

REVIEW

Rhinoviral infection and asthma: the detection and management of rhinoviruses by airway epithelial cells

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Summary

Human rhinoviruses (HRV) have been linked to the development of childhood asthma and recurrent acute asthma exacerbations throughout life, and contribute considerably to the healthcare and economic burden of this disease. However, the ability of HRV infections to trigger exacerbations, and the link between allergic status and HRV responsiveness, remains incompletely understood. Whilst the receptors on human airway cells that detect and are utilized by most HRV group A and B, but not C serotypes are known, how endosomal pattern recognition receptors (PRRs) detect HRV replication products that are generated within the cytoplasm remains somewhat of an enigma. In this article, we explore a role for autophagy, a cellular homeostatic process that allows the cell to encapsulate its own cytosolic constituents, as the crucial mechanism controlling this process and regulating the innate immune response of airway epithelial cells to viral infection. We will also briefly describe some of the recent insights into the immune responses of the airway to HRV, focusing on neutrophilic inflammation that is a potentially unwanted feature of the acute response to viral infection, and the roles of IL-1 and Pellinos in the regulation of responses to HRV.

Introduction

Human rhinoviruses (HRVs) are an important pathogen, infection with which is associated with significant morbidity and even mortality, particularly in the elderly [1–4]. HRV infection is common in association with community-acquired pneumonia [5–7]. There is increasing evidence linking HRV infection with the development of childhood asthma and recurrent acute asthma exacerbations throughout life. These areas have been the focus of several recent excellent review articles, which we highlight below [8–10]. In this article, we will briefly describe some of the recent insights into the immune responses of the airway to HRV, focusing on the detection of HRV by epithelial cells, and the roles of IL-1 and Pellinos in the regulation of responses to HRV.

Rhinoviral infection, bronchiolitis and asthma

Whilst the role of viral infections in the development of asthma remains a complicated and controversial area, the potential for severe lower respiratory tract infections in young children to have long-term effects

remains a concern [11]. HRV are recognized as the second most common cause of bronchiolitis in young infants, with only respiratory syncytial virus (RSV) detected more frequently [12, 13]. Transient wheeze with viral infections in childhood is mostly not associated with the later development of asthma [11] though more severe episodes of infection causing hospitalization are being linked to risks of establishment of atopic asthma [11], and atopic individuals may have worse responses to HRV [14].

In asthma, acute exacerbations have a major impact on quality of life and are rarely associated with mortality. Their frequency and requirement for emergency interaction with health services means they also contribute considerably to the economic burden of this disease. HRV are the dominant viral pathogen detected in as many as 60% of virally induced asthma exacerbations and the major pathogen associated with exacerbation related hospital admissions [15–17]. Despite this, how HRV infections trigger exacerbations, and the link between allergic status and HRV responsiveness, remains incompletely understood (reviewed in [8]). The distinction between upper and lower respiratory tract infection by HRV remains controversial, but current

opinion is that it is related to the health status of the individual. In non-asthmatic subjects, symptoms are usually confined to the upper respiratory tract and are those of the common cold, for example, rhinorrhea, nasal obstruction and occasionally coughing. In contrast, patients with underlying asthma or other chronic airways disease appear more likely to develop lower respiratory tract symptoms, including cough, shortness of breath, chest tightening and wheezing [18, 19]. One potential mechanism for this enhanced vulnerability is the finding that production of interferons (IFN) type 1 (IFN- β) and type 3 (IFN- λ), crucial anti-viral cytokines that modulate the host response to viral infection, is impaired in human bronchial epithelial cells (HBEs) from asthmatic donors, and that this deficiency is likely to be independent of the atopic state of the individual [20–24]. Interestingly, IFN deficiency has very recently been linked to increased HRV susceptibility in pregnant women and patients with cystic fibrosis [22, 25]. However, others have failed to show differential expression of IFNs in HBEs from asthmatic compared with normal subjects [26, 27], or enhanced viral replication during an infection [28]. Alternate theories linking HRV to acute exacerbations have been proposed, including a role for synergistic interactions between allergens and HRV leading to wheeze and amplification of airway inflammation, whilst enhanced neutrophilic infiltration in response to HRV infection, especially into the lower airways, has also been reported to aggravate asthma through enhanced airway oedema and smooth muscle contraction leading to greater airway inflammation (reviewed in [9]). Of note, HRV is commonly isolated alongside other bacterial and viral pathogens and thus may play a major role in predisposing the airway to concomitant or subsequent infections by modulating the hosts innate immune response, especially in patients with underlying airways disease [5, 29–30].

HRV serotypes and cellular entry

HRV are non-enveloped, positive, single-stranded RNA (ssRNA) viruses of the *Picornaviridae* family, with a genome in the region of 7.2 kb enclosed within a protein capsid of approximately 27 nm diameter. At present, 148 HRV serotypes have been identified [33–35]. These serotypes can be classified in multiple ways but there are two main methods. The first is via the receptor utilized to enter the host cell. Major group HRVs (88 serotypes) bind intercellular adhesion molecule-1 (ICAM-1), and in some instances make use of heparin sulfate proteoglycans (HSPG) for cellular entry [36], whilst minor group HRVs [12 serotypes bind low-density lipoprotein receptor (LDLR)]. A new and distinct group of HRV was discovered in 2007 [37], but

difficulty in culturing these viruses within a lab environment has limited the amount of information currently known about them, including the receptor that they bind to. HRV serotypes can also be divided into three phylogenetic groups, HRV-A (74 serotypes), HRV-B (25 serotypes) and HRV-C (> 50 serotypes), based upon sequence homology [38]. Over 90% of the known HRV-A and HRV-B serotypes utilize ICAM-1 to gain cellular entry, LDLR is used by 12 members of HRV-A, whilst the receptor for the new HRV-C group is currently unknown [38, 39]. However, methods to culture and produce HRV-C *in vitro* have very recently been developed, and should enable the unique features of HRV-C replication and biology to be identified [40–42]. HRV-C serotypes are emerging as a group of interest in younger children, particularly in those with asthma [43–45]. In addition, older children identified as positive for HRV were more likely to be infected with HRV-C serotypes [46].

It is now established that HRV enter host cells via receptor-mediated endocytosis, with the drop in pH within the endosome causing rapid viral uncoating and release of the ssRNA into the cytoplasm where viral replication and the generation of double-stranded (ds) RNA intermediates occurs. Two main methods of viral entry have been proposed (reviewed in [47]). The first is dependent upon clathrin-coated vesicles and may predominate for minor group viruses that utilize LDLR [48]. The second more recently reported method requires ceramide-enriched membrane platforms and is independent of clathrin and dynamin and predominates for major group viruses utilizing ICAM-1 [49, 50]. Interestingly, both entry pathways can trigger replication-independent responses to HRV infection, leading to direct and rapid activation of PI-3 kinase and downstream activation of Akt and NF- κ B, the generation of IL-8 [51–54], and presumably through this the tissue neutrophilia that is common after HRV infection [55, 56].

HRV detection by PRRs

Once HRV have gained entry to the host their early detection depends on the innate immune response and their identification by pattern recognition receptors (PRRs) within the airway, which have the ultimate aim of triggering pro-inflammatory and antiviral responses that result in the eradication of invading pathogens. These PRRs include Toll-like receptors (TLRs), as well as cytoplasmic RIG-like helicase receptors (RLRs) such as retinoic acid inducible protein I (RIG-I) and melanoma differentiation associated gene 5 (MDA5). The HRV capsid can be recognized via TLR2 on the epithelial cell surface [32, 57], but once HRV have gained entry to the HBEs early recognition is thought to predominantly be

via detection of HRV double-stranded (ds) RNA replication products by the endosomal sensor TLR3 [58–60]. This subsequently induces production of cytoplasmic RIG-I and MDA5, which also recognize dsRNA intermediates, to elicit the maximal response to viral infection [59, 60]. Although evidence strongly points to TLR3 having the major role in responses to HRV, a recent report has also suggested an alternative mechanism in which the endosomal single-stranded (ss) RNA sensors TLR7/8 are involved in rhinoviral recognition by HBECS [57]. However, we [61, 62] and others [59, 63, 64] have shown lung bronchial epithelial, alveolar and airway smooth muscle cells are relatively unresponsive to TLR7/8 ligands. It remains possible that TLR7/8 expression is induced upon HRV infection and subsequently plays a role in viral detection, but currently the dominant receptors involved in recognition appear to be TLR3 and the RLHs. The relative total contribution of each TLR and RLH to viral responses remains to be clarified. At present, relatively few studies have explored the biology of the primary airway cell in disease, and normal primary cells, epithelial cells from patients with COPD, or cells from patients with different asthma phenotypes or severities, may all have different levels of RLR/TLR expression and thus favour one or other recognition system at different points in the HRV replication lifecycle. Exposure to cigarette smoke also appears to be an exceptionally powerful modulator of the response to HRV in primary human bronchial epithelial cells [65]. In mouse models of chronic viral infection, there is also evidence that TLRs and RLRs play separate roles in the induction of local IFN responses and development of chronic antibody responses [66]. Recently, the act of viral fusion with the cell membrane has been shown to be capable of activating IFN responses and potentiation of TLR signalling via the protein STING [67], but its roles in HRV infection remain unknown. It is also important to note that the marked differences in the responses of the immune system in children and adults to viruses may have profound impacts on the nature of the responses to HRV. Chronic viral infection in children is handled very differently to adults (see review of [68]). Additionally and importantly, the timing and nature of viral infections experienced by the young may have longstanding and profound impacts on local viral and bacterial immunity [69, 70].

The mechanisms associated with the reported ability of TLR3 to detect HRV remain somewhat of an enigma, since its very location on the internal surface of the endosomal membrane and requirement for an acidified environment negates it 'seeing' the dsRNA viral replication products it is known to detect but which are generated within the cytoplasm. Autophagy has recently been proposed as a pathway by which cyto-

plasmic viral RNAs are presented to the endosomal TLRs.

Autophagy and PRR detection of HRV

Autophagy is a cellular homeostatic process that allows the cell to encapsulate its own cytosolic constituents within double-membrane organelles called autophagosomes. These can fuse with endosomes to produce amphisomes, which then acidify and fuse with lysosomes to acquire hydrolases and allow recycling or degradation of the sequestered material that includes long-lived proteins, cellular macromolecules and whole organelles (Fig. 1a) [71]. Autophagy is involved in numerous aspects of cell physiology and provides a defence against multiple forms of cellular stresses, including those involved in controlling infection, inflammation and immunity (reviewed in [72]). Recent reports suggest this may now extend to viral pathogen sensing and handling.

TLR activation (including TLRs 3 and 7/8) has been shown to up-regulate autophagy [73–75], however, the signalling pathways downstream of TLRs that trigger

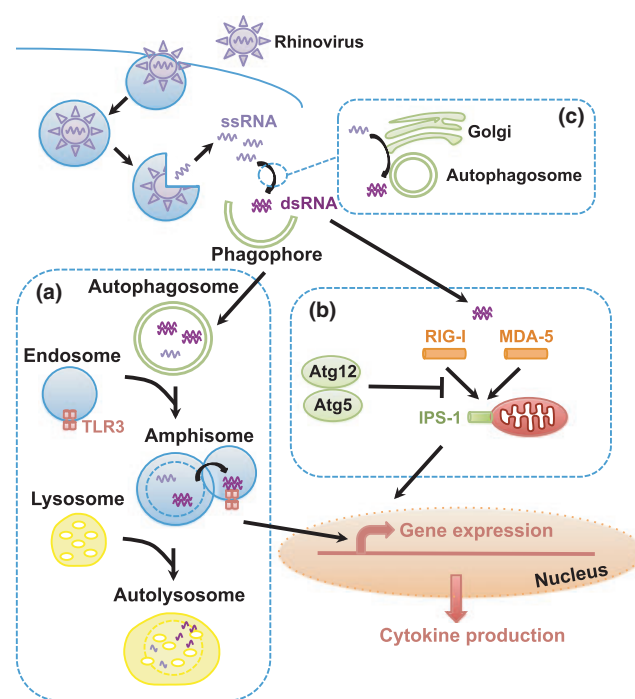


Fig. 1. Overview of the potential areas of crosstalk between autophagy and viruses. Human Rhinoviruses (HRV) enter host cells via endocytosis, ssRNA is released into the cytoplasm where viral replication occurs. (a) Autophagy transfers viral replication products from the cytoplasm to the endosomal compartment containing Toll-like receptor 3 (TLR3) to allow downstream signalling. (b) Autophagy proteins can also inhibit the association between RIG-like helicase receptors (RLRs) (RIG-I/MDA-5) and their signalling adapter IPS-1 resulting in a reduction in signalling. (c) Finally, RV subverts host cell membranes to enable their own replication.

autophagy have yet to be fully determined. In response to TLR stimulation the TLR adaptor proteins MyD88 and TRIF can both interact with Beclin-1, a key component of a class III phosphatidylinositol 3-kinase complex (PI3KC3) that initiates autophagosome formation [76]. Furthermore, this interaction recruits Beclin-1 to the receptor complex allowing TRAF6-mediated K63-linked ubiquitination of Beclin-1, which is crucial for its ability to initiate autophagy [77]. The link between autophagy and TLRs is further supported by recent publications showing that inhibition of cellular autophagy reduces type I interferon (IFN) production in response to TLR3 [78] or TLR7 [79] activation (Fig. 1a). Conversely, two studies have suggested an atypical role for autophagic proteins as suppressors of the RLR triggered antiviral immune response (Fig. 1b) [80, 81]. The heterodimeric autophagy protein 5 (Atg5)–Atg12 conjugate, a key autophagy regulator, associates with RIG-I and IPS-1 via their CARD domains and inhibits downstream signalling [80], whilst Atg5-deficient cells exhibit increased production of reactive oxygen species (ROS) and type 1 IFN [81]. To introduce a note of caution extrapolating these data to HRV, it should be noted that all of the above studies did not look directly at rhinoviral infection or use airway epithelial cells, but were restricted to predominantly murine cells or non-physiological overexpression strategies.

Limited studies have revealed that HRV can indeed regulate autophagy, although this is again not straightforward. HRV-2 and HRV-14, members of the minor and major serotype groups, respectively, both induce autophagosome formation [82, 83]. In contrast a second minor group virus, HRV-1A, is thought to be unable to promote autophagy [83]. Interestingly, it is now well-established that many viruses subvert autophagosome membranes to promote their own replication and release (Fig. 1c) [84], including HRV-2 [83], although initial work had suggested autophagy did not affect HRV-2 production [85]. Conversely, HRV-1A induces fragmentation of the Golgi apparatus to provide a site for viral replication [86]. However, these data were again not obtained in the natural host cell of HRV, the human airway epithelium. We have not observed major roles for autophagy in the mediation of responses to HRV in a human airway epithelial cell line (Ismail et al., in submission), but these data do not rule out a role for autophagy in HRV handling in other circumstances, e.g. the stressed epithelium of disease.

Indeed evidence supporting a role for autophagy in human lung disease is growing [87]. It is well-known that HRV infection is also a major cause of acute exacerbations in COPD patients, with augmented airway and systemic inflammation reported [88]. A recent seminal study described increased autophagy in clinical tissue samples from the lungs of COPD patients, which was

maintained throughout disease progression (GOLD stage 0 to 3/4) [89]. Evidence of increased autophagy was also observed *in vivo* in the airways of mice subjected to chronic smoke inhalation, and *in vitro* in human bronchial epithelial cells exposed to cigarette smoke extract (CSE) [89]. This work and subsequent studies identified autophagy as an early response event to cigarette smoke that may precede and ultimately trigger epithelial cell death, and thus COPD pathogenesis [89–91]. With regards to asthma the role of autophagy is less clear. Th1 (IFN γ) and Th2 (IL-4, IL-13) cytokines have been shown to differentially regulate autophagy in macrophages [92, 93], and asthma is often characterized as a Th2-driven inflammatory disease [94]. In addition, Atg5 mRNA expression was very recently shown to be increased in the human nasal epithelial cells of acute asthmatics compared to stable asthmatics and non-asthmatic controls [95]. This group also identified two single-nucleotide polymorphisms (SNPs) in the Atg5 gene, including one that caused enhanced promoter activity, and which they demonstrated to be functionally relevant and associated with childhood asthma [95]. Studies of autophagy in the different asthma variants of eosinophilic and non-eosinophilic disease, with all their varying subgroups [96], have yet to be undertaken. Overall, it is likely that the stressed epithelial cells of patients with COPD or various asthma phenotypes may show different levels of autophagy.

HRV and the induction of innate inflammatory responses

The vital and essential role of IFNs in the induction of effective responses to HRV is not in doubt or dispute, but has been excellently covered elsewhere [97–99]. However, recent work has also given new insights into other mechanisms that may result in the airway inflammation that is a potentially unwanted feature of the acute response to HRV. Here, we will summarize some work from our own group and others on the roles and regulation of IL-1 and IL-8 in HRV disease.

Induction of an allergic-type eosinophilic inflammatory response in response to viral infection may be at least in part dependent upon production of IFNs, since chemokines such as RANTES/CCL5 are heavily dependent upon IFN production for their transcription in this setting. Curiously, patients with asthma are at risk of severe eosinophilic exacerbations of asthma during a viral infection, though there are also good data that patients with asthma are deficient in the production of IFNs when infected with HRV, summarized above. The mechanism accounting for the decreased production of type 1 and type 3 IFNs during viral infection in asthma remains poorly understood. The production of IFNs is important in control of viral infection. HRV also infects

fibroblasts, and these cells are highly deficient in IFN production [100]. Exogenous IFN protects fibroblasts and epithelial cells from HRV-induced death [100, 101], and may also limit viral replication and adverse consequences of HRV infection in the epithelium [101]. Indeed, early trials are underway that are seeking to replace IFNs to limit viral replication. A recent publication revealed that RV-1B infection of mice lacking the MDA-5 receptor had delayed type 1, and attenuated type 3, IFN production with a concomitant transient increase in viral load [102]. However, of note, RV-infected MDA-5 null mice with allergic airways disease had viral titres comparable with wild-type mice despite their deficient IFN response, and interestingly the MDA-5 null mice had decreased RV-induced neutrophilic infiltration and airway hyperresponsiveness [102]. Thus, although IFN production may in part be linked to allergic airways inflammation and/or Th2-type T cell recruitment, neutralization of this inflammatory arm is likely to have complex effects on host recovery from HRV infection, particularly during airway disease.

Of interest, innate lymphoid cells (ILCs) are a heterogeneous cell population that have recently been ascribed a critical role in controlling inflammation and tissue homeostasis at mucosal sites, including the airway [103, 104]. Three functionally distinct subsets of ILCs have been identified: natural killer (NK) cells (cytotoxic ILCs), Ror γ t⁺ ILCs and type 2 ILCs (ILC2). Within the airway ILC2 appear to be the significant cell type involved in regulating viral infection [105, 106]. ILC2 accumulate within the lung in a murine model of influenza viral infection, and whilst they have little impact on viral load they do support maintenance of the airway epithelial barrier [106]. As discussed above, viral infections exacerbate symptoms in patients with asthma and a second study has identified ILC2 as essential mediators of influenza virus-induced airway hyperactivity (AHR), when methacholine was utilized to precipitate AHR, via a novel pathway involving the IL-13-IL-33 axis [105]. It seems likely that ILC2s will also play a role in HRV-induced immune responses, especially within the asthmatic airway given their propensity to drive Th2-mediated immunity and disease [107], and research exploring the ability of these cells to contribute to airway inflammation elicited by HRV is eagerly awaited.

HRV is also associated with a neutrophilic inflammatory response that may also contribute to the pathology of severe and life threatening asthma. The activation of TLR3, RIG-I and MDA5 by respiratory viruses all couple to production of neutrophil-recruiting chemokines such as IL-8, at least in part via activation of NF- κ B [62, 108–111]. There is a

well-recognized clinical association of viral and bacterial infections: in COPD, coinfections commonly occur [31, 112, 113] and in asthma, a viral exacerbation is not uncommonly followed by bacterial chest infection. We have shown that bacterial agonists most efficiently activate IL-8 production from epithelial cells when presented to these cells in the presence of innate immune cells such as monocytes [61, 62, 114, 115]. Such coculture models are probably relevant to asthma and COPD, where acute inflammation causes recruitment of monocytes as well as other leucocytes. We have recently shown that simple models of coinfection can be established in which HRV is allowed to infect epithelial cells in the presence of monocytes and LPS. These models show that the combination of HRV infection and LPS exposure in the presence of monocytes causes markedly enhanced production of IL-8 [109]. This amplified production of IL-8 can be significantly reduced by antagonism of IL-1, a key mediator released from LPS-activated monocytes [62, 114, 116]. HRV infection can also directly induce IL-1 production [117], and there are strong links between viral infection and activation of the inflammasome and IL-1 processing, recently summarized by Jacobs and Damania [118]. Thus, it may be that inhibition of IL-1 could reduce the lung inflammatory response to HRV, especially in the context of a coinfection. The many pathways regulating inflammasome activation, for example including the role of TRIMs [119] hints at further levels of complexity to come.

We have recently found that inhibition of epithelial TLR/IL-1 signalling by knockdown of the key signalling molecule MyD88 or treatment with IL-1 receptor antagonist reduces IL-8 generation in response to HRV even in the absence of monocytes or endotoxin [109]. However, such strategies are also associated with a moderate increase in viral replication, perhaps suggesting that targeting this axis may have some unwanted effects on viral control, and that antagonism of IL-1 may not be without adverse consequences.

Interest therefore has begun to focus on targets that allow downregulation of innate immunity as exemplified by downregulation of IL-8 production, without inhibition of IFN production or unwanted permissive increases in viral replication rates. The increasing evidence for links between IL-1 signalling and IFN production or signalling, as shown by our recent study, make such targets challenging. Recently, we have found that an E3 ubiquitin ligase Pellino-1 may be one such target. Pellino-1 was originally described as a regulator of IL-1 signalling by control of IRAK1 ubiquitination [120]. However, in mice Pellino-1 regulates late NF- κ B activation induced by TLR3 via the kinase

RIP1 [121]. In primary human epithelial cells, we have shown that Pellino-1 knockdown reduces IL-8 production in response to HRV infection, without allowing increases in rhinoviral replication or inhibiting type 1 and type 3 IFN production [108]. This control of IL-8 production appears to be independent of control of IL-1 signalling. These data may suggest that Pellino-1 will be a valuable therapeutic target to limit viral inflammation in the airways. To add complexity, in mice Pellino-1 expression is regulated by the transcription factor IRF3 [122], a key component of the IFN signalling pathway, so future studies may uncover critical links between IL-8 production and IFN signalling mediated by Pellino-1 or other members of the Pellino family.

Conclusion

Recent developments in the identification of pattern recognition receptors involved in detection of HRV infection, and their downstream signalling pathways, may allow separate manipulation of specific components of the response to HRV, limiting the inflammatory response without undermining the antiviral immune response. The contribution of additional cellular systems such as autophagy to the handling and control of HRV infection remains more obscure, and is likely to be dependent on disease phenotypes and severity.

Conflict of Interest

The authors declare no conflict of interest.

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