Innate Immunity in the Airways to Respiratory Viruses

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1 Introduction

The human airways are vital to respiration, allowing access of the atmosphere to the delicate gas exchange interface of the alveoli. However in doing so they also act as a gateway for a wide range of noxious particulates and micro-organisms to enter into the lungs. Respiratory viruses are the most common cause of infection in humans worldwide and some of the most clinically important respiratory viruses are listed in Table 1. Many of these viruses have been recognised as human pathogens for some time, others such as the human SARS coronavirus (SARS-CoV) have only recently emerged with devastating though brief consequences in 2003, while viruses like influenza and respiratory syncytial virus (RSV) result in marked morbidity, mortality and socio-economic burden annually. Respiratory viral infection often causes a wide range of diseases from mild symptoms such as cough and sore throat to severe life-threatening pneumonia. The consequences of infection are the result of a complex interplay between the virulence of the pathogen and the ability of the host to resist and mount an effective immune response.

Human immune system is a complicated and yet structured biological process that protects the host from infection. Advances in molecular immunology have provided valuable insight into the functional characterization of novel signalling proteins and cytokines that are involved in human immunity. The innate arm of human immune system has become a major research focus since the discovery of pattern recognition receptors, overthrowing the theory of non-discriminative innate immune responses against self and non-self entities. The ability of innate immune system to recognize different foreign pathogens further identifies innate immunity as the most important first line of defence that not only limits viral replication and spread, but also directs appropriate pathogen-specific adaptive immune activation for efficient clearance of viruses.

Similarly, over the course of virus-human co-evolution numerous respiratory viruses have also developed immune evasion strategies for efficient infection in the host. Advances in reverse genetic engineering has discovered novel viral virulence factors, and characterized their function and interaction with immune cells, thereby further delineating the mechanism of viral infection and uncovering novel therapeutic strategies. Although innate immune signalling networks have been extensively investigated, there are still numerous signalling proteins, regulatory and intercepting pathways, and transcriptional mechanisms that are still poorly defined. This review will therefore explore current understandings of innate immune responses to respiratory viruses in the human airways, and how these responses can be altered by the invading viruses. In addition, in the context of virus infection the host immune response can also be as damaging as the direct effects of infection. This is highlighted particularly in the case of those with chronic airways diseases such as asthma and chronic obstructive pulmonary disease (COPD), whom not only have abnormal regulations of immune responses but also are more susceptible to viral infection and suffer more severe symptoms and exacerbation of their pre-existing conditions following infection. The molecular mechanisms underlying the increased susceptibility and severe infection outcome in those with chronic airways diseases are largely unknown, this review will also discuss potential abnormalities in the innate immunity and how respiratory viral infection can further worsen the infection outcome in these individuals.
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Table 1: Viruses that are known to cause diseases in the human airways. * indicates clinically important respiratory viruses.

2 Viral Infection and Innate Immunity in the Airways

2.1 Airway Epithelium

As the portal to the external environment, the mucosal epithelium that lines the respiratory system is an important barrier that defends against foreign pathogens. As the first point to contact, respiratory viruses therefore primarily infect and replicate in the airway epithelial cells (AECs).

Before infection can be established a virus must bind to glycoproteins on the host cell surface, tricking host cells to initiate receptor-mediated endocytosis. The specific host cell receptors to which viruses attach vary greatly, however these host receptors are usually ubiquitously expressed on human cells. For example, SARS-CoV binds to angiotensin-converting enzyme 2 (ACE2) to be endocytosed into the cells (W. Li et al., 2003), while influenza viruses bind to glycoproteins with terminal sialic acid (SA) residues of specific configurations. Human rhinoviruses of major group receptor bind to intracellular adhesion molecule (ICAM) -1 and that of minor group bind to low-density lipoproteins (LDLs), all of which are expressed on airway epithelial cells (Papi & Johnston, 1999; Suzuki et al., 2001).

Binding diversity within the same virus family has been most thoroughly studied in influenza, as different strains of which may bind with its surface protein haemagglutinin (HA) to SA residues of different modifications, and will be discussed here. SA are monosaccharide molecules found ubiquitously as terminal residues on the glycan chain of many polysaccharide and glycoproteins on human epithelial cell surface, secreted glycoproteins, as well as surface glycolipids (Suzuki et al., 2005). Diversity in SA presentation includes the position of SA branching from the carbon backbone of glycoproteins, modification of hydroxyl group, and different α-linkages from the 2-carbon to the sugar chain. Different strains of influenza bind to SA residues with different linkage to the carbon backbone of glycoproteins. Human influenza viruses preferentially bind to SAα2,6Gal terminal linkages, which are predominantly found on epithelial cells of the upper respiratory tract, whereas avian
influenza viruses bind to terminal SAα2,3Gal linkages in the lower airways (Baum & Paulson, 1990; T. Ito et al., 1997; Rogers & Paulson, 1983; Ryan-Poirier et al., 1998). The difference in their binding specificities are partly attributed to the amino acid residue at position 226 and 228 of the HA glycoprotein. The HA of human influenza viruses contains a 226Leu and 228Ser that results in the preferential binding to SAα2,6Gal residues. In contrast, the HA of avian influenza viruses have a 226Gln and 228Gly, which binds to SAα2,3Gal linkages (Connor et al., 1994; Rogers & D’Souza, 1989). This difference in amino acid residues and distribution of SA residues in the airways may explain why highly pathogenic avian influenza virus H5N1 is currently incapable of sustainable human-to-human transmission, as mutations at neither sites were found in H5N1 strain isolated from infected individuals (Yamada et al., 2006).

Two recent studies further investigated the molecular changes required for H5N1 to become efficiently transmissible amongst humans, and identified a number of mutations that transformed SAα2,3Gal binding property of H5 HA protein to SAα2,6Gal binding. One study found that Gln226Leu/Gly228Ser mutations increased the binding of H5 HA to SAα2,6Gal while retaining the binding capacity to SAα2,3Gal. However together with mutations at Asn158Asp/Asn224Lys/Gln226Leu/Thr318Ile this fully converted the virus with mutant H5 HA in a 2009 pandemic H1N1 backbone to become efficient in aerosol transmission in ferret model, a standard and widely accepted animal model for influenza transmissibility studies (Imai et al., 2012). Another study identified another set of mutations in H5N1 that transformed this highly pathogenic avian influenza with inefficient human-to-human transmission to sustainable airborne transmission (Herfst et al., 2012). Herfst et al. showed that mutant H5N1 virus with mutations at Gln222Leu, Gly224Ser, His103Tyr, and Thr156Ala in H5N1 HA protein, and Glu627Lys in the viral polymerase protein PB2 were able to transmit efficiently between ferret models via aerosols. This clearly illustrates the importance of these mutations in HA and PB2 protein for efficient transmission and spread amongst humans, although it remains unclear if any influenza viruses can become airborne with these mutations. These results provide valuable insight into new vaccine design strategies, and current and future avian influenza surveillance network.

While the binding specificities of influenza viruses have been well established using various techniques such as structural biology and glycan microarray, the viruses can still cause infection in the absence of their respective receptors. This therefore raises questions as to whether viruses can enter into airway epithelial cells via other mechanisms. Supporting this hypothesis, a study showed that human influenza virus was able to infect and replicate to a similar titre in the lungs of mice lacking SAα2,6Gal linkage receptors compared to wild type mice (Glaser et al., 2007). In addition, we have also recently shown that despite low levels of SAα2,3Gal linkage on BECs, a low pathogenic avian influenza virus H11N9 was still able to endocytose into the BECs at similar efficiency as human strain and replicated to high viral titre (A. C. Hsu et al., 2011). The highly pathogenic avian influenza H5N1 was also able to replicate to even higher extent compared to H3N2 and H11N9 (A. C. Hsu, Parsons, K., Barr, I., Lowther, S., Middleton, D., Hansbro, PM., Wark, PAB., 2012).

There are several potential host surface glycopolymers with other modifications such as fucosylation that have been shown to be bound by influenza HA proteins (Rapoport et al., 2006), however recent evidence suggests that phosphotidylinositol-3-kinase (PI3K) signalling pathway is heavily
involved in viral endocytosis. PI3K is a ubiquitous signalling pathway that controls cellular metabolism and proliferation [26], and also highly regulates an internalization process called macropinocytosis (Araki et al., 1996; Hewlet et al., 1994; West et al., 2000). Macropinocytosis is a non-specific, receptor-independent internalization process that forms endocytic vesicles and brings the attaching virus into the host cells, though the mechanisms of how viruses activate this process remain unknown. Nevertheless a number of respiratory viruses have been shown to utilize the PI3K pathway for viral entry, including influenza and rhinoviruses. Influenza is able to activate PI3K pathway that facilitates viral endocytosis into BECs, while inhibition of PI3K results in reduced viral endocytosis and viral replication (de Vries et al., 2011; Ehrhardt et al., 2006). This PI3K activation was shown to be due to the influenza non-structural (NS) 1 protein that binds and activate PI3K for efficient viral endocytosis and infection. There is also evidence of PI3K-mediated viral entry of human rhinovirus and adenovirus into the host cells, although the exact mechanism of this viral-PI3K-mediated macropinocytosis is still unclear (Lau et al., 2008; E. Li et al., 1998). This indicates that viral entry into host cells is not completely dependent on the receptor-mediated endocytosis, but also involves receptor-independent macropinocytosis, and PI3K signalling appears to be important in this process. Receptor blocking therapeutics already exist on the market, such as oseltamivir that inhibits the influenza surface protein neuraminidase (NA) which cleaves HA-SA interaction and releases the newly made virions. Although drug resistance is rapidly emerging, investigation in the molecular mechanisms of virus-induced macropinocytosis may reveal potential therapeutic targets for respiratory viral infections, particularly for influenza.

Following successful endocytosis viruses must utilize the host cells to replicate and then spread to neighbouring cells. As BECs are the primary site of infection for respiratory viruses, they often initiate the early immune response to infection, which is likely to have a profound effect on the subsequent inflammatory and immune response. Innate immunity is an evolutionary conserved first line of defence that is initiated in infected cells in an attempt to limit viral replication and spread, concomitantly induce the development of pathogen-specific adaptive immunity for viral clearance. During infection, replicating viruses produce RNA or DNA intermediate products, which can be recognized by host intracellular pattern recognition receptors (PRRs), and then initiate early innate immune responses to contain viral infection at the site of infection. Toll-like receptors (TLRs) are a family of membrane-spanning PRRs that recognize components that are broadly shared by pathogens but are distinctive from host molecules, and are collectively called pathogen associated molecular patterns (PAMPs). There are currently 12 members of TLRs identified in mammals, three of which play an important role in viral innate immunity and viral pathogenesis.

Most respiratory viruses are ssRNA viruses, though they also produce dsRNA intermediates during replication. Viral ssRNA and dsRNA are recognised as foreign entity by different families of PRRs (Figure 1). TLR3 is a membrane spanning PRR located in the endosomal membrane that recognizes viral dsRNA and predominantly initiates inflammatory response (Alexopoulou et al., 2001). Upon binding TLR3 initiates a signalling cascade that activates cytoplasmic nuclear factor κB (NF-κB), which is then translocated to the nucleus where it induces the expression of pro-inflammatory cytokines such as CXCL-8 and tumour necrosis factor (TNF) -α, leading to acute inflammation including the infiltration and activation of neutrophils (Chaouat et al., 2009). Viral-mediated activation
of NF-κB also drives the expression and release of another class of innate immune protein called human defensins, which is classified into α and β sub-family. Human α defensins (HDs) are mainly induced by blood immune cells such as neutrophils (Ganz, 2003; Rehaume & Hancock, 2008), whereas human β defensins (HBDs) are mainly produced by lung epithelial cells and immune cells (Ganz, 2003; Yang et al., 2004). Respiratory viruses including rhinovirus, influenza viruses, and RSV have all been shown to induce HBD2 and HBD3 in a NF-κB and TNF-α-dependent manner (Duits et al., 2003; Kota et al., 2008; Leikina et al., 2005; Proud et al., 2004), and their functions are mainly involved in the inhibition of viral entry and disruption of viral envelope. HBD2 has been shown to bind and disintegrate RSV envelope under electron microscopy, leading to the fragmentation of the virus (Kota, et al., 2008). Similarly HBD3 was shown to inhibit viral-host membrane fusion during influenza infection leading to reduced viral entry (Leikina, et al., 2005). Together with other HDs and HBDs such as HD5 that also inhibits viral entry step of another virus family herpes simplex virus (HSV) (Hazrati et al., 2006), this indicates that the defensins may be broadly involved in the antiviral responses to invading viruses at the early phase of infection.

The second set of PRRs that also recognizes foreign pathogens are the nucleotide-binding domain and leucine-rich-repeat-containing (NLR) family. NLR protein 3 (NLRP3) can recognize dsRNAs and ssRNA, and can be induced by viral infection including influenza (Allen et al., 2009) and rhinovirus (Schneider et al., 2010) (Figure 1). Upon binding to viral RNAs, NLRP3 interacts with an adaptor protein Apoptotic Speck protein containing caspase activation and recruitment domain (CARD) (ASC) and forms a complex known as the NLRP3 inflammasome. This complex then activates pro-caspase 1 into functional caspase 1, which in turn activate pro-IL-1 into its biological active form IL-1β, which binds to toll/IL-1 receptor (TIR) that shares signalling with TLRs and up-regulates inflammatory cytokines (Takeda et al., 2003). IL-1β also recruits neutrophils to the site of infection and further promotes inflammatory mediators (Akira et al., 2006; Allen, et al., 2009; Ting et al., 2008).

The third family of PRRs called retinoic acid-inducible gene-I (RIG-I)-like receptors (RLRs), including RIG-I, melanoma-differentiation-associated gene-5 (MDA-5), and laboratory of genetics and physiology 2 (LGP2), are also able to recognize different forms of viral RNAs, and are primarily responsible for the induction of type I and type III interferons (IFNs), a critical component of the innate antiviral responses that limit viral replication (Figure 1). RIG-I preferentially recognizes ssRNAs with 5’ triphosphate (5’ppp ssRNA) that are only generated during influenza, RSV, and human metapneumovirus replication (Le Goffic et al., 2007; Loo et al., 2008; Pichlmair et al., 2006; Saito & Gale, 2008). In contrast MDA-5 is critical in the detection of dsRNA viruses or those with dsRNA intermediates including human rhinoviruses (Kato et al., 2006; Satoh et al.). LGP2 is a positive regulator that aids the viral RNA binding by RIG-I and MDA-5 (Satoh, et al.). After initial binding to viral RNAs by RIG-I/MDA-5/LGP2, the adaptor protein tripartite motif protein 25 (TRIM25) ubiquitinates the first CARD domain of RIG-I/MDA-5. This results in a conformational change that allows the second CARD domain to associate with the adaptor protein IFN-β promoter stimulator 1 (IPS-1; also known as MAVS, VISA or CARDIF) on the mitochondrial membrane. Tumour Necrosis Factor (TNF)-receptor-associated factor (TRAF3) then associates with this complex and activates TRAF family member-associated NF-κB activator (TANK)-binding kinase 1
Figure 1: RIG-I/MDA-5/LGP2, TLR3 and NLRP3 inflammasome pathway leading to the expression and release of type I/III IFNs and inflammatory cytokines in epithelial cells.

(TBK1) and inducible IκB kinase (Oganesyan et al., 2006). These kinases phosphorylate interferon regulatory factor 3 (IRF3) and IRF7, which then translocate to the nucleus where they initiate the expression of type I and type III IFNs (Doyle et al., 2006; Fitzgerald et al., 2003; Kotenko et al., 2003).

Type I (IFN-α-β) and type III (IFN-λ1/2/3) IFNs are potent innate antiviral mediators that are released by epithelial cells upon viral infection. These secreted IFNs then bind to type I IFN (IFNAR1/2) and type III IFN (IL28Rα/IL-10Rβ) receptor on the same or neighbouring cells (Kotenko, et al., 2003; Pestka et al., 1987; Sheppard et al., 2003), and induce the expression of over 300 IFN-stimulated genes (ISGs), through the activation of signal transducer and activator of transcription (STAT)-1 and -2 (Figure 2) (Levy & Garcia-Sastre, 2001; G. R. Stark et al., 1998). This promotes the establishment of the full antiviral responses of epithelial cells and provides positive reinforcement of IFN responses.

Many ISGs have been identified to have roles in host defence against viral infection. Protein kinase RNA-activated (PKR) is a dsRNA-activated cellular enzyme that not only induces a rapid and potent expression of IFN proteins (Kujime et al., 2000; Kumar et al., 1994), but also induces apoptosis upon viral infection, thus preventing virus replication (Garcia et al., 2006; Gil & Esteban, 2000; Zhang & Samuel, 2007). PKR also phosphorylates the elongation initiation factor eIF2, which results in a rapid inhibition of viral mRNA translation (G. R. Stark, et al., 1998). Proteins such as 2’, 5’ – oligoadenylate synthetase (OAS) and MxA protein elicit an antiviral state in the infected cells by inhibiting the viral replication. OAS can be activated by viral RNA, which in turn activates an endoribonuclease RNase L that
can cleave extensively viral RNA (Chen et al., 1999). MxA also interferes with viral protein synthesis and inhibits viral replication by promoting host cell apoptosis (Mibayashi et al., 2002; Pavlovic et al., 1992; Zhou et al., 1997; Zurcher et al., 2000). Positive regulators such as RIG-I, MDA-5, and IRF7 further amplify the antiviral responses (Garcia, et al., 2006; Kujime, et al., 2000; Pavlovic, et al., 1992; Slater et al., 2010).

Despite the distinctive viral recognition and signalling outcomes of TLRs and RLRs, recent characterization of these PRRs also revealed cross-regulation of these signalling pathways. Human rhinovirus is a ssRNA virus that generates dsRNAs during replication, which can be recognized by TLR3 and MDA-5, stimulating an inflammatory and antiviral response. In contrast influenza, also a ssRNA virus, only produces ssRNAs with 5’ triphosphate (5’ppp-ssRNAs), which is recognized by RIG-I, and not dsRNAs during replication (Pichlmair, et al., 2006). Although influenza has been demonstrated to induce inflammatory response via TLR3 signalling, it is questionable as how influenza 3’ppp-ssRNAs are recognized by TLR3, and may possibly involve other pathways such as NLRP3. NLRP3 has been shown to recognize influenza RNAs and induce inflammatory response, although the specific RNA configurations that NLRP3 binds are yet to be characterized. TLR3 and NLRP3 may therefore both contribute to the inflammation in the lung of infected individuals (Jiang et al., 2004; Le Goffic, et al., 2007). This is especially true with the highly pathogenic avian influenza H5N1. A hallmark feature of H5N1 infection in the infected individuals is a massive pro-inflammatory cytokine storm, leading to severe toxic shock and multi-organ failure with a fatality rate of approximately 60% (Chotpitayasunondh et al., 2005; Tran et al., 2004).

Although the PRRs that results in this inflammatory cytokines release in H5N1 infection are not known, NF-κB has been shown to play a critical role, while inhibition of NF-κB has resulted in a marked reduction in the production of these cytokines (Schmolke et al., 2009). Interestingly preventing this cyto-

**Figure 2**: Type I and type III IFNs mediated induction of ISGs expression in epithelial cells.
kine storm in H5N1-infected knockout mice model did not lead to reduced pathogenicity or improved mortality (Droebner et al., 2008; Salomon et al., 2007). It then appears that that the removal of damaging effects of inflammatory cytokines is not enough to reduce high mortality associated with H5N1 infection.

TLR3 signalling pathway, typically involved in the inflammatory responses, has also been shown to regulate the expression of antiviral proteins via NF-κB. This transcription factor downstream of TLR3 is normally involved in the induction of inflammatory cytokines, however Thomson, et al. identified a cluster of NF-κB binding sites on human IFN-λ1 promoter, demonstrated that NF-κB was critical in IFN-λ1 induction (Thomson et al., 2009). IFN-β on the other hand can also be up-regulated by a complex of transcriptional factors. IFN-β gene enhancer can be recognized by three major transcription factors, nuclear factor (NF) -κB, interferon-regulatory factors (IRFs), and activating transcription factor (ATF) -2. These three factors then bind to a structural protein called high mobility group (HMG) -1 protein, forming a multi-component transcription factor-enhancer complex named the IFN-β enhanceosome. HMG-I promotes and stabilizes of the binding to transcription co-activator cAMP response element-binding (CREB) binding protein (CBP)/p300, and increases the formation of another complex called transcription pre-initiation complex (PIC) that facilitates the positioning of RNA polymerase II over the gene transcription start site, denatures DNA thereby initiating the transcription of IFN-β gene (Kim & Maniatis, 1997; Yie et al., 1999) (Figure 3). Although the formation kinetics of IFN-β enhanceosome during respiratory viral infections is still unclear, the formation of IFN-β enhanceosome is critical in the induction of IFN-β during viral infection.

This differential binding of transcription factors therefore ensures dynamic up-regulation of these important antiviral proteins following viral infection, especially when the invading viruses such as influenza possess antiviral evasion strategies to maximize replication. The multiple layers of host innate defences attempt to restrict replicating viruses, and at the same time signal for other important immune cells to the site of infection for further clearance of viruses.

2.2 Airway immune cells

Neutrophils are important innate immune cells that respond to signals of tissue damage and infection (Cowburn et al., 2008). These effector cells express the surface receptor CXC Chemokine Receptor 1 and 2 (CXCR1 and 2), which are attracted by CXCL-8 produced by the infected BECs and recruited to the site of infection down a chemokine-induced gradient (Woolhouse et al., 2002). Respiratory viral infections result in a rapid infiltration of neutrophils into the lung, and once at the site of infection neutrophils, via cluster of differentiation (CD) 11a/CD18, adhere to ICAM-1 on BECs, and phagocytose infected and/or damaged cells, resulting in the formation of phagosome (Tate et al., 2008; Tate et al., 2011). Within which toxic proteases (including neutrophil elastases, matrix metalloproteinases (MMPs), and myeloperoxidase (MPO) are produced to convert reactive oxygen species (ROS) such as hydrogen peroxide into hypochlorous acid that then degrades ingested viral proteins (Lambeth, 2004; Yamamoto et al., 1991). This event is called neutrophil oxidative burst and provides a secondary defence against foreign pathogens. Neutrophils therefore play a critical role in the clearance of replicating virus, and this importance was further demonstrated in the reduced survival to influenza infection in mice that had been depleted of neutrophils (Tate, et al., 2008). Furthermore, BECs infected by influenza, parainfluenza, rhinovirus and RSV have been shown to increase ICAM-1 expression that allows enhanced neutrophil adherence to the infected cells, thereby further enhancing viral clearance (Ratecliffe et al., 1988; J. M. Stark et al., 1992; S. Z. Wang et al., 1998). Concomitantly neutrophils themselves are also capable of inducing
Figure 3: Formation of IFN-β enhanceosome in IFN-β induction and IRF3/7 and NF-κB co-regulation of IFN-λs induction. Transcriptional factor IRF3/7, NF-κB, and ATF-2 forms a large complex with HMG-I, which then binds to CBP/p300 and drives the transcription of IFN-β. The IRF-3/7 and NF-κB binding site on the IFN-λ promoter allows for IFN-λs gene transcription.

Macrophages are another type of phagocytic cells that scavenge foreign pathogens and dead cellular debris in the lung, while also participate in inflammation. These scavenger immune cells are of haemopoietic origin and migrate to the respiratory tract via blood and lymphatic vessels. Pro-monocytes migrate from bone marrow into the circulation and mature into monocytes, which then differentiate into macrophages when they reach their destined organs. In the lungs, alveolar macrophages are also an important component of pulmonary defence that are recruited to the site of infection by chemokines including chemokine (C-C motif) ligand (CCL3, also known as Macrophage Inflammatory Protein (MIP) -1α) and CCL5 (also known as Regulated And Normal T cell Expressed and Secreted (RANTES)) released from virus-infected cells. They are then capable of recognizing externalized phospholipid phosphatidyl-
serine, a hallmark feature of apoptosis, on the surface of infected cells and initiate phagocytosis (Hashimoto et al., 2007; Piccolo et al., 1999; Shiratsuchi et al., 2000; Shukaliak & Dorovini-Zis, 2000). CCL2 (or Monocyte Chemotactic Protein (MCP) -1) can also be induced by macrophages to recruit monocytes, and DCs to the site of inflammation (Xu et al., 1996). The inductions of these chemokines are dependent on the activation of NF-κB (Qin et al., 2012; Rimbach et al., 2000; Werts et al., 2007). Macrophages also appear to have similar innate immune signalling system, and many respiratory viruses such as influenza, rhinoviruses, and RSV are able to infect and replicate in macrophages and induce similar innate immune responses as that found in BECs. Infection of macrophages with these respiratory viruses has been shown to induce TLR3 and NF-κB with up-regulated pro-inflammatory cytokines such as CXCL-8 and TNF-α. RIG-I/MDA-5 was also induced and led to increased type I IFN responses after infection (Cheung et al., 2002; Laza-Stanca et al., 2006; Senft et al., 2010; J. Wang et al., 2012). Macrophages also produce SP-A that binds to influenza HA and NA, RSV F (fusion) and G (attachment) protein that results in enhanced viral uptake and phagocytosis by the macrophages (Barr et al., 2000; Ghildyal et al., 1999; Malhotra et al., 1994).

Natural Killer (NK) cells are a class of cytotoxic lymphocytes that play a crucial role in innate antiviral responses to viral infections. NK cells can be activated by cytokines including type I IFNs, CCL2, CCL3, and CCL5 induced by viral infection. At the site of infection NK cells are able to recognize the natural cytotoxicity receptor such as NKp46 on the virus-infected cells and release intracellular proteases including perforin and granzymes, leading to the induction of apoptosis of infected cells (Biron et al., 1999; Mandelboim et al., 2001). NK cells are also capable of secreting cytokines such as TNF-α, further contributing to the inflammatory response after viral infection. While NK cells appears to be an important effector immune cells against viruses such as and parainfluenza virus and RSV, influenza viruses have been shown to infect and replicate in NK cells, after which kills the infected NK cells by inducing apoptosis via unknown mechanism (Anderson et al., 1989; Mao et al., 2009).

Another important class of immune cells are the dendritic cells (DCs), which play a pivotal role in both the innate response to infection, as well as initiating the adaptive immune response in response to infection. There are several phenotypes of DCs in the lung with varying immune functions. Plasmacytoid (p)DCs, on the other hand, are potent IFN producers against viral infection. pDCs share similar immune signalling pathways as BECs, however RIG-I appears to be dispensable during viral infection and instead rely on TLR7/8 and 9 in the endosome to recognize viral RNAs and initiate IFN responses via IRF7 and inflammatory response via NF-κB (Honda & Taniguchi, 2006; Takeda & Akira, 2005). Interestingly, human influenza H3N2 and H1N1, human rhinovirus, RSV, and human metapneumovirus have all been shown to have restricted replication due to potent type I IFN responses in pDCs (Boogaard et al., 2007; Fonteneau et al., 2003; Schrauf et al., 2009; Smed-Sorensen et al., 2012). It is possible that the high antiviral responses exerted in the pDCs circumvent the need for cytoplasmic PRRs such as RIG-I. High pathogenic avian influenza H5N1 on the other hand was able to replicate efficiently by heavily suppressing this type I IFN responses in the infected pDCs (Thitithayanont et al., 2007). The IFNs produced by BECs and pDCs not only establish antiviral responses and contain viral replication, but can also promote DC maturation and their ability to stimulate virus-specific cytotoxic T cells response for efficient viral clearance (Luft et al., 1998; Spadaro et al., 2012). Myeloid (m)DCs, as well as macrophages, are the sentinel cells that capture viral antigens that are released from infected BECs, and present the antigens to naïve T cells in the lymph node, leading to an virus-specific cytotoxic T cell response to efficiently clear the invading virus and memory T cell response in preparation for future re-infection (Cerwenka et al.,
DCs have also been discovered to interact with unique subsets of T lymphocytes called invariant natural killer T (iNKT) and Th17 cells, which have been shown to contribute to innate inflammatory response to viral infections. For example, influenza infection has been shown to induce IL-1β via RIG-I, TLR7 and NF-κB in DCs, which then binds to IL-1 receptor (IL-1R) on DCs and can up-regulate the expression of IL-6 and TNF-α via NF-κB, further driving inflammatory response to infection (Boehm et al., 1997; Cogswell et al., 1994; Ho et al., 2008; Paget et al., 2012). IL-1β can also activate iNKT cells to release immune-modulatory cytokines via NF-κB, including type II IFN, IFN-γ, that further stimulate BECs and DCs to produce inflammatory cytokines that recruit macrophages and neutrophils to the site of infection and inflammation (Boehm, et al., 1997; Ho, et al., 2008; Paget, et al., 2012). Influenza infected DCs also release IL-1β, IL-6, TNF-α, IL-10, and IL-23, which differentiate and expand Th17 population that then release IL-17 and IL-22, also via NF-κB, and further enhance the inflammatory responses in DCs and BECs (Cho et al., 2006; Dong, 2008; Hamada et al., 2009; Laan et al., 2001; Manel et al., 2008; Yao et al., 1995). This plethora of innate immune cytokines and complex innate immune network therefore contribute to the efficient viral clearance, nevertheless most viruses have also developed strategies to either evade or suppress host immune systems.

3 Viral Evasion of Innate Immunity

Co-evolution of viruses and animals has resulted in the development of multiple layers to the innate defensive response with the aim of containing invading viruses at the initial site of infection. On the other hand respiratory viruses have also evolved abilities to evade and inhibit host immune responses to promote survival.

Amongst the respiratory viruses, influenza is one of the best characterized viruses in their immune evasion capabilities and is therefore discussed here. Influenza viruses undergo frequent mutations (~1/10^4 bases per replication cycle) due to the relatively low proof-reading property of the influenza RNA-dependent RNA polymerase (Zambon, 2001). These genetic changes often occur in the gene encoding for the HA and NA, resulting in a new strain that is able to evade host antibodies generated from previous infection or vaccination. This phenomenon, known as antigenic drift, is the reason for annual update to influenza vaccine (Webster et al., 1992). A more significant antigenic change occurs when different virus strains from two or more host species co-infect a single host, allowing genetic reassortment to occur in the infected host and generate a new strain of influenza virus to which the population may have little or no immune memory (Webster, et al., 1992). This process, known as antigenic shift, may result in a viral strain with pandemic potential due to it unpredictable pathogenicity (Zambon, 1999). Antiviral drugs such as influenza M2 ion channel blockers (adamantane and rimantadine) and NA inhibitors (oseltamivir and zanamavir) are also available. M2 ion channel is critical in driving the uncoating of the virus after entry into the host cell, and M2 blockers therefore inhibits early step of the replication cycle. NA inhibitors block the detachment of newly made influenza virions from the host cell surface. These drugs have similar efficacy when given in season prophylaxis and post-exposure against influenza infection (Hayden et al., 1999; Jefferson et al., 2006; Monto et al., 1999). Due to the high use of antiviral drugs and fast mutation rate of influenza these viruses have acquired resistance to current drugs (de Jong et al., 2005; Kiso et al., 2004; Monto et al., 2006; Ward et al., 2005). Our recent report of the rapid emergence of oseltamivir-resistant 2009 H1N1 pandemic virus (A/Newcastle/89/2011) in Australia further highlights
the danger of a heavy reliance on current antiviral drugs and discovery of novel therapeutic options (Hurt et al., 2011; Hurt et al., 2012).

Influenza viruses also encode proteins that suppress the transcription and function of important antiviral proteins including type I and III IFNs. The non-structural (NS) 1 protein of influenza is a protein with molecular mass of approximately 26,000, and is a multi-functional protein expressed very rapidly to help the establishment of viral infection by interfering with host mRNA processing and translation, as well as inhibiting host immune responses, especially the antiviral system. Influenza NS1 protein is able to bind, with its effector domain, to essential host mRNA processing and translation protein, cleavage and polyadenylation specificity factor subunit 30 (CPSF30) and poly A binding protein (PAB) II, shutting down the host protein synthesis to gain control of the host machinery required for viral protein synthesis (Figure 4).

![Figure 4: Influenza NS1 inhibition of host mRNA processing.](image)

The IFN antagonistic property of NS1 occurs at multiple stages of the IFN signalling cascade. NS1 interacts with the viral sensor RIG-I, and can also inhibit the downstream activation of MAVS and nuclear translocation of IRF3, thereby inhibiting IFNs response (Mibayashi et al., 2007; Talon et al., 2000). The NS1 protein specifically inhibits TRIM25-mediated RIG-I ubiquitination, which is crucial for maximal IFNs expression during viral infection (Gack et al., 2009). This NS1-TRIM25 binding event is dependent on the arginine and lysine at position 38 and 41 respectively in the RNA binding domain, and glutamic acid at position 96 and 97 in the effector domain of NS1 (Bornholdt & Prasad, 2008; X. Wang et al., 2002). Beside the inhibitory role in IFNs expression, NS1 protein also inhibits cellular proteins that establish the antiviral state of infected cells. The RNA-binding domain of NS1 can bind to viral RNA to prevent detection by PKR (Y. Lu et al., 1995). It also binds to PKR itself via the effector domain (residue...
123 – 127) and inhibits PKR-mediated viral mRNA suppression and PKR-induced apoptosis (Figure 5) (Bergmann et al., 2000; Dauber et al., 2006; S. Li et al., 2006; Min et al., 2007).

![Diagram of PKR](image)

**Figure 5:** Known binding targets of influenza NS1 protein in relation to amino acids involved.

The frequent mutations of the influenza genome often generate variant strains with different pathogenesis, and therefore the strength of NS1-mediated antiviral inhibition also varies with different strains of influenza. We have shown that primary BECs (pBECs) elicited reduced type I and III IFNs responses to a human influenza virus H3N2, when compared to a low pathogenic avian influenza H11N9 (A. C. Hsu, et al., 2011). This differential response observed with these two strains was due to the more effective NS1 of H3N2 that effectively inhibited the IFN responses, while the NS-1 of H11N9 was relatively weak in this inhibition. This is likely to represent another example of the adaptation of the H3N2 to its human host, in which it had been circulating for approximately 50 years. The high pathogenic avian influenza H5N1 on the other hand completely abolished the type I and III IFN responses, resulting in higher viral replication compared to H3N2 infection and cell death (A. C. Hsu, Parsons, K., Barr, I., Lowther, S., Middleton, D., Hansbro, PM., Wark, PAB., 2012). A number of studies have identified that the NS1 of H5N1 is a very potent inhibitor of RIG-I-mediated signalling and type I and III IFN responses (Bornholdt & Prasad, 2008; Jackson et al., 2008; Jiao et al., 2008). Further studies revealed that while human influenza is sensitive to exogenous treatment of type I and III IFNs regardless of the inhibition by NS1, the H5N1 strain was able to survive regardless of IFNs treatment (A. C. Hsu, et al., 2011; Hyland et al., 2006). Normally IFN-induced antiviral proteins including PKR can degrade NS and other viral genes, but NS1 of H5N1 is able to bind to and effectively inactivate these antiviral proteins (Seo et al., 2002). This antiviral resistance of NS1 was found to require a glutamic acid at position 92. Human influenza engineered with NS1 of H5N1 showed an enhanced replication in the presence of IFNs, whereas the wild type and the mutant virus containing a mutation at residue 92 failed to replicate under the same condition (Seo, et al., 2002).
While similar NS1-mediated antiviral inhibition was also observed in immune cells including macrophages and pDCs, especially with high pathogenic avian H5N1 virus (Fernandez-Sesma et al., 2006; Jia et al., 2010), other respiratory viruses also have developed similar antiviral inhibitory capability. For example, adenovirus contains an early region 4 (E4) that encodes proteins involved in efficient viral replication, and E4 open reading frame 3 (E4 ORF3) specifically inhibits type I IFN responses and enhances viral replication (Ullman et al., 2007). RSV has been shown to utilize its NS1/2 protein to degrade STAT2 and impair the type I IFNs mediated antiviral response in tracheobronchial epithelial cells (Elliott et al., 2007; Ramaswamy et al., 2004; Ramaswamy et al., 2006). Similarly SARS-CoV was recently found to encode a highly basic nucleocapsid (N) protein that inhibits the synthesis of type I IFN response, although the mechanism of this suppression remains unclear (X. Lu et al., 2011). As immune cells such as pDCs have similar innate immune signalling system as BECs, respiratory viruses would exert IFN inhibition in a similar fashion as that in BECs. This demonstrates the importance of early innate immune evasion strategies in viruses that are successful as human respiratory pathogens.

Type I IFNs are an important antiviral cytokines that are induced by viral infection and amplify antiviral responses through type I IFN receptors and Jak/STAT pathway and eradicate replicating viruses. One would assume that these potent IFNs are only induced upon viral infection (inducible IFNs) and subside once infection is resolved. However several studies, including our recent findings, have found the expression of constitutive IFN-β, which played a pivotal role in containing viral replication, even when faced with potent IFN suppressive effect of viral virulence factors such as influenza NS1 (Bacci, Muscettola, et al., 1985; Bacci, Paulesu, et al., 1985; De Maeyer-Guignard et al., 1988; A. C. Hsu, et al., 2011; A. C. Hsu, Parsons, K., Barr, I., Lowther, S., Middleton, D., Hansbro, PM., Wark, PAB. , 2012; Sato et al., 2000; Taniguchi & Takaoka, 2001).

Bacci (1980) first observed the presence of IFN-β in un-infected tissues and this low levels of IFN-β exerted antiviral activities (Bacci, 1980), and later IFN-β mRNA and protein was also found in tissues of healthy mice maintained in specific pathogen-free conditions (Gresser, 1990; Tovey et al., 1987). We also found that BECs secrete low levels of IFN-β when viral infection is absent, and when host protein synthesis was inhibited at translational step with an inhibitor cycloheximide, to determine if IFN-β could still be made and secreted, an enhanced release of IFN-β was observed with and without influenza infection (A. C. Hsu, Parsons, K., Barr, I., Lowther, S., Middleton, D., Hansbro, PM., Wark, PAB. , 2012). This increased IFN-β was then found to be due to the release of pre-formed IFN-β from within the cytosol. While blocking of the IFN-β receptor IFNAR2 reduced the effect of this enhanced IFN-β and led to increased influenza replication.

The mechanism of the production of constitutive IFN-β is yet to be fully elucidated, however Hata et al. (2001) found that deletion of IRF3/7 did not affect constitutive IFN-β production, and this IFN-β release would then stimulate the expression of IRF7 and further induce the ISGs expression following viral infection. The transcription factor NF-κB has also been implicated in the induction of constitutive IFN-β. NF-κB is composed of dimerized subunits of p50, p52, p65, and RelB, and this complex is normally under inhibition by IκB kinase. Once stimulated by various factors including viral infection, IκB kinase is degraded by ubiquitination, allowing the NF-κB complex to translocate into the nucleus and induce the expression of inflammatory and type I IFNs (Chaouat, et al., 2009; Jacobs & Harrison, 1998). The subunit p65 has recently been demonstrated to drive the constitutive production of IFN-β, and when p65 was absent in murine embryo fibroblasts, this constitutive IFN-β was significantly diminished, and subsequently failed to induce efficient antiviral response to vesicular stomatitis virus (VSV) infection.
These findings provide novel insights into the possible mechanisms of constitutive IFN-β production, which is able to counter-act some of the suppressive effects by influenza NS1 protein via IFN-β-IFNAR2 signalling and induce early antiviral responses following infection. While our observation of pre-formed IFN-β protein that is stored within the cells is also novel, the exact mechanism by which IFN-β is released when host protein synthesis was blocked at a translational level is unclear. The enhanced IFN-β production associated with cycloheximide may reflect that influenza-induced inhibition of host protein synthesis is a potential trigger for this constitutive release of IFN-β via a novel pathway.

These observations are in agreement with the notion of a “revving up model” first proposed by Taniguchi and Takaoka (Bocci, Muscettola, et al., 1985; Bocci, Paulesu, et al., 1985; De Maeyer-Guignard, et al., 1988; Sato, et al., 2000). Constitutive IFN-β and its downstream signalling primes BECs to exert a more robust antiviral response to viral infection, whereas in the absence of this signal epithelial cells become hypo-responsive to this IFN stimulus (Sato, et al., 2000; Taniguchi & Takaoka, 2001) (Figure 6). Nevertheless it is currently unknown if type III IFNs are also constitutively expressed with type I IFNs for synergistic antiviral effects.

![Diagram](image)

**Figure 6**: Constitutive release of IFN-β leads to a robust antiviral response via IFNAR1/2 receptor and Jak/STAT1/2.

### 4 Virus Infection in Chronic Airways Diseases

Respiratory viral infections in healthy individuals usually results in symptoms that normally subside within seven days, those with chronic airways diseases including asthma and chronic obstructive pulmonary disease (COPD) are more susceptible to the effect of infection, leading to more severe symptoms following infection.

Asthma and COPD are both characterized by airflow obstruction, which is reversible in asthma but is progressive and not fully reversible in COPD. These diseases are associated with an increase in sensitivity of the lung to a variety of stimuli, for asthma these include allergens such as house dust mite
and air pollutions. COPD is primarily caused by chronic cigarette smoke, however inhaling of industrial toxins, fumes, and chemicals as well as prolonged exposure to air pollution and second hand smoke can also lead to this disease. This high sensitivity to foreign pathogens and particulates then leads to heightened inflammation in the airways of those with chronic airways diseases. Despite the similarities in the clinical features of asthma and COPD, there are also differences in these diseases. Inflammation in asthma is primarily observed in the large and small airways, whereas in COPD inflammation mainly affects small airways and lung parenchyma leading to emphysema, although large airways may also be affected (chronic bronchitis) (Jeffery, 2000). The heightened inflammation is thought to lead to a cycle of continuous airway injury and repair, leading to a vicious cycle that further enhances inflammation. The enhanced inflammatory cytokines including CXCL-8 from the BECs and neutrophils can release these inflammatory mediators and further drive excessive inflammation. This results in symptoms of shortness of breath, persistent cough, sputum production, wheezing, chest tightening, which are often worsened by recurring respiratory viral infection.

Respiratory tract viral infections are associated with the majority (40% - 60%) of the acute exacerbations in asthma (Johnston et al., 1995) and COPD subjects (Seemungal et al., 2001). They lead to more severe lower airway symptoms and are associated with increased hospitalizations (Johnston et al., 1996). A recent study demonstrated that asthmatic children are more susceptible to 2009 H1N1 influenza virus compared to non-asthmatics, and suffered loss of asthma control during infection (Kloepfer et al., 2012). Interestingly, another recent study showed that while individuals with asthma had greater respiratory symptoms due to influenza infection compared to non-asthmatics at the time of hospital admission, asthmatics were not more likely to have severe outcome of infection (Myles et al., 2012). Management of asthma condition with corticosteroid use correlated with this decreased likelihood of severe symptoms. However influenza vaccination status of the recruited subjects in the study was not recorded and may explain this decreased infection outcome in the asthmatics.

The specific pathways that lead to this susceptibility to viral infection and the severe outcomes in these individuals, though are still poorly defined, may be attributed to the abnormal innate immune responses in the lungs of those with chronic airways diseases. Viral infection in asthma and COPD results in heightened airway inflammation with the release of inflammatory cytokines including TNF-α and CXCL-8 from the infected cells and further recruits neutrophils and other immune cells to the site of infection. IL-17 has also been shown to induce the release of CXCL-8 from the infected cells, contributing to the already exaggerated inflammation in the airways and resulting in destructive changes in those with asthma and COPD (Wiehler & Proud, 2007). This therefore indicates that while inflammation may be protective against viral infections, in those with chronic airways diseases this does not appear to efficiently clear the replicating virus and instead cause severe complications that increase mortality.

As antiviral responses are critical in controlling viral infections at the site of infection, the early innate antiviral response to viral infection therefore may also be altered in asthma and COPD. Recent studies have demonstrated that subjects with chronic airways diseases have an impaired antiviral response to infection with rhinovirus, which leads to increased susceptibility to infection and more severe clinical disease. Rhinovirus-infected primary BECs (pBECs) from those with asthma and COPD induced heightened inflammatory cytokines such as CXCL-8 and a lower level of IFN-β and IFN-λ1 response compared to healthy controls (Contoli et al., 2006; Mallia et al., 2011; Uller et al., 2010; Wark et al., 2005). This directly impaired the ability of the infected host cells to undergo early apoptosis, leading to increased virus replication and ultimately cytolysis of the infected cells (Contoli, et al., 2006; Wark, et al., 2005).
This impaired type I and type III IFN responses appear to be a common feature in those with chronic airways diseases, as pBECs from subjects with cystic fibrosis, a genetic disorder that mostly affect the lung, have also been shown to have reduced antiviral IFN responses to rhinovirus infection (Vareille et al., 2012). The early innate IFN response to respiratory viral infection is therefore crucial in limiting the severity of infection. This was further shown in STAT1 knockout mice, which are unable to respond to virus infection with type I IFNs, consequently these mice were 100 times more sensitive to influenza infection and developed disseminated infection, while wild type mice had infection confined to the lungs and more mild clinical disease (Garcia-Sastre et al., 1998).

As those with COPD also share an exaggerated inflammatory response in the airways with asthma, it is plausible that those with COPD may also have reduced antiviral responses to respiratory viral infection, leading to severe complications following infection with respiratory viruses. Indeed, a recent study experimentally infected individuals with COPD with human rhinovirus, and showed impaired antiviral responses compared to healthy subjects (Mallia, et al., 2011). Rhinovirus infection in those with COPD resulted in an intense inflammatory CXCL-8 response in the lungs, with deficient IFN-β protein production compared to the infected healthy individuals. This finding therefore provide important insight into the mechanisms underpinning the high susceptibility to the effect of viral infection in those with chronic airways diseases, and the specific pathways leading to this impaired antiviral response in asthmatics and those with COPD is currently under intense investigation.

The increased susceptibility and severe outcomes of infection in asthma and COPD may be attributed in part to the chronic exposure to chronic inflammation in the airways. Chronic inflammation is known to alter the epigenetics within the cells and tissues, resulting in DNA hyper-methylation and gene silencing, and often precedes the development of many types of cancer (Brower, 2011). In fact, COPD is associated with reduced expression and activity of histone deacetylase 2 (HDAC2) that suppresses inflammatory gene expression (Cosio et al., 2004; K. Ito et al., 2000; K. Ito et al., 2005). HDAC is a family of nuclear enzymes that tightly modulate gene transcription by regulating the “tightness” of chromatins that are wrapped around the histones, and HDAC2 was found to be reduced in the lung tissues of those with COPD, and led to uncontrolled expression of inflammatory cytokines (K. Ito, et al., 2005), which may be further enhanced during infection with respiratory viruses. Similar epigenetic controls such as HDAC1 that regulate antiviral responses may also be abnormal in those with chronic airways diseases (Nusinzon & Horvath, 2003; Roger et al., 2011). In addition, cigarette smoke, the primary cause of COPD, has been shown to impair RIG-I and JAK/STAT pathway, leading to reduced type I IFN response after influenza and RSV infection in human epithelial cells (Modestou et al., 2010; Proud et al., 2012; Wu et al., 2011).

The chronic cigarette smoke exposure in the airways may therefore be progressively driving continuous inflammation, tissue damage/repair, while altering the innate antiviral responses to viral infection. Inflammation, viral infection, and cigarette smoke can also independently lead to the generation of oxidative stress in the airways. The level of reactive oxygen species (ROS), have been found to be higher in the lungs of those with chronic airways diseases (Morcillo et al., 1999; Rahman et al., 1996). Acute and/or chronic inflammation resulted from viral infection and/or cigarette smoke can generate ROS, such as superoxide anion, at a rate that outpaces the effect of endogenous antioxidants, leading to oxidative stress (Fridovich, 1999). Oxidative stress itself can also activate NF-κB and enhance the induction of inflammatory response (Rahman et al., 2001), and also inhibit Jak/STAT1/2 signalling, leading to reduced type I IFN-mediated ISGs response (Di Bona et al., 2006).
Furthermore respiratory viruses such as influenza have also developed strategies to evade and suppress host antiviral responses. The combined level of ROS generated from infection, chronic inflammation and cigarette smoke exposure therefore worsens the symptoms by heightening inflammation and reducing antiviral immunity, and ultimately leading to increased mortality.

**Figure 7:** Oxidative stress from high inflammation, respiratory viral infection, and cigarette smoke enhances the induction of inflammatory cytokines, and inhibits antiviral signalling proteins and response.

## 5 Concluding remarks

Innate immunity is crucial in the defence against respiratory viral infections, especially influenza viruses, and abnormalities in these early immune responses have an important impact on the outcome of infection. Different respiratory viruses have different binding target for efficient endocytosis, however PI3K-mediated macropinocytosis appears to be an evolutionary conserved path into the host cells used by several viruses, signifying a potential therapeutics exploration, especially for viruses with pandemic potential.

The human innate immunity is a complex system that includes the detection of conserved pathogen associated molecules that then result in an immediate inflammatory and antiviral response that ensures the clearance of invading viruses. Conversely viruses have evolved highly effective strategies to compromise this response, allow the establishment of infection and the consequent development of in-
flammation. Substantial research is being performed to elucidate the complex interactions of the human immune system with respiratory viruses, including exploration of additional antiviral signalling pathways and functional studies of un-characterized ISGs. Reverse genetic engineering and profiling of other additional functions and binding targets of viral virulence factors may also reveal previously un-identified host antiviral proteins. The use of more relevant cells such as primary airways cells or more suitable animal models to further understand host immunity and viral infection and pathogenesis, identifying novel and potentially viable therapeutic strategies that can be used to fight against drug-resistant viruses and those with pandemic potentials.

Furthermore subjects with chronic airways diseases such as asthma and COPD have chronically inflamed airways, and are more susceptible to respiratory viral infections, particularly with seasonal and pandemic influenza. Viral infection further exacerbates the pre-existent chronic inflammation and lead to more severe complications following infections with increased mortality. Although studies have uncovered the reduced antiviral immunity in pBECs of those with asthma and COPD, the molecular mechanisms underlying this impaired antiviral response are currently being investigated intensely in hope to identify novel therapeutic targets that may calm the inflammation and enhance the antiviral responses to respiratory viral infection.

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