

Rhinoviruses in the pathogenesis of asthma: The bronchial epithelium as a major disease target

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In the 1950s and early 1960s, David Tyrrell described newly identified small RNA viruses, human rhinoviruses (HRVs), as the cause of the common cold. With more than 100 serotypes comprising major and minor subgroups, HRVs preferentially infect nasal epithelial cells through the intercellular adhesion molecule 1 and low-density lipoprotein receptors, respectively. Virus internalization also involves activation of the acid sphingomyelinase, which transforms membrane rafts into ceramide-enriched membrane platforms, followed by activation of p38 mitogen-activated protein kinase by the small G protein Rho A. Until the 1990s, HRVs could only be identified by their cytopathic effect on cultured He La cells; however, the advent of improved nucleic acid-based detection methods has provided a diagnostic tool for this and other respiratory viruses.^{1,2} Stress and a lowering of body temperature predispose to HRV infection, but usually the symptoms are mild and transient, being limited to coryza and pharyngitis,³ with virus nucleic acid being detected in nasopharyngeal,⁴ tonsillar,⁵ and middle ear⁶ fluids.

The ability to identify respiratory viruses by their nucleic acid signatures in nasal and lower airway secretions using PCR-based technology led to a series of studies demonstrating the presence of respiratory viruses in 60% to 93% of acute exacerbations of asthma in children and adults with a particular propensity during the fall, winter, and early spring months, when, at a community level,

Abbreviations used

HRV: Human rhinovirus

OR: Odds ratio

RSV: Respiratory syncytial virus

infection with these viruses is most prevalent.⁷ The majority of respiratory virus infections are caused by HRVs. Experimental infection of subjects with mild asthma followed by bronchial biopsy has demonstrated virus RNA to be largely restricted to epithelial cells but also present in some mononuclear cells and fibroblasts.^{8,9} *In vitro*, HRV has also been shown to infect smooth muscle cells, but as in the case of macrophages, despite enhancing certain cellular functions,^{10,11} HRVs are unable to replicate in these cells.

A high proportion of infant wheezing is associated with HRV infection.¹² The children with HRV infection, when compared with those with respiratory syncytial virus (RSV) infection, were older (13 vs 5 months) and presented more frequently with atopic dermatitis (odds ratio [OR] 16.7; 95% CI, 2.22-100) and blood eosinophilia (OR, 2.22; 95% CI, 1.04-50), suggesting a pathophysiologic link to asthma. A French study of 192 infants age 3 years or less hospitalized for acute bronchiolitis¹³ revealed a causative virus in 72.5% of cases comprising RSV (30%), HRV (21%), enterovirus (9%), influenza A (6%), and human metapneumovirus (4%), with some mixed infections.

In this issue of the *Journal of Allergy and Clinical Immunology*, Malmström et al¹⁴ have evaluated the presence of HRV in the lower respiratory tracts of corticosteroid-naïve infants age 3 to 26 months with recurrent respiratory symptoms by using *in situ* hybridization applied to lower airway biopsy specimens. They detected HRV RNA localized to the epithelium in 45% of biopsy specimens associated with abnormal lung function measured as decreased airway conductance in 86% of HRV-positive versus 58% of HRV-negative infants. For ethical reasons, the authors were unable to use healthy control subjects. However, acknowledging this limitation, these unique findings strengthen the potential pathophysiologic role for HRV in infant wheezing and asthma. In a further

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study of 81 children in Finland who had been hospitalized for wheezing at less than 2 years of age, evaluation during the third year of life revealed asthma in 40% of children, of whom 90% were atopic. In this study early predictors of asthma were atopic dermatitis (OR, 3.5; 95% CI, 1.2-10.1), specific IgE (OR, 3.5; 95% CI, 1.2-10.1), and inhalant allergy (OR, 11.3; 95% CI, 1.9-67.6). The authors also reported that RSV during the wheezing episodes in infancy was relatively rare, but in contrast, HRV infection was common (58%).¹² An important feature of this study was the fact that the infants subjected to bronchoscopy had been symptomatic for a median period of 6 months before airway biopsy and therefore were at special risk of asthma. A recent study by Xatzipsalti et al¹⁵ has shown that in hospital admissions for wheezing illness, those infants who had asthma when compared with those without asthma, were at greatest risk of having circulating HRV detected by means of PCR for up to 6 weeks after infection, suggesting that in asthma there is a defect in epithelial barrier function. An association between HRV infection in infancy and the later development of asthma has been reported by Lemanske et al,¹⁶ who have shown that in a population of 285 children genetically at risk for asthma studied during the first 3 years of life, HRV infection in the first year was the greatest risk factor for persistent wheezing in the third year (OR, 10.0), with 63% of infants who wheezed during RV seasons continuing to wheeze in the third year when compared with only 20% of all other infants (OR, 6.6). Other respiratory viruses, including RSV, also increased the risk of persistent wheezing at age 3 years but to a substantially lesser degree than observed with HRV. The authors suggest symptomatic HRV illness during infancy is likely to be an important risk factor for the development of asthma. Heymann et al¹⁷ came to a similar conclusion that viral infections, especially HRV, were the dominant risk factor for wheezing among children hospitalized before age 3 years, whereas in those aged 3 to 18 years, this was replaced by atopy as the dominant risk factor.

A key question arising from these studies is whether atopy and associated allergic inflammation is the cause of asthma or whether some primary abnormality in the airways epithelium, possibly revealed by virus infection, predisposed individuals to express their atopy as asthma. The recent discovery that airway epithelial cells are deficient in their capacity to generate interferon- β (IFN- β) when infected with HRV¹⁸ raises the possibility that a defect in innate immunity might underlie the origins and persistence of asthma. The normal response of the airway epithelium to virus infection is the induction of primary IFNs, such as IFN- β , through activation of the Toll-like receptor 3, which recognizes viral double-stranded RNA, leading to apoptosis that is able to effectively eliminate the infected cell and therefore limit viral replication and release. However, in asthmatic epithelial cells a major defect in this pathway leads to enhanced viral replication and virus-induced cell cytotoxicity. Evidence that this pathway might be relevant to the persistence of asthma, as well as exacerbations, comes from the demonstration

that in asthmatic patients HRV can persist up to 6 weeks after infection^{19,20} and that in patients with severe asthma HRV is detectable in airway biopsy specimens between exacerbations.²¹ There is also evidence to suggest that in asthma the coexistence of atopy enhances the clinical effect of HRV infection and both prolongs and enhances airway hyperresponsiveness.²² The mechanisms through which HRV leads to altered airway function associated with exacerbations is not known for certain, but in addition to the enhanced cell cytotoxicity observed in asthmatic epithelial cells,¹⁸ infection with HRV induces mucin production,²³ delays epithelial repair,²⁴ induces cytokine and chemokine release to recruit secondary effector cells,²⁵ and promotes the production of growth factors.²⁶

The inability of the asthmatic airway epithelium to effectively defend the lung against what is normally an innocuous virus raises the possibility of this being part of a broader epithelial defect relevant to asthma pathogenesis. The recent discovery of filaggrin as a key molecule involved in maintaining epithelial integrity and its dysfunction in atopic dermatitis and asthma²⁷ adds support to the view that diseases such as eczema and asthma are disorders of the epithelium rather than primarily being diseases of the adaptive immune response. A high proportion of the novel genes identified by positional cloning in asthma are epithelial in origin (eg, ESE3, DPP10, HLA-G, GPRA, PCDH-1, and filaggrin). A reduced ability of the airway epithelium to defend itself against multiple environmental insults could help explain why so many different environmental factors are risk factors for the development of asthma (eg, domestic chemicals, environmental tobacco smoke, outdoor and indoor air pollutants, allergens, and viruses). In addition to an impaired innate response to virus infection, asthmatic airway epithelial cells cultured and differentiated on an air-liquid interface exhibit reduced transepithelial electrical resistance that, when compared with normal epithelial cells, decreases further on exposure to irritants, such as tobacco smoke extract.²⁸ This decrease in transepithelial electrical resistance is explained by deficient formation of tight junctions and organizations of their constituent proteins that include occludin, ZO1, and the claudins. Such a fundamental barrier defect would serve to facilitate the passage of inhaled environmental materials from the surface into the airway wall, thereby augmenting inflammatory process in the airway wall. Thus, by interacting with environmental insults, such as virus infections and pollutants, the airways in asthma might be reacting as a chronic wound in which defective epithelial repair delays healing and maintains inflammation.^{29,30} Recent studies showing that continuous or intermittent treatment with inhaled corticosteroids, although suppressing symptoms, have no effect on the natural history of asthma in asthma-prone infants up to 2 years of age suggests that inflammation alone is insufficient to explain the origins of asthma in childhood and may even reduce lung growth.³¹⁻³³ These findings could be explained by a primary defect in the epithelial mesenchymal trophic unit that is engaged both during

branching morphogenesis of the developing fetal lung and in asthma.³⁴

This different view of asthma pathogenesis could lead to the development of a new approach to asthma prevention and treatment, in which greater emphasis is placed on trying to protect the airways against environmental insults rather than solely focusing on the suppression of inflammation. One recent example of a similar approach being adopted in inflammatory bowel disease has been the use of growth factors, such as epidermal growth factor³⁵ to accelerate mucosal healing and keratinocyte growth factor to protect the oral mucosa from ulceration (mucositis) during cancer chemotherapy.³⁶ One therapy currently being investigated in asthma is synthetic surfactant, inhalation of which has been shown to protect against allergen-induced bronchoconstriction,³⁷ possibly by acting as a barrier because endogenous surfactant production is also deficient in asthma.³⁸

Finally, with the population continuing to age, it should not be forgotten that HRV infection is a major cause of lower airway problems in the elderly,^{39,40} especially in those with chronic obstructive pulmonary disease.⁴¹ The recent discovery that the airway epithelial cells of patients with chronic obstructive pulmonary disease and smokers also deficient in IFN- β production and are even more sensitive to the cytotoxic effects of HRVs⁴² raises the possibility that with smoking or aging, the IFN- β pathway is compromised, thereby increasing the risk of severe, acute lower airway infection.

Although generally a benign virus, HRV is teaching us that both in the origins and exacerbations of asthma, as well as in the elderly and those who smoke, altered innate immunity increases the risk of significant common lung diseases. Asthma is a complex disorder that in many cases involves allergic pathways, but increasingly, research is steering us away from simply thinking of the disorder solely in terms of allergic inflammatory mechanisms and redirecting us to the target organ, which is likely to be the prime origin of disease susceptibility.

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