Exogenous IFN-β has antiviral and anti-inflammatory properties in primary bronchial epithelial cells from asthmatic subjects exposed to rhinovirus

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Background: Rhinoviruses are the major cause of asthma exacerbations. Previous studies suggest that primary bronchial epithelial cells (PBECs) from asthmatic subjects are more susceptible to rhinovirus infection because of deficient IFN-β production. Although augmenting the innate immune response might provide a novel approach for treatment of virus-induced asthma exacerbations, the potential of IFN-β to modulate antiviral and proinflammatory responses in asthmatic epithelium is poorly characterized.

Objectives: We sought to compare responses of PBECs from nonasthmatic and asthmatic subjects to exogenous IFN-β and test the inflammatory effects of IFN-β in response to rhinovirus infection.

Methods: PBECs were treated with IFN-β and infected with a low inoculum of human rhinovirus serotype 1B to simulate a natural viral infection. Expression of interferon-responsive genes and inflammatory responses were analyzed by using reverse transcription–quantitative real-time PCR, cytometric bead arrays, or both; viral titers were assessed by using the 50% tissue culture infection dose.

Results: Expression of IFN-β–stimulated antiviral genes was comparable in PBECs from nonasthmatic or asthmatic donors. Exogenous IFN-β significantly protected PBECs from asthmatic donors against rhinovirus infection by suppressing viral replication. Interferon-inducible protein 10 (IP-10), RANTES, and IL-6 release in response to rhinovirus infection was triggered only in PBECs from asthmatic donors. Although exogenous IFN-β alone stimulated some release of IP-10 (but not IL-6 or RANTES), it significantly reduced rhinovirus-induced IP-10, RANTES, and IL-6 expression when tested in combination with rhinovirus.

Conclusions: PBECs from asthmatic donors have a normal antiviral response to exogenous IFN-β. The ability of IFN-β to suppress viral replication suggests that it might limit virus-induced exacerbations by shortening the duration of the inflammatory response. (J Allergy Clin Immunol 2011;127:1148-54.)

Key words: *Innate immunity, picornaviridae infections, type I interferons, asthma exacerbation*

Asthma is a common respiratory tract disease affecting 300 million persons worldwide (approximately 5%),[1] with approximately 5.2 million sufferers in the United Kingdom. It is estimated that respiratory virus accounts for between 44% to 80% of asthma exacerbations in children[2-6] and adults,[7] with rhinovirus being the most common pathogen. The respiratory epithelium is the primary target of rhinovirus infection and is of key importance in initiating the host response.[8,9] Although the risk of rhinovirus infection is not increased in asthmatic subjects, these subjects succumb to more frequent lower respiratory tract infections with more severe and longer-lasting symptoms than seen in nonasthmatic subjects,[10] suggesting key differences in the way the lower airways of asthmatic subjects respond to respiratory tract viral infection. These might be linked to many factors, including increased expression of IL-8 and MUC5AC, increased eosinophil numbers, or altered Toll-like receptor (TLR) expression, which can occur in asthmatic airways in response to viral infections (reviewed in Mallia and Johnston[11]). A defect in the ability of asthmatic bronchial epithelial cells (BECs) to produce IFN-β, IFN-λ, or both in response to viral infection might also contribute to the abnormal lower airway response.[12,13]

Interferon was first described as an activity secreted by virus-infected cells, so named because it "interfered" with viral capabilities, preventing further infection of cells that were exposed to the virus.[14] Since then, 3 classes of interferon, type I, type II, and type III, have been described. There are 5 human type I interferons, but the predominant forms are IFN-α and IFN-β, which play key roles in innate responses to viral infection in nonhematologic cells.[15,16] IFN-α and IFN-β are genetically and structurally very similar and use the same receptor.[17] The receptor complex is composed of 2 subunits, IFN-α, β, and ω receptor (IFNAR)-1 and IFNAR-2, and in the absence of either subunit, there is no high-affinity ligand binding and no biological effect.[18,19] Type I interferons were first used therapeutically in the clinical setting in 1986. Since then, IFN-α has been used for the treatment of malignant and viral diseases and IFN-β for relapsing remitting multiple sclerosis.[15]

In response to infection with a single-stranded RNA virus, such as rhinovirus, type I interferons are initially expressed after recognition of double-stranded RNA, the replicative intermediate of the virus, by cellular pattern-recognition receptors, such as melanoma differentiation-associated gene 5 (MDA-5; also known as Ifih1) and TLR3,[20,21] leading to activation of interferon

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Abbreviations used
BEC: Bronchial epithelial cell
IP-10: Interferon-inducible protein 10
IRF-7: Interferon regulatory factor 7
MDA-5: Melanoma differentiation-associated gene 5
PBEC: Primary bronchial epithelial cell
PKR: RNA-dependent eIF-2a protein kinase
qPCR: Quantitative real-time PCR
RIG-I: Retinoic acid–inducible protein I
RV1B: Human rhinovirus serotype 1B
STAT: Signal transducer and activator of transcription
$TCID_{50}$: 50% Tissue culture infection dose
TLR: Toll-like receptor

regulatory factor (IRF) 3 and induction of IFN-β expression. Secreted IFN-β then functions in either an autocrine or paracrine manner to activate the Janus kinase–signal transducer and activator of transcription (STAT) pathway, inducing a wave of *de novo* transcription of antiviral genes, as well as expression of more type I interferons. This secondary wave amplifies the initial primary antiviral response, resulting in apoptosis of infected cells, prevention of viral replication, and limitation of viral spread.

Previous studies with BECs from asthmatic donors revealed a dampened interferon response that allowed viral replication to continue unchecked,[13] suggesting that this might prolong the duration of infection and sustain the inflammatory cascade. Because exogenous IFN-β protected against infection in this model, it has been proposed that type I interferons might have therapeutic potential for the treatment or prevention of virus-induced asthma exacerbations. However, it is not known whether the ability of BECs from asthmatic donors to amplify the antiviral response downstream of the type I interferon receptor is normal. Furthermore, the potential for IFN-β to amplify or modulate inflammatory responses in the context of rhinovirus infection is poorly understood. Therefore we compared responses of BECs from nonasthmatic and asthmatic subjects to exogenous IFN-β and tested the antiviral and inflammatory effects of IFN-β in response to rhinovirus infection.

METHODS

Primary cell culture

Bronchial brushings were obtained by means of fiberoptic bronchoscopy from subjects with or without asthma according to current guidelines.[22] Subjects were selected from a volunteer database. Details of patients' phenotypes are in Tables E1 and E2 (available in this article's Online Repository at www.jacionline.org). All procedures were approved by the Southampton and South West Hampshire Research Ethics Committee (REC no. 05/Q1702/165). Brushings were processed for primary bronchial epithelial cell (PBEC) culture in bronchial epithelial growth medium, as previously described.[23] PBECs were plated on collagen-coated wells (30 μg/mL PureCol; Inamed Biomaterials, Fremont, Calif) and used at passage 2. Before infection, the bronchial epithelial growth medium was replaced with basal medium (BEBM containing insulin [5 μg/mL], transferrin [5 μg/mL], and sodium selenite [5 ng/mL; ITS; Sigma, Poole, United Kingdom] with 0.1% BSA), and the cells were incubated overnight before use.

Viral culture

Human rhinovirus serotype 1B (RV1B; a gift from Professor Sebastian L. Johnston, Imperial College, London) was amplified in HeLa cells, as previously described.[24] Viral strain was confirmed by means of quantitative real-time PCR (qPCR; pathogen detection kit; Primer Design, Southampton, United Kingdom), and infectivity was determined with a HeLa titration assay as 50% tissue culture infective dose ($TCID_{50}$) per milliliter.

Infection of cells with rhinovirus

PBECs were infected with RV1B (1×10^3 $TCID_{50}$ units/10^5 cells) for 1 hour at room temperature, after which the cells were washed to remove residual viral particles and the cultures were replenished with basal medium. Where indicated, cultures were pretreated with IFN-β (100 IU/mL) for 24 hours before infection and were retreated with fresh IFN-β after infection. Controls of UV-irradiated RV1B (1200 mJ/cm^2 on ice for 50 minutes), medium alone, and IFN-β only were included in all experiments. Culture supernatants were stored at −80°C for analysis.

Extraction of total RNA and mRNA quantification

After treatments, cells were harvested into TRIzol reagent (Invitrogen, Paisley, United Kingdom), and total RNA was isolated with standard protocols. RNA (1 μg) was reverse transcribed by using the Precision reverse transcription kit (Primer Design, Southampton, United Kingdom), according to the manufacturer's instructions. cDNA was amplified by means of PCR with Perfect Probe or Double Dye assays (Primer Design). Expression levels of mRNA were calculated relative to the housekeeping genes glyceraldehyde-3-phosphate dehydrogenase and ubiquitin C by using the ΔΔCT method. RV1B viral RNA copy number was determined by using a standard curve.

Cytokine analysis

Interferon-inducible protein 10 (IP-10) and RANTES protein secretion in the cell supernatant was measured with the BD Cytometric Bead Array (CBA; BD, Franklin Lakes, NJ), according to the manufacturer's instructions. Analysis was performed with a FACAria with FCAP array version 1 software.

Statistical analysis

Data were tested for normal distribution and then analyzed with appropriate tests. For normally distributed data, the Student *t* test was used, whereas nonparametric data were analyzed with the Wilcoxon signed-rank test for within-group comparisons or the Mann-Whitney *U* test for between-group comparisons. A *P* value of .05 or less was considered statistically significant. Correlations were performed with SPSS software (SPSS, Inc, Chicago, Ill).

RESULTS

Sensitivity of PBECs from asthmatic or nonasthmatic donors to IFN-β and upregulation of antiviral genes

Initially, we investigated the phosphorylation of STAT1 and STAT2 in PBECs in response to exogenous IFN-β using Western blotting. Time-course and dose studies showed phosphorylation of both STAT1 and STAT2 to be maximal by 60 minutes, with maximal phosphorylation occurring at greater than 50 IU/mL IFN-β. This was seen in PBECs taken from asthmatic and nonasthmatic volunteers. Analysis of STAT1 and STAT2 phosphorylation in PBECs from 3 nonasthmatic and 3 asthmatic donors treated with IFN-β at 100 IU/mL for 1 hour revealed responses in each case, and semiquantitative analysis suggested no differences in overall responses to exogenous IFN-β (see Fig E1 in this article's Online Repository at www.jacionline.org).

To more accurately quantify activation of the Janus kinase–STAT pathway by IFN-β, we measured downstream induction of mRNA for interferon-responsive genes by using RT-qPCR. Time and dose responses for induction of IRF-7 by IFN-β showed