

# Respiratory Viruses and Asthma

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## Abstract

### Keywords

- ▶ asthma
- ▶ viruses
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- ▶ respiratory syncytial virus
- ▶ rhinovirus
- ▶ airway microbiota
- ▶ genetic susceptibility

Asthma remains the most prevalent chronic respiratory disorder, affecting people of all ages. The relationship between respiratory virus infection and asthma has long been recognized, though remains incompletely understood. In this article, we will address key issues around this relationship. These will include the crucial role virus infection plays in early life, as a potential risk factor for the development of asthma and lung disease. We will assess the impact that virus infection has on those with established asthma as a trigger for acute disease and how this may influence asthma throughout life. Finally, we will explore the complex interaction that occurs between the airway and the immune responses that make those with asthma so susceptible to the effects of virus infection.

## Early-Life Exposure to Viruses and the Development of Asthma

### Viral Bronchiolitis and the Development of Asthma

Acute lower respiratory tract infections resulting in bronchiolitis remain among the most common cause of hospital admission in the developed world for children younger than the age of 2 years. In those younger than 1 year, bronchiolitis caused by respiratory syncytial virus (RSV) is the primary cause,<sup>1</sup> while for those older than 1 year, infection with rhinovirus (RV) is more prominent.<sup>2</sup>

RSV bronchiolitis has been associated with an increase in subsequent wheezing illness, and it has long been considered that this association may play a causative role in the development of asthma. Research has now shown that severe early-life infection with RSV is associated with an increased risk of infrequent wheeze (odds ratio: 3.2 [95% confidence interval [CI]: 2.0–5.0],  $p < 0.001$ ), and frequent wheeze (4.3 [2.2–8.7],  $p \leq 0.001$ ) by the age of 6 years, but was no longer associated with episodes of wheeze by the age of 13 years.<sup>3</sup> There was also no association between RSV lower respiratory

tract illnesses and development of allergic disease, though RSV lower respiratory tract illnesses were associated with significantly lower lung function at the age of 13 years.<sup>3</sup> In support of these observations, Lu et al also went on to show that the risk of an asthma diagnosis at the age of 6 years was determined by the severity of the acute RSV episode along with a family history of asthma highlighting interaction with atopy.<sup>4</sup> Similarly, severe RSV bronchiolitis resulting in hospitalization was associated with a six- to eightfold increase in admission for wheeze in the first 2 months after RSV hospitalization, but this was no longer increased a year later.<sup>5</sup> However, in those with a diagnosis of asthma, the risk for hospitalization with RSV infection was increased threefold, and this risk was not time dependent, suggesting host factors associated with a diagnosis of asthma at this age increase susceptibility to RSV, but the effect RSV has alone on airway symptoms such as wheeze is transitory.<sup>5</sup>

RV infection is the second most common virus associated with bronchiolitis, and the most common virus detected in association with wheezing illnesses in children by the age of 1 to 2 years.<sup>6–8</sup> Three species of RV (human RV [HRV]-A, HRV-B,

and HRV-C) are now recognized to include 150+ antigenically distinct virus subtypes. Most of these are likely to also represent serotypes explaining life-long susceptibility to RV infections.<sup>9</sup> Differences in association of RV species with disease have been observed, with RV-A and the recently discovered RV-C responsible for more severe lower respiratory tract illnesses.<sup>10–12</sup> HRV-A and HRV-B can be grown in standard laboratory cell lines enabling isolation from clinical samples and classification according to neutralizing antibody responses (serotypes). This occurred in the 1960s and 1970s and led to the identification of approximately 100 strains. HRV-C was only discovered in 2006,<sup>13</sup> as they could not be cultured using standard techniques and hence has gone unappreciated as a pathogen. HRV-C has now been shown to infect both the youngest children and to cause the most severe acute RV-induced lower respiratory tract disease.<sup>12</sup> Once researchers were able to culture HRV-C using respiratory mucosal explants or air-liquid interface differentiated bronchial epithelium, they determined that it used the human transmembrane protein, cadherin-related family member 3 (CDHR3) for virus binding and replication.<sup>14</sup> Serendipitously, it had recently been shown that there were four distinct alleles associated with CDHR3 expression, with one of these representing a single nucleotide polymorphism (G→A) that converts a residue cysteine to tyrosine at position 529 (Cys<sub>529</sub>→Tyr, rs6967330). This leads to a rare, but phenotypically dominant, asthma-related A-encoded Tyr529 variant of the CDHR3 gene that was linked to greater airway epithelial cell (AEC)-surface expression of CDHR3, and an increased risk of wheezing illnesses and hospitalizations for children 2 to 5 years.<sup>15,16</sup> Bochkov et al then went on to show that compared with wild-type CDHR3, cells transfected with the asthma susceptible CDHR3-Y529 variant had a 10-fold increase in HRV-C binding, increasing infectious virus yields.<sup>14</sup> These findings are the first to link genetic susceptibility to respiratory tract infection with the development of childhood asthma.

### Allergy Virus Infection and the Risk of Asthma

Allergic sensitization is also intertwined in the risk of developing asthma and virus-induced wheezing. Children who develop sensitization to multiple aeroallergens at a younger age are more prone to develop severe acute asthma needing hospitalization.<sup>17</sup> The relationship between virus infection and allergic sensitization was elegantly demonstrated by the investigators of the Childhood Origins of Asthma study (COAST) study who showed that children sensitized to aeroallergens and who wheezed with RV during the first 3 years of life had the greatest risk for developing asthma.<sup>18</sup> The investigators went on to demonstrate that there was a sequential relationship with allergic sensitization leading to viral wheezing, especially wheezing was associated with RV, but there was no evidence that viral wheezing itself led to allergic sensitization.<sup>19</sup> In fact, the impact of RV infection on early life has been the subject of a meta-analysis of 15 original studies, 10 reported on the results of 4 longitudinal cohort studies with different follow-up periods. RV wheezing illness in the first 3 years of life was associated with an increased risk of wheezing/asthma in

later life (relative risk [RR] = 2.00, 95% CI: 1.62–2.49,  $p < 0.001$ ). In subgroup analysis done by age at follow-up, the association was significant in those <10 years (RR = 2.02, 95% CI: 1.70–2.39,  $p < 0.001$ ) and  $\geq 10$  years (RR = 1.92, 95% CI: 1.36–2.72,  $p < 0.001$ ).<sup>20</sup> Therefore, as has been seen with RSV, it is a host susceptibility linked to atopy, as demonstrated by a family history of allergic asthma that confers a significant risk of RV-induced wheeze. The impact of viral infection in early life and the role this then plays in the inception of asthma was best summarized by Gern and Busse, when they stated “severe infections at this stage can result in 2 non-exclusive outcomes, the first being that they may impair normal lung development and the second being that they may trigger wheezing illnesses in susceptible individuals.”<sup>21</sup>

### Preventing Virus-Induced Wheeze

Given the close association with viral bronchiolitis and childhood asthma, interventions to prevent or at least reduce the severity of the disease, potentially could have important and long-lasting effects. Although this is not possible for RV infection, the development of a humanized monoclonal antibody that targets the RSV F protein, palivizumab, allows passive immunization that reduces the susceptibility and severity of RSV bronchiolitis in preterm, high-risk infants.<sup>22</sup> For this reason, investigators randomized 429 otherwise healthy preterm infants to treatment with palivizumab or placebo.<sup>23</sup> The treated infants had a 61% (95% CI: 56–65) relative reduction in total number of wheezing days in the first year of life, and the proportion of infants with recurrent wheeze was 10% points lower in those treated with palivizumab (11 vs. 21%,  $p = 0.01$ ).<sup>23</sup> In a similarly sized study that looked at outcomes up to 6 years of age, following palivizumab treatment in the first year of life, there were fewer recorded episodes of wheeze, but no difference was seen in doctor diagnosis of asthma.<sup>24</sup> It will be interesting to determine whether this intervention will impact on either lung function or asthma at later ages.

### The Airway Microbiome and Asthma in Early Life

The interaction between the developing immune system and viral infections in early life also occurs against the backdrop of exposure to bacteria or the airway microbiota. Until recently, the healthy lower airway was not considered to harbor bacteria. Our understanding of respiratory microbiota has changed with the advent of culture-independent techniques involving high-throughput sequencing of the 16S rRNA gene, a highly conserved locus of the bacterial genome. This has revealed a complex microbial community that varies between states of health and respiratory disease.<sup>25</sup> A relationship between bacterial colonization and early-life asthma was first demonstrated in the Copenhagen birth cohort that showed a heightened risk of wheeze and asthma in children whose nasopharynx was colonized with *Streptococcus pneumoniae*, *Moraxella catarrhalis*, and *Haemophilus influenzae* at 1 month of age. These same bacteria were also present during acute episodes of wheeze associated with viral infections and conferred a similar degree of risk for acute wheeze.<sup>26</sup>

Teo et al collected nasopharyngeal aspirates from children at 2, 6, and 12 months of age and within 48 hours of a clinical respiratory tract infection and applied modern methods of 16S rRNA gene sequencing.<sup>27</sup> They then went on to assess changes in the upper airway microbiota and chronic wheeze at the age of 5 years. *Staphylococcus* and *Corynebacterium*, organisms known to be part of the skin microbiome, were the dominant species in the first 2 months of life before changing to *Alloicoccus* or *Moraxella* at 6 to 12 months, concomitant with a stabilization of the microbial population. They speculated that infants are likely to be colonized initially with skin bacteria from their parents and these populations are replaced over time by *Moraxella* or *Alloicoccus*. This upper respiratory microbiota then remains stable over time in healthy individuals. However, in infants who had virus respiratory infections, there was a greater abundance of *Streptococcus*, *Moraxella*, and *Haemophilus*. While early *Moraxella* colonization was associated with a younger age for the first viral upper respiratory infection, *Streptococcus* colonization was associated with earlier initial lower respiratory infection. The level of subsequent asthma risk then was found to inversely relate to the age at which initial *Streptococcus* colonization occurred. The investigators speculated that lower airway infection with RSV or RV contributes to an altered microbe featuring *Streptococcus* and the resulting effect on the airway and/or developing immune system predisposes to the development of asthma.<sup>27</sup> This intriguing area obviously requires further work, but it is fascinating to see how exposure in early life to pathogens, either viral or bacterial can alter both the airways and immune response so profoundly.

### The Hygiene Hypothesis and Protection against Asthma

The hygiene hypothesis was developed to explain the relationship between microbial exposure in early life and protection against allergic and autoimmune diseases that continue to increase in prevalence particularly in westernized societies. It proposed that improvements in housing and changes to an urban lifestyle may be responsible for a lack of exposure to bacterial pathogens in early life resulting in an immune response that becomes skewed to the development of hypersensitivity and allergy.<sup>28,29</sup> Apart from infection, exposure to multiple bacterial antigens can occur from the indoor and outdoor environment. Bacterial products, such lipopolysaccharide (LPS) or endotoxin, are recognized as foreign by the innate immune system and their exposure, especially in early life activates a developing immune system. In support of this concept, high-level chronic exposure to LPS has been shown experimentally to induce immune tolerance.<sup>30</sup> This hypothesis was supported by German investigators who examined samples of dust from the mattresses of children and then related the levels of endotoxin to the prevalence of asthma and allergies and to serum levels of specific immunoglobulin E (IgE). They found that children exposed to "farm dust," containing high levels of LPS were associated with a significant decrease in the risk of hay fever, atopic sensitization, atopic asthma, and atopic

wheeze in childhood.<sup>31</sup> This work has been supported by strong epidemiological studies confirming early-life exposure to farming and bacterial LPS significantly reduces the risk of asthma.<sup>31,32</sup> Recently, the mechanisms behind this regulation have started to be understood, with AECs and the regulator protein, A20, playing a key role.<sup>33</sup> A20 is a deubiquitinase that is an essential negative regulator of nuclear factor- $\kappa$ B (NF- $\kappa$ B) activation and the subsequent induction of inflammatory responses.<sup>34</sup> A20 forms a unique ubiquitin-editing complex with other proteins that is required for the activation of NF- $\kappa$ B.<sup>35</sup> A20 knockout mice are highly susceptible to exposure to inflammatory mediators and LPS.<sup>36</sup> Recently, an important role has emerged for A20 in the regulation of early asthma. Mice when exposed to low levels of LPS in early life do not develop features of asthma, despite sensitization to house dust mite (HDM). Using knockout mice with selective A20 deficiency in the AECs, the protective effect of LPS was diminished, with increased epithelial expression of inflammatory mediators, chemokine (C-C motif) ligand (CCL)-20 and granulocyte macrophage colony-stimulating factor (GM-CSF) following HDM.<sup>36</sup> In human AECs, LPS treatment also blunted the inflammatory response (CCL-20, GM-CSF, and interleukin [IL]-1 $\alpha$ ) following HDM exposure.<sup>37</sup> Bringing these findings together, Schuijs et al demonstrated that LPS-exposed epithelial cell cytokines are responsible for activating dendritic cells (DCs), and suppressing type 2 immunity to HDM, while loss of the ubiquitin-modifying enzyme A20 in lung epithelium abolished this protective effect. They also demonstrated that a single-nucleotide polymorphism in the gene encoding A20 was associated with allergy and asthma risk in children growing up on farms.<sup>33</sup>

While preventing exposure to RSV may be a risk for early-life asthma, exposure to bacterial polyproteins may conversely be protective. Some preliminary evidence hints at this. Recent studies have been performed using an extract from bacterial products OM-85 BV that can safely be given as an oral preparation. Preliminary studies suggest that children treated with OM-85 BV demonstrated an immune response that was skewed away from type 2 immunity, with increased interferon (IFN)- $\gamma$ /IL-4 ratios.<sup>38</sup> A small clinical trial has also shown that its use decreased asthma exacerbations; in 1 to 6 years old, attacks were reduced 37.9% and 2 days shorter for OM-85 BV versus placebo ( $p < 0.001$ ).<sup>39</sup> In fact, OM-85 BV reduced attacks to a greater degree than inhaled corticosteroids.

## Respiratory Viruses as Triggers of Asthma Exacerbations

### Epidemiology of Acute Asthma

While viral infection is highly linked with wheeze in early life, and the subsequent development of asthma, the association between viral infections and exacerbation of asthma in adolescents and adults with an established diagnosis of asthma is particularly strong. Currently, the Global Initiative for Asthma (GINA) describes an exacerbation as an acute change in symptoms or lung function, typically requiring a change to management, though consensus on what defines

an exacerbation is poor.<sup>40</sup> Acute exacerbations are the leading cause of asthma deaths and hospitalization. It is estimated that 80% of the costs associated with the treatment of asthma are due to exacerbations, despite them happening in a minority of patients.<sup>41</sup> Given the gravity associated with asthma exacerbations, there has been much work on the etiology of these acute events. It has been identified that viral infections are responsible for the majority of asthma exacerbations (80% in children and 76% in adults).<sup>42,43</sup> Thus, we will discuss the role of viruses in the epidemiology, risk factors to exacerbation, and influence on asthma control.

In general, infection with respiratory viruses remains the most common cause of upper respiratory tract infection (URTI). Of these infections, RV is the most common, representing up to 50% of documented cases.<sup>44</sup> Following this are infections with coronavirus, parainfluenza, influenza, RSV, enterovirus, and adenovirus. While the majority of these viruses tends to involve AEC infection of the upper respiratory tract and subsequent inflammation, influenza and RSV are distinguished by a propensity to infect the lower respiratory tract and cause particularly severe damage to the airway epithelial layer and, as such, lead to increased disease severity.<sup>44</sup>

RV, while being the most common cause of URTI (and the most common viral infection of man), is also most common virus associated with exacerbations of asthma. Indeed, while upwards of 76 to 80% of acute exacerbations of asthma have been linked with virus infection, 60% of these are due to RV.<sup>42,43,45</sup> This represents a higher proportion of patients affected by RV than that found in the general population associated with URTI, which has led to insights that patients with asthma may actually be more susceptible to viral infection.<sup>46,47</sup> It has been observed that patients with asthma are more likely to develop more severe lower respiratory tract symptoms in the setting of viral infection than healthy individuals.<sup>48</sup> This is not uniformly associated with all RVs as there is heterogeneity associated with the responses to RV infection<sup>49</sup> among more than 150 strains identified.<sup>45</sup> In particular, it has been noted that lower respiratory tract infection with RV occurs a few days after initial symptoms begin, which correlates well with the timing of asthma exacerbations.<sup>45</sup> RV-induced asthma exacerbations tend to occur in autumn and spring, mirroring increasing incidence of infection in the general population.<sup>47</sup>

RSV and influenza make up most of the remaining viral-induced asthma exacerbations.<sup>50</sup> As previously noted, RSV is a particularly important pathogen in children younger than 2 years of age in winter,<sup>51</sup> but in the past has thought to be less common in adults with acute exacerbations of asthma.<sup>48</sup> However, with more widespread testing of adults in hospital settings, it is becoming increasingly recognized as a serious and common cause of lower respiratory tract infection, especially in the elderly.<sup>52</sup> Influenza most commonly causes asthma exacerbations during the winter months, as this is also when it is most prevalent.<sup>53</sup> Asthma in the setting of influenza infection is associated with worse disease severity, and higher risk of hospitalization, need for intensive care, and mortality.<sup>53</sup> Although it is implicated in a very small

proportion of asthma exacerbations (0.7–2.5%), adenovirus has similarly been linked with more severe disease.<sup>48</sup>

### Those at Risk of Acute Exacerbations of Asthma

Asthma is a heterogeneous disease of airway inflammation and airway hyperresponsiveness (AHR). Its multidimensional nature means we should look at the disease from many angles to assess how individuals will respond to a particular insult. This is especially true in the difference in response to viral infection between patients with asthma and healthy patients. In general, compared with healthy individuals without asthma, Corne et al showed in a community cohort study that while patients with asthma were no more susceptible to getting RV infection than healthy individuals, they were much more prone to develop lower respiratory tract involvement and more severe symptoms.<sup>54</sup> Differences in the immune response in asthma may account for this and there may be a complex interaction between the inflammation seen in asthma and the ability respond to virus infection in the airways.

### Deficient Innate Immune Response in Asthma

While not a risk factor that usually distinguishes patients with asthma from each other, asthmatics have been demonstrated to have deficient immune response to viral infection.<sup>55</sup> In vitro experiments with RV have shown that patients with asthma have higher levels of viral replication following infection than control subjects. Indeed, experimental studies imply there are deficiencies in IFN-mediated antiviral responses in patients with asthma that are contributory to this process,<sup>47,56</sup> though these findings have not always been observed, particularly in those with mild disease.<sup>57,58</sup> By their nature, all these studies have had limited numbers and findings in clinical asthma strongly suggest that the risk of acute asthma varies and could be restricted to a certain “at risk of acute asthma” phenotype.<sup>59</sup> This concept is supported by the finding that deficient type I IFN responses are found in those with severe asthma.<sup>60</sup> Recently, the hypothesis that correcting deficient airway antiviral responses would improve outcomes in acute asthma was tested by randomizing asthmatics to receive nebulized IFN- $\beta$  after developing cold symptoms.<sup>61</sup> Subjects had moderate-to-severe asthma, and a history of cold induced acute asthma. While 91% of subjects developed a cold, the majority did not develop acute asthma and treatment with nebulized IFN- $\beta$  overall did not result in a significant improvement. However, in those with poor control despite treatment, “cold” symptoms led to a greater worsening in asthma and nebulized IFN- $\beta$  led to a significant attenuation. This may be partly explained by alterations in the immune system due to active inflammation since atopic children have been shown to be particularly at risk of more severe viral exacerbations, and this is mitigated by anti-IgE therapy.<sup>62</sup> Other risk factors that require further exploration include low vitamin D levels and higher levels of stress. It is suspected that these both play their part in reducing immunity against viral infection, contributing to risk of exacerbation.<sup>27</sup> Resultant higher levels of viral replication and insufficient IFN production and the



associated airway inflammation that occurs concurrently could certainly explain this propensity for frequent exacerbations.

The importance of acute asthma is increasingly acknowledged in international guidelines that seek to minimize its risk.<sup>63</sup> The clinical factors that have been associated with an increased risk of acute asthma are (1) poor asthma symptom control, (2) more severe asthma (as defined by GINA and based on a lower forced expiratory volume in 1 second [FEV<sub>1</sub>]), and (3) smoking.<sup>64</sup> Exacerbation frequency also has been linked to poor asthma control<sup>59</sup> and there appears to be a complex relationship between poor asthma control and susceptibility to virus infection. The majority of research into asthma exacerbations has included well-controlled individuals. Jackson et al demonstrated that uncontrolled asthmatics experience more frequent lower respiratory tract symptoms, a worse fall in FEV<sub>1</sub> and slower recovery compared with well-controlled individuals during exacerbation, independent of asthma severity.<sup>65</sup> Bateman et al have shown a clear link between asthma control and predicting future risk of exacerbation but have so far not been able to demonstrate improvement in exacerbation rate with better control.<sup>66</sup> Kupczyk et al have shown that in a cohort of patients with severe asthma, frequent exacerbations were correlated with markers of ongoing inflammation such as exhaled nitric oxide and higher sputum eosinophils.<sup>59</sup> We also have shown that asthmatics with moderate-to-severe disease and persistent eosinophilic inflammation showed reduced toll-like receptor (TLR)7 expression, along with impaired IFN expression, in endobronchial biopsies.<sup>67</sup> In support of the concept that poor asthma control along with persistent asthmatic inflammation is responsible for the increased susceptibility to virus infection, investigators demonstrated in a cohort of children at high risk for acute exacerbations of asthma that the addition of omalizumab (a monoclonal antibody to IgE), when added to a regimen of guidelines-based therapy further improved asthma control, nearly eliminated seasonal peaks in exacerbations previously shown to be associated with virus infection.<sup>68</sup> They also went on to show in a subgroup that treatment with omalizumab improved *in vitro* IFN responses to RV, and in those who demonstrated the greatest improvement, there was the greatest reduction in exacerbation frequency.<sup>69</sup>

### Smoking and Pollution

A history of smoking has been identified as a risk factor for frequent exacerbation.<sup>59</sup> Indeed, *in vitro* work has confirmed that cigarette smoke exposure is associated with impaired IFN production, suggesting a plausible method for higher exacerbation frequency due to impaired antiviral responses, thus predicting exacerbations.<sup>70</sup> Passive exposure to cigarette smoke has also been linked with risk of exacerbations in children. This extends to pollution in the environment in general, as exposure to pollutants, such as sulfur and nitrogen dioxide, is also a risk factor for viral exacerbation of asthma.<sup>27</sup>

### The Impact of Virus Infection on Asthma Control

Well-controlled asthma is defined by the current GINA guidelines as that which causes infrequent symptoms, needs

for reliever therapy, and does not limit exercise tolerance, while optimizing adherence, psychosocial factors, exposures, comorbidities, and markers of inflammation.<sup>40</sup> A subset of patients who frequently exacerbate has been identified, and it is postulated that viral exacerbation may be one of the inciting factors in this recurrent loss of control and cycle of exacerbation.<sup>71</sup>

In addition, there is evidence that in children who presented to hospital emergency rooms, persistence of RV was noted at 6 weeks in 44% of patients. This was associated with worse peak flow at presentation and likely contributes to severity of disease and thus, impacts on subsequent control.<sup>72</sup> In contrast, Chang et al have looked at the effects of documented viral infection on the course of asthma exacerbation and subsequent recovery. While viral infection did predict an increased severity of asthma exacerbations for clinically defined viral illness, it did not correlate with features of subsequent outcome. In particular, pediatric asthma quality of life, asthma, and cough diaries were not worsened by the presence of viral infection in the setting of acute asthma exacerbation after 6 weeks.<sup>73</sup> As a result, at present, while there are some suggestions of an impact of viral exacerbation on subsequent asthma control, this is not yet entirely defined and further research is needed in the area to be able to make any further conclusions on the effects on asthma control of viral exacerbation.

## Bidirectional Interaction of Asthma and Virus Infection—The Mechanisms as to How One Predisposes to the Other

The airway epithelium acts as a physical and active immune barrier between the internal environment of the lung and the external environment that contains potentially noxious gases, agents, and pathogens including viruses. The pathogen-associated molecular patterns (PAMPs) such as viral or bacterial nucleic acids, fungal products, and bacterial endotoxins such as LPSs interact with the airway epithelium. These PAMPs are efficiently recognized by diverse range of innate germline encoded receptors in the cell surface or in the cytoplasm of the AECs and able to elicit a strong immune response. These receptors are known as pattern recognition receptors (PRRs).<sup>74,75</sup>

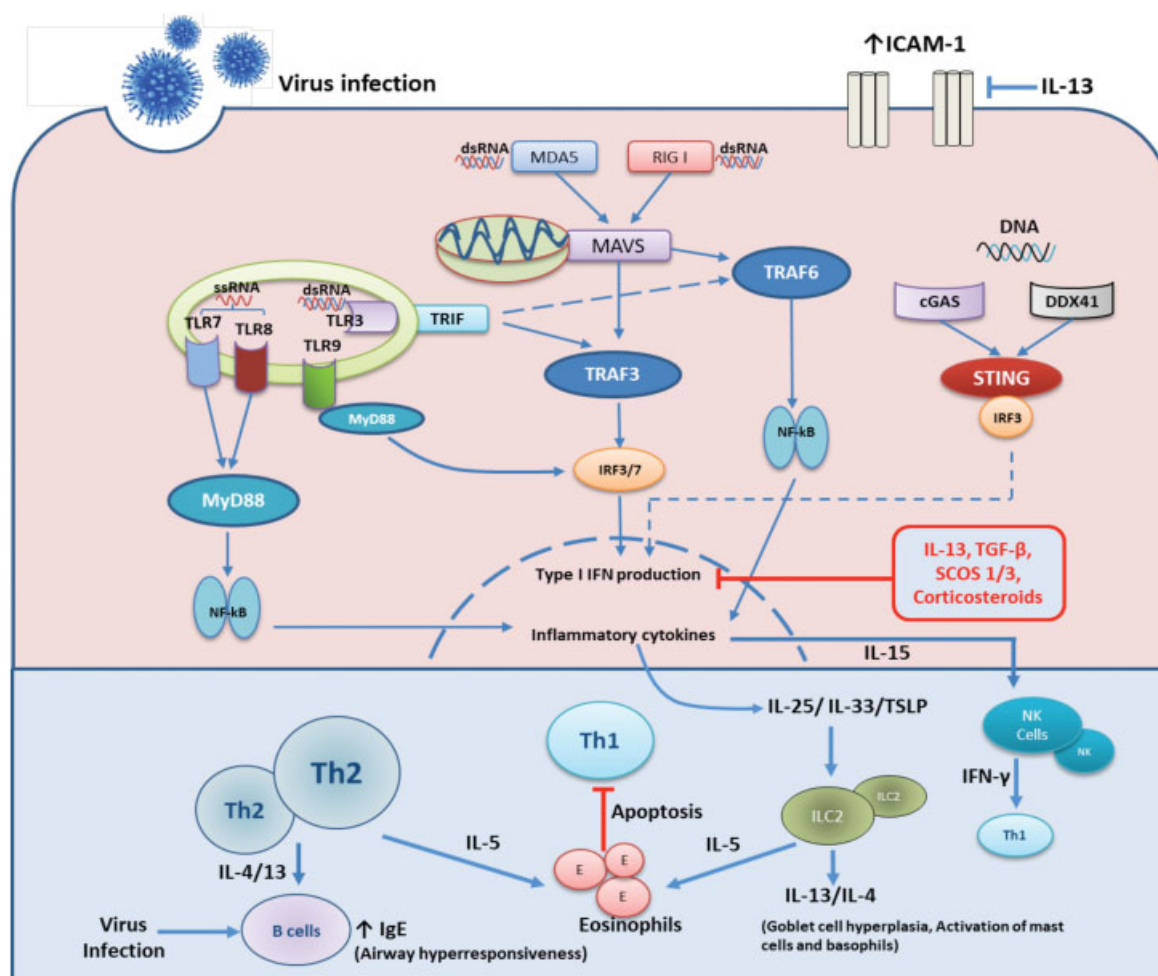
Respiratory viruses such as HRVs, RSV, coronaviruses, and influenza virus are the most common infectious illnesses in the airways and usually self-limiting and confined to the respiratory tract.<sup>44</sup> The presence of these RNA virus infections are sensed via PRRs such as the TLRs, retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs), and nucleotide-binding oligomerization domain-like receptors. Among TLRs, TLR3 expressed in the intracellular endosomes of bronchial epithelium cells and responds to the presence of double-stranded RNA, which is produced during replication of these viruses.<sup>76</sup> TLR3 triggers signaling via TIR-domain-containing adaptor-inducing IFN- $\beta$  (TRIF) and then associates with tumor necrosis factor (TNF) receptor-associated factor 3 (TRAF3) and TRAF6. This leads to the activation of IFN regulatory factor 3, which translocates into the nucleus

resulting in production of type I IFNs. TRIF also interact with receptor interacting protein 1 which activates NF- $\kappa$ B and production of inflammatory cytokines.<sup>77</sup> TLR 7/8 are also found within endosomes, activated by single-stranded RNA and leads NF- $\kappa$ B activation via MyD88-dependent pathway.<sup>78</sup> TLR4 is mainly responsible for the detection of bacterial endotoxins; however, it has been reported that some viral proteins (F protein of RSV) are detected by TLR4 and induce type I IFNs and proinflammatory cytokines via activating IRF-3, NF- $\kappa$ B, and activating protein 1.<sup>78</sup>

RLR signaling plays a major role in detecting several picornaviruses, influenza virus, and RSV (**Fig. 1**). RIG I

recognizes 5'-triphosphate (5'PPP) RNA and dsRNA during virus infection and melanoma differentiation-associated protein 5 detects long dsRNA of > 2 kb.<sup>79</sup> The components of viral RNA are recognized by these RLR receptors and initiate type I IFN production.<sup>80,81</sup>

Type I and type III IFNs have a crucial role in controlling virus infection in the airway epithelium. Type I IFNs and IFN- $\alpha/\beta$  act on the type I IFN receptor and via JAK-STAT1/2 pathway activates a series of IFN-stimulatory genes which produce potent antiviral proteins and eliminate virus infections. Type III IFNs ( $\lambda$  IFNs) such as IFN  $\lambda$  1, 2, and 3 (also known as IL-29, IL-28A, and IL-28B) also elicit an antiviral



**Fig. 1** Innate and adaptive immune responses to respiratory virus infections in the airways. Under normal circumstances the innate immune response to virus infection is triggered by infection of airway epithelial cells and recognition of viral pathogen associated molecular patterns (PAMPs) by both the infected epithelium and resident immune cells. These cells contain various pathogen recognition receptors (PRRs) that recognise a diverse range of PAMPs and elicit innate then adaptive immune responses to eliminate virus infections. Viral dsRNA are recognised by RIG I, MDA5 or TLR3 receptors while sRNA are mainly sensed by TLR7/8. Various DNA viruses are recognised by cytoplasmic DNA sensors; cGAS, DDX41 or by endosomal TLR9. Upon recognition downstream signaling cascades are initiated to produce type I interferons via IRF3/7 activation that induces antiviral proteins that inhibit viral replication within infected cells and spread to neighboring cells. Inflammatory cytokines production via NF- $\kappa$ B activation, results in release of IL-6, CXCL8 and the recruitment and activation of neutrophils and macrophages. Infected cells release IL-15 that activates NK cells that produce IFN- $\beta$  and that target infected cells. This supports a robust Th1 environment for recruitment of TH-1 and type 1 innate lymphoid cells (ILC1), resulting in viral clearance. In asthma there is a pre-existent state of active airway inflammation. In the case of active type 2 inflammation, the presence of increased type 2 lymphocytes, both TH-2 cells and ILC2 cells promote this abnormal state. Following infection of the asthmatic epithelium there is heightened release of IL-25/IL-33 and TSLP that further activate ILC2 cells. Activated ILC2s and Th2 cells largely produce more IL-4/13 and IL-5 all of which activate other inflammatory cells such as eosinophils and this results in worsened inflammation of the airways. IL-4/13 also enhance the IgE production by B lymphocytes which may further impair activation of the innate immune cells such as dendritic cells to virus infection. Increased expression of type 2 cytokines, TGF- $\beta$  and SOCS1/3 negatively regulates type I interferon production while type 2 cytokines also enhance ICAM-1 expression all of which results increased virus replication.

response; however, their cellular receptor distribution on different tissues is limited.<sup>82,83</sup>

Besides IFNs, there are several cytokines and chemokines produced by airway epithelium during virus infections. These cytokines/chemokines possess different roles in innate and adaptive immunity. Cytokines such as IL-6, TNF- $\alpha$ , granulocyte colony-stimulating factor (G-CSF), and granulocyte macrophage-CSF (GM-CSF) are secreted upon various respiratory virus infections. IL-6 is a proinflammatory cytokine that modulate lung neutrophils, monocytes, and T cells activity.<sup>84</sup> Increased secretion of IL-6 can result in excessive inflammation and damage. In contrast, the absence of IL-6 in the setting of influenza infection results in increased viral replication and reduced antiviral T cell activity.<sup>85</sup> Therefore, proper balance of IL-6 in the lung is necessary for the balance of innate and adaptive immunity. G-CSF modulates production and recruitment of neutrophils. GM-CSF involves in expansion and activation of pulmonary DCs and macrophages. Also, GM-CSF has shown a protective role against influenza virus infection.<sup>86</sup> TNF- $\alpha$ , while a potent proinflammatory cytokine, also has demonstrated an antiviral activity against influenza and RSV infections.<sup>87,88</sup>

Chemokines such as CXCL-8/IL-8, CXCL-10, and RANTES/CCL-5 are also induced by respiratory virus infections. CXCL-8/IL-8 mainly recruits neutrophils into lungs and modulates their activity. In addition, it has various functions on other types of immune cells as well.<sup>89</sup> IL-8 is induced by various virus infections such as RSV, influenza A, and RV in airway epithelium.<sup>90-92</sup> CXCL-10/inducible protein-10 is produced by several cells such as monocytes, neutrophils, eosinophils, epithelial, endothelial, and fibroblast with response to IFN- $\gamma$ . It can be induced by RV, RSV, influenza virus, etc., and have shown a protective role against pathogen infections.<sup>93-96</sup> CCL-5/RANTES is a chemokine induced by viral infections.<sup>97</sup> It recruits monocytes, T cells, and eosinophils into the lungs<sup>98</sup> and induces viral clearance.

As already discussed, deficient innate IFN responses in asthmatic epithelial cell release of IFN- $\beta/\lambda$  have been described<sup>47,56</sup> though not in all cases.<sup>58,99,100</sup> Some studies suggest this impairment correlates with the disease severity and more prominent in severe, less controlled asthma.<sup>61</sup> However, the molecular mechanisms of impaired innate immune responses in asthma still remain obscure. It has been proposed that the Th2/type 2 airway inflammatory environment present in the asthmatic airway may have a role in impaired innate antiviral immunity. Treatment with recombinant IL-13 in epithelial cells has been shown to enhance RV replication and suppress types I and III IFN production.<sup>101,102</sup> In addition, IL-13 has been shown to increase Intracellular adhesion molecule (ICAM)-1 expression on epithelial cells resulting increased RV infection.<sup>103</sup> Transforming growth factor- $\beta$  (TGF- $\beta$ ) is a potent mediator of airway remodeling in asthma and is highly expressed in the asthmatic epithelium.<sup>104</sup> Expression of TGF- $\beta$  can suppress both IFN- $\beta/\lambda$  in airways<sup>105</sup> and its overexpression enhances RV infection.<sup>106</sup> Suppressor of cytokine signaling (SOCS) proteins are negative regulators of cytokine signaling. Intriguingly, SOCS1 and SOCS3 appear to be increased in

asthma.<sup>107,108</sup> Moreover, RV infection and Th2 cytokines further increased the SOCS1 and SOCS3 in asthmatic individuals resulting dampened IFN production.<sup>109</sup> Continuous corticosteroid treatments have also demonstrated a negative effect on innate antiviral effect. Peripheral blood mononuclear cells pretreated with budesonide promoted RV infection by reducing type I IFN production.<sup>110</sup> In addition, influenza A and RV demonstrated enhanced viral replication in AECs treated with glucocorticosteroids, while exogenous IFN adjuvant markedly reduced the glucocorticosteroid amplified virus infections.<sup>111</sup>

### Respiratory Virus Infection and Activation of Innate Lymphoid Cells and Natural Killer Cells in Asthma

Following viral infection, AECs release numerous immune activating mediators (► Fig. 1). It is reasonable to suggest that the composition of the epithelial immune response will have a profound impact on the downstream immune pathways that regulate inflammation and ultimately disease severity. Relevant to infections in asthma are reports showing that epithelium expresses mediators that can drive both innate type 2 and antiviral immunity. For example, IL-25 and IL-33 activate group 2 innate lymphoid cells (ILC2),<sup>112,113</sup> while release of IFN- $\alpha/\beta$  and IL-15 activates natural killer (NK) cells.<sup>114</sup>

ILC2 is recently discovered innate immune cells of lymphoid origin that lack lineage-specific markers including T and B cell receptors.<sup>115,116</sup> They are an important source of type 2 cytokines. ILC2 expresses an array of cytokine receptors and upon activation with airway epithelial derived cytokines such as IL-33, IL-25, and thymic stromal lymphopoietin, ILC2s produce large amounts of type 2 cytokines such as IL-4, IL-5, IL-9, and IL-13.<sup>117</sup> Type 2 cytokines play an important role in the activation of mast cells, eosinophils, and basophils; induce goblet cell hyperplasia and mucus production; and thus directly stimulate the characteristic pathological responses in asthma.<sup>118</sup>

RV infection of the AECs stimulates the release of IL-25 and IL-33 both of which activate NF- $\kappa$ B and STAT5 pathways that drive the expression of IL-5 and IL-13 from ILC2.<sup>119</sup> Influenza virus can also induce AHR by activating ILC2 via IL-33 secretion from alveolar macrophages<sup>120</sup> and NKT cells both of which stimulate ILC2-mediated production of IL-5 and IL-13 and accumulation of eosinophils in the lungs.<sup>121</sup>

NK cells are another class of innate immune cell that arises from a common lymphoid progenitor. These play an important role in defense against infectious agents including viruses.<sup>122</sup> Following viral infection, NK cells produce large amounts of IFN- $\gamma$  that supports a Th1/type 1 immune environment in the lung that promotes viral clearance, and plays a critical role in the subsequent development of an effector CD4 Th1 response. This may occur indirectly through NK cell priming of DCs; with bidirectional cross-talk, IFN- $\gamma$  released by NK cells activates DCs to produce IL-12, which in turn feeds back on the NK cell to further amplify IFN- $\gamma$  secretion.<sup>123</sup> However, when NK cell responses are impaired to viral infection, reduced levels of IFN- $\gamma$  may promote type 2 immune environment in the lungs with increased inflammation, viral replication, and clinical features

of asthma.<sup>124,125</sup> Interestingly, impaired NK cell responses appear to be a strong feature of acute severe RSV bronchiolitis,<sup>126</sup> and as we have previously discussed, this is an independent predictor for recurring wheeze up to the age of 6 years.

During RV infection, IL-15 is expressed by macrophages, DCs, and epithelial cells.<sup>127</sup> IL-15 mediates the activation, expansion, and recruitment of NK cells to the lungs to eliminate the virally infected cells by producing granzyme and IFN- $\gamma$ .<sup>128</sup> Impairment in IL-15 signaling can affect the NK cell protective feature against the virus, hence promoting asthma. Influenza virus can worsen airway inflammation in a murine asthma model of H1N1 strain. NK cells similarly play an important role in the response to influenza, with increased NK cells infiltrating within the first few days of infection with influenza virus.<sup>129</sup> NKp46 is an important NK cell receptor that binds directly with influenza virus HA protein resulting in cell activation and virus clearance.<sup>130</sup>

## Conclusion

Respiratory virus infections, especially RSV and RV, are a major cause of early childhood wheezing illnesses. As acute events, these infections are enough to trigger recurring episodes of wheeze (particularly with recurrent RV infections), are likely to influence permanently lung development, and in those susceptible, predispose to the development of asthma in later life. In established asthma, viruses remain important triggers of acute exacerbations, with those most susceptible tending to have either pre-existent poor asthma control or more severe underlying asthma. The reason for this susceptibility to respiratory virus infection remains unclear, but is very clearly closely related to the imbalanced inflammatory process active in asthma that can involve impaired type I/III IFN production coupled with excessive expression of cytokines that activate neutrophilic and type-2/eosinophilic inflammatory pathways (–Fig. 1). Originally thought to be primarily involved in control of viral replication, there is growing evidence that type I IFN is also an important negative regulator of inflammation. The role of the airway epithelium as the site of viral replication and initiator of the inflammatory pathways that cause exacerbations is now well established. Given the diversity of viruses that can precipitate an asthma exacerbation, antivirals and vaccines are unlikely to be feasible treatment options. As a result, research is now focused on redressing the asthmatic airway epithelial innate immune response to viral infection.

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