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Influenza-induced innate immunity: regulators of viral replication, respiratory tract pathology & adaptive immunity

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Abstract

Influenza virus infections usually cause mild to moderately severe respiratory disease, however some infections, like those involving the avian H5N1 virus, can cause massive viral pneumonia, systemic disease and death. The innate immune response of respiratory tract resident cells is the first line of defense and limits virus replication. Enhanced cytokine and chemokine production following infection, however, appears to underlie much of the pathology that develops after infection with highly pathogenic strains. A so-called 'cytokine storm' can damage the lung tissue and cause systemic disease, despite the control of viral replication. By summarizing current knowledge of the innate responses mounted to influenza infection, this review highlights the importance of the respiratory tract epithelial cells as regulators of innate and adaptive immunity to influenza virus.

Keywords

antiviral immunity; cytokine; cytokine storm; influenza A; innate immunity; lung epithelium; respiratory tract; type I IFN; virulence

Influenza virus remains a major cause of morbidity and mortality worldwide. Between 5 and 15% of Americans acquire upper respiratory infections during annual epidemics, costing the US economy alone billions of dollars each year [101]. More severe infections affect millions of people worldwide and cause 250,000–500,000 deaths yearly. A member of the Orthomyxoviridae family, influenza virus contains a single-stranded, segmented, negative-sense RNA genome. They are separated into three types: A, B and C. Their highly variable surface proteins hemagglutinin (HA) and neuraminidase (NA) are used to further group the influenza A and B strains into serotypes. Influenza types A, B and C can all cause human disease; however, group A and B strains are the most significant. Group A strains have been associated with the most severe pandemics, including the most recent: the 2009 'swine-origin flu' (A/H1N1) pandemic.

Influenza A infects a variety of species including humans, birds, pigs, horses, mink, cats and dogs and is endemic in water fowl, thought to be the main reservoir of the virus from which

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new endemics and pandemics emerge. The zoonotic potential of influenza virus has been exemplified by human outbreaks caused by avian H5N1 infections following close contact of afflicted individuals with infected poultry. Antigenic drift can lead to HA mutations, resulting in viruses with slightly different antigenic profiles to which pre-existing immunity may be ineffective. Antigenic shifts usually occur through re-assortment of genome segments from two or more influenza viruses. These novel strains have the potential to rapidly spread through immune-naïve populations, resulting in global pandemics.

Following aerosol infection, influenza causes a mild to moderately severe respiratory disease but can occasionally cause systemic disease [1]. In general, infections with seasonal and less-pathogenic strains cause lesions in the upper respiratory tract. In contrast, more virulent strains cause viral pneumoniae with epithelial necrosis and inflammation, extending into distal airways and alveolar air spaces with grave consequences for the host [2,3]. Diffuse alveolar damage characterized by alveolar epithelial necrosis, hyaline membrane formation, pulmonary edema and intra-alveolar hemorrhage is often a consequence of infection with virulent strains, including H5N1 and 1918 H1N1 [2].

The pathogenesis of these virulent infections is incompletely understood. One current widely accepted model is that infections with highly pathogenic influenza strains lead to a dysregulation of the host innate immune response, resulting in excessive release of proinflammatory cytokines and chemokines. This 'cytokine/chemokine storm' then leads to a large influx of inflammatory cells into the respiratory tract in an attempt by the immune system to control the viral infection, but which instead causes 'collateral' damage to the lung tissue and inhibits oxygen exchange in the alveoli. The development of therapies aimed at facilitating viral clearance with standard antiviral drugs, while also dampening and/or regulating the immune response to limit the associated damage of the airways, is an attractive treatment model. However, we currently lack a detailed understanding of the mechanisms by which innate responses result in overshooting responses, and the specific innate responses that provide protection versus destruction.

This article summarizes current knowledge on the induction of innate cytokine and chemokine responses in the local environment of the respiratory tract following influenza virus infection. We will detail the responsible cell types and the quality of the innate response and summarize how the induction of innate responses controls antiviral adaptive immunity. The review highlights the importance of the respiratory tract epithelial cell as a primary regulator of the antiviral immune response to influenza virus infections.

Innate response by airway epithelial cells

Airway epithelial cells are the primary target of influenza infection and thus play a critical role in instigating the innate response. Influenza infection begins when the HA protein on the viral surface binds to sialic acid residues expressed on the host cell surface. Avian influenza strains (e.g., H5N1) preferentially bind to α 2,3 sialic linkages, while human strains (e.g., H1N1) bind to α 2,6 linkages, which are particularly abundant on airway epithelial cells [4]. Viral endocytosis is followed by activation of intracellular pattern recognition receptors. Influenza virus activates the endosomal Toll-like receptors (TLRs; TLR3 recognizing dsRNA and TLR7 recognizing ssRNA) and cytoplasmic retinoic acid-induced gene 1-like receptor (RIG-1), melanoma differentiation-associated gene 5 and NOD2 [5–10]. While all receptors will trigger type I interferon (IFN) secretion, activation of these receptors may induce different immune responses. TLR3 stimulation strongly induces NF- κ B-dependent inflammatory responses after influenza infection [6], RIG-I induces antiviral responses [7], NOD2 activates the inflammasome complex [5] and TLR7 was shown to regulate the induced B-cell response [11].

Pattern-recognition receptor binding initiates signal transduction events, which result in the production and release of antiviral type I IFN (IFN- α and IFN- β) and type III IFN (IL-29 [IFN λ 1], IL-28A [IFN λ 2] and IL-28B [IFN λ 3]), as well as other cytokines and chemokines. In contrast to previous assumptions, recent studies suggest that type III IFN is the predominant antiviral cytokine produced following intranasal infection of mice [12], explaining the normal degree of virus clearance previously observed in mice that lack the type I IFN receptor (IFNAR; [13,14]).

Airway epithelial cells express IFN- β within 3–6 h of influenza infection [10,15]. IFN- β initiates the transcription of antiviral genes, including RNA-dependent protein kinase (PKR), 2'-5'-oligoadenylate synthetases, Mx protein and GTPases in neighboring cells through binding of the IFNAR1/IFNAR2 receptor and activation of the IFN-stimulated gene factor 3 transcription factor (see reviews [16–18]). This same pathway also enhances production of IL-28/29 [12]. Together, these proteins have broad but non-specific functions in inducing a so-called 'antiviral state'. This influenza virus infection-induced rapid antiviral cytokine response is able to reduce the initial viral replication and viral spread, as mice deficient in crucial signaling molecules downstream of type I and III IFN (STAT-1/STAT-2) succumb to the infection more readily than wild-type mice [12,19]. As we outline below, the NS-1 protein of influenza virus can inhibit the induction of IFN, thereby counteracting this potent innate immune response. This rapid induction of antiviral activity allows the host to mount the more slowly developing adaptive immune responses.

Consistent with the engagement of multiple pattern-recognition receptors, *in vitro* studies demonstrate that tracheobronchial and alveolar epithelial cells are capable of producing cytokines and chemokines other than IFN in response to influenza infection [20]. Within 3–6 h following infection, airway epithelial cells upregulate IL-1, IL-6, CCL5 (RANTES) and CXCL10 expression [10,15,21,22]. Other chemokines, including TNF- α , IL-8 and CCL2 (MCP-1), are expressed 18–24 h postinfection [10,15,22].

Chemokine expression undoubtedly controls leukocyte emigration into the airway microenvironment by creating a gradient to guide the migration out of blood vessels and through the airway epithelium and also by inducing necessary adhesion molecules. For example, airway epithelial production of CCL2 creates a gradient for emigration of blood-derived monocytes into alveolar airspaces, a cell type that strongly increases following influenza infection [23,24]. Lack of alveolar macrophage recruitment reduces antigen presentation and induction of influenza-specific CD8 T-cell responses [25]. Thus, these responses are also crucial for efficient induction of adaptive immunity. Furthermore, TNF- α and IL-1 upregulate adhesion molecules, including ICAM-1 and E-selectin on endothelial cells, important for the transendothelial migration of neutrophils [26]. *In vivo* evidence for a role for these cytokines in neutrophil migration comes from mice that lack IL-1R expression. Following infection of these mice with influenza virus, greatly reduced numbers of neutrophils accumulated in the respiratory tract [27]. Finally, TNF- α enhances monocyte and neutrophil transmigration across influenza-infected and normal epithelium [23,28], likely through upregulation of β 2 integrins (CD11a, CD11b and CD11c/CD18), β 1 integrins (VLA-4, -5, -6 and CD47) and ICAM-1 and VCAM-1 on epithelial cells [29].

Taken together, the infection-induced elaboration of cytokines and chemokines by respiratory tract epithelial cells orchestrates the earliest leukocytic response. Changes and alterations to these responses are likely to have profound effects not only on the quality and magnitude of the leukocytic response, but also on subsequent events such as control of viral replication, development of an effective adaptive immune response, further elaboration of leukocyte-derived cytokines and chemokines and eventually the regulated repair of the epithelium. However, despite this important regulatory function of the epithelium, we

currently lack a clear understanding of how their responses are induced and regulated. Such knowledge is crucial for eventual therapeutic intervention.

Innate responses regulate the early induction of adaptive immunity

Increasing amounts of data point to the close connection between the innate immune responses that are triggered following respiratory tract infections with influenza virus and the induction of adaptive immunity locally in the respiratory tract-draining lymph nodes. Given the close proximity between the epithelial cells of airways and lungs with the local lymph nodes and the multiple functions cytokines play in regulating various aspects of immune responses, it is not surprising that adaptive immune response induction is influenced by the quality and magnitude of the innate response. This has been documented in particular for some aspects of the influenza infection-induced antibody response (see later). More recent studies point to important direct effects on T cells as well [30], while the indirect effects through the activation of dendritic cells have long been appreciated.

As previously outlined, influenza virus infection triggers IL-1 β and IL-18 secretion through the engagement of RIG-I and possibly NLRP3-triggered inflammasome activation (reviewed in [5]). A recent study provided evidence that induction of the inflammasome pathway is required for maximal production of antiviral serum IgM and IgG and intranasal IgA [31]. In apparent contrast, studies in IL-1R^{-/-} mice revealed a distinct deficit in influenza-specific IgM but not IgG or IgA secretion following influenza infection [Waffarn EE, Baumgarth N, Unpublished Data] [27]. However, others failed to find any evidence for the effects of caspase-1 or cryopyrin, another component of the inflammasome complex, on B-cell response outcome [32]. Differences in the outcomes of these studies might be due, at least in part, to the use of distinct influenza strains or doses of infection. Together, it seems that these inflammatory cytokines do have some effects on the induction of humoral immunity to influenza, although currently available data, gained exclusively in mouse models, are not always consistent and the precise mechanisms of these effects are currently unknown.

A clearer picture emerges from studies on the role of type I IFN in directly [13,33,34] and indirectly [35,36] shaping the antiviral humoral response to influenza infection. Type I IFN was shown to increase regional lymph node sizes after infection [13,33] and the development and size of germinal centers [Baumgarth N, Unpublished Data]. IFN can also drive plasma cell generation [13,34] via induction of IL-6 production by antigen-presenting cells [35]. The effects of type I IFN on B cells are direct and remarkably localized, in that type I IFN seems to be the first signal that all regional lymph node B cells are exposed to after infection, while B cells in the spleen and lung are unaffected [13]. The outcome of direct type I IFN receptor signaling is a massive change in gene expression of the B cells [33], resulting in increased antibody responses and changes to antibody quality. While these effects are clearly documented, the mechanisms underlying these immune enhancing effects of IFN-mediated direct B-cell stimulation remain to be identified. The induction of TLR3 and TLR7 expression by B cells [13] indicates that this cytokine can enhance the sensitivity of B cells to viral RNA recognition following B cell-receptor-mediated internalization of the virus, which in turn might enhance virus-specific antibody responses and alter the antibody-isotype profile generated [11]. On the other hand, increased TLR7 expression has been linked to autoimmunity [37]. Thus, these innate cytokine-driven changes in the anti-viral B-cell response must be under tight control, so as to avoid the induction of autoreactive B cells that might be inadvertently stimulated at the site of infection, where many autoantigens are exposed due to the cytolytic nature of the influenza virus infection. Given the array of innate cytokines produced by epithelial cells and leukocytes at the site of infection after influenza virus infection, it is likely that other innate cytokines affect adaptive T- and B-cell

responses. Their identification might provide important clues for the development of adjuvants that can mimic adaptive immune responses observed following a natural infection.

Innate response by airway macrophages & dendritic cells

Although productive viral replication only occurs in the airway epithelial cells, influenza virus nonproductively infects alveolar macrophages and dendritic cells [2,3,24,38,39]. Furthermore, viral proteins have been identified in many other cell types, including neutrophils, T cells, NK and B cells [40]. Similar to airway epithelial cells, *in vitro* experiments with blood-derived monocytes and dendritic cells, demonstrate that these cells produce a variety of cytokines and chemokines in response to influenza infection. Human macrophages express type I and type III IFNs, IL-1 α , IL-1 β , IL-6, TNF- α , CXCL8, CCL2 (MCP-1), CCL3 (MIP-1 α), CCL4 (MIP-1 β), CXCL9 and CXCL10 following infection with low (seasonal H1N1) and high (H5N1, 2009 H1N1) pathogenic strains, with evidence for induction of ERK1/2, p38 and JNK signal transduction pathways in their production [38,41–46].

TNF- α is a dominant cytokine produced by macrophages following influenza infection. As a master proinflammatory cytokine, TNF- α production activates the NF- κ B transcription factor and amplifies the expression of other pro-inflammatory cytokines and chemokines within the microenvironment [47,48]. In fact, mice deficient in TNF- α and IL-1R exhibit reduced mortality, with diminished cytokine production and inflammation following infection with a lethal H5N1 virus, indicating that TNF- α widely influences the inflammatory response to influenza infection [49]. Furthermore, transcriptional profiling of TNF receptor-1 knockout mice infected with the reconstructed 1918 virus demonstrated delayed or decreased expression of genes involved in antiviral and innate immune pathways, thus, suggesting that signaling through this receptor contributed to the severity of the 1918 infection [50] and that systemic TNF- α levels might be a crucial marker for disease progression.

In addition to producing proinflammatory cytokines and chemokines, recent studies indicate that macrophages are important in re-establishing an anti-inflammatory environment late during the course of infection. Mice depleted of macrophages have prolonged pro-inflammatory responses with increased lung levels of IL-1 β , IL-6 and TNF- α [51,52]. Indeed, macrophages can upregulate anti-inflammatory proteins, including SOCS1 and SOCS3 and IL-1R antagonist following influenza infection, which inhibit IFN-stimulated gene expression and IL-1 receptor signaling, respectively [42,44]. While the great plasticity of macrophages and their responses are now well-understood [53], the mechanisms by which local macrophage populations switch from a pro- to an anti-inflammatory profile remain to be detailed. The changing milieu of a regenerating epithelium might provide signals that alter the functionality of the macrophages, or allow the recruitment of macrophages with regulatory functions.

Conventional dendritic cells reside within the airway epithelium and play an important role in initiating the adaptive immune response by migrating to regional lymph nodes where they present antigen to initiate the adaptive immune response. Furthermore, bone marrow-derived plasmacytoid dendritic cells migrate into the lungs following infection and are prime producers of IFN- α [54–57]. In general, conventional dendritic cells respond to influenza infection in a manner similar to macrophages, with upregulation of type I IFN, type III IFN, CXCL10 and TNF- α [38,56,57], while plasmacytoid dendritic cells elicit stronger IFN- α responses [54,56,57] with lesser production of CXCL10 and TNF- α [56]. They also appear to be more resistant to influenza infection and replication [57] compared with macrophages.

In a comprehensive study of human blood-derived plasmacytoid and myeloid dendritic cell responses to influenza exposure *in vitro*, Piqueras and colleagues noted three distinct waves of chemokine production [58]. During the first 2–4 h postinfection chemokines such as CXCL16, CXCL1, CXCL2 and CXCL3 were produced that are capable of attracting neutrophils, cytotoxic T cells and natural killer cells. By 8–12 h postexposure these cells were producing chemokines able to attract effector memory T cells such as CXCL8, CCL3, CCL4, CCL5, CXCL9, CXCL10 and CXCL11. Finally, at 24–48 h postexposure, when dendritic cells would likely reside in lymphoid tissue, their chemokine profile indicated their ability attract naive T and B lymphocytes. Thus, both resident and migrating dendritic cells contribute to the coordinated production of cytokines and chemokines within the airway microenvironment, thereby influencing leukocyte emigration and contributing to antiviral defenses. Continued chemokine production of dendritic cells after their migration to regional lymphoid tissue can regulate the adaptive response.

Differences in innate immune responses with viral virulence

For reasons that are incompletely understood, some influenza strains including, A/H5N1, the 1918 A/H1N1 and the pandemic 2009 A/H1N1, can incite severe pulmonary injury and inflammation in some patients. The H5N1 avian influenza strain, which was first isolated from humans in Hong Kong in 1997 [59], is associated with an extremely high mortality rate (56%) [101]. Affected patients die from acute respiratory distress and multiple organ failure, indicating that cytokine dysregulation is important in the pathogenesis of this disease. The 1918 influenza pandemic was the worst in recorded history, killing an estimated 50 million people worldwide [60]. Molecular reconstruction of the 1918 virus from archived human lung samples has led to a better understanding of the pathogenicity of this strain [61]. It is now widely accepted that a cytokine storm contributed to the severe pathology associated with this and other more virulent influenza virus strains. Whether this dysregulation occurs at the level of the epithelial cell response and/or the leukocytes that are residing in or are recruited to the site of infection remains an important open question for investigation.

In support of dysregulation of the host's innate immune response as a mechanism of enhanced viral pathogenicity, *in vitro* studies have demonstrated a positive correlation between viral pathogenicity and enhanced cytokine and chemokine expression (TABLE 1). Comparing H5N1 with a seasonal H1N1 strain in human macrophages, Lee and colleagues found enhanced gene expression of cytokine/chemokine, immune and inflammation-related pathways with H5N1 infection. Importantly, the exaggerated host response was not correlated with an enhanced viral replication rate [42]. Numerous studies with H5N1 influenza virus have made similar observations, demonstrating enhanced expression of TNF- α , IL-1 β , CXCL10, RANTES, MIP-1 α , MIP-1 β , MCP-1, IFN- λ 1, MCP-3 and IL-6 compared with low-pathogenicity influenza viruses and enhanced levels have correlated with increased expression of IRF3, a transcription factor important in regulating chemokine and cytokine gene transcription [15,44,45,62,63]. Importantly, not all H5N1 viruses are created equal. Different H5N1 clades induce quantitative differences in cytokine (IL-12, IL-16, IL-9 and TNF- α) and chemokine expression [43,44,64]. Thus, there are high and low cytokine-inducing clades even within the same pathogenic influenza strain.

Interestingly, H5N1 infection was also shown to prolong IFN- γ production by macrophages and is important in sustaining the cytokine/chemokine storm [45]. Consistent with this, results from our laboratory demonstrate that IFN- γ acts with the influenza virus to synergistically upregulate CXCL10 transcription in airway epithelial cells, which could result in excessive infiltration of effector T cells to airways [OSLUND KL, MANUSCRIPT IN PREPARATION]. In support of such a conclusion, our earlier studies have demonstrated that

blocking IFN- γ *in vivo* results in a selective reduction in T-cell infiltration during sublethal influenza virus infection of mice [65].

Animal models also corroborate that dys-regulation of the immune response plays an important role in the pathogenesis of H5N1 and reconstructed 1918 H1N1 infections (TABLE 2). Expression of type I IFN and genes important in innate immunity was markedly increased and prolonged in H5N1-infected cynomolgus macaques [66]. IFN- α , IFN- β , TNF- α , CXCL10, IL-6 and IFN- γ were among the most highly induced genes. Similarly, ferrets infected with the H5N1 virus demonstrated enhanced expression of type I IFN-related genes with marked induction of CXCL10 [67]. Pulmonary levels of proinflammatory genes, including type I IFN, TNF- α , IL-6 and IL-1 β were upregulated in mice and cynomolgus monkeys infected with the reconstructed 1918 virus [60,68].

The mechanisms underlying this strongly enhanced and potentially overshooting host response are unclear. To reiterate, several studies demonstrate that the exaggerated cytokine/chemokine secretion cannot be explained solely by an increase in viral replication [42,44,69]. Rather, there appears to be a fundamental difference in the host response to these more pathogenic influenza virus strains. Viral adhesion to different sialic acid residues could play a role, possibly affecting both viral distribution within the lung and in the microenvironment of the host cell responses. Recombinant influenza strains capable of binding α 2,3 sialic acid residues, such as H5N1 strains, seem to induce higher levels of pro-inflammatory cytokines and IFN-inducible genes compared with strains binding α 2,6 sialic acid residues [4]. Taken together, these data suggest that it is not the infection *per se*, but either the location of the virus within the lung, or the exposure of particular cell subsets that induces qualitatively and quantitatively distinct host responses.

Viral determinants of pathogenicity

The influenza A genome contains eight RNA segments that encode nine structural proteins and two nonstructural proteins, NS-1 and PB1-F2. Some studies have associated an enhanced cytokine response with specific viral genes. Viral determinants including H5N1 polymerases and, to a lesser extent, the NS gene have been correlated with enhanced cytokine secretion from macrophages [62,64]. However, the influenza NS-1 protein blocks key pathways of the innate immune response, enabling the virus to become established by blocking the induction of type I IFN from both airway epithelial cells and dendritic cells, and delaying the induction of immune responses [39,70,71]. NS-1 plays a significant role in antagonizing type I IFN through several mechanisms:

- Inhibition of RIG-1, the cytoplasmic receptor that senses single stranded viral RNA [72];
- Inhibition of STAT phosphorylation and the transcription factors IRF3, AP-1 and NF- κ B, which are important in IFN- β transcription [39,73–78];
- Binding to cellular proteins, including cleavage and polyadenylation specificity factor (CPSF) to block cellular mRNA processing in influenza infected cells [76].

A net outcome of the NS-1 action includes attenuation of dendritic cell maturation and activation, resulting in suboptimal T-cell and B-cell stimulation [13,30,33,70,71].

Numerous studies have demonstrated that NS-1 contributes to disease pathogenesis. Insertion of the NS gene segment from the highly pathogenic H5N1/97 strain into a less pathogenic A/PR/8 strain enhanced the virulence, as measured by delayed viral clearance and enhanced expression of inflammatory cytokines [79]. Interestingly, the mutation

associated with this enhanced virulence, a glutamic acid residue at position 92, has only been identified in the highly virulent Hong Kong H5N1/97 human isolates and in closely related avian viruses [79]. Likewise, the 1918 NS-1 gene induced expression of proinflammatory chemokine and cytokine genes important in attracting and activating lymphocytes and neutrophils while suppressing antiviral proteins including PKR and 2'-5'-oligoadenylate synthetase [73]. While NS-1 is certainly the best studied influenza viral protein, and appears dedicated to providing immune evasion from host responses, it is likely that alterations in other viral proteins also cause enhanced and/or altered host responses.

Conclusion

The innate immune response orchestrated within the airway epithelial microenvironment following infection with influenza virus is critical for limiting early influenza replication, permitting the host time to mount an adaptive immune response. A variety of studies using animal models and *in vitro* approaches demonstrate quantitative, and possibly qualitative, differences in the innate response to more pathogenic influenza strains. The development of a 'cytokine storm' following infection with highly pathogenic influenza strains has repeatedly been observed, which could result in 'collateral' damage to pulmonary tissue and cause systemic disease. The mechanisms underlying this overshooting response are incompletely understood but seem to be related to inherent differences in the virus-host interaction rather than simply increased viral replication. Like many viruses, influenza has evolved strategies to attenuate the innate immune response, which are primarily mediated by NS-1 inhibition of the type I IFN and the inflammasome response.

Future perspective

To date, studies investigating influenza pathogenesis have focused on *in vitro* cellular responses or whole-lung assays from animal models of disease. While this work has contributed to a broad understanding of the innate immune response to seasonal and virulent influenza infections, complex cellular interactions cannot be recapitulated with *in vitro* studies and subtle effects within the airway epithelial microenvironment cannot be appreciated with whole-lung assays. As the primary site of viral infection and resulting pathology, the airway epithelial microenvironment is critically important to the outcome of influenza infection. Subsequent investigations into influenza pathogenesis should focus on this complex microenvironment. Delivery of aerosolized therapeutics to this microenvironment could offer options to dampen an overzealous immune response in virulent influenza infections and improve clinical outcome. In addition, a detailed understanding of the pathogenesis within the airway microenvironment could identify new prognostic markers to improve treatment options earlier in the course of virulent influenza infections. It will be difficult to meet these goals without a comprehensive understanding of the interaction between influenza virus and host response within airways. Recent advances in influenza surveillance, rapid sequencing and reverse genetic approaches will facilitate the rapid testing of multiple viral mutants and are likely to enhance the discovery of other mutations that influence influenza-host interactions and thereby also enhance our understanding of the mechanisms by which innate responses are induced and regulated.

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Executive summary

Innate response by airway epithelial cells

- Airway epithelial cells are the primary target of influenza infection and replication and they participate in the innate immune response by producing a variety of cytokines and chemokines to limit viral replication and coordinate localized inflammatory cell migration.
- The role of airway epithelial cells in regulating *in vivo* regional inflammatory cell populations and resulting pathology in different pulmonary microenvironments (e.g., conducting airways and parenchyma) require thorough investigation to further our understanding of influenza pathogenesis and the development of improved therapeutics.

Innate responses regulate the early induction of adaptive immunity

- Production of innate cytokines profoundly affects local populations of B and T cells.
- Type I interferons provide a positive stimulus affecting both the magnitude and the quality of the regional B-cell response.
- Identification of this and other innate signals shaping adaptive immunity to influenza virus infection might provide leads for the development of adjuvants that could induce responses that mimic the highly protective immune responses that are induced following natural infection.

Innate response by airway macrophages & dendritic cells

- Resident and migrating macrophages and dendritic cells influence and sustain the regional inflammatory response through production of cytokines and chemokines.
- These cells play an important role in bridging innate to adaptive immune responses and in re-establishing an anti-inflammatory environment late in infection.

Differences in innate immune responses with viral virulence

- Some influenza strains induce severe pulmonary injury and inflammation extending into more distal airways, enhancing morbidity and mortality.
- Quantitative and qualitative differences in chemokine and cytokine generation from cells within the airway microenvironment in response to these virulent strains have led to the cytokine/chemokine storm hypothesis.
- Detailed investigations into innate responses, which are protective versus destructive are needed to tailor therapy towards enhancing viral clearance while dampening excessive inflammation.

Viral determinants of pathogenicity

- The influenza nonstructural protein, NS-1, attenuates the innate immune response by several mechanisms and also contributes to a proinflammatory response.
- Advances in molecular techniques are expected to lead to new insights into influenza–host cell interactions and the regulation of innate responses.

Table 1Cellular production of cytokines and chemokines following *in vitro* influenza infection.

Strain	Experimental model	Experimental outcome	Ref.
H5N1	Human airway epithelial cells	H5N1 > H9N2, H1N1 expression CXCL10, TNF- α and CCL5	[22]
Low pathogenic avian H9N2		H1N1 persistent elevation of TGF- β 2	
Seasonal H1N1			
H5N1	Human airway epithelial cells and alveolar cells	H5N1 > H1N1 expression CXCL10, IFN- β , CXCL5 and IL-6	[15]
Seasonal H1N1		Induction without differences in viral load	
H5N1	Human blood-derived plasmacytoid dendritic cells	H5N1 > H1N1, H3N2 and IFN- α No strain differences in TNF- α and CXCL10 induction	[56]
Seasonal H1N1, H3N2			
H5N1	Plasmacytoid dendritic cells	High levels of IFN- α production. Produced TNF- α	[57]
H5N1	Human monocyte-derived macrophages	H5N1 > H1N1 induction of an inflammatory response including CCL8, CCL5, type I IFN genes, CXCL10, CXCL9, CXCL11 and TNF- α	[42]
Seasonal H1N1			
H5N1	Human monocyte-derived macrophages	H5N1 strongly activated IRF3	[63]
Seasonal H1N1			
H5N1	Human monocyte-derived macrophages	H5N1 > pandemic H1N1 and seasonal H1N1 induced IL-2R, IL-6, IFN- α , MIP-1 α , MIP-1 β , RANTES and MCP-1	[45]
Pandemic H1N1 2009			
Seasonal H1N1		H5N1 prolonged induction of TNF- α , IFN- γ , IL-6 and MCP-1	
H5N1	Human monocyte-derived macrophages	H5N1 > seasonal, induced CXCL10, RANTES, IL-6, MIP-1 α , MIP-1 β and MCP-3 pandemic H1N1 2009 > seasonal IL-6, MCP-1, IL-9 and MCP-3	[44]
Pandemic H1N1 2009			
Seasonal H1N1 and H3N2			
Pandemic H1N1 2009	Human monocyte-derived macrophages	H5N1, H3N2 > pandemic H1N1 2009 > seasonal H1N1 induced CXCL10, CXCL8, CCL3, IL-6 and TNF- α	[41]
H5N1			
Seasonal H1N1 and H3N2			
H5N1	Human monocyte-derived macrophages	H5N1 > H3N2, H1N1 proinflammatory cytokines including TNF- α and IFN- β	[62]
Human H3N2			
Human H1N1			

Table 2

Chemokine and cytokine production in animal models of influenza infection.

Strain	Species	Experimental outcome	Ref.
H5N1 2004 human Vietnam isolate 1918 reassortment virus (2–3 genes)	Cyno macaque	H5N1 > 1918 reassortment virus induced marked and sustained expression of type I interferons, inflammatory and innate immunity genes including IFN- α , IFN- β , TNF- α , CXCL10, IL-6 and IFN- γ	[66]
H5N1 2004 human Vietnam isolate Reconstructed 1918 virus	Cyno macaque	1918 H1N1 > H5N1 expression of inflammatory and cell-death genes including IL-1 β and TNF- α	[68]
Pandemic 2009 H1N1	Cyno macaque	Enhanced MCP-1, MIP-1 α , IL-6, IL-18 correlating with enhanced pathology compared with seasonal H1N1	[80]
H5N1 2004 human Vietnam isolate H3N2	Ferret	H5N1 > H3N2 enhanced interferon-responsive genes especially CXCL10	[67]
Pandemic 2009 H1N1	Ferret	High induction of proinflammatory cytokines including IL-6, TNF- α and IFN- α	[81]
Pandemic 2009 H1N1 A/CA/07	Ferret	Pandemic H1N1 > seasonal H1N1 enhanced early expression of CCL2, CCL8 and CXCL7	[82]
Seasonal A/Brisbane/59/2007		CXCL10 and interferon-stimulated genes	
Pandemic 2009 H1N1	Human	Enhanced CCL2, CCL3, CCL19, CXCL8, CXCL10, CXCL11, IL-6, IL-17, IL-1 A, IL-1 β and TNF- α in lavage. Disease severity correlated with increased plasma levels of IL-1RA, IL-2, IL-6, IL-10, CCL3 and CCL4	[69]
H5N1	Human	High pharyngeal viral load correlated with high plasma levels of IL-6, IL-10 IFN- γ , CXCL9 and CXCL10	[83]
H5N1	Human	TNF- α expression in lung	[84]
H5N1	Human	Elevated IL-6, IFN- γ and soluble IL-2 receptor	[85]
H5N1	Human	High serum levels of CXCL10 and CXCL9	[86]
Reconstructed 1918 virus	Mouse	Increased and accelerated activation of proinflammatory and cell death-related genes	[60]
Pandemic 2009 H1N1	Mouse	High levels of IFN- γ , IL-4, IL-5, IL-12, MIP-1 α , MIP-1 β and RANTES compared with seasonal H1N1	[80]
H5N1	Mouse	H5N1 and 1918 H1N1 prolonged IFN- γ production and enhanced production of KC, IL-6, MCP-1, MIP-1 α 4 days postinfection	[24]
1918 H1N1			
Seasonal H1N1			