Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Review

CellPress

Ion flux in the lung: virus-induced inflammasome activation

Kathy Triantafilou and Martha Triantafilou

Institute of Infection and Immunity, School of Medicine, Cardiff University, Heath Park, Cardiff CF14 4XN, UK

Innate immunity has a primary role in lung antimicrobial defenses. The inflammasome has evolved for this purpose and is an important surveillance system that, when triggered, fights infection and eliminates pathogens. However, there is growing evidence that the inflammasome also plays a role in the pathogenesis of acute and chronic respiratory disease. Inflammasomes contribute to both the clearance of the pathogen as well as its pathogenesis – depending on the amount of inflammation triggered. How respiratory viruses trigger inflammasome activation remains unclear. Emerging evidence shows that ion flux is responsible for triggering inflammasome activation in the lung, causing lung pathology and disease exacerbations. Viroporins, encoded by all common respiratory viruses, are responsible for the changes in intracellular ion homeostasis that modulate inflammasome activation. This is a novel mechanism by which respiratory viral infection activates inflammasomes, and identifies sensing of disturbances in intracellular ionic concentrations as a novel pathogen-recognition pathway in the lung.

Viral infections of the respiratory tract

The cells and organs of the human body require a constant stream of oxygen to stay alive. The respiratory system provides oxygen to the cells of the body while removing CO_2, a waste product that can be lethal if allowed to accumulate. Similarly to other organs, such as the skin and gastrointestinal tract, the respiratory tract is constantly exposed to microbes and particles by inhalation [1,2].

Viral infections of the respiratory tract are the most common triggers of bronchiolitis, wheezing and acute asthma exacerbations. These viruses have evolved to colonize and replicate successfully on or within the lung epithelial cells. Previous studies have shown that respiratory syncytial virus (RSV), human rhinovirus (HRV), human influenza A virus (IAV), and human parainfluenza virus (HPIV) are detected in the acute exacerbation of asthma and chronic obstructive pulmonary disease (COPD), leading to rapid decline in lung function and increased mortality [3–5]. However, the mechanisms by which these respiratory viruses induce exacerbations of chronic respiratory disease are incompletely understood but are likely related to inflammatory mediators including interleukins and cytokines produced by the host innate immune response.

The severity of the symptoms produced are not only due to direct virus-induced damage to the lung but also to the triggering of inflammation and release of cytokines from airway epithelial cells, thereby attracting inflammatory cells to the airways [6–9]. Enhanced interleukin-1β (IL-1β) levels and inflammasome activation in the lung correlates with the worsening of respiratory physiology [10–12].

Respiratory viruses have evolved and developed an array of mechanisms to facilitate cell entry and successful viral replication as well as immune evasion strategies to avoid destruction by the host immune system. One such strategy used by HRVs, RSV, and IAV is to modify cell membrane permeability by the use of viroporins which create pores at biological membranes to permit the passage of ions and small molecules and facilitate virus entry [13].

However, the host has developed an immune system with a wide spectrum of pattern recognition receptors (PRRs) to detect infection. One such mechanism evolved by the host are the inflammasomes. These constitute a novel pathogen-recognition pathway that operates by sensing disturbances in intracellular ionic concentrations (either as K^+ efflux or Ca^+ fluxes) [14,15] to trigger the highly inflammatory cytokines IL-1β and IL-18, which are central in mediating lung inflammation and clearing viral infection [16].

In this review we discuss the relevance of viral ion channels in the activation of the inflammasome, and the potential clinical benefit of therapeutic interventions that target inflammasome assembly and activity, as well as drugs that block viral ion channels in respiratory disease.

Immune responses to pathogens

The innate immune system needs to react promptly to potential dangers posed by microbes and particles, while at the same time avoiding extensive tissue damage. This is achieved through an array of PRRs that reside on the cell surface or in specific subcellular compartments, and PRRs can bind pathogen-associated molecular patterns (PAMPs). PRRs also recognize self-molecules (non-microbial ligands) that are released after cell damage or death, known as danger-associated molecular patterns (DAMPs) [17]. PRR activation rapidly targets invading pathogens and infected host cells for elimination through immune cell recruitment and by the induction of transcriptional and post-translational mechanisms that lead to the production of inflammatory cytokines. Although the activation of PRRs leads to host defense pathways in infectious

Corresponding author: Triantafilou, M. (TriantafilouM@cardiff.ac.uk).

Keywords: ion channels; viroporin; inflammasome; respiratory viruses; human rhinovirus; respiratory syncytial virus; severe acute respiratory syndrome coronavirus; influenza A virus.

0966-842X/

 http://dx.doi.org/10.1016/j.tim.2014.06.002

diseases, it can also contribute to tissue injury by excessive release of cytokines [18].

The inflammasome

Proinflammatory cytokines of the IL-1 family are particularly potent inducers of inflammation. By virtue of the potentially destructive proinflammatory effects of uncontrolled IL-1β release, its production is tightly regulated. Pro-IL-1β must be processed by caspase-1 into its bioactive form IL-1β before its release from cells [19]. Although the signaling pathways and inflammatory outcomes of IL-1β activation have long been known, the mechanisms by which immune cells produce this cytokine have come to light only in the past decade with the discovery of inflammasomes, a key component of the innate immune response of the host. Inflammasomes are cytoplasmic innate immune sensors that act as multicomplex molecular platforms and control the activation of the proteolytic enzyme caspase-1 [20,21]. Caspase-1 in turn regulates maturation of the proinflammatory cytokines IL-1β and IL-18 as well as the rapid inflammatory form of cell death, termed 'pyroptosis', that is triggered by diverse ligands and by various stress signals associated with microbial infection or damaged self [22]. Inflammasomes contain a member of the Nod-like receptor (NLR) family and the adaptor ASC (apoptosis-associated speck-like protein containing a CARD), which is common to most inflammasome complexes [23]. ASC operates as a molecular platform for protein–protein interactions during inflammasome activation by oligomerizing into large disc-like structures, although ASC-independent complexes also form in particular cases [24].

The NLR family comprises 22 members in humans, and even more in mice, that are characterized by the presence of a central nucleotide-binding and oligomerization (NACHT) domain, which is commonly flanked by C-terminal leucine-rich repeats (LRRs) and N-terminal caspase recruitment (CARD) domains [25]. The pivotal involvement of inflammasomes in sensing external viral insults, and then responding accordingly by driving exaggerated inflammation, is becoming increasingly appreciated in the context of respiratory disease exacerbated by infection [26].

Inflammasomes and especially NLRP3 (NLR family, pyrin domain-containing 3) seem to respond to a wide range of pathogens and DAMPs [27]. The mechanisms by which these structurally distinct molecules trigger NLRP3 activation have been debated. It has been shown that two or potentially more signals are required for full NLRP3 activation. The first or priming signal can by triggered from a transcriptionally active Toll-like receptor (TLR), NLR, RIG-I-like receptor (RLR), or a cytokine receptor; this leads to transcriptional activation of the genes encoding pro-IL1β and pro-IL18 [28]. The second signal is triggered in response to various stress signals associated with damaged self [16].

Various models have been proposed and supported in literature for inflammasome activation. Inflammasomes can sense falls in cytosolic potassium levels [29–31], and membrane perturbations causing potassium efflux by microbial toxins such as aerolysin and nigericin have been shown to activate the NLRP3 inflammasome [32]. Extracellular ATP serves as a danger signal that alerts the immune system by binding to the purinoceptor P2X7 and inducing NLRP3 recruitment [33]. The release of lysosomal contents caused by lysosome disruption [34,35] or the production of reactive oxygen species (ROS) [36] has also been shown to trigger NLRP3 activation. However, intracellular ion fluxes seem to be the most important danger signal and amplifier of inflammasome recruitment. Intracellular Ca^{2+} signaling has been shown to be important in NLRP3 activation in response to a wide range of stimuli [15,37] including UV irradiation [38], sublytic membrane attack complex formation [39] (which generates pores that allow the influx of Ca^{2+} ions and increases the cytosolic Ca^{2+} concentration), as well as infections by viruses such as HRVs – which infect the cells and increase intracellular Ca^{2+} by generating membrane-integral pores, thus reducing the Ca^{2+} content of the endoplasmic reticulum (ER) and Golgi complex [40].

In addition, new studies implicate K^+ efflux as the common denominator for inflammasome activation by various stimuli including ROS, extracellular Ca^{2+}, mitochondrial or lysosomal damage, proposing that the fall in cytosolic content of K^+ acts as a trigger for NLRP3 engagement [14]. These studies show that inflammasomes are tightly regulated by intracellular ion concentrations and that ion imbalances provide the main trigger for their activation.

Viroporins

To escape killing by the host immune system, viruses employ strategies to enhance attachment and entry into the host cells and organs and promote efficient replication. Thus a common hallmark used by viruses during infection is alterations in cellular ion homeostasis [41]. The vast majority of RNA animal viruses encode cytotoxic pore-forming proteins known as viroporins, which modify membrane function at late stages of infection and facilitate budding of virions from infected cells. The existence of viroporins was initially suggested by observing that virus-infected cells became permeable to ions and small molecules. Viroporins are small, non-glycosylated, highly hydrophobic viral polypeptides which interact with cell membranes and increase their permeability to ions and other low molecular weight compounds [42,43]. A current hypothesis suggests that viroporins insert into the host cell membrane and subsequently oligomerize to form an aqueous channel. Usually, the ion channels formed by viroporins conduct at least one of the physiological relevant ions such as Na^+, K^+, Ca^{2+}, Cl^-, and H^+ [44].

Modification of host cell membrane permeability via viroporins seems to be a common feature of infections with animal viruses. Viroporins are encoded by a range of RNA and DNA animal viruses of clinical interest, including hepatitis C virus (HCV), HIV-1, JC polyoma virus, human papilloma virus (HPV), IAV, coronaviruses, picornaviruses (such as polioviruses), and togaviruses.

Although viroporins have been shown to participate in cell entry and genome replication, the main activity of viroporins is their involvement in virion assembly and release from infected cells. Viruses defective in viroporins are unable to accomplish proper assembly and release from

cells [45,46]. Another important aspect of viroporin function is that several viroporins can trigger programmed host cell death. Viral ion channels can participate in apoptotic cell death because perturbation of ion homeostasis is a common hallmark of apoptosis, giving rise to depolarization of the plasma membrane associated with intracellular cation overload and cell volume decreases as a result of anion and H_2O efflux [47]. Coronavirus viroporin E (envelope) protein has been described as an inducer of apoptosis [48]. In addition, the picornavirus 2B protein results in increase in the intracellular Ca^{2+} concentration which can induce apoptosis [49], thus playing a key role in host cell death.

Overall, it seems that viroporins have a central role in promoting viral pathogenesis. They inflict several cytopathic effects during the viral life cycle. Most importantly, they alter the plasma membrane potential. They dissipate the ionic gradient across the membrane by conducting the flux of different ions (e.g., Na^+ and K^+) across the membrane, leading to depolarization and helping the budding process of the virus. They alter Ca^{2+} homeostasis, induce intracellular protein remodeling, and can also dissipate the proton gradient in the Golgi and *trans*-Golgi network. Although these cytopathic effects are advantageous for the viral life cycle, promoting entry, assembly, and release of virus particles, the host innate immune system seems to have also developed 'sensors' to detect such ion dysregulation and trigger activation signals to fight off the infection. Inflammasomes seem to have a role in recognizing the ion dysregulation that is caused intracellularly by viroporins. They are able to sense the ion flux and trigger inflammasome activation and subsequently IL-1β and IL-18 production as a mechanism of defense against the virus. Interestingly, it is becoming apparent that most respiratory viruses trigger inflammasome activation and overzealous IL-1β and IL-18 production via viroporins. It is emerging that viroporins are central in the cytopathic effect of most of the common respiratory viruses, causing airway inflammation and exacerbations of airway disease. In the following sections we focus on the most common respiratory viruses and how they trigger inflammasome activation via ion flux triggered by viroporins.

Common respiratory pathogens that induce ion flux by viroporins

Several viruses that infect the respiratory tract (Table 1) cause inflammasome activation through ion flux disturbances.

IAV

IAV is a negative-sense single-stranded RNA virus that is a major public health problem. IAV is responsible for annual epidemics that cause severe illness in 5 million people worldwide, and novel IAV strains emerge sporadically as pandemic viruses. Viral influenza is estimated to cause 36 000 deaths and more than 200 000 hospitalizations annually in the USA alone [50]. In the UK it is estimated that influenza is associated with 12 500 excess deaths per year [51]. The most frequent serious complications of influenza are pneumonia, and exacerbations of chronic pulmonary diseases [52], leading to acute respiratory distress syndrome (ARDS) [53]. The unpredictable nature of emergent pandemic strains creates an urgent need to develop vaccines and therapeutics that can rapidly be prepared and deployed to prevent or treat the next influenza pandemic. The outcome of IAV infection is determined by the host immune response against the virus. Thus, understanding the mechanism by which IAV infection is detected and cleared by the immune system will provide a useful basis for the development of effective vaccines.

Table 1. Common respiratory viruses[a]

Family	Virus	Genome
Picornaviridae	Human rhinoviruses A, B, or C	ssRNA
Orthomyxoviridae	Influenza viruses A and B	ssRNA
Paramyxoviridae	Human respiratory syncytial viruses (RSV) A and B Human metapneumoviruses 1–4 Human parainfluenza viruses 1–4	ssRNA ssRNA ssRNA
Coronaviridae	Human coronavirus (SARS-CoV)	ssRNA
Herpesviridae	Cytomegalovirus or human herpes virus type 5 Varicella–zoster virus or human herpes virus type 3	dsDNA dsDNA
Adenoviridae	Human adenovirus	dsRNA

[a]Abbreviations: ds, double-stranded; ssRNA, single-stranded.

IAV triggers inflammasome activation and produces IL-1β and IL-18 upon infection, a response that can be blocked by a caspase-1 specific inhibitor [54]. The involvement of inflammasome in influenza-induced asthma exacerbation has also been investigated where infected bronchial epithelial cells from volunteers with and without asthma with IAV were examined for inflammasome activation. The results showed enhanced caspase-1 activation and IL-1β secretion, suggesting that inflammasome activation in bronchial epithelial cells may contribute to pathogenesis of virus-induced asthma exacerbation [55].

Several studies have shown that NLRP3 plays a role in IAV recognition and inflammasome assembly [56–58]. Inflammasomes are essential in host defense against IAV. NLRP3 has been shown to be a crucial component in the host response to IAV through sensing of viral RNA, and its deficiency in mice resulted in very high rates of lethality after AV infection [57]. However, in some patients with pre-existing airway inflammation, the influx of cytokines caused by the viral infection could lead to additive and synergic effects and exacerbations of airway disease. The activation of the NLRP3 inflammasome and production of IL-1β usually requires two signals [59]. In the case of IAV, viral RNA is a trigger [57] and TLR7 signaling is required for the transcription of pro-IL-1β, whereas the IAV M2 channel was shown to trigger signal 2 for the activation of NLRP3 inflammasome [60]. The M2 channel is a viroporin involved in viral genome replication and assembly, as well as virus particle entry into and release from infected cells. M2 also allows the entry of protons into virions, promoting virus uncoating in endosomes [61]. In addition to ion channel activity, it also regulates the pH balance between the acid lumen of the *trans*-Golgi complex (TGN) and the cytoplasm in influenza-infected cells. M2 reduces the acidification of intracellular vesicles and even cellular organelles [62]. NLRP3 monitors changes in the concentration or the subcellular localization of endogenous

danger signals. Therefore, the proton specificity of the M2 channel creates imbalances in the concentration of H^+, in addition to disturbances in the concentrations of Na^+ and K^+ cations, and acts as a trigger for inflammasome activation.

Rhinovirus

HRV is the most common respiratory virus associated with asthma exacerbations. HRV is responsible for up to 80% of acute asthma attacks and is also implicated in the majority of COPD exacerbations [63–65]. Furthermore, a pivotal study by Johnston *et al.* in children aged 9–11 years old with history of asthma found that 80–85% of asthma exacerbations were due to HRV [64].

The severity of the symptoms produced is not due to the direct virus-induced damage to the epithelium. Instead, rhinovirus infection induces inflammation and release of cytokines from airway epithelial cells, thereby attracting inflammatory cells to the airways. In patients with pre-existing airway inflammation, the influx of cytokines and inflammatory cells caused by HRV infection would lead to additive or synergistic effects and exacerbations of airway disease. Repeated asthma exacerbations caused by HRV infection can lead to airway remodeling which cannot be reversed by current pharmacological treatment, and consequently leads to decline in lung function. Thus, it is crucial to understand rhinovirus-induced airway inflammation in asthma and infectious exacerbations.

There is evidence indicating that rhinoviral infection acts as a trigger to enhance IL-1β levels within the airways of patients with COPD or asthma [10–12]. A study by Stokes *et al.* showed that epithelial cell responses to rhinovirus are modulated by IL-1β signaling via MyD88 [12]. Furthermore, there is evidence supporting rhinoviral infection as a trigger of acute inflammatory exacerbations in patients with underlying airway disease; IL-1β levels are enhanced [66] within the airways of patients with COPD or asthma [10–12] and this can aggravate symptoms. A new study by Dolinay *et al.* using a mouse model shows that inflammasome-regulated cytokines IL18 and IL-1β are crucial mediators of acute lung injury [67]. Their secretion was elevated in the plasma of patients with ARDS and served as a novel biomarker of intensive care unit morbidity and mortality [67]. These studies implicate the inflammasome pathway and its downstream cytokines in playing crucial roles in respiratory inflammation.

However, the mechanism of rhinovirus detection and activation of the inflammasome was unclear until it was shown that rhinovirus triggered inflammasome activation by affecting the balance of Ca^{2+}intracellular homeostasis [40]. The rhinovirus 2B protein, one of the nonstructural proteins involved in viral RNA replication, forms membrane-integral pores and increases cytosolic Ca^{2+} by reducing ER and Golgi Ca^{2+} levels [42,68,69]. NLRP3, in synergy with NLRC5, senses the imbalances in Ca^{2+} intracellular homeostasis and triggers IL-1β secretion.

Infection of primary bronchial cells with HRV leads to NLRP3 and NLRC5 activation and IL1β secretion. The overlapping biologic functions and pathogen specificity of NLRC5 with NLRP3 suggests that these proteins might act in a cooperative manner during inflammasome assembly. NLRP3 expression is inducible following infections by several bacteria or viruses [27,70]. However, there could be a tissue-specific role for the NLRC5 inflammasome in host sensing and immune defense – detection of rhinovirus respiratory infections by both NLRP3 and NLRC5 could explain why such respiratory infections lead to greater airway inflammation and more severe exacerbations.

When the Ca^{2+} channel inhibitor verapamil and the Ca^{2+} chelator BAPTA-AM [acetoxymethyl ester derivative of BAPTA, bis(aminophenoxy)ethane-tetraacetic acid] were used to inhibit inflammasome activation, IL-1β secretion induced by HRV infection was inhibited, thus indicating that calcium inhibitors could be used in the future to control exacerbated IL-1β production [40].

RSV

RSV is the primary cause of hospitalization in the first year of life for children in most parts of the world and is associated with significant morbidity and mortality. Forty percent of those children discharged from hospital have recurrent, repeated respiratory symptoms and wheezing for at least 10 years [71]. The infection is also important in the elderly and immune-compromised individuals. The spectrum of clinical manifestations ranges from mild upper respiratory tract illness, croup, to apnoea in premature infants, pneumonia, and bronchiolitis [72,73]. RSV RNA can also be detected from lower airway samples of some patients with COPD. RSV RNA detection has been associated with greater airway inflammation and accelerated disease progression in these patients. These findings suggest that RSV may play a role in the natural history of stable COPD [74].

RSV can also certainly lead to asthma exacerbations in older children and adults. Rhinoviruses are commonly found during acute exacerbations of COPD, but there are intriguing preliminary data suggesting that RSV may also be present in some asthma patients during remission [75,76].

One cytokine that is associated with RSV infection is IL-1β, and this cytokine has been shown to be secreted by RSV-infected airway cells [77]. Furthermore, *in vitro* studies have shown that RSV triggers NLRP3/ASC inflammasome activation. The first signal for NLRP3 inflammasome formation is activation of the TLR2/MyD88 (myeloid differentiation primary response 88)/nuclear factor κB (NF-κB) pathway for pro-IL-1β synthesis, followed by ROS and potassium efflux as the second signal. Both of these signals lead to caspase-1 activation and subsequent IL-1β secretion during infection [78]. The RSV small hydrophobic (SH) viroporin, which induces membrane permeability to ions or small molecules by the formation of a pore or channel in the plasma membrane [79], is essential in triggering the NLRP3 inflammasome in RSV infections of primary lung epithelial cells. Furthermore, RSV mutants lacking the viroporin SH were unable to trigger inflammasome activation and IL-1β secretion. Pharmacological treatment of RSV-infected cells with drugs that inhibit viral ion channels, or with lipid raft disruptors, blocked inflammasome activation – showing that lipid raft structure in intracellular compartments plays an important role in inflammasome activation. Upon RSV infection, the RSV SH

viroporin accumulates in the Golgi within lipid raft structures, forming ion channels selective for monovalent cations (Na^+ and K^+), which trigger the translocation of NLRP3 from the cytoplasm to the Golgi and its activation [80].

Coronaviruses

Severe acute respiratory syndrome (SARS) is caused by a novel coronavirus (SARS-CoV). SARS-CoV causes severe pneumonic disease in humans with an overall mortality rate of 10% [81]. Elderly patients have a poor prognosis, with higher mortality rates of up to 50% [82]. The clinical course of SARS-CoV-induced disease is characterized by fever, cough, dyspnea, hypoxemia, and radiographic evidence of pneumonia. Severely affected people experience respiratory failure and may need mechanical ventilation.

The first onset of symptoms is likely caused by the increase in viral replication and cytolysis. However, as the disease progresses there is a decrease in viral replication that correlates with the onset of immunoglobulin G (IgG) conversion. Interestingly, it is also in this phase that severe clinical worsening is seen, and this cannot be explained by uncontrolled viral replication. It has been hypothesized that the diffuse alveolar lung damage in this phase is caused by an over-exuberant host response and up to one-third of the patients develop severe inflammation of the lung, characterized by ARDS [83,84].

Studies show that there is excessive release of proinflammatory cytokines, referred to as 'cytokine storm', in SARS. Patients show highly elevated levels of interferon-γ (IFN-γ), IL-6, monocyte chemotactic protein 1 (MCP1), and IFN-γ-inducible protein 10 (IP-10), as well as significantly higher IL-18 and IL-1β levels not only in the blood but also in both lung and lymphoid tissues, indicating increased inflammasome activation in patients who died versus those who survived [84,85]. Moreover, pediatric SARS patients show markedly elevated circulating IL-1β levels, which suggests selective activation of the caspase-1-dependent pathway. Other key proinflammatory cytokines, IL-6 and tumor necrosis factor α (TNF-α), showed only mildly elevated levels at the initial phase of the illness [86]. These findings link the excessive inflammation seen in patients with inflammasome activation. However, the mechanisms of inflammasome activation in SARS have not yet been revealed. The virus encodes an E protein, a small transmembrane protein of 76–109 amino acids in length. This protein is characterized as a viroporin because it self-associates to generate an oligomeric structure that forms an ion-conductive pore in planar lipid bilayers. Where the bilayer membrane was permeabilized, the channel showed symmetric ion transport properties either for positive or negative applied voltages, and its conductance was not regulated by voltage. Interestingly, when E protein was reconstituted in negatively charged lipid bilayers, the ion channel became slightly more selective to cations than to anions [87]. SARS-CoV also encodes 3a, a 31 kDa protein which forms an ion channel and modulates virus release; however, this protein is not essential for virus viability [45]. It is possible that, as previously seen with other respiratory viruses, inflammasomes sense the cation imbalances caused by the viroporin E and 3a in SARs-infected cells and become activated.

Concluding remarks

NLRs represent a group of key sensors for microbes and damage in the lung. There has been a growing body of evidence showing that inflammasomes act as airway epithelium danger sensors and play key roles in the inflammation observed in respiratory diseases. By similarity to possibly most immune receptors, they can exert a protective or pathologic role by overzealous production of IL-1β and IL-18 depending on the magnitude and the context of their activation.

Emerging evidence is pointing towards the fact that ion flux is responsible for triggering inflammasome activation, and the subsequent IL-1β production in the lung causes lung pathology and disease exacerbations. Viroporins, which are encoded by all the common respiratory viruses, are responsible for the changes in the intracellular ion homeostasis that modulate inflammasome activation and cell death in the lung. Generalized permeabilization both of intracellular compartments (such as the Golgi and *trans*-Golgi network) and of the plasma membrane by viroporins provokes this ionic imbalance, leading to disruption of the permeability barrier. Respiratory viruses encode several viroporins which are able to conduct the flux of different ions (such as Na^+ and K^+) across the membrane (Figure 1), reducing the transmembrane potential, and this essentially affects: (i) the concentration of ions inside and outside the cells, (ii) the permeability of the membrane to these ions, and (iii) the activity of electrogenic pumps (for example the Na^+/K^+/ATPase and Ca^{2+} transport pumps). Some of the viroporins are also able to target intracellular compartments: one example is HRV 2B which is endowed with a potent capacity to cause profound and rapid alterations in Ca^{2+} release from intracellular stores (such as the ER and Golgi) and subsequent Ca^{2+} influx to the cytoplasm. Changes in the ion levels intracellularly (either an increase or decrease of ion concentration) in turn trigger inflammasome activation and IL-1β production. Inflammasome activation is one of the beneficial and non-specific defense mechanisms of the body against tissue damage and viral stimuli. However, repeated or persistent infections by these respiratory viruses trigger this vicious cycle which leads to damage in the lung. A question that arises is – why does the host retain such a destructive mechanism of activation and cytokine production? The answer is that the host does not have a choice. It senses the 'invasion' and tries to clear the infection. We have reached this situation because the coexistence of viruses and their hosts imposes an evolutionary pressure on both the virus and the host immune system. Viruses need to escape killing by the host immune system, and therefore employ viroporins to enhance attachment and entry into host cells and organs and promote efficient replication, but by contrast the host must retain a highly sensitive surveillance system to combat the infection.

Many further questions remain (Box 1). If respiratory viruses trigger inflammasomes through viroporins, is this a common phenomenon in viruses? There are viruses which encode viroporins – such as HIV-1 Vpu ('viral protein

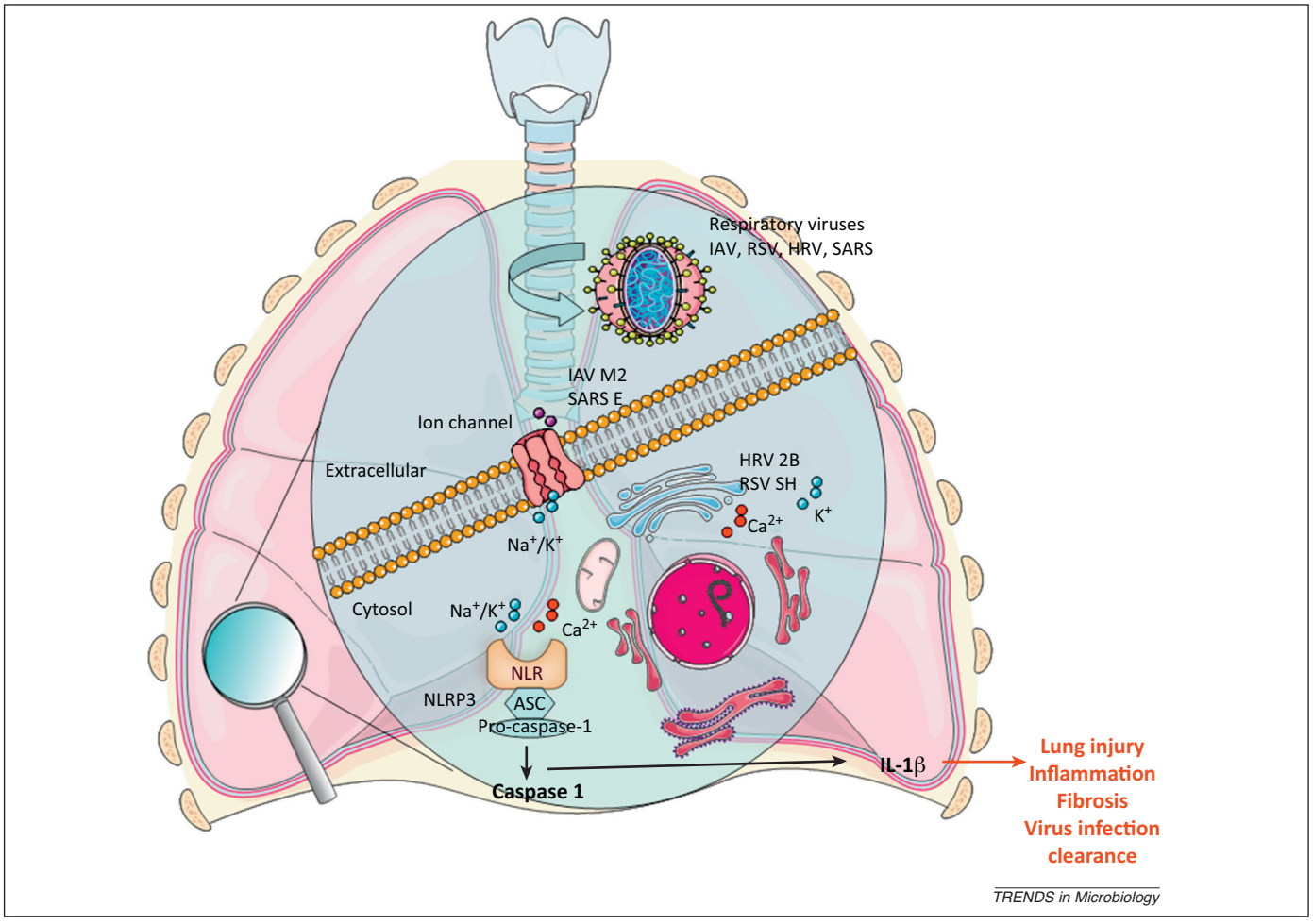

Figure 1. The NLRP3 inflammasome in lung inflammation and injury from respiratory viruses. Recent evidence suggests that viroporins such as IAV M2, RSV SH, HRV 2B, and SARS E protein, cause dysregulation of ions (such as Na^+, K^+, or Ca^{2+}), leading to NLRP3 inflammasome activation. NLRP3 activation leads to IL-1β secretion and lung injury, inflammation and virus clearance. Some viroporins permeabilize the plasma membrane (such as the IAV M2 and SARS E proteins), whereas others permeabilize intracellular compartments such as the Golgi (RSV SH and HRV 2B). Generalized permeabilization of both intracellular compartments (such as the Golgi and *trans*-Golgi network) as well as the plasma membrane by viroporins provokes this ionic imbalance. This ion influx triggers the NLRP3 inflammasome activation and causes disease pathology in the lung. Abbreviations: HRV, human rhinovirus; IAV, human influenza A virus; NLRP, NLR (Nod-like receptor) family, pyrin domain-containing 3; RSV, respiratory syncytial virus; SARS, Severe acute respiratory syndrome.

unique') which functions as an ion-conducting channel [88] and the HCV p7 protein which is selective for monovalent cations (Na^+ and K^+) [44]; however, these proteins do not stimulate NLRP3 inflammasome. Can only respiratory viruses do this?

Viroporins insert into the host cell membrane and regulate the components and structures of organellar and plasma membrane lipid bilayers by forming oligomers which, by tilting or bending, can adapt to changes in the lipid bilayer thickness or shape [89]. Viroporin structure and membrane topology are not the same for all viruses; the structure governs the pore opening and closing mechanisms, and possibly also the kinetic rate of ion permeability and the changes in ion concentrations – factors that could affect inflammasome activation – thus explaining why not all viroporins can trigger inflammasome formation.

Targeting the inflammasome in combination with viroporin inhibitors in virus-induced respiratory disease is an attractive potential therapeutic target (Figure 2). Amantadine is one of the best-studied and famous inhibitors for the influenza A virus M2 channel and has been used clinically as an anti-influenza drug for several years [90]. A drawback of amantadine is the high doses necessary to affect influenza. However, targeting the NLR inflammasome pathway in combination with ion channel inhibitors could be the way forward and could lead to beneficial treatments of viral respiratory disease.

Box 1. Outstanding questions

- Because inflammasome activation can lead to pathogenic effects, what are the regulatory mechanisms that the host must have in place for this sensor?
- In view of the fact that respiratory viruses trigger inflammasome activation through viroporins, is this a common phenomenon among viruses?
- Do viroporin structure and membrane insertion topology play a role in pore structure and the ability of the viroporin to activate the inflammasome?
- Could targeting the inflammasome in combination with viroporin inhibitors in virus-induced respiratory disease be an attractive potential therapeutic target in the future?

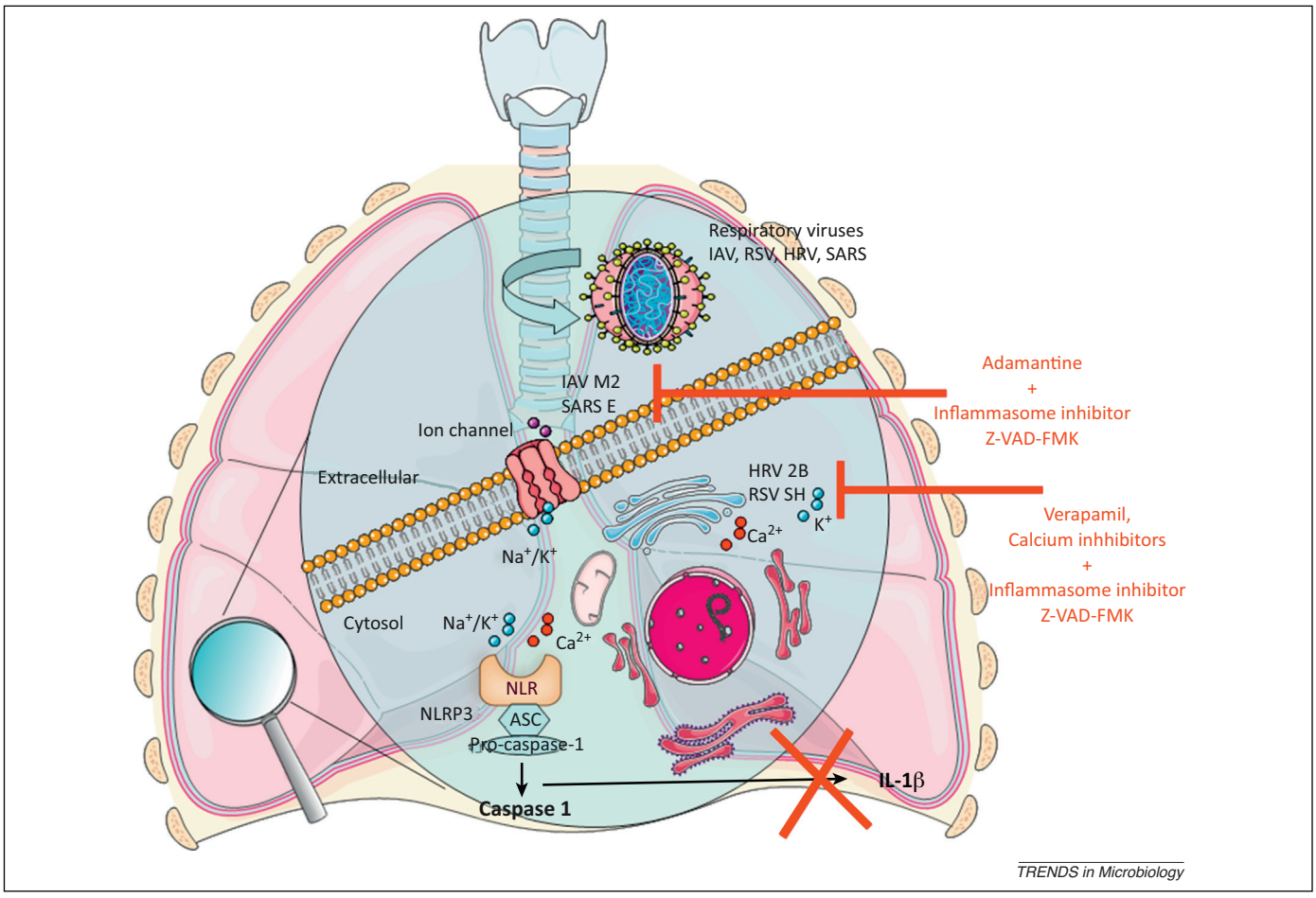

Figure 2. Therapeutic interventions in the lung. Recent evidence suggests that viroporins, such as IAV M2, RSV SH, HRV 2B, and SARS E proteins, cause dysregulation of ions (such as Na^+, K^+, or Ca^{2+}) that leads to NLRP3 inflammasome activation. NLRP3 activation leads to IL-1β secretion and lung injury, inflammation, and virus clearance. Targeting the inflammasome in combination with viroporin inhibitors in virus-induced respiratory disease is an attractive potential therapeutic target. We could use Amantadine, one of the best-studied and famous inhibitors for the influenza A virus M2 channel, in combination with an inflammasome inhibitor such as Z-VAD-FMK to target Na^+ ion channels, whereas we could use calcium inhibitors (such as verapamil) in combination with inflammasome inhibitors to block Ca^{2+} triggering of the inflammasome. Abbreviations: ASC, apoptosis-associated speck-like protein containing a CARD; Z-VAD-FMK, *N*-benzyloxycarbonyl-Val-Ala-Asp-fluoromethylketone; Figure 1 legend for other abbreviations.

References

1 Martin, T.R. and Frevert, C.W. (2005) Innate immunity in the lungs. *Proc. Am. Thorac. Soc.* 2, 403–411
2 Boyton, R. and Openshaw, P.J. (2002) Pulmonary defences to acute respiratory infection. *Br. Med. Bull.* 61, 1–12
3 Donaldson, G. *et al.* (2005) Airway and systemic inflammation and decline in lung function in patients with COPD. *Chest* 128, 1995–2004
4 Openshaw, P.J. and Tregoning, J.S. (2005) Immune responses and disease enhancement during respiratory syncytial virus infection. *Clin. Microbiol. Rev.* 18, 541–555
5 Rothberg, M.B. *et al.* (2008) Complications of viral influenza. *Am. J. Med.* 121, 258–264
6 Bochkov, Y.A. *et al.* (2010) Rhinovirus-induced modulation of gene expression in bronchialepithelial cells from subjects with asthma. *Mucosal Immunol.* 3, 69–80
7 Koto, H. *et al.* (1996) Mechanisms of impaired β-adrenoceptor-induced airway relaxation by interleukin-1β in vivo in the rat. *J. Clin. Invest.* 98, 1787
8 Pype, J. *et al.* (2001) Mechanisms of interleukin 1beta-induced human airway smooth muscle hyporesponsiveness to histamine. Involvement of p38 MAPK NF-kappaB. *Am. J. Respir. Crit. Care Med.* 163, 1010–1017
9 Shore, S.A. *et al.* (2014) Effect of IL-1 beta on responses of cultured human airway smooth muscle cells to bronchodilator agonists. *Am. J. Respir. Cell Mol. Biol.* 16, 702–712
10 de Kluijver, J. *et al.* (2003) Interleukin-1β and interleukin-1ra levels in nasal lavages during experimental rhinovirus infection in asthmatic and non-asthmatic subjects. *Clin. Exp. Allergy* 33, 1415–1418
11 Terajima, M. *et al.* (1997) Rhinovirus infection of primary cultures of human tracheal epithelium: role of ICAM-1 and IL-1beta. *Am. J. Physiol.* 273, 749–759
12 Stokes, C.A. *et al.* (2011) Role of interleukin-1 and MyD88-dependent signaling in rhinovirus infection. *J. Virol.* 85, 7912–7921
13 Gonzaleza, M. and Carrasco, L. (2014) Viroporins. *FEBS Lett.* 552, 28–34
14 Muñoz-Planillo, R. *et al.* (2013) K^+ efflux is the common trigger of NLRP3 inflammasome activation by bacterial toxins and particulate matter. *Immunity* 38, 1142–1153
15 Murakami, T. *et al.* (2012) Critical role for calcium mobilization in activation of the NLRP3 inflammasome. *Proc. Natl. Acad. Sci. U.S.A.* 109, 11282–11287
16 Martinon, F. *et al.* (2009) The inflammasomes: guardians of the body. *Annu. Rev. Immunol.* 27, 229–265
17 Matzinger, P. (2002) The danger model: a renewed sense of self. *Science* 296, 301–305
18 Akira, S. (2001) Toll-like receptors and innate immunity. *Adv. Immunol.* 78, 1–56
19 Dinarello, C.A. (2009) Immunological and inflammatory functions of the interleukin-1 family. *Annu. Rev. Immunol.* 27, 519–550
20 Martinon, F. and Tschopp, J. (2007) Inflammatory caspases and inflammasomes: master switches of inflammation. *Cell Death Differ.* 14, 10–22

21 Keller, M. *et al.* (2008) Active caspase-1 is a regulator of unconventional protein secretion. *Cell* 132, 818–831
22 Davis, B.K. *et al.* (2011) The inflammasome NLRs in immunity, inflammation, and associated diseases. *Annu. Rev. Immunol.* 29, 707–735
23 de Alba, E. (2009) Structure and interdomain dynamics of apoptosis-associated speck-like protein containing a CARD (ASC). *J. Biol. Chem.* 284, 32932–32941
24 Van Opdenbosch, N. *et al.* (2014) Activation of the NLRP1b inflammasome independently of ASC-mediated caspase-1 autoproteolysis and speck formation. *Nat. Commun.* 5, 3209–3214
25 Martinon, F. *et al.* (2002) The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. *Mol. Cell* 10, 417–426
26 Chaput, C. *et al.* (2013) NOD like receptors in lung disease. *Front. Immunol.* 4, 393–397
27 Kanneganti, T.D. (2010) Central roles of NLRs and inflammasomes in viral infection. *Nat. Rev. Immunol.* 10, 688–698
28 Bauernfeind, F.G. *et al.* (2009) NF-kappaB activating pattern recognition and cytokine receptors license NLRP3 inflammasome activation by regulating NLRP3 expression. *J. Immunol.* 183, 787–791
29 Petrilli, V. *et al.* (2007) Activation of the NALP3 inflammasome is triggered by low intracellular potassium concentration. *Cell Death Differ.* 14, 1583–1589
30 Kahlenberg, J.M. *et al.* (2004) Mechanisms of caspase-1 activation by P2X7 receptor-mediated K^+ release. *Am. J. Physiol. Cell Physiol.* 286, C1100–C1108
31 Franchi, L. *et al.* (2007) Differential requirement of P2X7 receptor and intracellular K^+ for caspase-1 activation induced by intracellular and extracellular bacteria. *J. Biol. Chem.* 282, 18810–18818
32 Mariathasan, S. *et al.* (2006) Cryopyrin activates the inflammasome in response to toxins and ATP. *Nature* 440, 228–232
33 Kanneganti, T.D. *et al.* (2007) Pannexin-1-mediated recognition of bacterial molecules activates the cryopyrin inflammasome independent of Toll-like receptor signaling. *Immunity* 26, 433–443
34 Halle, A. *et al.* (2008) The NALP3 inflammasome is involved in the innate immune response to amyloid-beta. *Nat. Immunol.* 9, 857–865
35 Hornung, V. *et al.* (2008) Silica crystals and aluminum salts activate the NALP3 inflammasome through phagosomal destabilization. *Nat. Immunol.* 9, 847–856
36 Dostert, C. *et al.* (2008) Innate immune activation through Nalp3 inflammasome sensing of asbestos and silica. *Science* 320, 674–677
37 Lee, G.S. *et al.* (2012) The calcium-sensing receptor regulates the NLRP3 inflammasome through Ca^{2+} and cAMP. *Nature* 492, 123–127
38 Feldmeyer, L. *et al.* (2007) The inflammasome mediates UVB-induced activation and secretion of interleukin-1beta by keratinocytes. *Curr. Biol.* 17, 1140–1145
39 Triantafilou, K. *et al.* (2013) The complement membrane attack complex triggers intracellular Ca^{2+} fluxes leading to NLRP3 inflammasome activation. *J. Cell Sci.* 126, 2903–2913
40 Triantafilou, K. *et al.* (2013) Rhinovirus-induced calcium flux triggers NLRP3 and NLRC5 activation in bronchial cells. *Am. J. Respir. Cell Mol. Biol.* 49, 923–934
41 Nieva, J. *et al.* (2012) Viroporins: structure and biological functions. *Nat. Rev. Microbiol.* 10, 563–574
42 Agirre, A. *et al.* (2002) Viroporin-mediated membrane permeabilization pore formation by nonstructural poliovirus 2B protein. *J. Biol. Chem.* 277, 40434–40441
43 Fischer, W. and Sansom, M. (2002) Viral ion channels: structure and function. *Biochim. Biophys. Acta* 1561, 27–45
44 Wang, K. *et al.* (2011) Viral proteins function as ion channels. *Biochim. Biophys. Acta* 1808, 510–515
45 Lu, W. *et al.* (2006) Severe acute respiratory syndrome-associated coronavirus 3a protein forms an ion channel and modulates virus release. *Proc. Natl. Acad. Sci. U.S.A.* 103, 12540–12545
46 Iwatsuki-Horimoto, K. *et al.* (2006) The cytoplasmic tail of the influenza A virus M2 protein plays a role in viral assembly. *J. Virol.* 80, 5233–5240
47 Franco, R. *et al.* (2006) Potential roles of electrogenic ion transport and plasma membrane depolarization in apoptosis. *J. Membr. Biol.* 209, 43–58
48 An, S. *et al.* (1999) Induction of apoptosis in murine coronavirus-infected cultured cells and demonstration of E protein as an apoptosis inducer. *J. Virol.* 73, 7853–7859
49 Madan, V. *et al.* (2008) Viroporins from RNA viruses induce caspase-dependent apoptosis. *Cell. Microbiol.* 10, 437–451
50 Heron, M. *et al.* (2009) *Deaths: Final Data for 2006* (*National Vital Statistics Reports*, Vol. 57, No. 14) Centers for Disease Control and Prevention
51 Fleming, D.M. (2000) The contribution of infl uenza to combined acute respiratory infections, hospital admissions, and deaths in winter. *Commun. Dis. Public Health* 3, 32–38
52 Thompson, W.W. *et al.* (2004) Influenza-associated hospitalizations in the United States. *JAMA* 292, 1333–1340
53 Short, K. *et al.* (2014) Pathogenesis of influenza-induced acute respiratory distress syndrome. *Lancet* 14, 57–69
54 Pirhonen, J. *et al.* (1999) Virus infection activates IL-1beta and IL-18 production in human macrophages by a caspase-1-dependent pathway. *J. Immunol.* 162, 7322–7329
55 Bauer, R.N. *et al.* (2012) Influenza enhances caspase-1 in bronchial epithelial cells from asthmatic volunteers and is associated with pathogenesis. *J. Allergy Clin. Immunol.* 130, 958–967
56 Ichinohe, T. *et al.* (2009) Inflammasome recognition of influenza virus is essential for adaptive immune responses. *J. Exp. Med.* 206, 79–87
57 Allen, I.C. *et al.* (2009) The NLRP3 inflammasome mediates in vivo innate immunity to influenza A virus through recognition of viral RNA. *Immunity* 30, 565
58 Im, H. and Ammit, A. (2013) The NLRP3 inflammasome: role in airway inflammation. *Clin. Exp. Allergy* 44, 160–172
59 Schroder, K. and Tschopp, J. (2010) The inflammasomes. *Cell* 140, 821–832
60 Ichinohe, T. *et al.* (2009) Influenza virus activates inflammasomes via its intracellular M2 ion channel. *Nat. Immunol.* 11, 404–412
61 Mould, J.A. *et al.* (2000) Permeation and activation of the M2 ion channel of influenza A virus. *J. Biol. Chem.* 275, 31038–31050
62 Sakaguchi, T. *et al.* (1996) The ion channel activity of the influenza virus M2 protein affects transport through the Golgi apparatus. *J. Cell Biol.* 133, 733–747
63 Nicholson, K.G. *et al.* (1993) Respiratory viruses and exacerbations of asthma in adults. *BMJ* 307, 982–986
64 Johnston, S.L. *et al.* (1995) Community study of role of viral infections in exacerbations of asthma in 9-11 year old children. *BMJ* 310, 1225–1229
65 Seemungal, T. *et al.* (2001) Respiratory viruses, symptoms, and inflammatory markers in acute exacerbations and stable chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* 164, 1618–1623
66 Hakonarson, H. *et al.* (1999) Rhinovirus-mediated changes in airway smooth muscle responsiveness: induced autocrine role of interleukin-1beta. *Am. J. Physiol.* 277, L13–L21
67 Dolinay, T. *et al.* (2012) Inflammasome-regulated cytokines are critical mediators of acute lung injury. *Am. J. Respir. Crit. Care Med.* 185, 1225–1234
68 van Kuppeveld, F.J. *et al.* (1997) Coxsackievirus protein 2B modifies endoplasmic reticulum membrane and plasma membrane permeability and facilitates virus release. *EMBO J.* 16, 3519–3532
69 de Jong, A.S. *et al.* (2003) Determinants for membrane association and permeabilization of the coxsackievirus 2B protein and the identification of the Golgi complex as the target organelle. *J. Biol. Chem.* 278, 1012–1021
70 Kanneganti, T.D. *et al.* (2006) Critical role for Cryopyrin/Nalp3 in activation of caspase-1 in response to viral infection and double-stranded RNA. *J. Biol. Chem.* 281, 36560–36568
71 Simoes, E.A.F. (1999) Respiratory syncytial virus infection. *Lancet* 354, 847–852
72 Ogra, P.L. (2004) Respiratory syncytial virus: the virus, the disease and the immune response. *Paediatr. Respir. Rev.* 5, S119–S126
73 Ruuskanen, O. *et al.* (2011) Viral pneumonia. *Lancet* 377, 1264–1275
74 Wilkinson, T. *et al.* (2006) Respiratory syncytial virus, airway inflammation, and FEV_1 decline in patients with chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* 173, 871–876
75 Seemungal, T. *et al.* (2001) Respiratory viruses, symptoms, and Inflammatory markers in acute exacerbations and stable chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* 164, 1618–1623
76 Openshaw, P.J.M. (2005) Antiviral immune responses and lung inflammation after respiratory syncytial virus infection. *Proc. Am. Thorac. Soc.* 2, 121–125

77 Schmitz, N. *et al.* (2005) Interleukin-1 is responsible for acute lung immunopathology but increases survival of respiratory influenza virus infection. *J. Virol.* 79, 6441–6448
78 Segovia, J. *et al.* (2012) TLR2/MyD88/NF-kB pathway, reactive oxygen species, potassium efflux activates NLRP3/ASC inflammasome during respiratory syncytial virus infection. *PLoS ONE* 7, 1–15
79 Bukreyev, A. *et al.* (2012) Recombinant respiratory syncytial virus from which the entire SH Gene has been deleted grows efficiently in cell culture and exhibits site-specific attenuation in the respiratory tract of the mouse. *J. Virol.* 71, 8973–8982
80 Triantafilou, K. *et al.* (2013) Human respiratory syncytial virus viroporin SH triggers inflammasome activation. *Thorax* 68, 66–75
81 Chan, H. *et al.* (2003) Coronavirus in severe acute respiratory syndrome (SARS). *Trends Mol. Med.* 9, 323–325
82 Peiris, J.S. *et al.* (2003) Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study. *Lancet* 351, 1767–1772
83 Peiris, J.S. *et al.* (2004) Severe acute respiratory syndrome. *Nat. Med.* 10, S88–S97
84 Huang, K.J. *et al.* (2005) An interferon-gamma-related cytokine storm in SARS patients. *J. Med. Virol.* 75, 185–194
85 Jiang, Y. *et al.* (2005) Characterization of cytokine/chemokine profiles of severe acute respiratory syndrome. *Am. J. Respir. Crit. Care Med.* 171, 850–857
86 Ng, P.C. *et al.* (2004) Inflammatory cytokine profile in children with severe acute respiratory syndrome. *Pediatrics* 113, 7–14
87 Verdiá-Báguena, C. *et al.* (2012) Coronavirus E protein forms ion channels with functionally and structurally-involved membrane lipids. *Virology* 432, 485–494
88 Schubert, U. *et al.* (1996) Identification of an ion channel activity of the Vpu transmembrane domain and its involvement in the regulation of virus release from HIV-1-infected cells. *FEBS Lett.* 398, 12–18
89 Phillips, R. *et al.* (2009) Emerging roles for lipids in shaping membrane-protein function. *Nature* 459, 379–385
90 Oxford, J.S. and Galbraith, A. (2009) Antiviral activity of amantadine: a review of laboratory and clinical data. *Pharmacol. Ther.* 11, 181–262