Rhinovirus 16–induced IFN-α and IFN-β are deficient in bronchoalveolar lavage cells in asthmatic patients

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Background: Asthmatic patients have defective rhinovirus-induced IFN-β and IFN-λ production from bronchial epithelial cells and IFN-λ from bronchoalveolar lavage (BAL) cells. Whether bronchoalveolar lavage cells have defective type I interferon responses to rhinovirus is unknown, as are mechanisms explaining defective rhinovirus interferon induction in asthmatic patients.

Objective: We sought to investigate rhinovirus induction of type I interferons in BAL and blood mononuclear cells from asthmatic patients and healthy subjects and to investigate mechanisms of any deficiency observed.

Methods: BAL and blood mononuclear cells from atopic asthmatic patients and healthy subjects were infected with rhinovirus *ex vivo*. Interferon proteins were analyzed by using ELISA. mRNA expression of key components of interferon induction pathways were analyzed by using quantitative PCR.

Results: Rhinovirus induction of type I interferon protein was delayed and deficient in BAL cells from asthmatic patients, and lower interferon levels were associated with greater airway hyperresponsiveness and skin prick test response positivity. Expression of Toll-like receptor (TLR) 3, TLR7, TLR8, retinoic acid–inducible gene I (RIG-I), melanoma differentiation–associated gene 5 (MDA-5), TIR domain–containing adapter-inducing IFN-β (TRIF), myeloid differentiation primary response gene 88 (MyD88), caspase recruitment domain adaptor inducing IFN-β (CARDIF), IL-1 receptor–associated kinase 4 (IRAK4), IκB kinase β (IKKB), IκB kinase ι (IKKI), interferon regulatory factors 3 and 7, and rhinovirus induction of expression of the virus-inducible molecules TLR3, TLR7, RIG-I, and MDA-5 were not impaired in these interferon-deficient BAL cells in asthmatic patients. Defective rhinovirus interferon induction was not observed in blood mononuclear cells.

Conclusions: Rhinovirus induction of type I interferons in BAL cells is delayed and deficient and might be a marker of more severe asthma. Defective rhinovirus interferon induction in asthmatic patients was not accompanied by differences in the expression or induction of key molecules implicated in viral induction of interferons. (J Allergy Clin Immunol 2012;129:1506-14.)

Key words: *Asthma, interferon, rhinovirus, bronchoalveolar lavage cells, peripheral blood mononuclear cells, airway hyperresponsiveness*

Rhinovirus infection is responsible for at least 50% of asthma exacerbations. Atopic asthmatic patients experience more severe and longer-lasting rhinovirus-induced lower respiratory tract symptoms than nonasthmatic patients,[1,2] and deficient rhinovirus induction of antiviral interferons is implicated in their increased susceptibility to rhinovirus infection.[3,4]

Airway cells produce type I antiviral IFN-α (13 subtypes in human subjects) and IFN-β and type III IFN-λ1 and IFN-λ2/3[5] in response to rhinovirus infection. Defective rhinovirus-induced interferon in asthmatic patients has been reported for 2 types of interferons in 3 different cell types: IFN-β in primary human bronchial epithelial cells (HBECs), IFN-α in PBMCs from children, and IFN-λ in HBECs and bronchoalveolar lavage (BAL) cells.[3,4,6,7] Deficiency has been associated with viral load *in vitro*[4] and viral load and markers of exacerbation severity *in vivo*.[3] Deficient IFN-α subtype 2 (IFN-α2) response to respiratory syncytial virus and Newcastle disease virus has been reported in PBMCs from atopic asthmatic adults and children.[8,9] These findings suggest that defective interferon induction in asthmatic patients is likely important in rhinovirus-induced exacerbations. The type I interferon response to rhinovirus stimulation in BAL cells is unknown, as are the mechanisms of defective interferon induction.

Viral RNAs are recognized by membrane-bound and endosomal Toll-like receptors (TLRs) 3, 7, and 8 and the cytosolic RNA helicases retinoic acid–inducible gene I (RIG-I) and melanoma differentiation–associated gene 5 (MDA-5) in airway and immune cells. Activation of these receptors results in association with their adaptor proteins, which triggers a complex downstream

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Abbreviations used
BAL: Bronchoalveolar lavage
CARD: Caspase recruitment domain
CARDIF: CARD adaptor inducing IFN-β
dsRNA: Double-stranded RNA
HBEC: Human bronchial epithelial cell
IKKB: IκB kinase β
IKKI: IκB kinase ι
IRAK4: IL-1 receptor–associated kinase 4
IRF: Interferon regulatory factor
MDA-5: Melanoma differentiation–associated gene 5
MyD88: Myeloid differentiation primary response gene (88)
PRR: Pattern-recognition receptor
RIG-I: Retinoic acid–inducible gene I
RV14: Rhinovirus 14
RV16: Rhinovirus 16
TLR: Toll-like receptor
TRIF: TIR domain–containing adapter-inducing IFN-β

signaling pathway resulting in interferon induction. TLR3 recognizes double-stranded RNA and induces interferon through the adaptor molecule TIR-domain–containing adapter–inducing IFN-β (TRIF) to activate inducible IκB kinase ι (IKKI) and then dimerization, phosphorylation, and nuclear translocation of interferon regulatory factor (IRF) 3, resulting in induction of interferon gene transcription.[10-13] IRF7 is highly homologous with IRF3, also induced by viral infection, and a potent inducer of IFNs in myeloid cells.[14] TLR7 and TLR8 recognize single-stranded RNA and induce interferon through a myeloid differentiation primary response gene 88 (MyD88)–dependent pathway using IL-1 receptor–associated kinase 4 (IRAK4), resulting in phosphorylation of IκB kinase β (IKKB) and activation of IRF7 and nuclear factor κB.[15] Activation of the cytosolic RNA helicases RIG-I and MDA-5 by short double-stranded RNA (dsRNA) and single-stranded RNA and long dsRNA, respectively, induces conformational change, allowing interaction with caspase recruitment domain (CARD) adaptor inducing IFN-β (CARDIF)[16] and also resulting in activation of IKKI, IKKB, nuclear factor κB, IRF3, and interferon induction (see Fig E1 in this article's Online Repository at www.jacionline.org).

This study aimed to determine whether deficient rhinovirus-induced type I interferon production was present in BAL cells and PBMCs from adult atopic asthmatic patients and to investigate the mechanisms of defective rhinovirus-induced interferon production by measuring expression and induction of TLRs and RNA helicases and key signaling molecules implicated in interferon induction in atopic asthmatic patients (asthmatic patients) and nonatopic nonasthmatic subjects (healthy subjects).

METHODS

Subject recruitment

Twenty-two asthmatic patients and 20 healthy subjects were recruited. All were nonsmokers, with no exacerbations or respiratory tract infections in the preceding 6 weeks. Atopy was defined as 1 or more positive skin prick test responses (see the Methods section in this article's Online Repository at www.jacionline.org). Asthma was defined in atopic subjects as a physician's diagnosis of asthma with bronchial hyperresponsiveness. Healthy subjects were nonatopic and had no previous relevant medical history and no bronchial hyperresponsiveness (Table I; see the Methods section in this article's Online Repository). The study was approved by the St Mary's Hospital Ethics Committee. All subjects provided written informed consent.

BAL cell and PBMC isolation and processing

BAL fluid was obtained by means of fiberoptic bronchoscopy with a Keymed BF260 bronchoscope (Olympus, Essex, United Kingdom) in accordance with British Thoracic Society guidelines.[17] BAL fluid was filtered through a 100-μm cell strainer (BD Falcon, Bedford, Mass), washed, and resuspended in RPMI, as previously described,[3] and cytospins were performed for cell differentials.

PBMCs were isolated from whole blood by means of gradient centrifugation in Histopaque (Sigma-Aldrich, Dorset, United Kingdom), and cells were washed and resuspended in RPMI 1640 medium (Invitrogen, Carlsbad, Calif), as previously described.[3]

Rhinovirus stimulation

BAL cells and PBMCs were infected with rhinovirus 16 (RV16; multiplicity of infection, 1). RV16 stocks were generated and titrated, as previously described.[3] As negative controls, cells were treated with medium alone or filtered control. RV16 was removed after 1 hour, cells were washed and resuspended in fresh medium, and cells and supernatants were harvested. PBMCs were harvested at 8, 24, and 48 hours; because of the limited numbers of BAL cells obtained, these cells were harvested at 8 and 24 hours. Similar experiments were also performed with the minor group virus RV1B (multiplicity of infection, 1).

RNA isolation and quantitative PCR

Total RNA was extracted from cells with the RNeasy MiniKit (Qiagen, Hilden, Germany) and used for cDNA synthesis by using the Omniscript RT kit (Qiagen). Reactions were analyzed with an ABI 7000 TaqMan (Applied Biosystems, Foster City, Calif), as previously described.[12] Results were normalized to 18S. For primer and probe sequences, see Table E1 and the Methods section in this article's Online Repository at www.jacionline.org.

Interferon protein measurement

IFN-β (Invitrogen), IFN-α (Invitrogen), and IFN-α2 (Mabtech, Nacka Strand, Sweden) release was measured by using ELISA, according to the manufacturer's instructions. The sensitivities of each assay were 10 pg/mL.

Statistical analysis

Statistical analysis was performed with GraphPad Prism software (version 4.3; GraphPad Software, San Diego, Calif). Because the majority of data were not normally distributed, the Kruskal-Wallis test with the Dunn correction was used, followed by between-group testing with the Mann-Whitney test if results were significant. Clinical correlations were analyzed by using the Spearman rank correlation coefficient except for PC_{20} and the number of positive skin prick test responses (because all healthy subjects had the same value: >16 μg/mL for PC_{20} and 0 for number of positive skin prick test responses). Associations between interferon levels and PC_{20} values and the number of positive skin prick test responses were assessed with the Fisher exact test on percentages. Data are presented as medians unless otherwise stated. A P value of less than .05 was considered significant.

RESULTS

BAL cell samples were successfully obtained from 22 asthmatic patients and 17 healthy subjects, and PBMCs were obtained from 22 asthmatic patients and 20 healthy subjects. Clinical characteristics of these participants are shown in Table I.

BAL cells were predominantly (approximately 95%) macrophages, and there was no significant difference in the proportion of macrophages, lymphocytes, and neutrophils isolated from asthmatic patients or healthy donors. Asthmatic patients had significantly more eosinophils in BAL fluid than healthy subjects ($P < .001$, see Fig E2 in this article's Online Repository at www.jacionline.org).