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Immune Responses in Rhinovirus-Induced Asthma Exacerbations

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Abstract

Acute asthma exacerbations are responsible for urgent care visits and hospitalizations; they interfere with school and work productivity, thereby driving much of the morbidity and mortality associated with asthma. Approximately 80 to 85 % of asthma exacerbations in children, adolescents, and less frequently adults are associated with viral upper respiratory tract viral infections, and rhinovirus (RV) accounts for ~60–70 % of these virus-associated exacerbations. Evidence suggests that it is not the virus itself but the nature of the immune response to RV that drives this untoward response. In particular, evidence supports the concept that RV acts to exacerbate an ongoing allergic inflammatory response to environmental allergens present at the time of the infection. The interaction of the ongoing IgE- and T cell-mediated response to allergen superimposed on the innate and adaptive immune responses to the virus and how this leads to triggering of an asthma exacerbation is discussed.

Keywords

Rhinovirus; Asthma; Pathogenesis; Viral-induced asthma exacerbations; Innate immunity; Adaptive immunity

Introduction

Acute exacerbations are a hallmark of urgent care visits and hospitalizations for asthma as they interfere with school and work productivity and as such drive much of the morbidity (and, presumably, all of the mortality) associated with asthma. Equally important, the presence of frequent exacerbations defines an asthma phenotype that is most often

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Compliance with Ethical Standards

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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associated with the progressive loss of lung function [1–3]. Several emergency department studies have been conducted to determine the cause of these exacerbations. Early on, it was recognized that viruses were a likely culprit, and subsequently, it was demonstrated that 80 to 85% of asthma exacerbations in children are associated with some form of upper respiratory tract viral infection [4]. Rhinovirus (RV) accounts for 60–70 % of these virus-associated exacerbations in children over the age of 3 [5–12]. And, while a less frequent an occurrence, RV infections remain a common cause of asthma exacerbations throughout adulthood [13•]. In our studies at the University of Virginia, RV infections were identified in 61 % of children aged 3–18 years hospitalized with an asthma exacerbation [14] with similar numbers observed in a follow-up study that was performed in Costa Rica [15].

RV infections are common throughout the year with most not producing exacerbations and many being asymptomatic ([16] and unpublished data). However, examination of emergency department visits consistently reveals a spike in admissions in the fall with a similar but smaller increase in the spring [7, 17]. The fall increase was originally thought to be due to children returning to school and acquiring infections, but this failed to account for the spring increase in exacerbation rate and that RV infections occur throughout the year. These spikes do correspond to the fall and spring allergy seasons in the northern hemisphere where the studies were conducted. Support for an allergic component to the seasonal increases in asthma exacerbations was provided from a study that demonstrated the ability of anti-IgE to decrease the fall and spring exacerbation rate [17, 18••]. This review will focus on the role that RV plays in inducing inflammation through innate and adaptive immune responses and how this contributes to asthma exacerbations in the face of allergic inflammation.

Rhinovirus Biology

RV was originally isolated in 1956 and since has been determined to be the agent responsible for most of the upper respiratory tract cold symptoms [19, 20]. The virus is a positive sense, single-stranded nonenveloped RNA virus belonging to the picornavirus family with more than 160 strains identified by classical serology and newer sequencing methods [21]. The serotypes (or “clades”) can be classified as either HRV-A, -B, or -C viruses based upon genetic homology [21–23], though HRV-A and -C are the serotypes responsible for most RV-induced asthma exacerbations (Table 1). And, more recently, the very closely related enterovirus family has also been increasingly associated with asthma exacerbations. The viral genome encodes for both structural (capsid) proteins and nonstructural proteins that are involved in viral replication and virion assembly. Four viral capsid proteins (VP1, VP2, VP3, and VP4) make up the outer protein coat that houses and protects the viral RNA genome of mature RV virions [24]. It is the amino acid differences in these capsid proteins that are responsible for the antigenic differences among various RV strains.

Intercellular adhesion molecule-1 (ICAM-1) is the main receptor used by HRV-A and -B (>90 %) to gain entry into the cell, while the minor group receptor, low-density lipoprotein (LDL), is used by a minority of the serotypes (<10 %) [25, 26]. Binding of HRV-A and -B to ICAM-1 occurs near the site of binding by the integrin CD11a/CD18, and upon binding, the protein capsids disassociate allowing for entry of the viral RNA into the cell. ICAM-1 exists

both as a membrane bound and a soluble form with some believing the soluble form acts as a scavenger to help limit RV infection. As a counter to this response and to increase the spread of infection, RV induces expression of the membrane-bound form of ICAM-1 following infection of the respiratory epithelium [27]. Following identification of HRV-C in 2006 [23] and given its divergent nature and structure compared to the groups A and B viruses, it was recognized that this virus used a different mode of cellular entry. This was based in part on structural modeling studies indicating that neither ICAM-1 nor the LDL receptor would recognize the HRV-C capsid protein along with the difficulty in growing the virus using standard techniques that supported HRV-A and -B replication [28]. Utilizing fully differentiated sinus epithelial tissue that supported HRV-C replication, cadherin-related family member 3 (CDHR3) was identified as one receptor that allowed entry of the virus into host cells [29••].

Rhinovirus-Induced Respiratory Pathogenesis

The most common symptoms of RV infection are often limited to the upper respiratory tract in nonasthmatic individuals, peaking at days 3–4 postinfection. The symptoms in asthmatics also peak at the same time; however, the magnitude of response is higher and the duration persists longer [30]. Multiple studies have demonstrated a positive correlation between nasal lavage concentrations of inflammatory cytokines, particularly CXCL8 (IL-8) and CXCL10 (IP-10), and symptom severity [31, 32]. These chemokines lead to a neutrophilic inflammatory response that is associated with increased vascular permeability and stimulation of mucus hypersecretion resulting in rhinorrhea and nasal obstruction. The excess mucus production can lead to cough that is a result of posterior pharyngeal drainage and irritation of the throat. During RV infection, sinus involvement has been demonstrated in many individuals by computed tomography, which resolves quickly after the infection is cleared [33].

RV infection can induce lower respiratory tract symptoms, including cough, shortness of breath, chest tightness, and wheezing in patients with underlying asthma or other chronic lung diseases [13•, 34–38]. As IgE levels rise, the severity of lower respiratory tract symptoms increases in the asthmatic group [30]. Examination of the cellular response in the lower respiratory tract has demonstrated a higher neutrophilic and eosinophilic infiltrate with augmented type 2 cytokine production in asthmatics compared to healthy controls [39–41]. The basis for these lower respiratory symptoms has been controversial as it is unclear if they were due to infection of the lower airway epithelium or a reflection of indirect influences related to the immune response to the upper airway infection and trafficking of cells back to the lower airway. One of the strongest arguments against lower airway infection was the temperature sensitivity of replication of RV as it expands optimally at 33 °C, a temperature significantly lower than that of bronchial airway epithelium, but one typical of the nares [42]. However, numerous studies have demonstrated the presence of RV in the lower airway [43, 44] including work showing RV by in situ hybridization after experimental RV16 infection [38] and, importantly, increased rates of lower airway infection are observed in asthmatics compared to nonasthmatics [12]. While HRV-A serotypes replicate optimally at 33 °C, the higher temperature of the lower airways is not an absolute barrier to RV expansion as replication can occur, just at diminished levels [45]. An additional *caveat* is that

these studies examined infection by HRV-A viruses. To date, the role of lower airway infection by HRV-C has not been examined and may be very different than that of HRV-A, leading to higher infection rates and worse inflammation.

Many respiratory viruses, including influenza and respiratory syncytial virus, destroy the airway epithelial barrier through direct killing of the cells; however, RV by itself does not appear to cause overt cytopathology in healthy subjects. This was demonstrated using monolayers of adenoid tissue that were infected with RV [46] and are consistent with observations that failed to identify cytopathology in RV-infected nasal or bronchial biopsy tissue. Failure to identify cytopathology may reflect the rapidity by which apoptotic cells are cleared via phagocytosis not only by scavenger cells (e.g., macrophages) but also by adjacent healthy epithelial cells [47]. Caution is warranted as the tissue in these studies was from healthy subjects and infection was with a group A virus; the response in asthmatics or in response to group C virus has not been reported and may be different. Infection with RV increases vascular leakage and mucus secretion due in part to disruption of the epithelial barrier tight junction proteins [48]. RV infection leads to decreased expression of zona occludens-1, claudin-1, and E-cadherin mRNA and protein levels in human nasal epithelial cells [49]. This is mediated in part by toll-like receptor (TLR) 3 recognizing the double-stranded RNA (dsRNA) of the virus, insofar as poly dI:dC (a synthetic dsRNA) disrupted the airway epithelial apical junctions in a similar manner [50]. Disruption of the epithelial barrier facilitates translocation of bacteria and their soluble products through the junctions to basolateral epithelial receptors inducing further inflammation [48]. This process is likely also important through its ability to promote access of allergens.

Innate Immune Response to Rhinovirus

As discussed above, the peak of symptoms is observed at days 3–4 postinoculation after which viral load drops fairly rapidly with no differences being observed between healthy controls or asthmatics, although symptoms are greater and persist longer amongst asthmatics [30, 51••]. The speed at which symptoms develop is too rapid to be ascribed to the development and influences of an adaptive immune (T or B cell) response directed against RV. A de novo T cell response would take 10–14 days, while induction of a memory cell response would be quicker, but would still take 5–7 days. Symptoms developing at this early stage must therefore be mediated by the innate immune response to the RV. Alternatively, and consistent with the central role of IgE-dependent allergic reactions in driving asthma exacerbations, the infection could enhance an *already established* adaptive immune response to seasonally relevant allergens.

RV infection of the epithelium is rapid, as we have demonstrated in our studies, and results in assembly of up to 100,000 virions per cell and leading to the appearance of type I interferon and a drop in airway pH that occurs less than 24 h after experimental infection [52] (and unpublished observations). Detection of RV by the innate immune system makes use of the pattern recognition receptor (PRR) signaling pathways. Specifically, TLR2 expressed on the epithelial surface recognizes components of the RV capsid proteins. Once infected and the virus-derived RNA starts to induce transcription, double-stranded RNA is formed. RV-associated ssRNA and dsRNA is recognized by endosomal TLR3, TLR7, and

TLR8, and additionally, dsRNA is recognized by melanoma differentiation-associated gene-5 (MDA-5) and retinoic acid-inducible gene 1 (RIG-I) receptors [53, 54]. Following receptor activation, type I (IFN- α /- β) and type III interferon (IL-28A, IL-28B, and IL-29) expression is induced and acts to restrict viral replication. Studies using bronchial epithelial cells collected from healthy and asthmatic subjects have indicated a deficiency in type III interferon production from the asthmatic subjects that correlated with an increase in viral titer [55]. A *caveat* to these finding is that the investigators used bronchial epithelial cells rather than sinonasal epithelial cells, and these data were generated from in vitro culture rather than in vivo infection. Using nasal wash samples, Miller et al. found lower basal levels of type III interferon in asthmatics, but following infection, viral titer, and viral species were no different than identified in healthy controls [56]. They speculate that while the titer may not be different, asthmatics may be more likely to be infected due to the deficient type III interferon production [56]. Similar impairments in IFN- β expression from asthmatic epithelial cells have been described [57]. Aside from restricting viral replication, the IFNs along with IL-12 and IL-15 play important roles in cytotoxic and natural killer cell recruitment and activation [58] and deficiencies in IL-15 production in asthmatic have been described [59]. Additional cytokines released by RV-infected epithelium that influence the immune response include IL-6, IL-1 β , and IL-11. Correlations have been observed between IL-11 concentrations in nasal aspirates and clinical bronchospasm [60].

Following RV infection, numerous growth factors (G-CSF and GM-CSF) and chemokines (CXCL8, CXCL5 (ENA-78), CXCL10, and CCL5 (RANTES)) are produced and secreted that enhance granulocyte recruitment, survival, and activation. The majority of infiltrating granulocytes in the sinuses and lungs in response to RV are neutrophils, but moderate numbers of eosinophils can also be identified [61, 62]. CXCL8 and CXCL10 appear rapidly in the nasal lavage fluid and serum of RV-infected patients with concentrations paralleling the increases in peripheral blood neutrophil levels. Originally, it was thought that neutrophilic infiltration was important for viral control and that higher levels would indicate better outcomes; however, high levels of CXCL8 and CXCL10 early in infection and the subsequent neutrophilia link to the presence of more symptomatic RV infections [63]. The presence of eosinophils following RV infection may represent a by-product of worsening concurrent allergic reactions (discussed below) or an active response aimed at controlling the viral infection through secretion of RNAses found in their secretory granules [64].

Three epithelial-derived cytokines have been described that play a pivotal role in altering the T cell immune response to one capable of inducing or exacerbating a type 2 cytokine response: IL-25, thymic stromal lymphopoietin (TSLP), and IL-33 [65]. Measurements of baseline IL-25 levels from the lungs of asthmatics identified higher levels than in nonasthmatic subjects [40]. Following RV infection, IL-25 levels further increased with the difference being maintained between asthmatic and nonasthmatic subjects. These results were confirmed in vitro, with epithelial cells from asthmatics displaying a higher intrinsic ability to produce IL-25 [40]. Similar to IL-25, IL-33 is induced by epithelial cells following RV infection to a greater extent in asthmatic subjects and IL-33 levels correlate with viral load, IL-5 and IL-13 levels [41]. Direct induction of IL-5 and IL-13 from T cells and type 2 innate lymphoid cells (ILC2s) was demonstrated using supernatants collected from bronchial epithelial cells infected with RV [41]. These results provide further evidence that

RV can induce a type 2 cytokine response that could result in an exaggerated allergic immune response and thereby exacerbations in asthmatic individuals. Less is known concerning the role of TSLP following RV infection, although studies have reported increased levels of TSLP in nasal secretions from asthmatics infected with RV that was not seen in noninfected nonasthmatics [66, 67].

Adaptive Immune Response to Rhinovirus

If the viral burden becomes too great for the innate immune response to control, RV-specific T cells become engaged in virus eradication. As previously mentioned, viral titers begin to decline ~5 days after RV infection which is too rapid for the de novo activation and expansion of naïve RV-specific T cells, a process that takes 10–14 days. As such, the T cell response must occur through activation of preexisting effector/memory T cells responding to common epitope(s) displayed by the infecting RV. Consistent with this, epitope mapping of RV targeting T cells had identified an HLA-DR4-restricted CD4-specific epitope of RV39 VP1 maps to a region that is conserved across RV groups A, B, and C [68••].

RV induces maturation of DCs isolated from the blood of nonasthmatics as measured by increases in cell surface expression of MHC class II, CD80, CD83, and CD86 [69••]. The RV-matured DCs were able to stimulate proliferation of CD4 and CD8 cells with IFN- γ production from both cell types and IL-4 mainly from CD4 cells (Fig. 1 and Table 2) [69••]. Our results support and expand upon previous work that demonstrated CD4-specific T cell responses from cloned T cells stimulated with various RV serotypes [70]. Together, these results indicate that a memory T cell response is present that is capable of engaging quickly following RV infection. In summary, these observations imply that CD4⁺ T cells induced by one RV strain are capable of responding to other strains, that it can drive the observed rapid and potent T cell recall response, and finally, that it can include expression of type 2 cytokines and thereby exacerbate an ongoing allergic inflammatory response (Table 2).

Interplay Between Allergy and RV Immune Response

Many studies have demonstrated a clear link in children and adolescents between RV-induced asthma exacerbations and the presence of high titers of IgE to relevant allergens [15, 71, 72]. In our Costa Rica emergency department study, subjects who had a mite allergen-specific serum IgE titer of 100 IU/ml had a ~95 % chance of having an asthma exacerbation if they became infected with RV [15]. As previously discussed, experimental RV infection induces expression of a type 2 cytokine signature in asthmatics that develops during the early stages of infection. The end result is an increased number of eosinophils and eosinophil-associated inflammatory mediators (ECP and CysLTs) in nasal secretions [30]. These observations support the concept that RV produces its untoward effects through its propensity to indirectly exacerbate an ongoing allergic response to bystander allergens.

Our ongoing experimental RV infection studies in asthmatics have found that almost all subjects begin to develop lower airway symptoms within 24–48 h after inoculation. The peak of viral symptoms is at days 3–4 postinoculation which, as discussed earlier, is too fast for a de novo adaptive immune response to RV or even a memory T cell response to shared

RV epitopes, a process that would take at least 5 days postinfection to develop. It is therefore impossible to ascribe symptoms developing this early to the presence of an adaptive immune response to the virus. However, if an infection occurred during the allergy season, allergen-specific T effector lymphocytes would already be present and activated in the airways and circulation. Studies using MHC class II tetramers demonstrate that allergen-specific T cells are present in the circulation at a frequency of $1\text{--}5/1 \times 10^6/\text{ml}$ out of allergy season and increase ~10-fold during the allergy season [73]. It is plausible that in the presence of an ongoing T cell- and IgE-mediated inflammatory response to allergen, somehow, the development of an RV infection leads to a surge in type 2 cytokine production and enhanced IgE-mediated hyperreactivity. And, that together, these processes synergize to drive asthma exacerbations in susceptible individuals. This concept is certainly consistent with the intriguing observation previously discussed regarding the apparent capacity of neutralization of IgE to prevent seasonal (presumably largely RV-driven) asthma exacerbations [17, 18•]. However, the exact mechanism of how this occurs remains under active investigation by many laboratories.

Conclusion

While it is recognized that RV is involved in driving asthma exacerbations, the how and why this occurs remains largely unanswered. Progress has been made demonstrating that both the innate (early in infection) and the adaptive (late in infection) immune responses coordinate to eliminate the virus. Most asthmatics never have an exacerbation despite having two to three RV infections a year. What is clear is that most often, RV-associated asthma exacerbations coincide with a relevant allergen exposure. The interaction of the ongoing IgE- and T cell-mediated response to allergen superimposed on the innate and adaptive immune response to the virus and how this leads to triggering of an asthma exacerbation remains to be elucidated.

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Fig. 1.

RV-specific T cell proliferation in healthy volunteer. Representative flow cytometry showing IL-4 and IFN- γ expression in T cells (CD4) that were co-cultured with RV-pulsed DCs for 7 days. The x -axis displays cell proliferation as measured by carboxyfluorescein succinimidyl ester (CFSE) dilution with the y -axis demonstrating intracellular cytokine expression. Adapted from [69••].

Table 1

Comparison of viral serotypes in Costa Rica emergency room admissions

	Asthma (<i>n</i> = 68)	Acute rhinitis (<i>n</i> = 32)	Control (<i>n</i> = 14)
qPCR for RV, % positive	57.1 *	56.2 *	7.1
RV-A, % positive	50	59	0
RV-C, % positive	50	35	100
RV-B, % positive	0	6	
Other viruses, % positive	21	6	

Adapted from [15]

*
 $p < 0.01$ compared to control

Table 2

Proliferation and cytokine expression in RV immune surveillance

	CD4	CD8
CFSE low (%)	8.34 ± 3.4	3.46 ± 0.74
IFN- γ ⁺ (% of proliferating cells)	74 ± 9	45 ± 10
IL-4 ⁺ (% of proliferating cells)	39 ± 8	6 ± 2

Adapted from [69••]