



Rhinovirus-induced asthma exacerbations and risk populations

Laura Coleman^{a,b}, Ingrid A. Laing^{a,b}, and Anthony Bosco^a

Purpose of review

This article discusses recent findings into the mechanisms that determine how viruses trigger asthma exacerbations.

Recent findings

Substantial progress has been made in our understanding of the pathogenesis of virus-induced asthma exacerbations. This includes new insights into the role of bacteria, the regulation of interferon responses, and the discovery of innate immune pathways that link viral infections with allergic inflammation. Progress has also been made in elucidating the genetic risk factors for asthma exacerbations, most notably the contribution of the ORMDL3/GSDMB locus on 17q, the mechanisms underlying the farming effect, and the discovery that CDHR3 binds to rhinovirus species C.

Summary

Asthma exacerbations are heterogeneous conditions that involve the complex interplay between environmental exposures and innate and adaptive immune function in genetically predisposed individuals. Recent insights into the interrelationships between these factors provide new opportunities for therapeutic intervention.

Keywords

asthma exacerbation, interferons, rhinovirus, wheeze

INTRODUCTION

Asthma is a chronic and heterogeneous disease of the conducting airways, which is highly prevalent in the developed world. It is characterized by reversible airflow obstruction, airways inflammation, airways hyperresponsiveness, and airways remodelling. The symptoms include shortness of breath, chest tightness, cough, and wheezing. Asthma exacerbations are acute episodes in which patients experience a progressive worsening of these symptoms. Exacerbations have been defined as 'a worsening of asthma requiring the use of systemic corticosteroids to prevent a serious outcome' [1].

Many children, particularly boys, will suffer from viral wheezing illnesses in early life. In most children, these illnesses are transient conditions associated with diminished airway function [2]. The frequency of exacerbations diminishes with increasing age and following puberty, girls are most at risk. A subset of children will develop persistent asthma with recurrent exacerbations. A diagnosis of asthma is difficult for children under 5 years of age. Indeed, identifying those children that will go on to develop persistent disease at the point of their first

wheezing episode is crucial, so that interventions can be targeted to the group most at risk. Multiple risk factors have been identified for asthma exacerbations, including age, sex, ethnicity, low socioeconomic status, obesity, sleep apnoea, gastro-esophageal reflux, stress, atopy, a recent severe exacerbation, low lung function, poor asthma control, medication noncompliance, blood/sputum eosinophilia, and exposure to allergens, viruses, second hand smoke, and pollutants [3–5]. This review will outline developments in this field in relation to the role of viruses, bacteria, host immunity, and genetic susceptibility.

^aTelethon Kids Institute, West Perth and ^bSchool of Paediatrics and Child Health, University of Western Australia, Perth, Western Australia, Australia

Correspondence to Anthony Bosco, PhD, Telethon Kids Institute, PO Box 855, West Perth, Western Australia, Australia. Tel: +61 8 9489 7777; e-mail: Anthony.Bosco@telethonkids.org.au

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KEY POINTS

- The presence of pathogenic bacteria during viral infections enhances risk of infection spread from the upper to lower airways and increases ensuing symptom severity.
- Multiple pathways are dysregulated in asthma that impact on interferon responses during rhinovirus infections.
- Rhinovirus upregulates IL-25 and IL-33 in airway epithelial cells, and this in turn drives Th2 cytokine production by Th2 and ILC2 and the pathogenesis of asthma exacerbations.
- The protective effect of farm exposure on risk for asthma/wheeze is modified by polymorphisms on chromosome 17q and TNFAIP3.
- CDHR3 is a susceptibility gene for severe asthma exacerbations and binds to rhinovirus species C.

ROLE OF VIRUSES

It has been known for some time that the main trigger of asthma exacerbations in children and adults are respiratory viruses, particularly human rhinovirus [6,7]. Moreover, rhinovirus wheezing in early life is strongly associated with the development of asthma [8]. Bizzintino *et al.* [9] showed that rhinovirus species C was the dominant trigger of asthma exacerbations in children, and was also associated with more severe illness. Follow-up of this cohort found that rhinovirus-C was also associated with an increased risk of recurrent exacerbations, especially in atopics [10]. Children hospitalized with bronchiolitis because of rhinovirus were also more likely to have recurrent wheeze [11]. The frequency of rhinovirus-C infections is much lower in the community [12], suggesting that asthmatic children are more susceptible to infection with rhinovirus-C, or alternatively experience more severe symptoms.

VIRAL LOAD

The measurement of rhinovirus load during wheezing illnesses is an emerging field of enquiry. One study [13[■]] investigated the impact of systemic corticosteroids in children having their first moderate-to-severe wheezing exacerbation. They found that among children with a high rhinovirus load, those treated with prednisolone had less recurrence. This suggests systemic corticosteroids may benefit a subgroup of children with high viral loads.

Rhinovirus loads were not different in children or adults with acute asthma/wheeze vs. controls

with uncomplicated colds or acute rhinitis [14,15[■]]. A potential caveat of these data is that the timing between the onset of infection and the assessment of viral loads may be variable during naturally acquired infections. To address this issue, viral loads were measured in adults with or without asthma after experimental rhinovirus infection, but again no differences were identified [15[■]]. In contrast, Jackson *et al.* [16[■]] reported that viral loads were increased and peaked earlier in asthmatics compared with nonasthmatics after experimental rhinovirus infection, and peak viral loads correlated with illness severity. This suggests that viral loads may be increased in a subset of patients.

INTERACTIONS BETWEEN VIRUSES AND BACTERIA

Bacterial pathogens (*Streptococcus pneumoniae*, *Haemophilus influenza*, *Moraxella catarrhalis*) were detected in neonates at increased risk for recurrent wheeze [17]. Moreover, a prospective study [18[■]] of asthmatic and nonasthmatic children who were monitored on a weekly basis over winter, found that rhinovirus infections were associated with increased detection of *S pneumoniae* and *M catarrhalis*, and children were at increased risk of moderate exacerbations when rhinovirus was detected together with these pathogens.

Teo *et al.* [19[■]] profiled viral and bacterial communities in the nasopharynx of high-risk children during all respiratory illnesses and also healthy visits in the first year of life. They found that the nasopharynx was dominated by six common genera; profiles dominated by *Haemophilus*, *Streptococcus*, and *Moraxella* were associated with increased risk of acute respiratory infection, whereas *Alloiococcus*, *Corynebacterium*, and *Staphylococcus* were associated with reduced risks. No bacterial or viral groups were associated with the presence of wheeze during lower respiratory illness. However, the presence of pathogenic bacteria at the time of viral infection enhanced risk of infection spread from the upper to lower respiratory tract, and increased severity of ensuing symptoms. Wheezing lower respiratory illness with rhinovirus-C in the first year of life or colonization with more *Streptococcus* sequences by 7 to 9 weeks were independently associated with wheeze in later childhood, particularly in those with early atopic sensitization. This suggests *Streptococcus* species together with rhinovirus-C are early-life risk factors for wheezing exacerbations in childhood.

A longitudinal analysis of almost 1000 children from birth to age 11 years found that antibiotic use in early life was associated with subsequent severe wheezing and asthma exacerbations, and reduced

antiviral responses in later life [20²²]. In this context, it is noteworthy that antibiotic use enhanced risk of detection of *Haemophilus*, *Streptococcus*, or *Moraxella* in the ensuing month [19²²]. These data highlight the importance of microorganisms in immune development and risk for asthma exacerbations.

INTERFERONS

The interferon system plays a crucial role in antiviral defence by inducing a robust antiviral state in infected and neighbouring cells preventing the spread of infection. Seminal work showed that bronchial epithelial cells from subjects with asthma had deficient interferon responses to rhinovirus in culture, and this was inversely related to viral control and more severe respiratory symptoms after experimental rhinovirus infection [21,22]. However, not all studies found that interferon responses were deficient in asthma [23]. These inconsistent findings can be explained by the growing understanding that asthma is a heterogeneous disorder comprising distinct molecular subphenotypes [24,25²]. Because interferons are modulated by multiple pathways that are dysregulated in asthma [26²], variations in interferon responses may reflect the nature of the inflammatory milieu that was present at the time of epithelial sampling rather than an intrinsic defect in the capacity to respond to viruses [27,28].

Rhinovirus infections can be mild or asymptomatic in asthmatics, suggesting that other environmental cofactors are important in triggering exacerbations in natural settings [29]. Interferon responses are upregulated in the upper and lower airways of children during naturally acquired asthma exacerbations, although responses were reduced in children with evidence of chronic airflow obstruction [30,31]. Interferon responses were also increased in asthmatic children who wheezed vs. those who did not during rhinovirus infections [32]. Moreover, interferon responses were increased in adults with asthma during viral exacerbations compared with uncomplicated colds [33]. Taken together, these studies highlight a dual role for interferons in asthma. On the one hand, interferons activate antiviral defence mechanisms that limit viral replication, whereas on the other hand they may also enhance the inflammatory pathways that determine expression of disease symptoms.

REGULATION OF INTERFERON RESPONSES

Parallel studies in humans and mouse models have been instrumental in unravelling the mechanisms that control interferon responses to rhinovirus.

Toll-like receptor 7 (TLR7) is a sensor for single stranded viral RNA. TLR7 expression was reduced in bronchial biopsies from asthmatics, regardless of atopy [34²²]. In mouse models, the absence of TLR7 impairs interferon responses to rhinovirus and results in increased viral replication, and exaggerated eosinophilic inflammation and airways hyper-reactivity [34²²]. Suppressor of cytokine signalling 1 (SOCS1) was upregulated in bronchial biopsy specimens from adults with atopic asthma, and SOCS1 suppressed rhinovirus-induced interferon responses but not proinflammatory responses [35²²]. Forkhead box A3 is a transcription factor that is upregulated in airway goblet cells from patients with asthma [36]. Forkhead box A3-induced goblet cell metaplasia, suppressed the expression of anti-viral genes, and inhibited viral clearance during rhinovirus infections.

Most immunological studies in asthma focus on one gene or pathway at a time. An alternative approach is to view a biological system as a network of interacting gene products [37]. This systems biology approach suggested that interferon regulatory factor (IRF) 7 was a major hub that controls expression of interferon gene networks during asthma exacerbations [31]. In mouse models, knockdown of IRF7 abolished interferon responses to rhinovirus [38²]. These data suggest that IRF7 plays a major role in the regulation of interferons during rhinovirus infections, and highlight the application of systems-level approaches to decipher biological responses.

INTERACTIONS BETWEEN INNATE ANTIVIRAL AND ATOPY-ASSOCIATED INFLAMMATORY PATHWAYS

Risk for severe asthma exacerbations is maximal in rhinovirus infected children who are atopic and exposed to high levels of allergen [39]. Clues to the underlying mechanism were provided by the finding of increased FCER1G and IL-4/IL-13-induced genes in peripheral blood from children with severe, virus-induced exacerbations [40]. It was subsequently reported that monthly anti-IgE therapy almost eliminated the seasonal spikes in exacerbations, thus confirming the importance of the IgE pathway [41]. Recent studies have extended this paradigm by identifying innate pathways that link viral infection with allergic inflammation. Beale *et al.* [42²²] reported that bronchial epithelial cells from asthmatic donors have exaggerated IL-25 responses after experimental rhinovirus infection, and this was related to atopic status [42²²]. Moreover, rhinovirus enhanced allergen-induced IL-25 in a mouse model, and blockade of IL-25 signalling attenuated rhinovirus-induced exacerbation of

allergic airways disease. Jackson *et al.* [16²²] reported that IL-33 was upregulated in the airways of asthmatics after experimental rhinovirus infection, and IL-33 expression was positively associated with increased viral loads and expression of respiratory symptoms. IL-25 and IL-33 drive the production of IL-5 and IL-13 in Th2 cells and type 2 innate lymphoid cells (ILC2). These pathways can be upregulated by both allergen and rhinovirus, thus explaining how concurrent exposure to these agents can drive the pathogenesis of severe exacerbations. Notably, IL-25 and ILC2 responses to rhinovirus are exaggerated in neonatal mice, which could explain in part why viral infections cause more respiratory illnesses in early life [43²²].

HOST RESPONSES AND VIRUS TYPE

The association between rhinovirus-C and severe exacerbations suggests that some rhinovirus species are more pathogenic than others [9]. Infection of differentiated sinus epithelial cells with different rhinovirus species revealed that rhinovirus-B species replicated more slowly, were less cytotoxic, and induced lower levels of proinflammatory cytokines compared with rhinovirus-A and C species [44²²]. Iwasaki *et al.* [45²²] showed that total IgG1 titres to rhinovirus-A and rhinovirus-C were increased in asthmatic children with exacerbations compared with nonasthmatic controls. In contrast, species-specific IgG1 titres (obtained after absorption of plasma with other species) to rhinovirus-C were much lower than responses to rhinovirus-A or rhinovirus-B in both groups of children. Together, these studies show that rhinovirus species can elicit differential immune responses.

GENETIC SUSCEPTIBILITY

The identification of asthma susceptibility genes was accelerated by the development of genome-wide approaches for genetic profiling. This is exemplified by the discovery of the 17q21 locus, which is the most replicated region for childhood asthma, and the strongest signal in the genome associated with severe asthma exacerbations [46,47]. Notably, variants on 17q are specifically associated with the development of rhinovirus wheezing but not RSV wheezing, and the effects of these variants on asthma susceptibility are mainly observed in children who wheeze with rhinovirus [48]. The 17q region contains 15 genes [49]. ORMDL3 and GSDMB are considered the most plausible candidate genes in this region, because their expression is correlated with 17q variants and is also inducible by rhinovirus

[48]. Functional studies [50,51] suggest ORMDL3 drives airways remodelling and hyperresponsiveness, via activation of the unfolded protein response and downregulation of sphingolipid synthesis. The role of GSDMB in asthma is unknown.

Children who are raised on a traditional dairy farm are protected from atopy, asthma, and wheeze [52]. Wheeze in the first year of life was positively associated with older siblings, and negatively associated with exposure to farm sheds, and these effects are only observed in carriers of risk alleles on 17q [53²²]. Data from mouse models indicate that exposure to farm dust mediates protection from asthma via upregulation of the ubiquitin-modifying enzyme A20 in the airway epithelium, which is a negative regulator of NF- κ B [54²²]. In addition, the protective effect of farm shed exposure on asthma risk is modified by genetic variation in the A20 gene [tumor necrosis factor alpha-induced protein (TNFAIP3)] [54²²].

Genome-wide association studies [47,55²²] have identified a few susceptibility genes for asthma exacerbations, including CTNNA3, SEMA3D, IL33, RAD50 Homolog, IL1RL1 (IL-33R), and CDHR3. The role of CTNNA3 and SEMA3D in asthma is unknown. RAD50 is located within the Th2 locus on chromosome 5q, between IL-5 and IL-13. The physiological function of CDHR3 is unknown; however, recent evidence indicates it binds to rhinovirus-C. Bochkov *et al.* [56²²] showed that CDHR3 is hyperexpressed in cells that are susceptible to rhinovirus-C infection compared with those that are resistant, and ectopic expression of CDHR3 in resistant cells conferred susceptibility to infection. They also showed that transfection of a CDHR3 variant associated with severe exacerbations (Cys₅₂₉ → Tyr, rs6967330) increased rhinovirus-C binding and replication by 10-fold, thus providing a plausible explanation for the association of these variants with exacerbations.

A powerful approach to discover genetic variation underlying biological traits is to map variants that explain variations in gene expression levels. This approach was employed to map genetic variation underlying peripheral blood mononuclear cell (PBMC) responses to rhinovirus in healthy individuals [57²²]. They found evidence for genetic regulation of 38 genes, and interestingly only a few of these genes (UBA7, IRF5, and OAS1) are known to be involved in antiviral immunity. This suggests there is much to learn about the molecular pathways underlying host responses to rhinovirus, and the application of these tools to airway epithelial cells is likely to unveil additional insights [58,59].

PREVENTING ASTHMA EXACERBATIONS

Current therapies can reduce the risk of exacerbations but cannot entirely prevent them, thus new treatments are urgently needed [60,61]. IFN β is an attractive option because it enhances antiviral immunity and inhibits Th2 and ILC2 responses [62,63[■]]. A trial of inhaled IFN β administered at the first signs of a cold did not reach its primary end point, but it did prevent loss of asthma control in a subset of patients with difficult to treat asthma [64[■]]. Another study [65] employed short-term targeted treatment with omalizumab (anti-IgE) prior to the start of school. Omalizumab halved the proportion of children who had at least one exacerbation, but was only superior to inhaled corticosteroid boost in a subgroup of patients who had an exacerbation during the run-in phase of the trial. Mepolizumab (anti-IL-5) reduced exacerbations by half in patients with severe eosinophilic asthma [66]. Benralizumab (anti-IL-5R) treatment within 7 days after a severe exacerbation reduced the rate and severity of subsequent exacerbations [67]. Lebrikizumab (anti-IL-13) reduced exacerbation rates by 60% in Th2-high patients with difficult to treat asthma [68]. Overall, these investigational studies demonstrate that targeted therapies can reduce exacerbations in a subset of patients. IL-25 and IL-33 represent promising targets for future clinical trials.

CONCLUSION

Asthma exacerbations are heterogeneous conditions that involve complex interactions between environmental exposures and immunological phenotypes in genetically predisposed individuals. Current medications and newer targeted therapies can reduce exacerbations in some patients but cannot entirely prevent them. The discovery of innate immune pathways that link viral infections with allergic inflammation will provide new opportunities for therapeutic intervention. The next challenge is to understand the interaction of these risk factors to identify asthma subphenotypes that will benefit most from next generation treatments, as well as identify the subgroup of children during their first wheezing illness who will go on to develop persistent asthma.

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Conflicts of interest

There are no conflicts of interest.

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The study showed that wheeze in early life was positively associated with older siblings, and negatively associated with exposure to farm sheds. They also showed that these effects were only observed in carriers of risk alleles on 17q, suggesting that this genotype confers both risk for wheeze and also allows environmental protection.

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The study showed that exposure to farm dust or low-dose endotoxin could protect mice from allergic asthma. They showed that this protection was dependent on expression of A20 in the airway epithelium. It was also demonstrated that genetic variation in TNFAIP3 (encodes A20) was associated with risk for asthma and allergy in children who grew up on a farm.

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The authors presented a meta-analysis of genome-wide association studies in children with asthma. They showed that variants in CTNNA3 and SEMA3D were associated with exacerbations.

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