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Apoptotic eosinophils in sputum from asthmatic patients correlate negatively with levels of IL-5 and eotaxin

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Summary
Background: Eosinophilic inflammation of the airways is a key characteristic of asthma. A defect in eosinophil apoptosis might contribute to the chronic tissue eosinophilia associated with asthma.
Objective: Our purpose was to examine whether the occurrence of apoptotic eosinophils in induced sputum from asthmatic patients correlate with interleukin (IL)-5 and eotaxin.
Methods: Thirty stable and 30 exacerbated asthmatic patients were recruited. Twenty healthy subjects were enrolled as a control group. Induced sputum was obtained from asthmatic patients and from control subjects. The number of apoptotic eosinophils in sputum was assessed by flow cytometry. In sputum supernatant, eosinophil cationic protein (ECP) was measured by sensitive radioimmunoassay, and IL-5 and eotaxin by sandwich enzyme linked immunosorbant assay.
Results: Levels of eosinophils, apoptotic eosinophils, IL-5, ECP and eotaxin from asthmatic patients were higher than those from healthy subjects. Thirty exacerbated asthmatics showed higher proportions of eosinophils (median 29.3%, range 13.4%–40.9%), more detectable levels of IL-5 (50.44, 32.99–67.01 pg/ml) and eotaxin (644.6, 197.4–937.7 pg/ml) in their sputum than the patients with stable asthma ($P<0.05$). There were significant inverse correlations between the levels of sputum IL-5 and the proportion of sputum eosinophil apoptosis in patients with exacerbated and stable asthma ($r=-0.85$ and -0.79, $P<0.01$ and $P<0.05$, respectively). Also inverse correlations were found between the levels of eotaxin and the proportion of sputum eosinophil apoptosis in exacerbated ($r=-0.85$, $P<0.01$), or stable asthma ($r=-0.69$, $P<0.05$). Additional positive corrlations between the levels of sputum IL-5 and eotaxin in either exacerbatated ($r=0.93$, $P<0.01$) or stable asthma ($r=0.82$, $P<0.05$) were observed.

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Conclusions: Apoptosis of eosinophils might be suppressed by proinflammatory cytokines and chemokines such as IL-5 and eotaxin leading to their accumulation in the lung. Stimulation of eosinophils in airway with IL-5 and eotaxin may play a crucial role in allergic inflammation.

Introduction

Eosinophils are known to play a pivotal role in asthmatic airway inflammation. Infiltration of eosinophils into the bronchial wall and respiratory epithelial damage are two distinctive features of asthma.[1] These bronchial changes involve four steps—namely, enhanced eosinophil production, recruitment to lung tissue, activation of eosinophils, and release of mediators. The prolongation of eosinophil survival is important in the pathogenesis of asthmatic airway inflammation. Apoptosis plays a central part in normal tissue homeostasis as well as having a role in various clinical diseases characterized by either increased or decreased cell survival.[2] It is thought to be critically important in promoting the clearance of inflammatory cells and the resolution of inflammation. Apoptosis of eosinophils may be clinically relevant in asthma, promoting the removal of airway eosinophils and contributing to clinical improvement.

The recruitment of eosinophils to the airway is complex, and involvement of a number of cytokine families has been considered. Understanding the mechanisms of eosinophil recruitment to the airway and the regulation of eosinophil chemotaxis and activation can potentially lead to further improvement in the treatment of asthma. Interleukin (IL)-5, a cytokine that attracts, activates, and prolongs the survival of eosinophils, is important in causing eosinophilic inflammation in the asthmatic airway and contributes to eosinophil viability in the sputum of asthmatic patients during attacks.[3] Eotaxin is a chemokine with potent and selective chemotactic activity for eosinophils.[4] Recent studies have reported increased eotaxin levels in the bronchial mucosa, bronchoalveolar lavage fluid and sputum[5,6] from asthmatic patients compared with healthy subjects. These findings suggest that besides IL-5, eotaxin may contribute to the pathogenesis of asthma.

This study was designed to determine whether the occurrence of eosinophil apoptosis in sputum reflects activity in patients with asthma. The relationships between number of eosinophils and percentage of eosinophil apoptosis in sputum and IL-5 and eotaxin in sputum supernatant were evaluated.

Methods

Subjects

Patients attending the respiratory outpatient clinic and hospitalized patients at Fifth Affiliated Hospital of Zhongshan University were screened for the study. Thirty stable and 30 exacerbated asthmatics were selected. All subjects were 18 years or older and the diagnosis of asthma was defined and assessed by means of the global initiative for asthma (GINA) updated in 2002.[7] The inclusion criteria were: nonsmoking subjects with clinically exacerbated or stable asthma; a documented reversibility $>15\%$ of baseline FEV_1 following inhaled salbutamol 200 μg and a percentage of eosinophils $\geqslant 7\%$ in induced sputum. Sputum samples containing more than 30% squamous cells were excluded from the analysis. Patients had not received corticosteroids, cromoglycate, theophylline, or other medications 6 weeks before presentation, except for rescue inhaled β_2-agonists. Inhaled β_2-agonists were withheld for 12 h before venipuncture. No subject had any obvious respiratory infection for at least 1 month prior to sputum collection.

A separate group of 20 healthy nonsmoking subjects selected from a health-examination served as controls. They had no evidence of allergic rhinitis, asthma as identified on the basis of history or other allergic diseases. They had normal total IgE levels and eosinophil counts in peripheral blood. Spirometry was within normal limits in these subjects.

All visits comprised a medical history, allergy skin tests, baseline spirometry, sputum induction and hypertonic saline challenge. All subjects gave their informed consent.

Clinical assessment

Skin-prick tests were performed with commercial extracts (Dome/Hollister-Steir, Bayer Pharmaceuticals, Sydney, Australia) for house dust mites (Dermatophagoides pteronyssinus and Dermatophagoides farinae), mold mix (Alternaria, Aspergillus mix, Hormodendrum, Penicillium mix), mixed grasses, cat fur (cat hair and epithelium), and cockroach, together with positive (histamine) and negative (glycerine) controls. A skin-prick test was defined as positive if the wheal diameter was 3 mm or greater at 15 min. A subject was considered atopic if a positive skin-prick test was recorded for any allergen. Spirometry was performed on Microspiro-HI 298, Chest (Tokyo, Japan) and the best of three FEV_1 and FEF_{25-75} percentage of predicted maneuvers were recorded. The specialist was asked to classify patients into one of the four severity categories based on a combination of symptoms and FEV_1 criteria and according to the GINA recommendations.[7]

Sputum induction and processing

Sputum was induced with an aerosol of hypertonic saline using a slight modification of the method described by Pizzichini et al.[8] Inhaled salbutamol at 200 μg was given via