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Original Research

ASTHMA

Airway Inflammation and Illness Severity in Response to Experimental Rhinovirus Infection in Asthma

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Background: **The nature of bronchial mucosal inflammation and its physiologic and clinical significance in rhinovirus-induced asthma exacerbations is unclear. We investigated bronchial mucosal inflammatory response and its association with physiologic and clinical outcomes in an experimental model of rhinovirus-induced asthma exacerbations.**

Methods: **We used immunohistochemistry methods to detect phenotypes of inflammatory cells infiltrating the bronchial mucosa before and after experimental rhinovirus infection in 10 subjects with asthma and 15 normal subjects.**

Results: **Compared with baseline, rhinovirus infection significantly increased the number of epithelial ($P = .005$) and subepithelial ($P = .017$) neutrophils in subjects with asthma only and subepithelial $CD68^+$ macrophages in both subjects with asthma ($P = .009$) and normal subjects ($P = .018$) but more so in those with asthma ($P = .021$). Numbers of $CD45^+$, $CD68^+$, and $CD20^+$ cells; neutrophils; and eosinophils at day 4 postinfection were positively associated with virus load ($r = 0.50$-0.72, $P = .016$-0.03). At acute infection in subjects with asthma, $CD4^+$ cells correlated with chest symptom scores ($r = 0.69$, $P = .029$), the fall in the 10% fall in FEV_1 (PC_{10}) correlated with neutrophils ($r = -0.89$, $P = .029$), the PC_{10} correlated inversely with $CD4^+$ ($r = -0.67$, $P = .023$) and $CD8^+$ cells ($r = -0.65$, $P = .03$), the 20% fall in FEV_1 was inversely associated with $CD20^+$ cells ($r = -0.65$, $P = .03$), and higher epithelial $CD8^+$ cell counts were significantly associated with a greater maximum fall in FEV_1 ($r = -0.72$, $P = .03$), whereas higher subepithelial mast cell counts were significantly associated with a lower maximum percent fall in peak expiratory flow ($r = 0.8$, $P = .024$).**

Conclusions: **In subjects with asthma, rhinovirus infection induces bronchial mucosal neutrophilia and more severe monocyte/macrophage infiltration than in normal subjects. Airway neutrophils, eosinophils, and T and B lymphocytes during infection are related to virus load and physiologic and clinical severity, whereas mast cells are related to greater lung function.**

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Abbreviations: PC_{10} = 10% fall in FEV_1; PEF = peak expiratory flow; RV = rhinovirus

Rhinoviruses (RVs) are the major cause of acute exacerbations of asthma.[1-3] Human experimental RV infection in volunteers with mild asthma is associated with augmented physiologic and inflammatory responses to allergen challenge,[4,5] reductions in peak expiratory flow (PEF)[6] and FEV_1,[7] and increases in bronchial reactivity.[8] In agreement, our own previously reported study showed that experimental RV16 infection in asthma induced significant increases in bronchial reactivity, lower respiratory tract symptoms, and lung function impairment and that significant changes in these outcomes did not occur in normal subjects.[9]

Regarding RV-induced airway inflammation, two previous studies of experimental RV infection reported increases in submucosal $CD3^+$ lymphocytes and eosinophils in asthmatic and normal groups combined[10] and in subjects with asthma alone.[11] The increased

number of mucosal CD3+ cells was accompanied by an increase in airway responsiveness and correlated positively with cold symptoms.[10,11] However, it is unclear whether RV-induced bronchial mucosal cellular inflammatory responses differ between subjects with and without asthma.

In this article, we extend our previous work[9] to compare the nature of the bronchial mucosa inflammatory response to experimental RV infection in subjects with asthma and in normal healthy subjects and explore its physiologic and clinical significance in asthma. We hypothesized that the bronchial mucosal cellular inflammatory response to RV infection will be exaggerated and of a distinctive predominant inflammatory cell phenotype in subjects with asthma compared with normal healthy subjects and that the inflammatory cells recruited to the bronchial mucosa will be associated with virus load and increased clinical symptoms, airways responsiveness, and airflow obstruction associated with an exacerbation.

Materials and Methods

Subjects

We extended our previously reported investigation of the same 10 subjects with atopic asthma and 15 subjects without atopic asthma (Table 1).[9] These subjects were human RV16 neutralizing antibody seronegative. This study was conducted in accordance with the amended Declaration of Helsinki. All subjects gave written informed consent, and the study protocol was approved by the St. Mary's NHS Trust Research Ethics Committee (99/BA/345). See e-Appendix 1 for other details.

Experimental Infection With Human RV16

Subjects were administered 10,000 tissue-culture infective dose 50% of RV16 on day 0 by nasal spray.[9] See e-Appendix 1 for virologic confirmation of RV16 infection.

Bronchoscopy and Clinical Data

Bronchial biopsy specimens were taken 14 days before infection (baseline), at day 4 (acute infection), and at 6 weeks postinfection (convalescence). For details about the physiologic and clinical data obtained, see e-Appendix 1.

Immunohistochemistry

CD45+ pan-leukocyte and inflammatory cells, eosinophils, neutrophils, mast cells, and CD3+, CD4+, CD8+, and CD20+ cells were immunostained using previously described methods.[12] For details on the immunostaining method, see e-Appendix 1.

Quantification

The areas of epithelium and subepithelium in bronchial biopsy specimens were assessed using NIH Image, version 1.55 software (US National Institute of Mental Health). The inflammatory cells were counted using a light microscope. The data for cell counts were expressed as the number of positive cells per square millimeter of the subepithelium and per 0.1 mm^2 epithelium. For details on the counting methods, see e-Appendix 1.

Statistical Analysis

Within-group differences in cell counts between baseline and infection were assessed with Wilcoxon matched pairs test. Mann-Whitney *U* test was used to compare differences between groups. Spearman rank correlation was used to test for correlations between the numbers of phenotypes of inflammatory cells and physiologic and clinical data. $P < .05$ indicated significance. For further details on the statistical analyses, see e-Appendix 1.

Results

Inflammatory cells were present in both the bronchial epithelial and the bronchial subepithelial compartments. Representative photographs are shown in Figures 1A-E. Elastase-positive neutrophils (Fig 1A) and CD68+ monocytes/macrophages (Fig 1B) appeared to be more frequent in the bronchial mucosa of subjects with asthma during acute infection compared with baseline (Figs 1C, 1D). Application of irrelevant antibodies for the inflammatory cell markers was negative (Fig 1E).

Absolute Counts of Inflammatory Cells

Total Leukocytes: The number of subepithelial CD45+ cells in subjects with asthma was significantly greater at both baseline ($P = .014$) and day 4 infection ($P = .025$) (Fig 2A) than in normal subjects at the same time points. Compared with their respective

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