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Review

A novel H1N1 virus causes the first pandemic of the 21st century

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A novel H1N1 virus of swine origin (H1N1v) is currently spreading in humans, giving rise to the first pandemic in 40 years. The disease is of moderate severity but has notable differences from seasonal influenza. In contrast to seasonal influenza, those over 60 years are relatively spared, a likely consequence of the presence of H1N1v cross-neutralizing antibody in this age group. Most patients appear to have mild influenza-like illness and many of the complications leading to hospitalization and mortality occur in those with underlying disease conditions or pregnancy. Studies in animal models suggest that the novel H1N1v pandemic virus causes a more severe illness and appears to have a greater predilection for the alveolar epithelium than seasonal influenza viruses. As there are as yet little data on the pathogenesis and immunology of H1N1v infection in humans, we have reviewed relevant data from past pandemics, from seasonal influenza and avian influenza H5N1 to highlight key issues pertaining to pathogenesis and immunology.

Key words: H1N1 · Immunology · Influenza · Pandemic · Pathogenesis

Introduction

A novel H1N1 virus originating in swine recently emerged as the first influenza pandemic of the 21st century [1]. As the 20th century saw three influenza pandemics, in 1918 (H1N1), 1957 (H2N2) and 1968 (H3N2), and the historical record indicates that there were approximately three to four influenza-like pandemics every century, going back to 1500 AD [2], the emergence of a pandemic 40 years after the last in 1968 was hardly a surprise. Its nature and origins however, were unexpected. This review summarizes the emergence of this pandemic, clinical features of the disease and its pathogenesis, and also highlights the key questions for immunological research.

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The virus and the birth of pandemics

Pandemic influenza is caused by type A influenza viruses and these are single-stranded RNA viruses with an eight-segmented genome. Type A influenza viruses are subtyped on the basis of antigenic relationships of the virus surface glycoproteins, the HA and neuraminidase (NA), into 16 HA and 9 NA subtypes. The HA and NA are the key antigens to which protective antibody responses are directed and there is minimal serological cross-protection across HA subtypes. All 16 HA and 9 NA subtypes are present in the aquatic avian reservoir while a more restricted range of subtypes are endemic in other species such as pigs (H1, H3) and horses (H3, H7). Pandemics were believed to arise when a virus with a novel HA subtype (sometimes together with a novel NA) adapts to efficient transmission in humans [3]. The recent H1N1 pandemic arose from a swine H1N1 virus adapting to human transmission without genetic reassortment with current

human influenza viruses, *i.e.* all eight gene segments of the animal virus adapted to human transmission [4, 5]. Aspects of the evolution of viruses in pigs and how these contributed to the genesis of the current pandemic virus have been reviewed elsewhere [6].

Since previous pandemics arose from a subtype different from the currently prevailing human influenza virus, it was expected that the next pandemic will arise from a subtype other than H1 or H3, which were endemic in human for many decades. In retrospect, it is apparent why H1N1 of swine origin was successful as a pandemic virus. When the H1N1 pandemic arose in 1918, that virus concurrently infected pigs, although which came first remains unclear. The 1918 H1N1 virus has remained endemic in swine ever since as the "classical swine" H1N1 virus [7]. Recent experimental infections of pigs with the genetically reconstituted 1918 virus has shown that it replicates and transmits in swine [8]. The 1918 H1N1 virus is also the progenitor of the contemporary seasonal influenza H1N1 virus and many of the current swine H1 virus lineages also descend from this common 1918 H1N1 precursor. During the last 90 years, "herd-immunity" in the human population forced the seasonal H1N1 virus to undergo substantial antigenic drift. As there was little herd immunity in pigs because of their relatively short life span and quick turnover, the classical swine H1 viruses remained antigenically relatively stable [7] until the past 10 years or so when a series of reassortment events led to the emergence of the "triple reassortant" swine viruses that appeared to be more promiscuous genetically and variable antigenically [7]. The net result was an increasing antigenic divergence between the swine H1 viruses and human H1N1 viruses. Thus, humans infected exclusively with recent human seasonal H1N1 viruses have no cross-neutralizing activity against recent swine H1-subtype viruses including H1N1v (H1N1v, variant of the seasonal H1N1 viruses), although humans born earlier in the 20th century (and exposed to human H1N1 viruses more closely related to classical swine H1N1) do. Approximately a third of the human population over 60 years of age have cross-neutralizing antibody to the pandemic H1N1 2009 virus while few of the younger individuals do [9].

The nomenclature of the new pandemic virus has led to some confusion. Currently WHO recommends the name "pandemic H1N1 2009", with the abbreviated form being "H1N1v" (v standing for variant, to differentiate it from the seasonal H1N1 viruses). In this review, unless otherwise stated, we will use H1N1v for the pandemic H1N1 2009 virus and H1N1 to denote the seasonal H1N1 virus.

Clinical presentation

In the early phase of the pandemic, the apparent case-fatality ratios being reported from Mexico was as high as 2% [10]. It is now clear that these estimates were distorted by selective diagnosis of severely ill patients and also the lack of reliable denominator data. As the virus has spread globally, it is clear that this H1N1v pandemic is one of moderate severity; it is not comparable with that seen with zoonotic disease caused by avian-flu H5N1 or the 1918 Spanish flu pandemic. However, the pandemic H1N1v is not "just another seasonal flu". While seasonal influenza is associated with significant excess mortality, such excess mortality is primarily concentrated in the elderly, a segment of the population that appears to be relatively spared in the current H1N1v pandemic (See *The virus and the birth of pandemics section*). Those over 60 years constitute approximately 2% of all H1N1v cases or hospitalizations and in Europe almost 80% of cases have been in those <30 years of age [11]. In California, the median age of all cases was 17 years, hospitalized cases was 26 years and fatal cases was 45 years. Most reported deaths worldwide have been in those ≥ 40 years of age [12]. While the overall morbidity and mortality may not be dramatically higher than that seen with severe seasonal influenza outbreaks, the impact is on a different age group of the population. As of July 10 2009, it is estimated that >1 million people have been infected with H1N1v in the USA, with a hospitalization rate of 0.3–0.4% and a case fatality ratio of 0.02% [12].

The clinical spectrum is broad, ranging from a mild upper respiratory tract illness with or without fever, sometimes associated with exacerbations of underlying conditions. Some patients present with gastro-intestinal symptoms of vomiting or diarrhea. The infection occasionally leads to severe primary viral pneumonia leading to acute respiratory distress syndrome, multiple organ dysfunction and death [13]. Many of those hospitalized with severe illness have been pregnant women, those with underlying diseases such as asthma, obstructive airways disease, diabetes, immunodeficiency, chronic cardiovascular disease (excluding hypertension), chronic renal failure, seizure disorders, malignancy and morbid obesity and those under 2 years of age [14]. Pregnancy, especially during the third trimester, may be associated with severe disease, intra-uterine fetal death or spontaneous abortion. Following H1N1v infection, pregnant women appear to have an approximately 13-fold higher risk of hospitalization than the general population [12, 14, 15].

Pathogenesis and immunity

It is still not clear whether H1N1v disseminates beyond the respiratory tract to replicate, for example, in the gastro-intestinal tract, as H5N1 virus can. The preliminary pathological findings of fatal H1N1v disease have been those of diffuse alveolar damage (the histological findings seen in acute respiratory distress syndrome), and a hemorrhagic interstitial pneumonitis with a lymphocytic cell infiltrate suggestive of a primary viral pneumonia. Secondary bacterial super-infection has occasionally been seen but is not common with only 14% of fatal cases in California having microbiological evidence of secondary bacterial or fungal infection. In some areas it was observed that the early wave of severe hospitalized cases are those of primary viral pneumonia typically in otherwise healthy young individuals; this is associated with a poor prognosis. A second wave of hospitalizations was seen, primarily in those who are older and with underlying risk factors for influenza

complications. Overall, the majority of fatal cases and hospitalized patients had one or more underlying conditions [12, 14].

Primary viral pneumonia can occur rarely with seasonal influenza but its pathology is not well described. The available descriptions of primary viral pneumonia therefore have been from the severe pandemic of 1918 [16, 17] and 1957 [18]. Virus antigen has been detected in alveolar epithelial cells and alveolar macrophages [19]. While primary viral pneumonia is the rare exception following H1N1v infection, it is relatively common in those rare individuals who develop avian influenza H5N1 disease [20, 21]. There continues to be controversy over whether the lung pathology of primary influenza viral pneumonia is solely due to a direct viral cytopathic effect or whether it is contributed to by innate immune responses [16]. Furthermore, it is unclear whether the pathogenesis of this condition in 1918 influenza and H5N1 infection is similar to that seen in the rare cases with primary viral pneumonia following H1N1v infection. Detailed data from autopsy studies are awaited.

The H1N1v virus does not possess any of the genetic virulence motifs associated with either the H5N1 or the 1918 pandemic viruses, for example, Lys at residue 627 or Asn at residue 701 in the PB2 gene, the multiple basic amino acid motif in the connecting peptide of the HA (HA_0), Ser 66 in the PB1-F2 gene, Glu 92 in the NS1 gene [22]. However, in animal models, H1N1v virus causes more severe illness and has a greater predilection for infecting the alveolar epithelial cells than does seasonal H1N1 influenza in ferrets [23–25], mice and macaques [23]. Comparing mice, ferrets and macaques infected with H1N1v or seasonal H1N1 virus, the upper respiratory tract had comparable viral titers but viral replication in the lungs was markedly greater in H1N1v-infected animals.

Preliminary data suggest that H1N1v virus preferentially binds sialic acid (Sia) α 2–6 receptors, similar to seasonal influenza [25]. While this observation was compatible with the H1N1v virus's replication in the upper respiratory tract, which contains an abundance of Sia α 2–6 receptors, such a receptor preference would not be expected to allow H1N1v to replicate without prior adaptation in the mouse respiratory tract, which mainly contains Sia α 2–3 [26]. In contrast, avian influenza viruses (*e.g.* H5N1) binds Sia α 2–3 receptors, which are found in birds but also found in the alveolar epithelium. One hypothesis to explain the severity of human H5N1 disease has been the targeting of the virus to the Sia α 2–3 receptors found on the alveolar epithelium [27, 28], but such a hypothesis fails to explain why H1N1v (which apparently binds Sia α 2–6) causes more infection of the alveoli than does seasonal influenza H1N1. In addition to the terminal sialic acid linkages, internal linkages as well as fucosylation, sulfation and sialylation at the inner oligosaccharide may also determine HA receptor recognition. These findings point to an unusual cell tropism of the H1N1v virus and a more detailed analysis of the receptor binding profile of H1N1v is awaited.

Role of innate immune responses

There are so far no data on host innate immune responses of H1N1v-infected patients. The lungs of H1N1v-infected mice had a markedly different profile in cytokine induction, especially greater levels of Th2 cytokines such as IL-4, IL-10 and the Th1 cytokine IFN-γ [23]. The lungs of H1N1v-infected macaques had higher levels of chemokines MCP-1, MIP-1α, IL-6 and IL-18 than that of seasonal influenza H1N1-infected animals [23]. These changes may well be a reflection of the more extensive replication of H1N1v virus in the lungs rather than an intrinsic property of the virus in eliciting aberrant host responses as has been demonstrated with 1918 or H5N1 virus infection. Comparison of host responses of H1N1v and seasonal H1N1 virus-infected primary human cells would help resolve this question of whether H1N1v differs from seasonal influenza virus in its replication competence or in its capacity to induce host responses, as, for example, is seen in H5N1 infection (see later in this section).

While the severity of disease in H1N1v-infected mice, ferrets and macaques is greater than that seen with seasonal influenza H1N1 viruses [23, 25], disease in these animal models is not as severe as that seen with highly pathogenic avian influenza (HPAI) H5N1 or the 1918 H1N1 viruses [29, 30] where there is evidence that pathogenesis is contributed to by dysregulated host responses. Although H1N1v may not share similar pathogenic mechanisms, it is pertinent to briefly review this information for comparison and as a strategy that could be used to investigate the pathogenesis of H1N1v. When compared with seasonal influenza viruses, HPAI H5N1 elicits exaggerated pro-inflammatory host responses from infected macrophages and respiratory epithelial cells *in vitro* [31–34], from infected lung tissues of experimentally infected animals *in vivo* [34], and in the serum and lungs of patients with H5N1 disease [35, 36]. In comparison with seasonal influenza viruses, HPAI H5N1 viruses show increased virulence in mice, ferrets and macaques, with evidence of increased viral replication and dysregulated host responses [34, 37–39] that were associated with recruitment of macrophages and neutrophils into the lungs contributing to acute lung inflammation [34]. Ferrets infected with H5N1 viruses had stronger induction of CXCL10 and interferon response genes in the lungs in comparison with H3N2 subtype seasonal influenza. Blocking of CXCR3, the cognate receptor of CXCL10, with the drug AMG487 resulted in amelioration of symptoms and delayed mortality [40].

Mice and macaques that were 1918 H1N1-infected also had more severe disease and dysregulated host responses when compared with seasonal influenza virus-infected controls [29, 30, 34].

There are at present no comparable data on the quantitative virology or host responses in human H1N1v infections or *in vitro* data from primary human cells for integration with the animal model data and such investigations are urgently needed.

Antibody-mediated protection

The role of antibodies in protection against influenza virus infection is well established and antibody titers remain the main correlate of protection for evaluation of conventional vaccines. Passive immunotherapy was used with apparent success during

the 1918 pandemic [41] and passive immunotherapy with human monoclonal antibodies have also been shown to protect mice from challenge with the highly pathogenic H5N1 virus [42]. The role of passive immunotherapy for pandemic H1N1v remains to be demonstrated experimentally and clinically and could potentially be an adjunct management option for patients with severe disease. More recently monoclonal antibodies that have neutralizing activity and ability to protect mice from challenge against infection with different subtypes of influenza A have been identified [43]. The binding site of these antibodies has been confirmed to be the fusion domain of the HA2 region of the HA, which is well conserved across many subtypes [44]. While the dominant neutralizing epitopes are found in the HA, antibodies to the NA and M2 ectodomain also have protective activity. A recent study using whole genome fragment phage display libraries has shown that this strategy can be used to identify human antibody-binding epitopes on all virus proteins so as to compile a systematic analysis of antibody-binding epitopes for a given virus [45].

Role of virus-specific $CD4^+$ and $CD8^+$ T-cell immune responses

There are no data on the virus-specific $CD4^+$ and $CD8^+$ T-cell responses to the current pandemic H1N1v 2009 so far but viral-specific CD4 and CD8 response play critical roles for host defenses against seasonal influenza A infection. Cross-subtype reactive $CD8^+$ T-cell responses affect the clearance of virus infection even in those who lack virus-specific antibody [46]. Once intracellular influenza A infection is established, viral clearance is mainly dependent on virus-specific $CD8^+$ T cells, *i.e.* CTL. CTL recognize MHC class I antigenic peptide complexes on virus-infected epithelial cells and destroy the virus-infected cells mainly by exocytosis of granules containing perforin and granzymes and also through Fas/Fas ligand dependent cytotoxic mechanisms [47, 48].

$CD4^+$ T cells are important for adaptive immunity to natural influenza A infection or vaccination by providing help to B cells for antibody production. A major form of help is CD40 ligand (CD154), which is expressed by activated $CD4^+$ T cells and engages CD40 on B cells to promote antibody production, isotype switching and memory B-cell generation [49]. $CD4^+$ T cells may also provide help to $CD8^+$ T cells for the generation of cytotoxicity [50]. *In vitro* studies demonstrated that CD154 is the key source of $CD4^+$ T-cell help for the expansion of virus-specific $CD8^+$ T cells following vaccination [51]. Animal models suggest that influenza A virus-specific $CD4^+$ T cells contribute to the protection against lethal infection even in cases of complete humoral immunodeficiency [52]. Isolated influenza A virus-specific human and murine $CD4^+$ T-cell clones can also mediate cytotoxicity [53]. In addition, virus-specific $CD8^+$ T cells are rich sources of effector cytokines, such as IFN-γ and TNF-α, which can promote APC function and have direct antiviral activity [54]. In humans, virus-specific Th1 responses to other viruses (*e.g.* cytomegalovirus) may be more important than virus-specific $CD8^+$ T cells in controlling virus replication [55].

Possible future course of the H1N1v pandemic

A number of imponderables remain about the future trajectory of the H1N1v pandemic. There is little doubt that the H1N1v pandemic will continue to spread worldwide and that infection will resurge during the winter in the Northern hemisphere. Whether this novel virus will replace the hitherto endemic influenza A virus subtypes H3N2 and H1N1 remains a question. In southern hemisphere regions, *e.g.* Chile, Australia and South Africa, this winter influenza season has been dominated by H1N1v, although H3N2 viruses do continue to circulate and, in fact, a novel drift variant of H3N2 virus has emerged. In past pandemics (*e.g.* 1957, 1968), the pre-existing type A influenza virus was completely supplanted by the novel pandemic virus, presumably because of its antigenic novelty and selective advantage in spreading within a immunologically naïve population. The mechanism of such exclusion is unclear. If one assumes that there is little serological cross-protection between the old and new vaccine subtypes, then such immunological competition and exclusion likely relates to high and synchronized levels of cross-reacting T-cell immunity or innate immune resistance in the population. Given the fact that a substantial segment of the population (*i.e.* those older than 60) appear to be relatively protected from the H1N1v virus, it may be unlikely that the pandemic virus will exclude the previous H3N2 and H1N1 viruses from this subset of the population. Thus, a scenario of co-circulation of three subtypes of influenza A remains real and one with substantial implications for future influenza vaccine production. In the short term, novel pandemic H1N1v vaccine will be a monovalent vaccine with the trivalent seasonal influenza vaccine being manufactured separately to contain influenza A subtypes H1N1 and H3N2 and influenza B. Would future influenza vaccines have to be quadrivalent?

Will the H1N1v pandemic remain as mild as it appears to be at present? In 1918, and also in some European countries in 1968, the severity of the pandemic increased in its second wave of circulation [56]. Such a change in virulence of the virus may be associated with the "better" adaptation of this swine-origin virus to humans or reassortment of its genome with existing seasonal human influenza viruses. The acquisition of antiviral (oseltamivir) resistance through *de novo* acquisition of resistance mutations (sporadic resistant isolates have been reported from a number of countries though it is not yet widespread) or *via* acquisition of the resistant NA through reassortment with seasonal H1N1 viruses that are at present largely resistant to this drug remains a concerning possibility. Acquiring antiviral resistance would not change the inherent virulence of the virus but would remove a major therapeutic option for those occasional patients with severe disease.

As the H1N1v virus almost certainly arose in swine, it very likely retains the capacity to re-infect and spread in swine.

Experimental infections of swine with the H1N1v virus demonstrate that it efficiently infects these animals and remains transmissible amongst pigs [57]. We may thus have to face the prospect that the H1N1v pandemic in humans may be accompanied by an H1N1v panzootic in swine. More recently, the H1N1v virus has also been isolated from turkeys. The readiness with which H1N1 virus will infect pigs and perhaps turkeys provides an alternative scenario for reassortment with other viruses in swine and also with avian influenza viruses that may transiently infect swine (*e.g.* H5N1, H9N2) [58]. The possibility of reassortment of H1N1v virus with the HPAI H5N1 would be the nightmare scenario. This is not particularly likely since human H3N2 viruses have been endemic in swine in southern China in the early part of this decade [58], coincident with the H5N1 panzootic in poultry, but a reassortant virus has failed to emerge. Experimental co-infection of ferrets with HPAI H5N1 and human seasonal H3N2 viruses led to the emergence of reassortant viruses but these had reduced fitness and failed to acquire transmissibility in the ferret model [59]. However, the H1N1v virus has the "triple reassortant internal gene cassette" (TRIG cassette) that has over the past decade demonstrated a promiscuity in acquiring different virus surface genes through genetic reassortment [7]. So it is not inconceivable that H1N1v may succeed where other human seasonal influenza viruses have not, in reassorting with other swine or avian viruses.

While the H1N1v viruses currently circulating are genetically and antigenically homogenous, likely reflecting the lack of significant herd immunity in the population (with the exception of those >60 years of age), this situation is likely to change in the future. The virus will then likely behave in the same way as other influenza viruses and undergo regular antigenic drift.

Challenges for immunologists

While seasonal influenza carries the greatest disease burden for the elderly, the efficacy of currently available influenza vaccines in this group is far from ideal and novel strategies to improve vaccine efficacy for this target group are urgently needed [60]. While the H1N1v virus currently spares the elderly, with continued antigenic drift, it will likely pose problems for this age group in due course. The importance of the phenomenon of "original antigenic sin", *i.e.* that immunological responses remain directed at those cross-reactive epitopes first encountered by the immune system at the expense of novel and variant epitopes, has remained controversial with regards to influenza [61, 62]. This may potentially result in poor protective antibody responses following H1N1v infection and vaccination in the young adult population.

The regular antigenic drift of influenza viruses necessitates annual updating of influenza vaccines and also the need for multiple vaccine viruses to be included to cover different influenza A subtypes. The challenge posed by poorly immunogenic and antigenically diverse avian influenza viruses (*e.g.* H5N1) led to a number of developments in adjuvents that provide enhanced immunogenicity as well as broad cross-clade protection and allow antigen-sparing strategies [63, 64]. The unpredictable nature of pandemics implies monitoring and preparedness to immunize against a range of potential pandemic candidates, knowing fully well that predicting the vagaries of influenza virus behavior is well-nigh impossible, as we saw with the unexpected emergence of H1N1v as a pandemic. Thus, the Holy Grail of a universal influenza A vaccine that provides cross-protection against drift variants within the same subtype and across different subtypes is one that needs to be pursued with renewed vigor. The ectodomain of the influenza viral M2 protein is well conserved within and across influenza subtypes and vaccines based on this have been pursued (reviewed in [65]). Neutralizing antibody epitopes that are conserved across some influenza A subtypes have been defined and human monoclonal antibodies that confer cross-subtype protection in experimental animal challenge experiments have been reported with potential for passive immunotherapy and immunoprophylaxis [43, 44]. Passive immunotherapy is likely to be a useful option for novel emerging infections of the future, including influenza pandemics.

CTL specific for influenza virus are broadly reactive for viruses of different subtypes [66], and adoptive transfer experiments have shown that $CD8^+$ T-cell memory can cross-protect across subtypes [67]. Memory T cells established by seasonal influenza can cross-react even with avian influenza H5N1 [68]. The targets for such CTL responses include the short viral peptides derived from the nucleoprotein, polymerases PA and PB1 and the matrix protein 1 [69]. Direct characterization of influenza ligands eluted from HLA class I molecules (HLA-B*0702) using mass spectrometry identified a modest number (3–6) of viral peptides being presented during infection with H1N1 and H3N2 influenza A viruses [70]. Based on predicted HLA-binding motifs and viral sequence conservation, a recent study has identified a set of 54 influenza virus-derived T-cell epitopes (38 HLA class I and 16 HLA class II) recognized by healthy human blood donors [71]. Thirty-five out of these 54 epitopes were conserved with the H1N1v pandemic virus (26 HLA class I and 9 HLA class II) (our unpublished data). The challenge of inducing cross-reactive CTL response is that natural influenza infection leads to better CTL induction, and conventional-inactivated vaccines do not induce CTL and only weakly boost existing memory $CD8^+$ T-cell response [72]. While induction of such CTL by infection may not be able to confer sterilizing immunity or protection from infection, it may provide reduction of morbidity and mortality, especially in a pandemic situation as shown in the Cleveland family study [72–74].

$\gamma\delta$ T cells have potent antiviral activities against diverse viruses and they can be selectively expanded by phosphoantigen isopentenyl pyrophosphate. Such $\gamma\delta$ T cells efficiently kill cells infected with a range of human or avian influenza viruses [75] this may potentially provide a novel therapeutic approach to seasonal, pandemic or zoonotic influenza.

The challenges for immunologists in relation to influenza in general and pandemic H1N1v in particular are summarized in Table 1.

Table 1. Summary of some key research needs in immunological aspects on pandemic H1N1v disease

Clinical research needs:
1. Risk factors contributing to severe disease outcome
2. Viral load and immunological (innate immunity, T cells, antibody) parameters in pandemic H1N1v *versus* seasonal influenza and in severe *versus* mild disease
3. Immunological correlates of protection
4. Mechanisms that protect the aged group (>65 years) from H1N1v infection
5. Autopsy studies on fatal influenza to define virus tropism and host responses

Pathogenesis:
1. Defining the role of innate immune responses in host defense and disease pathogenesis using *in vitro* and *ex vivo* models of infection in respiratory epithelium and in relevant animal models
2. Does the NS1 protein of H1N1v have comparable ability to evade host innate immune responses as does NS1 of seasonal influenza
3. Defining the role of NK cells and γδ T cells in host defense against influenza
4. Host genetic susceptibility factors for influenza

Vaccines and passive immunotherapy:
1. Effective vaccines for the elderly and the infant (<6 years of age)
2. Strategies for active and passive immunity that offers broad cross-protection, against (i) antigenic-drift variants and (ii) across subtypes (heterosubtypic immunity)
3. Defining the role of cell-mediated immune responses in protection against influenza
4. Developing the novel adjuvants, which could preferentially enhance vaccine-induced cellular immunity
5. Mapping antibody and T-cell epitope mapping for H1N1v
6. Investigating the role of passive immunotherapy for H1N1v

General aspects:
1. Better animal models for understanding influenza disease pathogenesis
2. Sequence data and tools to study the genomics, proteomics and immunological responses in relevant animal models (*e.g.* ferrets)

Concluding remarks

While the threat from the highly pathogenic H5N1 virus over the past few years had raised the levels of global pandemic preparedness, the emergence of a pandemic, even one as mild as that caused by H1N1v, posed a major stress to health-care systems and public heath responders worldwide. One can only speculate what may have happened if indeed a more severe pandemic, for example, one caused by H5N1, had emerged. As previously highlighted by SARS [76], the rapidity of the global spread of H1N1v worldwide re-enforces the fact that our global travel connectivity increases our vulnerability to novel emerging disease threats [77]. While the experience gained with the use of novel adjuvents and antigen-sparing strategies in development of vaccine to H5N1 hopefully translates into increased vaccine availability to H1N1v virus in a timely manner this winter, the issues related to equitable distribution of vaccines to those who need it most, irrespective of geography and affluence, provides a political and moral dilemma [78].

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Abbreviations: **H1N1v:** variant H1N1 virus of swine origin · **HPAI:** highly pathogenic avian influenza · **NA:** neuraminidase · **Sia:** sialic acid

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