.[10–12] An extensive study of the causes of severe influenza during the 2009 pandemic, including patients enrolled by the 'Mechanisms of Severe Acute Influenza Consortium' (MOSAIC) in the UK, identified a minor variant (SNP rs12252-C) of the gene encoding the innate restriction factor, interferon-inducible transmembrane protein (IFITM3), that appears to be more prevalent among patients requiring hospital admission secondary to increased virus replication and/or alteration of influenza-induced cytokine expression.[13] A hyperactivated proinflammatory, but suppressed adaptive immunity (Th1/Th17)-related, cytokine response pattern was found in patients with severe A(H1N1)pdm09 pneumonia, different to that seen in patients with seasonal influenza.[11]

A study of 49 previously healthy adults admitted to the National Hospital of Tropical Diseases, Vietnam with RT-PCR-confirmed A(H1N1)pdm09 infection showed that the severe symptoms which developed in 10 of the patients were associated with transient T and NK cell deficiency.[14] CD8 phenotype changes during mild influenza in the 39 other patients were consistent with a rapidly resolving memory response, whereas in severe influenza, activation was either delayed or excessive, and recruitment of effectors cells to the lung appeared to be impaired.

Pro-inflammatory cytokines, such as IL1β and IL18, are regulated by a complex cytoplasmic protein scaffold known as the Nalp3 inflammasome.[15] Nalp3, ICE, IL1β and IL18 genes are upregulated following infection with highly pathogenic avian influenza (HPAI) virus in chickens, indicating a strong Nalp3 inflammasome response is associated with severe mortality. Modulation of this pathway may represent another option to mitigate against the adverse consequences of HPAI.

Bacterial infections are a common complication of influenza and contribute to the severity and burden of illness (J. Deng, this issue). The innate immune response is critical for the control of influenza but may also influence the risk of secondary bacterial infections.[16] Some innate responses such as IFN-gamma are linked to increased risks of secondary bacterial infections. As for other TLR agonists, synthetic compounds containing the TLR 2 agonist, Pam2Cys, administered into the lungs of mice resulted in a TLR 2-dependent immune-enhancement, characterized by induction of neutrophils, macrophages, NK cells and lymphocytes, and Th1 and inflammatory cytokines (but not IFN-alpha), and protected mice against virulent A/PR/8/34(H1N1) infection and reduced

virus burden following challenge with A(H3N2) and A(H3N1) viruses as well as respiratory syncytial virus.[17] Furthermore, Pam2Cys prophylaxis prevented lethality associated with secondary *S. pneumoniae* infection.

As for the host, the specific virus contributes to disease pathogenesis. The A(H1N1)pdm09 virus appears to be better able to replicate in the lower airway, which may partly explain the higher incidence of pneumonia.[18] The virulence of viruses is a multigenic property and results from the complex interplay between various virus factors and host components. For example, the influenza viral polymerase complex (PB1, PB2, PA, NP, virus RNA) has been shown to associate with a network of more than 300 human host proteins. Functional genomics (RNAi) approaches allow the study of how these host factors regulate virus RNA synthesis, induction of innate immune responses and adaptation to human cells. A number of the cellular proteins with different activities were shown to modulate polymerase activity and some RNA-binding proteins, including the DEAD-box RNA helicase 17 (DDX17), also governed A(H5N1) polymerase activity and efficient infection of human or chicken cells according to the species-specificity determinant residue 627 of PB2.[19] Thus, this network of virus polymerase–host protein interactions in part controls adaptation, replication and pathogenicity of A(H5N1) viruses in humans. These would also be potential targets for therapeutic intervention.

In addition to the receptor binding and protease cleavage properties of the HA, the pH of activation of the HA is a determinant of A(H5N1) virulence in ducks and chickens and may influence interspecies transmission. Comparable studies in mice showed that while the wild-type virus with a fusion pH of 5·9 replicated efficiently, a Y23H substitution in HA1 destabilized the HA protein (activation pH of 6·3) and attenuated virus replication in mice, whereas a stabilizing substitution K582I, which lowered the activation pH by 0·5 pH units, resulted in greater virus replication in the lungs of mice and increased weight loss and mortality, in addition to enhancing replication in the upper respiratory tract of infected mice and ferrets.[20] These findings could help to optimize live-attenuated vaccines for better replication and thus immunity in the nasal cavity, or to identify a novel target for antiviral drug development.

Diagnosis

Identification of the agents responsible for respiratory disease and in particular pneumonia continues to present the physician with serious challenges, despite the rapid growth in molecular diagnostics. Rapid antigen-based and in particular nucleic acid-based tests are largely replacing culture-based methods. qPCR or chip-based microarrays are particularly adept at multiplex screening for a wide variety of organisms, but may be limited by mutations emerging through evolution or emergence of novel variants. At one end of the spectrum of complexity, the PathChip system, which can amplify 'all' viruses and bacteria, whatever their genetic diversity, and automatically detect the presence of any of 50 000 virus and 20 000 bacteria genomes in a single test of a clinical sample, was described. In one trial of more than 250 samples, comparing its performance with other multiplex respiratory diagnosis platforms and cell culture, the chip achieved an average specificity of 98% and average sensitivity of 83% for 11 groups of viruses, with an average negative predictive value of 98%. Such wide ranging tests also have applications in molecular epidemiology and virus discovery. At the other end of the spectrum, there is the need for simple, cost-effective and highly sensitive methods for rapid detection of human A(H5N1) infection. An 'RT-SmartAmp' assay has been developed that combines both reverse transcriptase (RT) and isothermal DNA amplification reactions in a single step, thereby avoiding RNA extraction and PCR reaction.[21] It provides a practical tool, which is highly sensitive for A(H5N1) (lower limit of detection of ~50 copies of virus RNA) with no cross-reaction with seasonal A (H1N1), A(H1N1)pdm09, A(H3N2) or B-type viruses. Incorporation of an excitation-controlled hybridization sensitive fluorescent primer with a high signal-to-noise ratio facilitates on-site visual end-point detection.

In an aetiological study of community-acquired pneumonia in Beijing, 954 patients (mean age 45, range 14–94 years) were screened using various tests for a wide range of bacteria and 15 respiratory viruses. Forty-one percent had at least one pathogen detected: *M. pneumoniae* (18%), influenza virus A (10%), bacteria (9%), human rhinovirus (4%) and adenovirus (4%). Co-infection was frequent (8%) and was associated with a higher risk of mortality. *M. pneumoniae* and adenovirus were seen more frequently in younger patients, while influenza A and bacterial pneumonias were more common among older patients. Empiric treatment should take account of local epidemiological data.

Antiviral effectiveness

A small number of studies described antiviral effectiveness, either observational studies of patients in Japan given one of the four available neuraminidase inhibitors (NAIs), oseltamivir, zanamivir, laninamivir and peramivir, or combination therapy studies using animal models. A study of 211 A(H3N2)-infected paediatric patients treated with one of the four NAIs showed that peramivir treatment achieved the fastest alleviation of fever, compared with the other three NAIs;[22] however, only four patients had received peramivir. Comparison of the effectiveness of oseltamivir and zanamivir in A(H1N1)pdm09-infected patients showed a similar time for the alleviation of symptoms. In a separate study, a single 300 mg intravenous infusion of peramivir was also shown to

be more effective than the other NAIs in reducing the duration of fever in patients infected with A(H3N2) or A (H1N1)pdm09 viruses. The effectiveness of all four NAIs was lower for treatment of influenza B than for influenza A virus infections, although treatment with peramivir or zanamivir resulted in faster alleviation of fever in influenza B-infected patients than that achieved with oseltamivir or laninamivir. Of concern is that over 50% of patients still shed influenza B virus after completion of the 5 day course of NAI treatment, demonstrating the need for new treatment options for influenza B, particularly for severely ill patients.

The benefits of combination antiviral therapy were reviewed; studies have shown that compared with monotherapy, combinations of certain antivirals can reduce both the likelihood of selecting resistant strains and the number of secondary cases. While the effect of combination treatment with two different NAIs has been shown to be antagonistic, synergism with drugs targeting different proteins or stages of replication has been demonstrated, although further clinical studies are necessary. A number of animal studies investigating antiviral effectiveness were reported. The effectiveness of peramivir against a seasonal A(H1N1) variant containing the H275Y NA mutation was investigated in mice. Despite demonstrating reduced sensitivity *in vitro*, peramivir was effective in preventing mortality in mice, while oseltamivir treatment had no effect (G. Boivin, this issue). Whether this applies to other N1-containing viruses with this mutation requires study. Another mouse study demonstrated the benefits of treatment with a combination of the polymerase inhibitor favipiravir (T-705) with either oseltamivir or laninamivir, compared with monotherapy. Following A(H1N1) pdm09 infection of immune-compromised mice and antiviral treatment durations ranging from 5 days to 4 weeks, the combination of favipiravir plus laninamivir showed superior effectiveness compared with favipiravir plus oseltamivir or any of the drugs given alone. In a separate study, laninamivir monotherapy was shown to be effective in delaying or preventing contact transmission in a guinea pig model.

The lack of mortality among pregnant women in Japan during the 2009 pandemic appears to have been due to aggressive use of antivirals and vaccines, on the advice of the Japan Society of Obstetrics and Gynecology (JSOG), which included as follows: early visit to a general practitioner when febrile; prompt use of antivirals; active use of antivirals for prophylaxis after close contact with an infected person; and vaccination against the pandemic virus once the vaccine became available. Data suggest that antiviral medications were given for prophylaxis to 40 000–50 000 pregnant women, and about 60% of pregnant women were vaccinated within 1·5 months of the availability of vaccine in Japan.[23] Apparently as a consequence, the rate of infection with the pandemic A(H1N1) virus was lower among pregnant women than in the general population (3·5% versus 12%), and outcomes of women with severe influenza were low compared with other countries.[7]

Antiviral resistance

Data were reported on the frequency of resistance to NAIs in both treated patients and in samples collected via human or animal surveillance programmes. The frequency of resistance in oseltamivir-treated patients has been determined in a prospective, multicentre study called IRIS and coordinated by Roche since 2008.[24] Analysis of A(H1N1)pdm09 viruses from patients undergoing oseltamivir treatment revealed that approximately 13% of paediatric patients aged <5 years shed oseltamivir-resistant virus with the H275Y NA mutation. The frequency of resistance was significantly lower in older age groups (approximately 1%). Furthermore, the frequency of cases shedding resistant virus was higher in 2011 than in the previous 2 years, despite a similar numbers of patients being enrolled over the 3-year period. Viral shedding was more prolonged in oseltamivir-treated patients shedding the H275Y variant than in those infected with an oseltamivir-sensitive virus, demonstrating the reduced effectiveness of oseltamivir against the variant strain. Infection with a H275Y variant was detected in two Dutch travellers returning from vacation in Spain, both of whom had not been treated with oseltamivir.[25] These oseltamivir-resistant strains also contained the three potentially 'permissive' NA mutations, V241I, N369K and N386S, suggested to facilitate the spread of resistant virus in a community cluster in Australia in 2011. New insights into the mechanism of oseltamivir resistance conferred by a I223R substitution in NA of A(H1N1)pdm09 viruses were provided by crystallographic analysis of the NA, which demonstrated that the I223R substitution causes a narrowing of a region of the enzymatic site which results in a greater reduction in oseltamivir than zanamivir binding.[26] In immune-compromised mice continually infected with A(H1N1)pdm09 viruses, oseltamivir treatment selected for variants with a H275Y NA mutation, while laninamivir treatment selected for variants with E119G and R152K NA mutations. No viruses with reduced susceptibility to favipiravir have been detected following treatment with this polymerase inhibitor.

Variation in NAI susceptibility among A(H5N1) viruses collected from wild birds and poultry in South-East Asia was described. Most notable was the 30-fold lower oseltamivir sensitivity of Indonesian clade 2·1 viruses than clade 1 viruses. Substitutions in the I222 residue of NA, I222M, I222T and I222V, detected in a number of strains from both Indonesia and Vietnam, conferred a reduction in oseltamivir susceptibility.[27] An A(H5N1) strain isolated from a duck in Vietnam was found to have a H275Y mutation in NA and consequently significantly reduced oseltamivir susceptibility.

To assist the coordination of reporting NAI susceptibility data derived using the NA inhibition assay, a set of criteria

has been proposed by a WHO expert committee, based on fold increases in IC_{50} compared with viruses with normal inhibition: normal (<10-fold), reduced (10- to 100-fold) and highly reduced (>100-fold) susceptibility for influenza A and greater than fivefold, fivefold to 50-fold and >50-fold increases in IC_{50}, respectively, for influenza B. In addition, tools to assist laboratories in conducting NAI susceptibility testing were discussed; these included the isirv-AVG panel of oseltamivir-sensitive and -resistant reference viruses (http://www.isirv.org/site/index.php/reference-panel) and curve-fitting software (JASPR) for the determination of IC_{50} values, from the CDC, USA.[28]

Fitness of NAI-resistant viruses

A number of studies reported on the effects of NAI resistance mutations on virus replication and/or transmission, including permissive mutations that play a role in offsetting the deleterious effects of resistance mutations. Two NA mutations, Q136K and E119G, which have previously been reported to confer zanamivir resistance were investigated using reverse genetics-derived viruses to better understand their effects on *in vitro* and *in vivo* fitness.[29] Both variants exhibited reduced cell surface NA activity and virus replication *in vitro*, reduced virus replication in mice and reduced virus replication and transmission in ferrets. Reversion of the E119G variant to wild type in ferrets further demonstrated its lack of fitness of this variant.

Two 'permissive' substitutions in residues 222 and 344 of NA, shown to play a role in enabling the seasonal A(H1N1) (2007–2008) viruses to acquire the H275Y mutation without compromising fitness, were investigated in the context of H275Y-containing A(H1N1)pdm09 and A(H5N1) variants. Substitutions in residue 222 increased affinity for substrate in both A(H1N1)pdm09 and A(H5N1) viruses, while the effects of changes in residue 344 varied depending on the amino acid substitution and the NA sequence. Three potentially 'permissive' substitutions in NA of the cluster of oseltamivir-resistant A(H1N1)pdm09(H275Y) viruses detected in Australia in 2011 were investigated in a ferret model for their effects on virus replication and transmission. Two of the substitutions, V241I and N369K, which are currently present in the majority of circulating A(H1N1)pdm09 viruses, were shown to improve the fitness of H275Y-containing variants in ferrets, thereby demonstrating the potential for these oseltamivir-resistant variants to emerge in a similar manner to that seen with seasonal A(H1N1) viruses in 2008.

Novel antiviral agents

A number of novel anti-influenza agents that are either in development or undergoing clinical trials were described. Nitazoxanide is a thiazolide compound that has been approved for treatment of cryptosporidium and giardia and has been shown to inhibit *in vitro* replication of a broad range of viruses including influenza and parainfluenza viruses. The mechanism of action has been attributed to stimulation of innate immunity via interferon-stimulated pathways, as well as a direct effect on inhibiting HA maturation during virus replication.[30] A phase II/III clinical trial demonstrated that a 600 mg dose bid for 5 days significantly reduced symptom duration and virus load compared with placebo, with minimal adverse events reported. Combinations of nitazoxanide with oseltamivir were synergistic in inhibiting replication of influenza viruses *in vitro*, and further Phase III studies of the combination are planned. BARDA recently approved $150 million to support further development of nitazoxanide for the management of influenza.

Studies of the mode of action of the polymerase inhibitor favipiravir were described. Serial passage *in vitro* of influenza viruses in the presence of increasing favipiravir concentrations resulted in reduced virus polymerase fidelity. The consequent increase in frequency of transversion mutations during replication led to a lack of viable virus.[31] Phase III clinical trials of favipiravir in treating uncomplicated influenza have recently been completed in Japan and are planned for other countries.

Reports from the 2009 and prior pandemics suggest that intravenous immunoglobulin (IVIG) may improve clinical outcomes of severe influenza.[32,33] This impact may result from neutralization (either homologous or heterologous reactivity) of infectious virus or modulation of the immune response, blunting the 'cytokine storm' seen in some patients. Two ferret models were utilized to investigate the role of IVIG on A(H1N1)pdm09 and A(H5N1) infection. A single dose of IVIG, harvested prior to 2009, given at the time of challenge prevented significant virus replication in the lung but not in the upper respiratory tract of the A(H1N1) pdm09-infected ferrets. A single dose of IVIG prevented mortality and significant morbidity following challenge with a lethal dose of A(H5N1) virus; the level of virus replicating in the lung correlated with the dose of IVIG. In a different study, polyclonal immunoglobulin, derived from plasma of horses immunized with inactivated A(H5N1) virus, was shown to have good cross-reactivity *in vitro* against different clades of A(H5N1) viruses and to fully protect A(H5N1)-infected mice.

There is particular interest in the potential therapeutic use of monoclonal antibodies (Mabs) targeting conserved regions of the HA, which have been demonstrated to be effective in prophylaxis and treatment for mice. A range of different human Mabs, that target the stalk region of the HA, was found to be effective in treating both seasonal influenza A and B and highly pathogenic A(H5N1) infections. Mab CR6261, which binds to the stalk of all group 1 HAs, outperformed oseltamivir when given to mice 2 days post A

(H5N1) infection (0% versus 88% mortality, respectively) and was shown still to be effective when given 5 days post-infection with A/WSN/33(H1N1)[34], and was effective, when used prophylactically and therapeutically, in protecting ferrets against lethal A(H5N1) infection.[35] Mab CR8020, which binds to the stalk of all group 2 HAs,[36] prevented infection and was effective in treating mice up to 3 days after infection with A/HK/68(H3N2). More recently, of three human Mabs against influenza B, two, CR8033 and CR8071, bound epitopes on the head of HA of all influenza B viruses, while CR9114, that binds a conserved epitope on the stem, protected mice against lethal challenge with either influenza A or B viruses.[37] Clinical trials with the antibodies, CR6261 and CR8020, will commence in 2013. Proof of concept studies for therapy of severe influenza guided by point-of-care diagnostics are expected to follow shortly thereafter.

Mab VIS410, designed using atomic interaction network analysis, also exploits a conserved region in the stem of HA and neutralizes all group 1 and 2 influenza A viruses. Studies in mice suggest that prophylaxis and treatment regimens of VIS410 prevent weight loss and result in reduction in virus titres after experimental exposure to H1, H3 or H5 subtypes. Phase I safety studies and proof of concept studies utilizing a human challenge model are currently planned with the hopes of starting Phase II studies in high risk (e.g. transplant recipients and nursing home residents) and severely ill, hospitalized adults in 2017–2018.

Exploiting the vital role that host proteins play in influenza replication may offer new therapeutic targets. The ability to decrease A(H5N1) virus replication by modulating the expression of host proteins that are involved in cellular pathways such as endocytosis has been investigated.[38] Downregulation of proteins of the coat protein complex, by siRNA against COPA or by Brefeldin A, achieved a modest reduction in A(H5N1) infection in human cells without any associated cytotoxicity and demonstrated the potential for influenza replication to be reduced by inhibition of these host proteins. In a separate study, using cDNA microarray and long non-coding RNA microarray analyses, two genes in A549 cells were shown to be differentially expressed following influenza infection and to be associated with transport of NA in infected cells, providing possible future antiviral targets.

Research ethics

In relation to a discussion of future research needs, it was emphasized that conducting public health and clinical research in the setting of an epidemic of a novel or re-emerging infectious disease is vital, but exceptionally challenging. A study conducted in the Oxford University Clinical Research Unit (OUCRU), Ho Chi Minh City, Viet Nam and three other hospitals in Viet Nam with experience of epidemics, by in-depth interviews and focus group discussions, involved people representing different constituents of the health system (from research staff to patients) and who have participated in or reviewed research projects on infectious diseases. Seven main themes emerged, concerning: (i) the dividing line between public health needs, medical practice and research; (ii) the vulnerability of research participants and family members; (iii) research information provision and factors influencing decisions of research participants/family members; (iv) challenges faced by IRBs and factors which might influence their review and oversight in the setting; (v) dynamics of research collaboration; (vi) multiple commitments of investigators and staff due to existing and emergency workloads; and (vii) the role of the media and wider society during such rapidly evolving epidemics. While some of the issues are of general concern, others are unique to rapidly evolving epidemics, and it was stressed that these considerations be addressed effectively before the next major epidemic or pandemic.

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