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Rhinovirus-Induced Airway Disease: A Model to Understand the Antiviral and Th2 Epithelial Immune Dysregulation in Childhood Asthma

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Abstract

Rhinovirus (RV) infections account for most asthma exacerbations among children and adults, yet the fundamental mechanism responsible for why asthmatics are more susceptible to RV than otherwise healthy individuals remains largely unknown. Nonetheless, the use of models to understand the mechanisms of RV-induced airway disease in asthma has dramatically expanded our knowledge about the cellular and molecular pathogenesis of the disease. For instance, ground-breaking studies have recently established that the susceptibility to RV in asthmatic subjects is associated with a dysfunctional airway epithelial inflammatory response generated after innate recognition of viral-related molecules, such as double stranded (ds) RNA. This review summarizes the novel cardinal features of the asthmatic condition identified in the past few years through translational and experimental RV-based approaches. Specifically, we discuss the evidence demonstrating the presence of an abnormal innate antiviral immunity (airway epithelial secretion of type I and III interferons), exaggerated production of the master Th2 molecule thymic stromal lymphopoietin (TSLP), and altered antimicrobial host defense in the airways of asthmatic individuals with acute RV infection.

Introduction

Rhinovirus (RV) infection is the most common cause of asthma exacerbations in children, and is associated with progression of the asthmatic condition beyond childhood (1, 2). Large birth cohort studies have identified that RV infection in the first 3 years of life is associated with an almost 10-fold increase in risk for asthma at age 6 (1, 2), which may be higher in individuals with genetic susceptibility (3). At first glance, it may seem logical to think that RV pathogenicity in asthma is entirely attributable to classical viral invasion and replication in the lungs leading to severe injury and inflammation of the airways; however, there is increasing evidence demonstrating that RV, more than an aggressive viral respiratory pathogen, is a potent environmental challenge that unleashes abnormal airway immune responses in susceptible hosts independently of active RV replication (4, 5). Interestingly, there is compelling data suggesting that genetic predisposition (3), host factors such as young age (6) and atopy (7), and environmental influences like winter season (8) positively regulate the pro-asthmatic effects of RV in airway immune responses. Nonetheless, the fundamental mechanism that underlies why asthmatics are more susceptible to RV-induced airway disease than otherwise healthy individuals is still largely unknown. This RV-related pro-asthmatic mechanism represents a potentially unique opportunity to investigate the molecular basis of one of the most phenotypically complex respiratory conditions seen in humans of all ages.

Recently, there has been increasing interest in developing airway models to investigate the RV-mediated activation of epithelial innate immunity in asthma. This innovative research approach has dramatically expanded the “Th2 bias” paradigm of the disease, providing new clues about its cellular and molecular pathogenesis. Indeed, ground-breaking studies have recently established that the susceptibility to RV in asthmatic subjects is associated with the dysfunctional airway epithelial inflammatory response that is generated after innate recognition of viral-related molecules such as double-stranded (ds) RNA that activate highly conserved pattern recognition receptors (i.e., toll-like receptors) located in the respiratory tract (9–11). This review summarizes the novel cardinal features of the asthmatic condition identified in the past few years through translational and experimental RV-/dsRNA-based approaches. Specifically, we discuss the evidence demonstrating the presence of an abnormal innate antiviral immunity (airway epithelial type I and III interferons), exaggerated production of epithelial-derived molecules that prime Th2 responses (i.e., thymic stromal lymphopoietin), and altered antimicrobial host defense in the airways during acute RV infection.

Abnormal Type I and III Interferon Responses to RV in Asthma

Interferon (IFN) signaling is crucial for innate antiviral host defense against respiratory viruses. There is evidence implicating IFN- β (type I) and IFN- λ (type III) as the most significant IFNs secreted by the airway epithelium during acute RV infection (12, 13). IFN- β and IFN- λ s signal via their respective receptors with downstream activation of interferon-related factors (IRFs) that subsequently initiate transcription of IFN-stimulated genes (ISGs). ISGs orchestrate RV elimination by interfering with essential processes for viral replication and invasion (i.e., virus-associated protein trafficking and/or virion assembly)

(14–16). IFNs also modulate apoptosis and generate an autocrine loop (positive feedback) to promote antiviral responses and/or initiate programmed death in uninfected cells (14–16). Interestingly, a recent study identified that RV interferes with innate IFN production via the induction of the transcription factor FOXA3, which inhibits IRF-3 phosphorylation and ISGs expression, leading to reduced IFN responses and inhibition of viral clearance (17).

Type I and III IFN airway production can be evaluated experimentally by ex vivo dsRNA stimulation of either human nasal epithelial cells (HNEC) or human bronchial epithelial cells (HBEC). HNEC and HBEC can be grown submerged as a cellular monolayer, or can be differentiated at air-liquid interface (ALI) to generate a pseudostratified polarized structure that closely resembles the in vivo human respiratory epithelium (18, 19). Ex vivo dsRNA exposure activates TLR-3, retinoic acid inducible gene (RIG-I), and melanoma differentiation-associated gene 5 (MDA5) signaling pathways in HNEC or HBEC, which downstream initiate transcription of IFN genes and other NF κ B-related pro-inflammatory genes (20–22). Through the use of ex vivo dsRNA stimulation approaches, it has been established that virally induced IFN- β and IFN- λ production is significantly reduced in airway epithelial cells harvested from adult asthmatics (11, 23). Moreover, abnormal type I and III IFN responses have been observed in both epithelial models of ex vivo and in vivo RV infection in adult asthmatic individuals, particularly in those with severe disease and/or atopic phenotype (24–26).

Recent studies investigating type I and III IFN responses in the pediatric population have also identified reduced IFN secretion after RV/dsRNA airway epithelial stimulation in mild/moderate atopic asthmatic and atopic non-asthmatic children compared with that seen in non-atopic non-asthmatic children (27). Interestingly, the link between IFN dysregulation and RV during childhood seems to be more complex. This was recently illustrated by a large study (n=409) which reported that wheezing and disease severity in asthmatic children (5–18 years) is associated with higher in vivo airway levels of type III IFN- λ during acute RV infection (28). This data suggests that more than a simple deficiency of IFN response, there seems to be an underlying dysregulation in the signaling pathway that controls IFN production in the asthmatic epithelium. Although more research is needed to elucidate this mechanism of IFN dysregulation, to date, there is compelling evidence supporting the notion that childhood asthma is associated with abnormal RV-induced innate IFN responses, and that this airway immune dysregulation may interplay with the phenotypical features of the disease in children.

RV-induced Thymic Stromal Lymphopoietin: The Molecular Link between Antiviral and Allergic Responses in Asthma

Thymic stromal lymphopoietin (TSLP) is a recently discovered epithelial-derived cytokine that plays a determinant role in regulating the initiation and maintenance of allergic immune responses. TSLP's main function is to prime the differentiation of naïve T0 cells into Th2 lymphocytes via activation of antigen-presenting cells (29). TSLP overexpression leads to asthma-type Th2-driven airway changes, and its selective inhibition attenuates the airway changes seen in animal models of allergic asthma (30–32). Notably, the strongest evidence of the link between TSLP and the pathogenesis of Th2 responses in asthma comes from a

recent randomized, double-blinded, controlled clinical trial which identified that a human anti-TSLP monoclonal immunoglobulin G2 λ administered to subjects with asthma dramatically reduced allergen-induced bronchoconstriction and indexes of airway inflammation before and after allergen challenge (33). This recent “proof of concept” study highlights the critical role of TSLP in the generation of the asthmatic phenotype.

TSLP is also considered a molecular link between viral respiratory illnesses and the characteristic Th2 responses of asthma. TSLP is induced by RV infection or exposure to ds-RNA in animal models (34), where it is linked to the secretion of strong Th2/atopic mediators such as IL4, IL13 and eotaxin (34). Interestingly, Nagarkar et al., using a murine model of allergic asthma, showed that RV induces increased pulmonary eosinophils, eotaxin and Th2 cytokines with no significant difference in RV viral load, suggesting that the pro-asthmatic Th2 inflammatory response driven by RV in the asthmatic state is independent of its replication rate (35). In addition, ex vivo models of harvested human airway epithelial cells obtained from adult asthmatics confirmed that dsRNA exposure triggers exaggerated production of TSLP accompanied by blunted IFN- β secretion relative to control subjects (11). Collectively, these data support the notion that the human asthmatic condition in adults is characterized by enhanced TSLP/Th2 responses and abnormal antiviral epithelial immunity, which together may result in airway hyperreactivity during acute RV infections.

In the context of RV-induced airway disease in early life, we have recently reported that children under 3 years of age with acute natural RV infections have disproportionately higher levels of TSLP in nasal lavage samples compared with controls (36). These increased TSLP levels were associated with higher Th2 cytokines (IL-4/IL-13) relative to antiviral Th1 cytokines (IFN γ /IL12). TSLP also showed moderate positive correlation with the Th2/Th1 cytokine ratio in RV-infected children (36). These observations suggest that RV plays an important role in promoting a shift in immune responses toward Th2 in early life, which may facilitate the development of pro-asthmatic biophenotype during the first 3 years of age. The augmented airway inflammation might not be related to an increase in the susceptibility to RV; rather, viral infection may set a pro-inflammatory cascade that outlasts the presence of the virus. Although more research is needed to elucidate the mechanisms of this predominant TSLP/Th2 response during RV infection, it seems clear that TSLP enhances and orchestrates the atopic Th2 responses characteristic of the asthmatic lung (29–33).

RV Alters the Antibacterial Host Defense of the Respiratory Tract

Microbes perform an important physiological function in shaping the immune response of the airway mucosa. Epidemiological studies in children have shown that exposure to microbes early in life (e.g., farm exposure) confers a protective role in asthma (37). Evaluation of the lung microbiome in various disease states has identified some changes in microbial diversity and/or enrichment with specific pathogenic or nonpathogenic bacteria (38). Thus, it is plausible that specific components of the lung microbiome may prime the immune system to have a beneficial or detrimental immune response. For instance, early microbial exposure might lead to inadequate “priming” of the immune system during maturation, resulting in Th1–Th2 cell subset imbalances, regulatory T cell deficiency, or

innate immune abnormalities. Interestingly, in parallel with recent advances in innate immunity, there has also been great progress in the generation of high-throughput sequencing technology to investigate microbial populations in the airway during health and disease (39). Most of this technology relies on common markers for bacterial 16S rRNA, which allow taxonomic classification using bioinformatics tools to integrate next-generation data with existing databases and computational algorithms that lead to accurate and rapid identification of species and strains of pathogens (40). Although the interactions between lung microbiome and innate immunity are largely unknown, recent studies investigating lung microbiome and airway inflammatory changes have revealed new potential links. For instance, it has been recently established that chronic obstructive lung disease (COPD) exacerbations occur after infection with a new bacterial strain or a change in bacterial load from the existing microbiota (e.g., *Haemophilus influenza*) (41), which in turn is associated with increased lung inflammation, as determined by cellular analysis and neutrophil elastase in the sputum (41).

Interestingly, RV-induced illnesses have recently been suggested to interact with changes in lung microbiome. Mallia et al. identified that after experimental RV infection, secondary bacterial overgrowth was detected in 60% of subjects with COPD (42). Secondary bacterial infection has been linked to RV infection during naturally occurring COPD exacerbations as well (43). In addition, Kloepper et al. recently reported that RV infection enhances detection of specific bacterial pathogens in the upper airway, particularly *Streptococcus pneumoniae* or *Moraxella catarrhalis* (44), which correlated with an increased risk of expressing illness symptoms during asthma exacerbations (44). The exact mechanism by which RV infection is associated with this change in the microbial environment is not known; however, there is new evidence suggesting that RV promotes the secretion of antibacterial peptides that could potentially play a role in modifying the microbiome. Rohde et al. (45) showed that RV infection in asthma leads to increased release of CXCL8/IL-8, thereby attracting neutrophils into the airways where they release HNP 1–3, which further enhances neutrophil activity (45). This information, together with earlier studies which established that neutrophil elastase is significantly increased only in RV-infected subjects with COPD and secondary bacterial infections (42), suggest that the secretion of RV-induced antibacterial peptides and chemotactic molecules may modify the microbiome balance in the airways, which in turn could lead to altered mucosal immune defense and consequent colonization or expansion of potentially pathogenic micro-organisms.

Summary

Epidemiological cohorts have established that asthma is associated with abnormal airway responses to respiratory viruses, particularly RV. Ex-vivo studies of human airways have identified that the asthmatic condition is characterized by a dysfunctional immune epithelial signaling response after innate recognition of viral-related molecules such as dsRNA. This abnormal epithelial response translates into abnormal innate antiviral immunity (airway epithelial type I and III interferons), disproportionate production of the “master Th2 molecule” TSLP, and altered antimicrobial host defense in the airways (Fig. 1). Further investigation of the signaling events associated with newly identified innate immune defects of the asthmatic condition are likely to reveal the mechanism that underlies why asthmatics

are more susceptible to RV-induced airway disease than otherwise healthy individuals. This new knowledge may generate new approaches to treat viral-induced asthma in all age groups.

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Figure 1. Model of immune dysregulation in the asthmatic epithelium

After rhinovirus infection there is epithelial recognition of viral-related molecules such as double stranded (ds) RNA that activate innate pattern recognition receptors (i.e. toll-like receptors). The airway epithelium of asthmatic subjects exhibit abnormal immune antiviral responses (secretion of type I and type III interferons), exaggerated secretion of thymic stromal lymphopoietin (TSLP) and abnormal release of antimicrobial peptides and chemotactic molecules (i.e. CXCL-8) that are likely to change the airway microbial environment.