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

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Asthma;  
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Interleukin-5;  
Eotaxin

Summary

\textbf{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.

\textbf{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.

\textbf{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.

\textbf{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 correlations between the levels of sputum IL-5 and eotaxin in either exacerbated ($r=0.93$, $P<0.01$) or stable asthma ($r=0.82$, $P<0.05$) were observed.
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. 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 viability in the sputum of asthmatic patients during attacks. 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.

Eotaxin is a chemokine with potent and selective chemotactic activity for eosinophils. Recent studies have reported increased eotaxin levels in the bronchial mucosa, bronchoalveolar lavage fluid and sputum 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. The inclusion criteria were: nonsmoking subjects with clinically exacerbated or stable asthma; a documented reversibility >15% of baseline FEV1 following inhaled salbutamol 200 μg and a percentage of eosinophils ≥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 β2-agonists. Inhaled β2-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 FEV1 and FEF25–75 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 FEV1 criteria and according to the GINA recommendations.

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. Inhaled salbutamol at 200 μg was given via
metered dose inhaler (MDI) 15 min before sputum induction. Hypertonic saline (3%, 4% and 5%), generated by an ultrasonic nebulizer (Ultraneb 2000; DeVilbiss, Somerset, PA, USA), was inhaled at each concentration for 7 min. The output of the nebulizer was calibrated at 2.5 ml/min. At the start and after each period of inhalation, FEV₁ was measured for safety reasons. After inhalation of the hypertonic saline, subjects were asked to blow their nose, rinse their mouth, and swallow water to minimize contamination from post-nasal drip and saliva. The subjects were then instructed to cough sputum into a sterile container.

The samples of sputum were then gently mixed using a vortex mixer and placed in a shaking water bath at 37 °C for 15 min to ensure complete homogenization. The resulting suspension was filtered through 48 μm nylon gauze. The filtrate was centrifuged at 2000 × g for 4 min, to separate the supernatant from the cell pellet. The supernatants were aspirated and stored in Eppendorf tubes at −70 °C in separate aliquots for later assay. During examination, the volume of the entire supernatant was measured and divided into two aliquots. One volume of 0.1% dithiothreitol (DTT) (Sputolysin 10%; Calbiochem Corp., San Diego, CA, USA) for DPBS. The cells were suspended in 10% fetal calf serum-containing RPMI 1640 solution. In all cases, cells were incubated (4 × 10⁵ cells per tube) for 15 min at room temperature in 100 μl containing 5 μl annexin V FITC and 10 μl PI (Bender medSystem GmbH Rennweg, Vienna, Austria) in the dark with agitation. Two hundred microliters of the sample was diluted to 500 μl with binding buffer just before flow analysis. Samples were analyzed with a FACScan (Becton–Dickinson, San Jose, CA) and CellQuest cell analysis software (Becton–Dickinson). Forward angle light scatter and side scatter were used to gate the eosinophil population to include changes in light scatter properties accompanying the onset of apoptosis and exclude debris. At each time point, an unlabeled control also was prepared to ensure that observed fluorescence was not due to any changes in autofluorescence. Color compensation was set to eliminate FITC spill into the FL2 detector and PI spill into the FL1 detector. Green ( Annexin V FITC ) versus red ( PI ) fluorescence was assessed to monitor onset of apoptosis. Analysis was accomplished by use of quadrant stats in CellQuest software. Quadrants were set so that cells in lower left (LL) quadrant were viable cells (unlabeled). Non-viable (dead) cells (UL) will be PI positive, FITC positive cells were indicative of early apoptosis (LR), and FITC/PI positive cells indicated late apoptosis (UR). Total FITC positive (LR + UR) was used for comparative quantitation and graphing of apoptosis among groups.

Eosinophil isolation

The sputum pellet was resuspended in 10 ml of saline in a 50-ml tube. Ten milliliters of Percoll working solution (9 ml of Percoll; Sigma P1644; 1 ml 10 × Dulbecco phosphate buffered saline (DPBS) without calcium/magnesium; saline adjusted to 1.079 g/ml) was layered underneath the cell suspension and the tube was centrifuged at 400 × g for 30 min at room temperature. The top layers, including the mononuclear cell-containing interface, were aspirated and the pellet, containing mostly eosinophils. Contaminating red blood cells were lysed by cold milli-Q water for 5–10 s. One milliliter of 10 × DPBS was immediately added and cells were pelleted at 300 × g. Cells were then washed with 10 ml of DPBS. The cells were suspended in 10% fetal calf serum-containing RPMI 1640 solution. In all cases, cells were > 95% in terms of both viability and eosinophil purity. Cell viability was determined by trypan blue exclusion. Eosinophil purity was assessed by Wright–Giemsa stained cytospins.

Assessment of eosinophil apoptosis

After 3 days of incubation at 37 °C in a 5% CO₂-containing incubator, cells were harvested and the differential analysis of apoptotic cells was performed by the analysis of annexin V binding and propidium iodide (PI) uptake. Cells were incubated (4 × 10⁵ cells per tube) for 15 min at room temperature in 100 μl containing 5 μl annexin V FITC and 10 μl PI (Bender medSystem GmbH Rennweg, Vienna, Austria) in the dark with agitation. Two hundred microliters of the sample was diluted to 500 μl with binding buffer just before flow analysis. Samples were analyzed with a FACScan (Becton–Dickinson, San Jose, CA) and CellQuest cell analysis software (Becton–Dickinson). Forward angle light scatter and side scatter were used to gate the eosinophil population to include changes in light scatter properties accompanying the onset of apoptosis and exclude debris. At each time point, an unlabeled control also was prepared to ensure that observed fluorescence was not due to any changes in autofluorescence. Color compensation was set to eliminate FITC spill into the FL2 detector and PI spill into the FL1 detector. Green ( Annexin V FITC ) versus red ( PI ) fluorescence was assessed to monitor onset of apoptosis. Analysis was accomplished by use of quadrant stats in CellQuest software. Quadrants were set so that cells in lower left (LL) quadrant were viable cells (unlabeled). Non-viable (dead) cells (UL) will be PI positive, FITC positive cells were indicative of early apoptosis (LR), and FITC/PI positive cells indicated late apoptosis (UR). Total FITC positive (LR + UR) was used for comparative quantitation and graphing of apoptosis among groups.

Measurements of eotaxin, IL-5 and eosinophil cationic protein levels in sputum

The levels of eotaxin and IL-5 in sputum supernatant were measured using enzyme immunoassays according to the manufacturer’s protocol (R&D Systems, Minneapolis, Minn., and Endogen, Inc., Woburn, MA, respectively). The level of eosinophils cationic protein (ECP) in sputum was measured using a sensitive radioimmunoassay (Kabi Pharmacia Diagnostics, Uppsala, Sweden). Sensitivities for eotaxin, IL-5 and ECP were 5, 5 and 2 pg/ml, respectively.

Statistical analysis

Data are expressed as means ± SEM or as median values, with a range from the minimal to the maximal values. When the variables showed normal distribution, the comparison was performed using one-way analysis of variance and Student’s t test. When the variables did not show normal distribution, they were compared using the Kruskal–Wallis test. The level of significance was set at P < 0.05.

Results

The age and gender were similar in each of the three groups. FEV₁ and FEV₂₅₋₇₅ (percentage of predicted) were both significantly lower, and the levels of IgE and eosinophils in blood were significantly higher in asthmatic patients than in healthy subjects, and FEV₁ and FEV₂₅₋₇₅ (percentage of predicted) were much lower in exacerbated asthmatics than in stable ones (Table 1).
Characteristics of the sputum

All subjects tolerated the sputum induction procedure well and sputum could be obtained from all 20 healthy subjects and all patients with asthma. Table 1 shows the results of cytological analyses and ECP levels in sputum. The median numbers of total cells and proportions of neutrophils in sputum did not differ significantly between the three groups. The median proportions of lymphocytes and epithelial cells were significantly lower in asthmatic patients with exacerbation (2.1% and 3.9%, respectively) than in the healthy subjects (4.5% and 7.2%, respectively) (P<0.05). The study showed that proportions of eosinophils in sputum were much higher both in exacerbated and in stable asthmatic patients (29.3%, 20.3%) than in healthy subjects (2.4%, P<0.01). In exacerbated asthmatics, the median percentage of eosinophils in sputum (29.3%) was significantly higher than in the stable asthmatic patients (20.3%, P<0.05). The median concentrations of ECP in sputum supernatant were much higher in patients both with exacerbated (5014.9 ng/ml) and stable asthma (5092.4 ng/ml) than in the healthy subjects (109.6 ng/ml, P<0.01).

IL-5, eosinaxin and apoptotic eosinophil levels in sputum

IL-5, eosinaxin and apoptotic eosinophils could not be detected in sputum supernatant from two of the healthy subjects. Hence, the results are given for 18 healthy subjects. The median levels of IL-5 in sputum were 50.44 (range 32.99–67.01), 39.29 (22.37–64.55) and 10.46 (7.94–17.47) pg/ml in exacerbated and stable asthmatic patients and healthy subjects, respectively (Fig. 1). More IL-5 was found in asthmatic patients with exacerbation than in the stable asthmatic patients (P<0.05). Even in the stable asthmatics, the level of IL-5 was significantly higher than in healthy subjects (P<0.01).

The median levels of eotaxin in sputum were 644.6 (range 197.4–937.7), 342.2 (89.6–779.4) and 95.0 (10.2–384.9) pg/ml in exacerbated and stable asthmatics and healthy subjects, respectively (Fig. 1). The level of eotaxin was significantly higher in both asthmatic patient groups compared to the healthy subjects (P<0.01). More eotaxin was found in the patients with exacerbated asthma than in those with stable asthma (P<0.05).

The median for the proportions of apoptotic eosinophils in sputum was 3.86 (range 2.04–5.98)% in exacerbated asthmatic patients, 3.72 (2.11–5.86)% in stable asthmatics and 1.04 (0.79–2.13)% in the healthy subjects (Fig. 1). Thus, in both groups of asthmatic patients, the proportion of apoptotic eosinophils was significantly higher than in the healthy subjects (P<0.01). The proportions of apoptotic eosinophils in sputum were similar in stable and exacerbated asthmatics.

Correlations between IL-5, eotaxin, ECP, apoptotic eosinophils, eosinophils in sputum and clinical findings in stable and exacerbated asthmatic patients

There were significant positive correlations between the levels of IL-5, eotaxin and ECP in sputum supernatant and the percentages of sputum eosinophils both in exacerbated and stable asthmatic patients. No correlations were noted in the patients with asthma between the proportions of eosinophils in sputum and either IgE in blood or lung function measurements (Table 2, Fig. 2).

In both the exacerbated and stable asthmatic patient groups, the levels of sputum IL-5, eotaxin and ECP in sputum supernatant were inversely correlated to the proportion of sputum eosinophil apoptosis (Table 2, Fig. 2). There was no

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Subject and sputum characteristics and ECP levels in the study.</th>
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<tbody>
<tr>
<td>Characteristics</td>
<td>Control group (n = 20)</td>
</tr>
<tr>
<td>Age (year)</td>
<td>47.5 (18–64)</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>9/11</td>
</tr>
<tr>
<td>Allergic rhinitis, n (%)</td>
<td>0</td>
</tr>
<tr>
<td>Serum IgE (IU/ml)</td>
<td>133.4 ± 43.4</td>
</tr>
<tr>
<td>Blood eosinophils (/μl)</td>
<td>151 ± 54</td>
</tr>
<tr>
<td>Atopy, n (%)</td>
<td>4 (20)</td>
</tr>
<tr>
<td>FEV1, % predicted</td>
<td>92.1 ± 8.4</td>
</tr>
<tr>
<td>FEF25–75, % predicted</td>
<td>88.4 ± 8.4</td>
</tr>
<tr>
<td>Sputum indices</td>
<td></td>
</tr>
<tr>
<td>Total cell count, ×10⁶/g</td>
<td>0.99 (0.19–2.99)</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>2.4 (0.2–3.7)</td>
</tr>
<tr>
<td>Macrophages (%)</td>
<td>70.9 (36.1–92.2)</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>16.2 (4.2–40.8)</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>4.5 (0.3–14.2)</td>
</tr>
<tr>
<td>Epithelial cells (%)</td>
<td>7.2 (0.8–21.5)</td>
</tr>
<tr>
<td>ECP (ng/ml)</td>
<td>109.6 (19.5–302.5)</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SEM or shown as median (range). *P<0.05, **P<0.01, compared with control. #P< 0.05, compared with stable group.
The correlation between the amounts of IgE in blood or lung function parameters, and the percentage of apoptotic eosinophils in sputum in the asthmatic patients.

The levels of sputum IL-5 in both stable and exacerbated asthmatics were positively correlated with the concentration of sputum eotaxin (Fig. 3).

There were no correlations between the proportions of eosinophils and of apoptotic eosinophils in sputum in stable and exacerbated asthmatics.

**Discussion**

Allergy is characterized by the accumulation of activated eosinophils at the site of inflammation. Inhibition of eosinophil apoptosis has been proposed as a key mechanism for the development of tissue eosinophilia in allergic disorders. IL-5, IL-3, and GM-CSF derived cytokines, dramatically increase the life span of purified eosinophils by inhibiting their spontaneous apoptosis in vitro. Moreover, these cytokines are overexpressed in inflamed allergic tissue and have been shown to increase eosinophil survival in vivo, thereby contributing to the accumulation of eosinophils in allergic inflammation. In this study, we have shown that exacerbated and stable asthmatics, in comparison to healthy subjects, had significantly higher proportions of eosinophils both in blood and sputum, higher proportions of apoptotic eosinophils in sputum, and higher levels of IL-5, eotaxin and ECP in induced sputum. These findings do not support the notion that decreased apoptosis is a cause of eosinophil accumulation.

Asthma results from complex interactions between inflammatory cells, mediators, and the cells and tissues of the airways. Eosinophils are known to play a major part in asthmatic airway inflammation. Recruitment from the blood to the airway initially involves adherence of eosinophils to endothelial cells and subsequent migration into an inflammatory site. In agreement with this concept, we also found that eosinophil percentages in peripheral blood and induced sputum were elevated in stable and exacerbated asthmatic patients in comparison to healthy subjects. IL-5 enhances the interaction between inflammatory cells and epithelial cells. In lung tissue release of ECP is fundamental to the proinflammatory role of eosinophils. We found that the eosinophil activation markers IL-5 and ECP were significantly increased in patients with stable and exacerbated asthma, and the levels both of sputum IL-5 and ECP correlated positively with the proportions of eosinophils in sputum in all asthmatics. This suggests that the higher percentage of eosinophils and increased levels of eosinophil activation markers in asthmatic sputum are due to recruitment of eosinophils and possibly degranulation of eosinophils in the

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**Table 2** Correlations between percentage of eosinophils, and proportion of apoptotic eosinophils and the concentrations of IL-5, eotaxin and ECP in sputum, and lung function changes in asthmatics irrespective healthy subjects.

<table>
<thead>
<tr>
<th></th>
<th>IL-5</th>
<th>Eotaxin</th>
<th>ECP</th>
<th>IgE</th>
<th>FEV₁</th>
<th>FEF₂₅-₇₅</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eosinophils (exacerbated asthma)</td>
<td>0.94**</td>
<td>0.93**</td>
<td>0.80**</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Eosinophils (stable asthma)</td>
<td>0.92**</td>
<td>0.79*</td>
<td>0.76*</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Apoptotic eosinophils (exacerbated asthma)</td>
<td>−0.85**</td>
<td>−0.85**</td>
<td>−0.74*</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Apoptotic eosinophils (stable asthma)</td>
<td>−0.79*</td>
<td>−0.69*</td>
<td>−0.75*</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
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Data showed *P<0.05, **P<0.01.
NS: not significant.
bronchi. IL-5, a cytokine that attracts, activates and prolongs the survival of eosinophils, is an important eosinophil-regulating cytokine in the pathogenesis of allergic inflammation and asthma, and its concentration in asthmatics correlates with markers of eosinophil activation and number of T lymphocyte. It also indicates that ECP is fundamental to the proinflammatory role of eosinophils.

In this study, the proportions of apoptotic eosinophils in the induced sputum were higher in stable and exacerbated asthmatics, which may indicate that apoptotic eosinophils are involved in allergic inflammation. Apoptosis or programmed cell death is an ordered process of fundamental biological importance which is as vital to homeostasis as are cellular proliferation, differentiation, and migration. 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. IL-5 has an inhibitory effect on eosinophil apoptosis by regulating bcl-2 expression.

![Figure 2](image-url) **Figure 2** Relationship between the percentages of sputum eosinophil apoptosis and the concentrations of IL-5, eotaxin in sputum supernatant from asthmatic patients. Correlations between proportions of apoptotic eosinophils and the levels of IL-5 in sputum from exacerbated (A) and stable (B) asthmatic, and the amount of sputum eotaxin in the patients with exacerbated (C) and stable (D) asthma.

![Figure 3](image-url) **Figure 3** Correlations between IL-5 and eotaxin in sputum supernatant in exacerbated (A) and stable asthmatic patients (B).
et al. reported that IL-5 enhances survival in vitro by abrogating apoptosis. We found that IL-5 concentration in sputum from asthmatic patients was negatively correlated to the proportion of eosinophil apoptosis, which also suggests that IL-5 inhibits the apoptosis of eosinophils. Since inappropriate accumulation of eosinophils and the subsequent release of their potent armory of mediators, including cytotoxic granule proteins, lipid mediators, cytokines and chemokines, are thought to contribute significantly to the airway inflammation underlying asthma pathogenesis, programmed cell death (apoptosis) is probably important to avoid excessive damage. The findings of an increased proportion of apoptotic eosinophils in sputum from our asthmatic patients seem to indicate a mechanism for the resolution of an asthma attack. Whether individual asthmatic patients with low levels of apoptotic eosinophils have a worse prognosis remains to be seen.

Eotaxin production is regulated by and produced by cells in the bronchi and may be important in the pathogenesis of asthma and has been reported to play a role in eosinophilic inflammation. Eotaxin mRNA is highly expressed in sputum cells from asthmatic patients. Thus chemokine networks seem to exist in eosinophilic inflammation. Our results revealed that sputum obtained from asthmatic patients showed detectable levels of eotaxin, and the concentration of eotaxin was high compared to healthy subjects. Sputum eotaxin correlated inversely with the proportion of apoptotic eosinophils in sputum, and positively with sputum eosinophils, indicating an important role for eotaxin in eosinophil accumulation. Therefore, increased inflammation may affect IL-5 (cytokine) and eotaxin (chemokine) expression, suggesting that not only IL-5 but also eotaxin, which acts as a hematopoietic rather than as a chemoattractant factor, play important roles in eosinophil accumulation in the airways.

Seton et al. reported that stimulus-dependent release of the granule protein ECP was increased in apoptotic eosinophils and hypothesized that the cytoskeleton of eosinophils is disrupted during the apoptotic process. Their results showed an increased propensity to release ECP in response to appropriate stimuli. This study demonstrated that in sputum there was a negative correlation between apoptotic eosinophils and ECP in stable and exacerbated asthmatics. We postulate that increasing ECP released from both eosinophils and apoptotic eosinophils reaches an upper critical level and inversely inhibits the propensity to eosinophil apoptosis in asthma patients.

Our results revealed a positive correlation between sputum IL-5 and sputum eotaxin obtained from exacerbated and stable asthmatics. Exacerbated asthmatic patients showed more elevated levels of IL-5 and eotaxin in sputum than patients with stable asthma. This indicates that IL-5 and eotaxin could play a part in activation of asthma and participate in the process of allergic inflammation although possibly through different mechanisms. There were no correlations between eosinophils or apoptotic eosinophils in sputum and FEV1 and FEF25.75 (percentage of predicted) in asthmatic patients. This finding suggests that eosinophils and apoptotic eosinophils are not markers of clinical severity in asthma.

In conclusion, this study showed elevated concentrations of IL-5 and eotaxin, and high proportions of apoptotic eosinophils and eosinophils in sputum from asthmatic patients. IL-5 and eotaxin in induced sputum also showed positive correlation to the proportion of eosinophils, and negative correlation to the percentage of eosinophil apoptosis. These findings suggest that caution should be applied when interpreting the presence of IL-5 and eotaxin as specific markers for eosinophilic airway inflammation.

IL-5 and eotaxin enhance eosinophil numbers possibly by influencing the apoptotic process of eosinophils in patients with acute and stable asthma.

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References


