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The pathogenesis of influenza virus infections: the contributions of virus and host factors

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Influenza viruses cause acute respiratory inflammation in humans and symptoms such as high fever, body aches, and fatigue. Usually these symptoms improve after several days; however, the 2009 pandemic H1N1 influenza virus [influenza A(H1N1) 2009] is more pathogenic than seasonal influenza viruses and the pathogenicity of highly pathogenic H5N1 viruses is still higher. The 1918 influenza pandemic virus caused severe pneumonia, resulting in an estimated 50 million deaths worldwide. Several virulence factors have been identified in these virus strains, but host factors are also responsible for the pathogenesis of infections caused by virulent viruses. Here, we review the contributions of both virus and host factors to the pathogenesis of these viral infections.

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Introduction

Influenza viruses possess RNA as their genome and belong to the family *Orthomyxoviridae* [1]. Influenza A viruses (IAV), together with influenza B viruses, cause respiratory illness in humans. Wild aquatic birds are the natural reservoir of IAV [2]. Influenza pandemics occur when humans are introduced to IAVs with hemagglutinin (HA) to which they are immunologically naïve [3]. We have experienced four pandemics since the beginning of 20th century: Spanish influenza (H1N1) in 1918/1919, Asian influenza (H2N2) in 1957, Hong Kong influenza (H3N2) in 1968, and H1N1 influenza in 2009. Of these pandemic viruses, the 1918 virus was the most devastating, causing massive acute pulmonary hemorrhage and edema [4]. As antibiotics were not available then, secondary bacterial pneumonia was a major cause of death among those infected with the virus [5]. Until recently, it has been difficult to precisely evaluate the pathogenicity of the 1918 virus relative to other influenza virus strains. However, in 1999, the reverse genetics of influenza virus was established, enabling us and others to generate the 1918 virus from cloned cDNAs [6]. Infection of cynomolgus macaques with 1918 virus generated by reverse genetics resulted in severe lung damage and high virus titers, as well as disruption of the macaques' antiviral immune responses [7]. These studies directly demonstrated that the 1918 virus possessed sufficiently high pathogenicity to cause fatal pulmonary disease.

The genome of IAV consists of eight RNA segments, encoding HA, neuraminidase (NA), nucleoprotein (NP), M1, M2, nonstructural protein (NS) 1, NS2, polymerase acidic protein (PA), polymerase basic (PB) 1, PB1-F2, and PB2. Recently, research has focused on using reverse genetics to elucidate the role of each viral protein in the pathogenicity of influenza viruses. The range of severity of diseases caused by genetically similar IAV in humans is extremely wide, indicating that host conditions play an important role in determining the pathogenesis of IAV. Experiments with mammals such as mice, guinea pigs, ferrets, and non-human primates, are employed to analyze the involvement of host factors in IAV infections, while gene-targeted mouse models are useful for testing the function of individual host genes *in vivo*. The secretion of type 1 interferon is induced by viral infection and produces antiviral factors; IFNβ knockout mice are susceptible to influenza virus [8]. Therefore, type I IFN is a key molecule in the innate immune responses to infection with influenza virus and the magnitude of the type I IFN response influences the pathogenicity of the virus. Thus, the pathogenesis of influenza virus infection in humans depends on a combination of virus and host factors.

Virulence factors

The influenza viral proteins play a role in the lung pathology of humans. Among these proteins, HA is responsible for targeting cells for infection (Table 1) [9–11]. The HA of seasonal IAV binds to α2-6 sialylated glycans, which are expressed on the surface of the epithelial cells of the upper respiratory tract in humans [12]. Because of the inflammation caused by seasonal IAV

Table 1

Mutations in viral proteins that influence viral pathogenicity.

Protein	Virus	Mutation	Pathogenic effect	Reference
HA	H7N7	A143T	Increased attachment to bronchial epithelial cells and alveolar macrophages in humans	[9]
HA	1918 virus	D190E, D225G	From α2,6 to α2,3 (loss of transmission ability)	[10]
HA	Pandemic A(H1N1) 2009	D222G	From α2,6 to α2,3 Infection of ciliated bronchial epithelial cells	[11]
NA	H3N2	R292K, E119V, N294S	Oseltamivir-resistant (R292K, loss of transmission ability)	[62,63]
NA	H5N1	H274Y	Oseltamivir-resistant	[64]
PB1-F2	1918 virus	N66S	Delay of innate immune responses	[28]
PB2	H5N1	T271A	Increased polymerase activity in mammalian cells	[18]
PB2	H5N1, H7N7	E627K	Increased replication in mammalian respiratory tract	[19,20]
PB2	H5N1	D701N	Increased ability to replicate in mice	[21]
PA	H5N2	T97I	Adaptation in mice	[22]
NS1	H5N1	P42S	Increase in IFN antagonism	[33]
NS1	H5N1	Deletion from 85-94	Impaired inhibition of IFN production	[34]
NS1	H3N8 (duck), WSN	R127K, V205I, N209D	Increased replication and lethality in mice (R127K, loss of PKR binding)	[35,36]
NS1	H5N1	D92E	Low sensitivity to IFN and TNFa	[37]

infection is mainly limited to the upper respiratory tract, the disease is mild. Nonetheless, the viruses spread easily among human populations mediated by nasal discharges that contain high titers of live virus. Highly pathogenic avian H5N1 influenza viruses (HPAIV), however, preferentially recognize α2-3 sialylated glycans and primarily infect type 2 pneumocytes in the human lung [12]. Therefore, HPAIV infection often results in severe pneumonia in humans [13]. Because the primary target cells of HPAIV are deep in the lower respiratory tract, it is difficult for HPAIV to cause widespread infection among humans. Mutations in the HAs of H5N1 viruses confer upon these mutants the ability to bind to α2-6 as well as α2-3 sialylated glycans [14]. In the case of influenza A(H1N1)2009, a D222G substitution in HA, which was observed in severe and fatal cases, changes the receptor binding specificity of the virus from α2-6 to α2-3 sialylated glycans [11,15]. A study using cultures of human tracheobronchial epithelial cells showed that influenza A(H1N1)2009 with the D222G substitution in its HA could infect ciliated bronchial cells [11]. This cell tropism alteration mediated by an HA mutation may increase the severity of pneumonia. Therefore, we must carefully monitor the HAs of avian H5N1 viruses for amino acid mutations that may alter their pandemic potential as well as the HA of influenza A(H1N1)2009 for mutations that produce strains with higher pathogenicity.

HA also influences pathogenicity via its susceptibility to host proteases. For influenza viruses to be infectious, their HAs must be cleaved into two subunits, HA1 and HA2 [16]. The HA of seasonal IAV possesses a single arginine at the cleavage site and is cleaved by trypsin-like proteases that are produced by respiratory and gastrointestinal cells. In contrast, the HA of HPAIV possesses multiple basic amino acids at the cleavage site and is susceptible to ubiquitous furin and PC6, which reside in the trans-Golgi network [17]. This is one reason why HPAIV cause severe systemic infection leading to multiple organ failure and death.

The viral RNA polymerase complex consists of PA, PB1, and PB2. This complex is responsible for the transcription and replication of the viral genome. Several mutations in PA and PB2 support better replication of avian viruses in mammalian cells (Table 1) [18–22]. Therefore, it is important to monitor mutations in the genes of the RNA polymerase complex to detect viruses that replicate well in humans. A/Vietnam/1203/04 (VN1203) H5N1 virus, which was isolated from a fatal human case, is highly lethal to ferrets and mice [23,24]. When the viral RNA polymerase genes of VN1203 were replaced with those of a low pathogenic H5N1 virus, the pathogenicity of VN1203 was dramatically reduced in these animals [24]. Watanabe *et al.* also demonstrated that the RNA polymerase complex and NP played a role in the pathogenicity of the 1918 pandemic virus [25•]. Thus, the viral RNA polymerase complex also contributes to IAV pathogenicity in mammals.

The PB1 segment encodes a 90-amino acid protein, PB1-F2 that preferentially localizes to the mitochondria of infected cells [26]. PB1-F2 induces apoptosis and is a known virulence factor [27]. The amino acid change N665S in PB1-F2 was found to be responsible for the high virulence of both the 1918 pandemic and H5N1 viruses [28]. This mutation increases the secretion of proinflammatory cytokines, such as TNF-α and virus titers in the lungs. Other viral proteins, such as NA and NS1, are also implicated in the virulence of IAV. NA is important for efficient viral replication [29], while NS1 antagonizes interferon production in virus-infected cells.

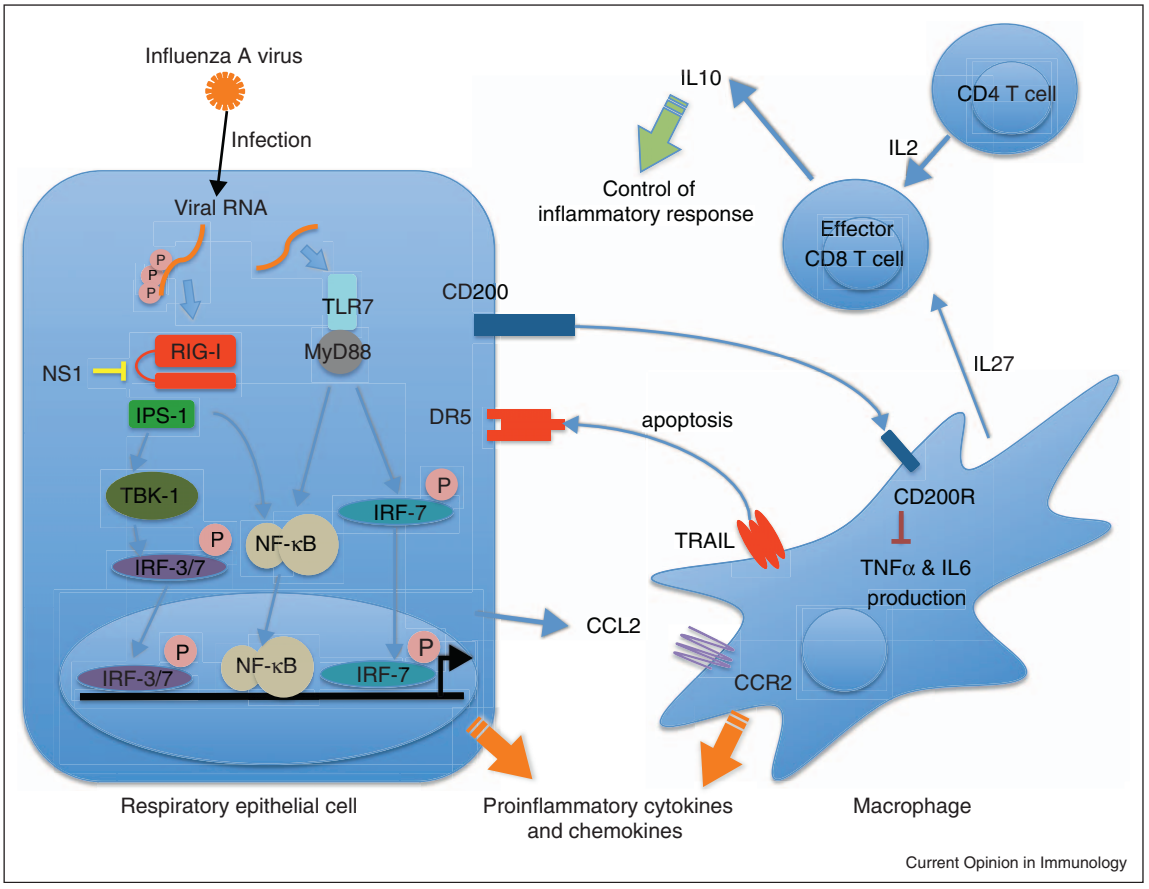
Host factors

The immune system protects the host from infection with influenza virus. Therefore, the pathogenesis of influenza virus depends on the function of the immune system. When IAV infect respiratory epithelial cells or alveolar macrophages, the single-stranded RNA of the influenza virus is recognized by toll-like receptor (TLR) 7 and retinoic acid-inducible gene-I (RIG-I) [30,31]. The signaling pathways of TLR7 and RIG-I induce the production of type I IFNs and activate antiviral host responses [32]. However, IAV can escape from the innate immune response by using NS1 to interfere with the RIG-I signaling pathway (Table 1) [33–37]. A recent study revealed that NS1 inhibits the function of tripartite motif (TRIM) 25 in the ubiquitination of RIG-I, which is an essential step in the type I IFN response [38••]. Because the NS1 of the 1918 virus efficiently suppressed the expression of IFN-regulated genes, NS1 is believed to contribute to pathogenesis by controlling antiviral innate immune responses [39]. NS1 also binds to protein kinase R (PKR), a well-known antiviral protein. The binding of NS1 and PKR inhibits the antiviral function of PKR by downregulating the translation of the viral mRNA, which is mediated by phosphorylation of eukaryotic translation initiation factor 2 alpha (eIF2α) [40]. The NS1 amino acids at positions 123–127 are essential for PKR binding and a mutation of these residues affects pathogenicity in mice [35,36]. In addition to the type I IFN response, RIG-I and TLR7 induce the production of inflammatory proteins mediated by NF-κB activation [41]. Therefore, influenza virus infection induces the upregulation of several inflammatory cytokines and chemokines, such as IL-1β, IL-6, IL-8, TNFα, CCL2 (MCP-1), CCL3 (MIP-1α), CCL5 (RANTES), and CXCL10 (IP-10) [42]. Among them, CCL2 recruits macrophages to the virus-infected lung [43]. CCR2-(a receptor of CCL2) positive macrophages express tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), which induces alveolar epithelial cell apoptosis [44]. CCR2 deficiency in IAV-infected mice inhibits macrophage migration to the lung and increases survival rates [45]. Thus, macrophages that migrate to influenza-infected lung play a pathogenic role in pulmonary inflammation. In lung infected with highly pathogenic IAV, such as the 1918 virus or avian H5N1 viruses, sizeable numbers of neutrophils are also recruited to the inflamed lung [42]. This suggests that neutrophils also contribute to IAV pathogenesis; however, the role of neutrophils in IAV infection remains unclear because neutrophils limit virus replication and lung inflammation [46•].

To maintain homeostasis during IAV infection, a regulatory immune system exists in the lung. CD200, a cell surface glycoprotein, is expressed on respiratory epithelial cells, and the CD200 receptor (CD200R) is expressed by myeloid cells, including macrophages, dendritic cells, and granulocytes [47,48]. In the uninfected state, CD200R expression on alveolar macrophages is maintained by IL-10 and TGFβ [49]. However, in lungs infected with IAV, CD200R expression is upregulated on these macrophages [49]. Experiments using CD200−/− mice revealed that CD200-mediated CD200R activation on lung macrophages inhibits the recruitment of immune cells, the production of proinflammatory cytokines, such as TNFα and IL-6, and inflammation in the IAV-infected lung [49]. As TNFα and IL-6 increase CD200R expression on alveolar macrophages, there is negative feedback of inflammatory responses controlled by CD200R. CD200R+ alveolar macrophages thus have an important role in resolving inflammation in IAV-infected lung [49]. IL-10 is known to be a major regulatory cytokine that inhibits inflammatory responses [50]. IL-10-producing effector CD8T cells are a major source of IL-10 in acute lung infection with IAV, and IL-10 produced by this CD8T cell subset controls the excessive lung inflammation caused by IAV infection [51••]. Furthermore, a recent study shows that IL-2, produced by CD4T cells, and IL-27 have a synergistic role in the generation of IL-10-producing CD8T cells [52••]. IL-27, a member of the IL-12 family, is produced by macrophages, dendritic cells, and neutrophils [53–55]. Thus, multiple cell-cell interactions regulate the immune response to IAV infection and maintain the homeostasis of the respiratory immune system (Figure 1).

As discussed above, the innate immune response is indispensable for the protection of the host against IAV infection. Therefore, a lack of type 1 IFN results in an increase in virus dissemination and susceptibility to IAV infection, including H5N1 viruses [56,57]. However, the unregulated response of proinflammatory cytokines and chemokines induced by TLR signaling can harm rather than protect respiratory organs. For example, virus clearance in the lung was better in CD200−/− mice than in wild-type mice because CD200−/− mice activated their innate response via their alveolar macrophages [49]. However, this uncontrolled innate immune response led to severe lung inflammation in the CD200−/− mice [49]. Therefore, innate immunity is like a two-edged sword with two distinct roles in the pathogenesis of IAV infection. In contrast, adaptive immunity, which involves viral antigen-specific antibodies and cytotoxic T lymphocyte activity, efficiently eliminates virus-infected cells and enables hosts to recover from viral infectious diseases. Immunodeficiency of B cells or T cells (RAG−/− mice) results in high susceptibility to IAV infection [58]. Therefore, adaptive immunity provides essential protection from IAV infection and effective prevention of repeat infection. Yet, a surprising number of severe diseases in middle-aged adults, who generally had normal immune function, were reported during the 2009 (H1N1) pandemic [59]. Interestingly, low affinity antibodies in sera and immune complexes with low affinity antibodies were detected in individuals with

Figure 1



A model depicting the multi-cellular interactions that regulate the inflammatory response during influenza virus infection. Influenza virus infection induces innate immune responses mediated by the TLR7 and RIG-I signaling pathways. Pulmonary macrophages migrate to infected epithelial cells in a CCL2-CCR2-dependent manner and induce apoptosis in the respiratory epithelial cells via TRAIL-death receptor 5 (DR5) interactions. On the other hand, the interaction of CD200 and CD200R downregulates the inflammatory response, including IL-6 and TNFα production by macrophages. Effector CD8T cells also inhibit the inflammatory response by IL-10. CD4T cells and macrophages produce IL-2 and IL-27, respectively, to support the regulatory function of IL-10-producing effector CD8T cells.

severe pneumonia [60••]. Furthermore, examination of lung sections from fatal cases of influenza A(H1N1)2009 infection revealed C4d deposition around the bronchi, indicating that an abnormal adaptive immune response may have contributed to the influenza pathogenesis.

Summary

The pathogenicity of influenza virus is dependent on the function of viral proteins and on host immune responses, including innate and acquired immunity, indicating the importance of both viral factors and the host immune system for influenza pathogenesis. A recent report showed that commensal microflora is important for the appropriate activation of pulmonary dendritic cells to induce influenza virus-specific immune responses [61••]. Therefore, the environmental conditions that surround the host and virus, including commensal microflora, must also be considered as factors contributing to viral pathogenesis. Despite extensive research on IAV pathogenesis, we still do not have effective therapies for IAV infection, except for antiviral drugs. Moreover, the emergence of drug-resistant viruses jeopardizes the effectiveness of these agents (Table 1) [62–64]. Controlling excessive host responses could serve as the basis of new strategies for the treatment of severe cases of IAV infection. A comprehensive understanding of how virus pathogenesis is mediated by various factors should assist in the development of new therapies to combat highly pathogenic IAV infections.

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