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Host response to influenza virus: protection versus immunopathology

JSM Peiris[1,2], Kenrie PY Hui[1] and Hui-Ling Yen[1,2]

Host responses play crucial roles in defense against influenza but sometimes these may contribute to immunopathology. Potentially, this may be more important in disease caused by viruses such as avian influenza A H5N1 or the 1918 H1N1 influenza virus rather than with seasonal influenza or pandemic H1N1 2009 (pdmH1N1). Understanding pathogenesis will help develop novel therapeutic options that minimize immunopathology without impairing beneficial host defenses.

Addresses
[1] Department of Microbiology, Li Ka Shing Faculty of Medicine, University of Hong Kong, Hong Kong, China
[2] HKU-Pasteur Research Center, Hong Kong, China

Corresponding author: Peiris, JSM (malik@hkucc.hku.hk)

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Introduction

Influenza viruses belong to the family Orthomyxoviridae and are enveloped RNA viruses with a segmented, negative-sense, single-stranded RNA genome. This review focuses on the pathogenesis of human disease caused by type A influenza viruses, the only ones that cause both epidemic and pandemic influenza. Particular attention is paid to recent data with relevant reviews being cited for earlier literature [1,2••,3].

The virus envelope antigens, haemagglutinin (HA) and neuraminidase (NA) are the major targets of the protective host antibody response. Influenza A is antigenically subtyped into 16 HA and 9 NA subtypes. Natural infection provides little long-term serological cross-protection between different subtypes and pandemics are associated with the emergence of novel subtypes to which the population is immunologically naive. Although this remains true for the immunodominant epitopes eliciting antibody responses after natural infection or vaccination, fusion inhibiting monoclonal antibodies that bind to conserved epitopes in the HA-2 and cross-protect against many virus subtypes have been identified [4].

The recent 'swine-origin' H1N1 pandemic of 2009 arose from a subtype (H1) already endemic in humans, albeit a novel one of zoonotic origin that was markedly different antigenically. While children had no immunity to pdmH1N1, a proportion of those born in the early part of the 20th century had neutralizing antibody and were relatively immune [5].

Pathogenesis in humans

Virus infection and cytopathology of the respiratory epithelium cause the respiratory symptoms of influenza while pro-inflammatory cytokines such as IL-6, TNF-α and interferons contribute to the systemic effects of fever and myalgia. These cytokine levels correlate with duration of hospitalization in patients more seriously ill with influenza [2••] Innate immune dysregulation and altered procoagulant activity are associated with other rare complications including acute necrotizing encephalopathy and increased risk of cardiovascular deaths [6,7]. Secondary bacterial infection was a major contributor of morbidity and mortality with seasonal influenza and past pandemics including the 1918 pandemic [3] as well as the recent pdmH1N1 2009 pandemic [8••] but did not play a major role in the severe primary viral pneumonia seen in zoonotic H5N1 disease [9] (Table 1).

Pandemic H1N1 2009

Clinical symptoms and viral load kinetics in the upper respiratory tract of patients with pdmH1N1 were comparable with those with seasonal influenza [10]. Patients with severe pdmH1N1 disease did not differ in viral load in the upper respiratory secretions at presentation, but viral load remained elevated for a prolonged period and they had higher levels of pro-inflammatory cytokines in the plasma [11]. Whether increased cytokine levels are a reflection of severe disease or part of its pathogenesis is unclear. As with seasonal influenza, severe disease was largely associated with pregnancy or underlying cardiac or respiratory disease or immunosuppression. However obesity was a risk factor not recognized in previous pandemics or seasonal influenza [12]. In mice with diet-induced obesity, influenza virus infection was associated with increased mortality, lung pathology and dendritic cell dysfunction and impaired memory T-cell function [13].

Autopsy of decedents of pdmH1N1 showed extensive tracheitis, bronchitis and diffuse alveolar damage (DAD). Secondary bacterial infection was seen in about half of the cases. Viral antigen was found in the tracheo-bronchial

Table 1

Comparison of pathogenesis of pandemic H1N1, avian H5N1 and 1918 H1N1 with seasonal influenza[*].

	Pandemic H1N1 2009	H5N1	1918 H1N1
Human clinical and autopsy data			
Viral load in upper respiratory tract	Viral load comparable to seasonal flu but severe cases have slower clearance of virus [10,11].	Higher and more prolonged viral load cf. seasonal flu [2••].	Not known
Pro-inflammatory cytokines in serum/plasma	Higher in patients with severe disease [11].	Higher in H5N1 vs. seasonal influenza (IP-10, MIG, MCP-1, IL-8, IL-10, IL-6, and IFN-gamma) and patients with fatal outcome have higher levels that survivors [2••].	Not known
Pathology of fatal disease	Primary viral pneumonia and diffuse alveolar damage (DAD) or secondary bacterial superinfection [8••].	Primary viral pneumonia and DAD. Secondary bacterial infection uncommon [2••,9].	Diffuse alveolar damage due to primary viral pneumonia or secondary to bacterial superinfection [3].
Extra-respiratory dissemination of virus	Rare	Occurs. But lung pathology remains major cause of death [2••,9].	Not known
Experimental animal infections studies			
Macaques	Higher viral titres and more lung pathology cf[+] seasonal flu. Higher and more prolonged elevation of MCP-1, MIP-1a, IL-6 and IL-18 [14••].	Severity of pathology H5N1>1918> seasonal flu. Targets type 2 pneumocytes. Stronger and more prolonged innate inflammatory responses (type 1 IFN, IL-1, IL-6) cf. seasonal flu [2••].	Severe respiratory disease with fatal outcome associated with a dysregulated innate immune response. Prolonged activation of IL-6, CCL11(eotaxin-1), CXCL6 (GCP-2) genes but weaker type 1 interferon response [2••] Seasonal influenza virus with 1918 HA and NA has increased pathogenicity and increased innate immune response cf seasonal flu [2••].
Ferrets	More virus replication in alveoli and more lung pathology>But less severe than H5N1 or 1918 [14••,15,16]	More innate immune and interferon signalling, IP-10, T and B cell-signalling pathways downregulated in H5N1 cf. seasonal H3N2 [2••].	Severe disease in ferrets [18].
Mice	Replicate more efficiently in lung and modestly more lung pathology and weight loss cf seasonal flu but not as severe as H5N1 or 1918 H1N1 [14••]. Lung cytokine induction modest and lower than induced by H5N1, 1918 or a previous triple reassortant H1N1 virus (A/Ohio/2/07) that infected humans [60].	Increased macrophage and polymorph infiltration and increased levels of pro-inflammatory cytokines (IL-6, interferon-γ, MCP-1, MIP-1α) in mouse lung [17••].	Severe disease in mice associated with high replication efficiency and activation of pro-inflammatory cytokines and apoptotic pathways [2••]. Increased macrophage and polymorph infiltration and increased levels of pro-inflammatory cytokines (IL-6, interferon-γ, MCP-1, MIP-1α) in mouse lung [17••].
Infection of primary human cells *in vitro*			
Macrophages	Viral replication and innate host responses comparable to seasonal influenza [38,39].	Stronger pro-inflammatory cytokine responses (TNF-α, interferon-α and β, IP-10, MCP-1, RANTES, MIP-1 α and β) cf seasonal flu [17••].	No evidence of increased induction of pro-inflammatory cytokines [17••].
Alveolar epithelial cells	Viral replication and innate host responses comparable to seasonal influenza [37••].	Stronger pro-inflammatory cytokine responses (interferon-β, IP-10, RANTES, IL-6) cf seasonal flu [2••,41].	Enhanced replication competence in human primary broncho-epithelial cells [61].

Bronchial epithelial cells	Marginally increased replication in differentiated cells. Enhanced viral replication at 33°C and comparable cytokine induction to seasonal influenza [37••].		
Dendritic cells	Viral replication and innate host responses comparable to seasonal influenza [38].	Stronger IFN-alpha responses cf seasonal flu in pDCs, differences in TNF-α less pronounced, comparable IP-10 induction [40] and enhanced replication competence [17••]. More efficient replication in microvascular endothelial cells [41]. Comparable replication to seasonal influenza but higher TNF-α, IL-6 production [42].	Comparable viral replication cf low pathogenic virus [17••].
Endothelial cells			
Neuronal cells			
Conjunctiva cells	Productive replication unlike seasonal influenza [37••].	Productive replication unlike seasonal influenza [37••].	

* If not otherwise specified, comparisons are made with seasonal influenza.
+ cf. compared with.

epithelium, type I and type II alveolar epithelial cells and alveolar macrophages [8••]. These findings were similar to those reported in the 1918 and 1957 pandemics and severe seasonal influenza [3].

Pathogenesis of zoonotic H5N1 disease

Key aspects of the pathogenesis of H5N1 disease in humans have been reviewed previously (Table 1) [2••]. Patients with H5N1 disease have higher and more prolonged viral load in respiratory specimens and higher plasma levels of pro-inflammatory cytokines and chemokines. Direct viral cytopathology, differential tropism toward the lower respiratory tract and differentially activated host responses contribute to the pathogenesis of H5N1 disease [2••].

Animal experimental studies

Compared to seasonal influenza, pdmH1N1 causes modestly increased disease severity in mice, macaques and ferrets with increased replication in the lower respiratory tract and elevated cytokine levels in the lung. However, such disease was not as severe as that caused by H5N1 or 1918 H1N1 viruses (summarized in Table 1) [14••,15,16]. Compared to infection with seasonal influenza, mice, macaques and ferrets infected with H5N1 or 1918 viruses had increased lung pathology and mortality and evidence of cytokine dysregulation (summarized in Table 1) [2••,17••].

Studies in mice defective in specific genes related to innate immunity

Mice deficient in IL-1 receptor or type 1 IFN genes have poorer survival following H5N1 virus challenge [2••] and the MX1 gene contributes to increased resistance to H5N1 and 1918 H1N1 virus infection [18]. In ferrets, virus strains with increased virulence appear to induce weaker IFN responses and stronger IL-6 responses detected in nasal fluids [2••].

On the other hand, IL-17RA knockout mice infected with influenza have improved survival, lower expression of pro-inflammatory cytokines and chemokines and reduced lung inflammation despite a higher viral burden compared to wild-type mice [19••]. Mice with defects in TLR-3, CCR2 or COX 2 (but not COX1) [2••] also have improved survival and reduced disease severity when challenged with seasonal influenza viruses. Mice with defects in TNF-α receptors or passive immunotherapy with TNF-α neutralizing antibodies have reduced weight loss although survival remains unchanged following challenge with some H5N1 virus strains [2••]. Inactivated H5N1 virus has been shown to induce oxidized phospholipids, which trigger an inflammatory response that leads to acute lung injury via TLR4 and the TRIF/TRAF6 signalling pathway [20]. These findings suggest that some innate immune responses contribute to immunopathology.

The role of adaptive immune response in protection and immunopathology

Cross-reactive CD4 and CTLs established by seasonal influenza A virus infection or vaccination provides protection against pandemic H1N1 or H5N1 influenza viruses by clearance of influenza virus from the lung [21–23]. Resident T cells may temper the early innate immune response. Rag-1 deficient mice that lack functional lymphocytes or mice depleted with CD4 or CD8 T cells produced more pro-inflammatory cytokines upon poly(I:C) or LPS treatment [24•]. In addition, effector and memory CD4 T cells abolish macrophage inflammasome-mediated caspase-1 activation and subsequent IL-1β release and thereby suppress potentially damaging inflammation [25••].

CD8 T cells

Peripheral T cells require a primary interaction with DCs which migrate to the draining lymph node and a subsequent interaction with pulmonary DCs including pulmonary plasmacytoid DCs, $CD8\alpha^+$ DCs, or TNF-α inducible nitric oxide synthase (iNOS) producing DCs (tipDC) thereby promoting increased T cell survival and accumulation [26,27]. While CD8 T cells play an important role in viral clearance, the release of cytotoxic molecules (e.g. granzyme and perforin) and antiviral cytokines (e.g. TNF-α and IFN-γ) can contribute to lung pathology. Transfer of HA-specific CD8 T cell clones leads to progressive lethal lung injury in the absence of active viral replication in transgenic mice expressing influenza HA antigen on the alveolar epithelial cells [1]. However, if these mice are deficient in Egr-1 (epithelial early growth response-1) they do not develop such lung injury suggesting a role for ERK kinases induced Egr-1 in CD8 cell mediated immunopathology [28].

CD4 T cells

Effector CD4 T cells are divided into subsets including Th1, Th2 and the more recently described Th17. Th17 is important against fungal infections, regulates inflammation and promotes autoimmunity [29]. Th1 and Th17 hypercytokinemia has been correlated with severe pandemic H1N1 influenza [30]. Interestingly, expression of Th17-associated cytokines in the lungs correlates with better survival of IL-10 knockout mice upon high-dose lethal challenge with influenza virus [31].

The role of IL-10 is controversial. IL-10 knockout mice showed better survival and increased viral clearance upon challenge with H1N1 (PR8) [31]; however, administration of monoclonal antibody to IL-10R to block IL-10 signalling resulted in increased and accelerated mortality as well as elevated inflammatory mediators [32•]. CD200 is highly expressed on resting airway epithelium and its interaction with its ligand CD200R on alveolar macrophages provides inhibitory signals to maintain immune homeostasis [33••]. CD200 knockout mice showed more weight loss, lung infiltration and lung endothelium damage following influenza infection and depletion of T cells prevented this increased immunopathology although with decreased viral clearance [33,34]. These studies suggest that both CD4 and CD8 T cells contribute to influenza clearance and immunopathology. Regulation between protection and pathological effects may rely on a panel of inhibitory regulators, such as IL-10 and the CD200R-CD200 interaction.

Host genetic factors and severity of influenza

Compared to C57BL/6J mice, DBA/2J mice have 1000–10,000-fold lower lethal-dose 50 for influenza infection [35,36]. Five gene loci on chromosomes 2, 7, 11, 15, and 17 are associated with resistance to H5N1 virus [36]. In response to PR8 infection, both DBA/2J mice and C57BL/6J mice upregulate IFN-responsive genes but DBA/2J mice showed a stronger expression of genes associated with inflammatory responses and prostaglandin-pathways [35]. Overall, these studies suggest that the susceptible DBA/2J mouse mounts a stronger innate immune response which may be detrimental to the host upon influenza virus infection.

In vitro and *ex vivo* human cells

Clinical observations and animal experiments indicate that severe H5N1 and 1918 H1N1 virus disease is associated with cytokine dysregulation. However, because effects of multiple cycles of viral replication and host responses are inextricably intertwined, it is not clear whether cytokine dysregulation is a consequence of the severe disease (caused by direct viral damage) or contributes to pathology in its own right. Studies with relevant primary human cells infected with defined virus dose can more clearly define intrinsic differences between viruses in inducing host responses and complement data from humans and animal models. Alveolar epithelial cells and alveolar macrophages are key target cells in primary viral pneumonia and diffuse alveolar damage [2••,3,8••,9]. The key differences in host responses elicited in immune cells (e.g. macrophages, DCs) and respiratory epithelial cells infected with pdmH1N1 and H5N1 viruses are summarised in Table 1. H5N1 viruses elicit markedly stronger pro-inflammatory responses in these cell types while seasonal and pdmH1N1 influenza viruses elicit broadly comparable host responses and show comparable viral replication competence [17••,37••,38–42]. The modest differences between seasonal influenza and pdmH1N1 correlate well with their observed virulence in humans. This contrasts with the greater differences in virulence between seasonal and pdmH1N1 viruses implied by animal model data [14••,15,16].

The differences in pro-inflammatory cytokine induction between H5N1 and seasonal influenza in macrophages are mediated in part by activation of p38MAPK and IRF-3 pathways [43]. Paracrine interactions between

macrophages and alveolar epithelial cells amplify and broaden these responses and COX-2 is a key regulator of the cytokine cascade [44]. Comparison of the host transcriptome showed that the differences between H5N1 and seasonal influenza (H1N1) viruses are quantitative rather than qualitative in nature with TNF and IFN signalling and Jak-Stat pathways being those more differentially activated [45]. The *in vitro* transcriptome data are comparable with that found in macaque and ferret lungs [46,47]. Analysis of recombinant viruses generated by reverse genetics shows that the viral polymerase genes rather than the HA, NA or NS1 are central to these differential host responses and they are not solely determined by viral polymerase activity [48]. Interestingly, preliminary analysis of yeast-two-hybrid screens has revealed that influenza virus polymerase subunits have multiple interactions with key cell-signalling pathways [49].

H5N1 activates NF-κB in endothelial cells more efficiently than low pathogenic influenza viruses [50]. Using a dominant negative mutant of IkappaB kinase 2, it was shown that most H5N1-induced genes are dependent on NF-κB activity. H5N1 viruses lead to apoptosis of neuronal cells and astrocytes (Table 1) [42]. The induction of apoptosis in NK cells may help the virus to evade some aspects of the innate immune response [51,52].

Therapeutic interventions

Anti-inflammatory agents such as gemfibrozil and sphingosine analog AAL-R reduce mortality in influenza-infected mice and alleviate pulmonary tissue injury, respectively [53,54]. Combination of a cyclooxygenase-2 (COX-2) inhibitor with mesalazine and the antiviral drug zanamivir resulted in improved survival in H5N1-infected mice [55]. A peroxisome proliferator-activated receptor-γ agonist pioglitazone moderates the deleterious effects of TNF-α inducible nitric oxide synthase (iNOS) producing DCs (tipDCs) in the lung airways and improved survival in influenza-infected mice [26]. Ferrets infected with H5N1 virus have strongly induced CXCL10 responses, and attenuating signalling via CXCR3, the receptor of CXCL10, reduced disease severity and delayed mortality [47].

The sympathetic nervous system increases pro-inflammatory cytokine release and exacerbates the pathogenesis of H1N1 (PR8) infection. Chemical sympathectomy reduced lung pathology, cellular infiltrates and cytokine induction in this experimental model and α-adrenergic agonists improve survival following a lethal virus challenge [56].

Protease activated receptors (PAR) are activated by extracellular proteases found in the lung. Activation of PAR_2 inhibits influenza virus replication via an IFN-γ independent pathway and PAR_2 agonists increase survival of influenza H1N1 infected mice and is associated with reduced neutrophil infiltrates, reduced RANTES and increased IFN-γ [57].

Conclusion

Mammalian innate and adaptive immune responses are complex, interconnected and crucial for host defense against infectious disease. However, in some situations, some of these responses may lead to deleterious consequences. It is sobering to note that while antiviral therapy remains the mainstay of treatment for H5N1 disease, oseltamivir treatment, even when commenced within 4 days of onset, was associated with mortality rates in excess of 50% in Indonesia [58]. This highlights the need for alternative and adjunctive therapeutic options that target host-responses. The challenge therefore is to selectively down-modulate harmful host responses without affecting beneficial ones which may permit viral replication to continue unchecked. Interventions that affect host cellular pathways that are crucial for viral replication as well as down-modulating targeted innate host responses post particular attraction. For example, inhibition of the Raf/MEK/ERK kinase cascade and activation of NF-κB lead to impaired virus replication as well as to dampening down of the host pro-inflammatory cytokine responses and they have potential therapeutic roles in influenza [59••]. Crucially, unlike antiviral drugs, strategies that target the host will preclude the emergence of drug resistant viruses.

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