Chemokine release from human rhinovirus–infected airway epithelial cells promotes fibroblast migration

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Background: Thickening of the lamina reticularis, a feature of remodeling in the asthmatic airways, is now known to be present in young children who wheeze. Human rhinovirus (HRV) infection is a common trigger for childhood wheezing, which is a risk factor for subsequent asthma development. We hypothesized that HRV-infected epithelial cells release chemoattractants to recruit fibroblasts that could potentially contribute to thickening of the lamina reticularis.

Objective: We sought to investigate whether conditioned medium from HRV-infected epithelial cells can trigger directed migration of fibroblasts.

Methods: Human bronchial epithelial cells were exposed to medium alone or infected with HRV-16. Conditioned medium from both conditions were tested as chemoattractants for human bronchial fibroblasts in the xCELLigence cell migration apparatus.

Results: HRV-conditioned medium was chemotactic for fibroblasts. Treatment of fibroblasts with pertussis toxin, an inhibitor of Gαi-coupled receptors, prevented their migration. Production of epithelial chemoattractants required HRV replication. Multiplex analysis of epithelial supernatants identified CXCL10, CXCL8, and CCL5 as Gαi-coupled receptor agonists of potential interest. Subsequent analysis confirmed that fibroblasts express CXCR3 and CXCR1 receptors and that CXCL10 and, to a lesser extent, CXCL8, but not CCL5, are major contributors to fibroblast migration caused by HRV-conditioned medium.

Conclusion: CXCL10 and CXCL8 produced from HRV-infected epithelial cells are chemotactic for fibroblasts. This raises the possibility that repeated HRV infections in childhood could contribute to the initiation and progression of airway remodeling in asthmatic patients by recruiting fibroblasts that produce matrix proteins and thicken the lamina reticularis. (J Allergy Clin Immunol 2016;138:114-22.)

Key words: *Airway epithelium, chemoattractants, fibroblasts, human rhinovirus, migration, airway remodeling, inflammation*

Airway remodeling is a characteristic feature of asthma that includes thickening of the lamina reticularis, increased smooth muscle mass, angiogenesis, increased numbers of fibroblasts/myofibroblasts, an altered epithelial phenotype, and goblet cell hyperplasia/metaplasia, leading to increased mucus production.[1] Collectively, these changes result in a thickened airway wall and have been suggested to underlie the development and persistence of airways hyperresponsiveness.[2,3]

Although asthma can manifest at any age, it is usually evident in childhood in the majority of patients.[4] Therefore it is notable that recent bronchial biopsy studies have demonstrated that several major components of airway remodeling are already present in preschool children with symptoms or airflow limitation, in some instances even before a formal clinical diagnosis of asthma is made.[5-7] Interestingly, although thickening of the lamina reticularis was observed in these studies, it was not observed in infants with airflow limitation,[8] suggesting that this feature of remodeling is induced as a consequence of some initiating stimuli experienced in early childhood.

Human rhinovirus (HRV) infections in early life are a common trigger for childhood wheezing illnesses,[9] and HRV-induced wheezing illnesses in the first 3 years of life are a major risk factor for subsequent asthma.[10,11] Longitudinal analysis has shown that preschool children have about 6 HRV infections per year[12] and that serial viral infections can lead to recurrent wheezing episodes.[13] Because airway remodeling begins to appear in this same early-life timeframe, this raises the concept that HRV infections also can play a role in initiation and progression of airway remodeling.

Thickening of the lamina reticularis results from increased deposition of extracellular matrix proteins believed to be deposited by fibroblasts, myofibroblasts, or both, which are present in increased numbers in the subepithelial region of the airway.[14] Because the airway epithelium is the primary site of HRV infection and replication in the airways, we hypothesized that infected epithelial cells release chemoattractants that could cause migration of human airway fibroblasts toward the epithelial layer.

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Abbreviations used
BEBM: Bronchial epithelial basal medium
BEGM: Bronchial epithelial growth medium
DMEM: Dulbecco modified Eagle medium
GPCR: G protein–coupled receptor
HBE: Human bronchial epithelial
HBF: Human bronchial fibroblast
HRV: Human rhinovirus
NGS: Normal goat serum
PDGF: Platelet-derived growth factor
PTX: Pertussis toxin

METHODS

Materials

The following reagents were purchased from the indicated suppliers: bronchial epithelial basal medium (BEBM) and additives to create serum-free bronchial epithelial growth medium (BEGM) were from Lonza (Walkersville, Md); HBSS, Dulbecco modified Eagle medium (DMEM), 0.25% Trypsin-EDTA 1X, Tryple Select enzyme solution, and FBS were from Life Technologies (Burlington, Ontario, Canada); WI-38 cells and HRV type 16 were from American Type Culture Collection (Manassas, Va); recombinant CXCL8, CXCL10, and CCL5, antibody for CXCL10 ELISA and neutralization experiments, as well as flow cytometric antibodies for CXCR1, CXCR2, CXCR3, and isotype controls were all from R&D Systems (Minneapolis, Minn); SCH 527123 and SCH 546738 were from MedChem Express (Princeton, NJ); and all other chemicals were purchased from Sigma-Aldrich (Oakville, Ontario, Canada).

Human lungs

Nontransplanted human lungs were obtained from a tissue retrieval service (International Institute for the Advancement of Medicine, Edison, NJ). Ethics approval to receive and use lung tissues was obtained from both the Conjoint Health Research Ethics Board of the University of Calgary and the Internal Ethics Board of Institute for the Advancement of Medicine. No personal identifying information was provided for any of the donors.

Epithelial cell culture

Primary human bronchial epithelial (HBE) cells were obtained by means of protease digestion of dissected airways (main stem bronchus to the fourth generation), as previously described.[15] These airways all contain a morphologically similar pseudostratified epithelium. For the current studies, cells were derived from 6 individual lung donors (5 male donors; age range, 13-63 years). All 6 donors died from head trauma or stroke, and none had any inflammatory lung disease. HBE cells were cultured until 80% to 90% confluence in BEGM at 37°C in 5% CO_2. Before experimentation, cells were cultured for 24 hours in BEGM from which hydrocortisone was removed. HBE cells were then treated with BEBM for 2 hours before stimulation.

Fibroblast cell culture

Human bronchial fibroblasts (HBFs) were also obtained from normal nontransplanted human lungs. For these studies, cells from 17 individual donors (10 male donors; age range, 13-63 years) were used. None of the donors had inflammatory lung disease. One donor died of a central nervous system tumor, and all others died of head trauma or stroke. Main stem bronchi were dissected into small pieces and suspended in DMEM supplemented with 10% FBS. Tissue plugs were placed in 24-well plates, and cells were allowed to grow out to confluence. Tissue plugs were removed and fibroblasts were suspended with Tryple Select and transferred to T-175 flasks (Corning, Corning, NY). This was designated as passage 1. Cells were suspended with Tryple Select and stored at passage 2 in liquid nitrogen. For experimentation, fibroblasts were thawed, placed in a T-175 flask, and grown in 10% FBS DMEM until approximately 90% to 100% confluence. Once confluence was reached, the cells were again suspended with trypsin and transferred into a cell migration apparatus. Cells were used up to passage 7. Fibroblasts used for migration experiments show typical fibroblast morphology and are negative for α-smooth muscle actin, which is robustly expressed in smooth muscle cells from the same lungs (see Fig E1 in this article's Online Repository at www.jacionline.org).

HRV-16 and viral infection of epithelial cells

HRV-16 viral stocks were propagated in WI-38 cells. Each viral stock was purified to remove soluble products from WI-38 cells by means of sucrose density centrifugation, as previously described.[16] Replication-deficient HRV-16 was produced by exposing aliquots of the same purified stock used for live virus experiments for 5 minutes to a Spectroline Model XX-15F high-intensity short-wavelength (254 nm) UV lamp (Spectronics Corp, Westbury, NY) at a distance of 5 cm. Inactivation was confirmed by the inability of stocks to replicate and cause cytopathic effects in WI-38 cells.

HBE cells from 6 individual tissue donors were exposed to 10^5 50% tissue culture-infective dose/mL of HRV-16, UV-treated HRV-16, or control medium (BEBM) for various lengths of time up to 24 hours at 34°C in 5% CO_2. Incubations were performed at 34°C because this is the optimal temperature for HRV replication and is routinely used in the field. After the treatment period had elapsed, conditioned medium from each of the 6 donors was removed and pooled into respective conditions to provide a single consistent stock of material from each condition for migration experiments. These conditions included the following: HRV-conditioned medium (HRV-infected HBEs), UV-HRV–conditioned medium (UV-infected HBEs), and control medium (BEBM-treated HBEs). These media were then used as chemoattractants for HBFs from the indicated number of individual tissue donors in each experiment (n = number of fibroblast donors).

Pharmacologic treatment of fibroblasts

Before treatment with pertussis toxin (PTX), HBFs were seeded at 10^6 cells per T-75 flask. Once confluence was reached, HBFs were treated with FBS-free DMEM or the same medium containing 3 ng/mL PTX for 12 hours before use for experimentation. The chemokine receptor antagonists SCH 546738 (CXCR3) and SCH 527123 (CXCR1/CXCR2) were used at final concentrations of 20 and 75 nmol/L, respectively, based on preliminary concentration-response experiments against recombinant cytokines.

Neutralizing antibody treatment of conditioned medium

Neutralizing antibodies to CXCL10 (R&D Systems) and CXCL8[17] or appropriate control antibodies at concentrations of 150 and 240 ng/mL, respectively, were added to HRV-16 condition media and incubated at room temperature for 2 hours before use as chemoattractants for HBFs.

Migration assays

Briefly, HBFs were suspended with trypsin and placed into the top wells of the migration assay chamber. Chemoattractants were placed in the bottom wells of the migration apparatus. Stimuli used were as follows: DMEM (negative control), HRV-16 alone at the same dose used to infect HBE, platelet-derived growth factor (PDGF) AB (positive control), and conditioned media removed from HBEs. Both the Boyden chamber (Neuroprobe, Gaithersburg, Md) and xCELLigence apparatus (ACEA Biosciences, San Diego, Calif) were used to complete these experiments. Detailed methods for both systems are provided in the Methods section in this article's Online Repository at www.jacionline.org.

Flow cytometry

Fibroblast expression of CXCR3, CXCR2, and CXCR1 was assessed by using flow cytometry after incubation of HBFs with specific antibodies or