Interferon response and respiratory virus control are preserved in bronchial epithelial cells in asthma

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Background: Some investigators find a deficiency in IFN production from airway epithelial cells infected with human rhinovirus in asthma, but whether this abnormality occurs with other respiratory viruses is uncertain.

Objective: To assess the effect of influenza A virus (IAV) and respiratory syncytial virus (RSV) infection on IFN production and viral level in human bronchial epithelial cells (hBECs) from subjects with and without asthma.

Methods: Primary-culture hBECs from subjects with mild to severe asthma (n = 11) and controls without asthma (hBECs; n = 7) were infected with live or ultraviolet-inactivated IAV (WS/33 strain), RSV (Long strain), or RSV (A/2001/2-20 strain) with multiplicity of infection 0.01 to 1. Levels of virus along with IFN-β and IFN-λ and IFN-stimulated gene expression (tracked by 2′-5′-oligoadenylate synthetase 1 and myxovirus (influenza virus) resistance 1 mRNA) were determined up to 72 hours postinoculation.

Results: After IAV infection, viral levels were increased 2-fold in hBECs from asthmatic subjects compared with nonasthmatic control subjects ($P < .05$) and this increase occurred in concert with increased IFN-λ1 levels and no significant difference in *IFNB1*, 2′-5′-oligoadenylate synthetase 1, or myxovirus (influenza virus) resistance 1mRNA levels. After RSV infections, viral levels were not significantly increased in hBECs from asthmatic versus nonasthmatic subjects and the only significant difference between groups was a decrease in IFN-λ levels ($P < .05$) that correlated with a decrease in viral titer. All these differences were found only at isolated time points and were not sustained throughout the 72-hour infection period.

Conclusions: The results indicate that IAV and RSV control and IFN response to these viruses in airway epithelial cells is remarkably similar between subjects with and without asthma. (J Allergy Clin Immunol 2014;134:1402-12.)

Key words: *Asthma, IFN, influenza A virus, respiratory syncytial virus, primary-culture airway epithelial cells*

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The IFN production and signaling pathway is critical for defense against viral infections because it is required for controlling viral replication.[1,2] Accordingly, there is considerable interest in whether variation in the IFN system might account for susceptibility to viral infection. In that regard, it has been recognized that individuals with asthma may be more susceptible to respiratory viral infections and more likely to exhibit slower viral clearance and worse respiratory symptoms, including exacerbations of their underlying disease.[3-7] In fact, respiratory viral infections are perhaps the most common trigger of asthma exacerbations.[5,7-10] Moreover, infection with some types of viruses, for example, respiratory syncytial virus (RSV) and human rhinovirus (HRV), is also implicated in the development of asthma in early childhood.[11-21] Thus, it is natural to consider that any possible increase in the incidence or severity of viral infection in asthma might be due to a deficiency in antiviral defense, and in particular a suboptimal pathway for IFN production or signaling.[6]

A further extension of the proposal for IFN deficiency in asthma derives from the role of the airway epithelial cell in host defense. For example, the IFN response of airway epithelial cells appears to be required for adequate defense against respiratory viruses in experimental mouse models.[22] However, the concept remains to be formally proven in experimental systems and the data for IFN deficiency in asthma remain controversial. Some

Abbreviations used

BEGM: Bronchial epithelial growth medium
hBEC: Human bronchial epithelial cell
HRV: Human rhinovirus
hTEC: Human tracheal epithelial cell
IAV: Influenza A virus
ISG: IFN-stimulated gene
MOI: Multiplicity of infection
MX1: Myxovirus (influenza virus) resistance 1
OAS1: 2′-5′-Oligoadenylate synthetase 1
RSV: Respiratory syncytial virus
UV: Ultraviolet

reports indicate that human bronchial epithelial cells (hBECs) isolated from asthmatic subjects and then placed in culture will produce lower levels of IFN-β and IFN-λ in response to infection with HRV-A16 and that this deficiency will lead to increases in viral level.[23-25] These observations have led to clinical trials of IFN treatment to prevent and/or attenuate the impact of viral infections in asthma. In contrast, a series of other reports shows no significant difference in IFN production or signaling in response to HRV-A16 infection in hBECs isolated and cultured from asthmatic compared with nonasthmatic subjects.[26-28]

There are many possible reasons for the difference in study outcomes, but one particular concern is the nature of the cell culture system used to assess the IFN system. In particular, the use of undifferentiated cells cultured under submerged conditions versus well-differentiated cells maintained under air-liquid interface conditions might have profound effects on the susceptibility to viral infection. It is uncertain whether well-differentiated cell cultures (that mimic physiologic conditions) or undifferentiated cultures (that might mimic epithelial damage in pathologic conditions) are more relevant to natural infection *in vivo*. The evidence for IFN deficiency in cells from subjects with asthma was found in undifferentiated cultures,[24] and at least 1 group was unable to confirm this abnormality despite similar culture conditions.[28] However, in both cases, the studies include only a limited number of conditions to optimize viral yield and the main end point of viral titer can be difficult to monitor if viral replication rates are low. Moreover, HRV replication varies significantly with temperature and strain,[29] adding additional complexity to defining any differences in HRV control. For example, studies reporting a defect in asthma used the HRV-A16 strain[23,24] whereas the study finding no difference used HRV-A1.[28] Furthermore, the previous studies aimed at HRV but did not address other types of viruses despite experimental evidence that paramyxovirus and influenza virus replicate at high efficiencies in the lower airways and in airway epithelial cell cultures and may cause more severe and longer lasting airway disease in experimental models studied *in vivo*[30,31] and perhaps in the setting of clinical infections as well.[16,21,32,33]

On the basis of these uncertainties, we reasoned that it would be useful to reexamine viral level and IFN response in airway epithelial cells under comprehensive conditions that are optimized for virus-induced IFN production. In addition, given the difficulties in reaching firm conclusions for HRV infection, we aimed to assess other types of respiratory viruses that are also sensitive to IFN actions and are implicated in asthma pathogenesis. We included RSV, which has not yet been assessed in this type of system despite its association with asthma. We also assessed influenza A virus (IAV), which was also implicated in asthma exacerbations and was found to disproportionately affect people with asthma in the latest US epidemic of influenza.[32,33] An initial study of IAV (using the H3N2 A/Bangkok/1/79 strain) found no significant difference in viral clearance and no loss of IFN production in hBECs from asthmatic compared with nonasthmatic subjects.[34] However, this study used well-differentiated hBEC cultures so the results cannot be compared directly with the original reports of IFN deficiency in asthma. It therefore remains uncertain whether reports of RSV and IAV link to asthma in humans might be a consequence of IFN deficiency and/or higher viral levels and/or more severe disease.

Here, we assess viral level and IFN production and action after inoculation with RSV and IAV in primary-culture hBECs obtained from asthmatic and nonasthmatic subjects and cultured under submerged conditions. We chose these culture conditions to best capture the difference in viral level and IFN production found in previous reports and to avoid intersubject differences in the degree of airway epithelial cell differentiation that might itself influence viral infection and antiviral response. We utilize well-characterized strains of RSV (RSV Long and RSV A/2001/2-20) and IAV (IAV WS/33), and we establish conditions for high-level viral replication and monitor both type I IFN (marked by IFN-β) and type III IFN (marked by IFN-λ) production as well as IFN-stimulated gene (ISG) expression over a full 72-hour time course of infection. Despite this comprehensive approach, we find a remarkable similarity rather than a significant difference between airway epithelial cells from asthmatic versus nonasthmatic individuals, suggesting that innate epithelial control over 2 key respiratory pathogens (RSV and IAV) is preserved in this disease state.

METHODS

Study subjects

Eighteen subjects (11 asthmatic subjects and 7 nonasthmatic control subjects) were recruited for the study and were characterized as summarized in Table I (for all asthmatic subjects) and detailed in Table E1 in this article's Online Repository at www.jacionline.org (for each asthmatic severity subset). For asthmatic subjects, the diagnosis and severity of asthma were based on National Asthma Education and Prevention Program (NAEPP) guidelines,[35] symptoms of asthma were reported within the past 12 months, and reversible airway obstruction (defined as ≥12% and 200 mL increase in FEV_1 with inhaled bronchodilator) and bronchial hyperreactivity (defined as a provocative concentration of methacholine causing a decline in FEV_1 of 20% or ≤16 mg/mL) were present on pulmonary function testing. The clinical characteristics of asthmatic and nonasthmatic subjects for each group of experiments were not significantly different from the group as a whole. For nonasthmatic control subjects, participants were required to be in good overall health with no history of asthma, allergy, or nasal or sinus disease. For all subjects, atopic status was defined as 1 or more positive allergy skin test result to a panel of 14 prevalent US-wide aeroallergens, or alternatively, a positive result to allergen-specific IgE (8 types) by ImmunoCAP assay if skin testing could not be performed. None of the study participants had significant bronchospasm, history of respiratory failure requiring intubation, bronchial or upper respiratory tract infection (including sinus infection) within the month before assessment, and history of tobacco smoking in the year before assessment. All subjects provided informed consent under a study protocol that was approved by the Human Studies Committee of the Washington University Institutional Review Board.

Cell isolation and culture

Primary-culture human tracheal epithelial cells (hTECs) were isolated as described previously[36] from samples obtained from lung transplant donors

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