Heterogeneity of Airway Inflammation in Persistent Asthma*

Evidence of Neutrophilic Inflammation and Increased Sputum Interleukin-8

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Study objectives: To identify the characteristics of airway inflammation in persistent asthma and to examine the role of neutrophilic inflammation in noneosinophilic persistent asthma.

Methods: Nonsmoking adults (n = 56) with persistent asthma and healthy control subjects (n = 8) underwent hypertonic saline solution challenge and sputum induction. Selected sputum portions were dispersed with dithiothreitol and assayed for total cell count, cellular differential, supernatant eosinophil cationic protein (ECP), myeloperoxidase, and interleukin (IL)-8.

Results: We identified two distinct inflammatory patterns. Typical eosinophilic inflammation occurred in 41% of subjects, whereas the remainder exhibited noneosinophilic asthma (59%). Both neutrophil percentage and absolute neutrophil counts were increased in subjects with noneosinophilic asthma (64%, 283 x 10^6/mL) compared to eosinophilic asthma (14%, 41 x 10^6/mL) and control subjects (34%, 49 x 10^6/mL; p = 0.0001). Myeloperoxidase was elevated in both noneosinophilic (250 ng/mL) and eosinophilic groups (254 ng/mL) compared with control subjects (82 ng/mL; p = 0.002). Sputum IL-8 levels were highest in subjects with noneosinophilic asthma (45 ng/mL) compared to eosinophilic asthma (9.6 ng/mL) and control subjects (3.5 ng/mL; p = 0.0001). Neutrophils correlated with IL-8 levels (r = 0.72). ECP was highest in subjects with eosinophilic asthma (2,685 ng/mL) compared with noneosinophilic asthma (1,081 ng/mL) and control subjects (110 ng/mL; p = 0.0001).

Conclusion: Induced-sputum analysis in persistent asthma identifies two different inflammatory patterns. The most common pattern is noneosinophilic, associated with a neutrophil influx and activation, which may be mediated by IL-8 secretion. There is heterogeneity of airway inflammation in persistent asthma, which indicates differing mechanisms and may impact on treatment responses.

Key words: asthma; eosinophil; induced sputum; inflammation; interleukin-8; neutrophil

Abbreviations: AHR = airway hyperresponsiveness; APAAP = alkaline phosphatase antialkaline phosphatase; ECP = eosinophil cationic protein; IL = interleukin; IQR = interquartile range; PD_{20} = provocation dose of hypertonic saline solution causing a 20% fall in FEV1

Airway inflammation with eosinophils is now accepted as a fundamental characteristic of asthma.1 There is, however, increasing recognition of noneosinophilic forms of asthma, particularly in subjects with severe disease.2–5 Noneosinophilic asthma has been reported in severe refractory asthmatic patients who are dependent on ingested corticosteroid,2,3 and may be associated with more frequent intubation episodes and greater expiratory airway collapse.3 Exacerbations can also be noneosinophilic in nature.5,6 The mechanisms of noneosinophilic asthma and the role of mucosal inflammation are not well understood. Some studies2,3 implicate neutrophils in the pathogenesis of noneosinophilic asthma, but others4,5,7 do not. Neutrophils are not generally recognized as part of the inflammatory process in stable asthma8,9; however, elevated neutrophils are seen in severe refractory asthma,2–3 and severe exacerbations.6,10–12 The pathogenic mechanisms of neu-
atrophil inflammation include an influx in response to the chemokine interleukin (IL)-8,6,13 with release of several potent proinflammatory enzymes, such as myeloperoxidase, that cause tissue damage.

Persistent asthma comprises up to 15% of cases of asthma, and is more common than severe refractory asthma.1 The characteristics of airway inflammation in subjects with persistent asthma are not well defined. In particular, the frequency of noneosinophilic disease and neutrophil influx is not known. We tested the hypothesis that induced-sputum analysis could be used to identify two different inflammatory subtypes in persistent asthma: a typical eosinophilic response and a noneosinophilic pattern. Furthermore, we hypothesized that persistent noneosinophilic asthma would be associated with evidence of neutrophilic inflammation characterized by increased sputum neutrophils, release of neutrophil myeloperoxidase, and the presence of increased amounts of IL-8 in sputum supernatant.

MATERIALS AND METHODS

Subjects

Nonsmoking adults with persistent symptomatic asthma and healthy control subjects were recruited from the Respiratory Outpatient Clinic of the John Hunter Hospital and by advertisement (control subjects). Subjects were identified from a list of consecutive attendances to the respiratory clinic and were assessed for participation in this study. We screened 78 subjects with a diagnosis of asthma receiving high-dose inhaled corticosteroids, and identified 56 subjects who met the inclusion criteria. Subjects were required to have persistent asthma defined as (1) recurrent wheeze or asthma symptoms on at least 2 days per week for at least 2 weeks in the previous month, or use of inhaled short-acting β2-agonist at least three to four times a week for relief of asthma symptoms; (2) current airway hyperresponsiveness (AHR) with provocative dose of hypertonic saline solution causing a 20% fall in FEV1 (PD20) of < 20 mL, and (3) the use of high-dose inhaled corticosteroids in the same dose for at least 4 weeks prior to study. The daily minimum doses for inhaled corticosteroids were as follows: beclomethasone dipropionate ≥ 1,000 μg, fluticasone propionate ≥ 500 μg, or budesonide ≥ 1,000 μg. All patients were receiving inhaled, short-acting, β2-agonist rescue medication on an as-needed basis for the relief of asthma symptoms. We excluded subjects if they failed to meet the inclusion criteria or if they had received any systemic corticosteroids during the previous 4 weeks, had any serious concurrent diseases, or had a recent (past 4 weeks) asthma exacerbation, clinical evidence of a respiratory tract infection, or oral candidiasis. Control subjects were asymptomatic, healthy subjects with normal spirometry findings and airway responsiveness. The Hunter Area Health Service Research Ethics Committee approved the protocol, and informed written consent was obtained from each participant.

Clinical Assessment

At the study visit, demographic details of age, sex, ethnic origin, height, weight, and history of atopic disease were collected. An asthma history was taken, including asthma duration, smoking history, the number of hospitalizations for asthma, and courses of oral steroids during the last year. Recent asthma symptoms (in the past 2 weeks) were assessed, including morning asthma, nocturnal waking due to asthma, asthma symptoms overall, any severe attacks, and frequency of rescue medication use. Any concurrent medical conditions and medications were recorded. Subjects were also assessed by history and clinical examination for the presence of a respiratory tract infection and oral candidiasis, and were excluded if either was present. A hypertonic saline solution bronchial provocation challenge and sputum induction were performed. Subjects subsequently recorded symptoms and peak expiratory flow in a daily diary for 2 weeks.

The severity of persistent asthma was classified as mild persistent (step 2), moderate persistent (step 3), or severe persistent (step 4), using National Heart, Lung, and Blood Institute guidelines.1 Subjects were placed in the highest severity category based on assessment of clinical and spirometric criteria. Subjects with mild persistent asthma exhibited any of the following: FEV1 > 80% predicted, nighttime symptoms more than twice per month, and/or symptoms more than twice per week but less than once a day. Moderate persistent asthma was defined by daily symptoms, and short-acting β2-agonist requirements, nighttime symptoms more than once per week, FEV1 > 60% and < 80% predicted, or peak expiratory flow variability > 30%. In severe persistent asthma, there were continual symptoms, limited physical activity, frequent nighttime symptoms, or FEV1 < 60% predicted.

Spirometry

Subjects withheld taking short-acting β2-agonists for 4 h before testing. Baseline spirometry was performed using a Minato Autospiro AS-600 (Minato Medical Science; Osaka, Japan). Subjects were asked to perform three reproducible FEV1 and FVC maneuvers while in a sitting position and wearing nose clips. Results were compared with published predicted values.14

Saline Solution Challenge

Hypertonic saline solution (4.5%) was inhaled for doubling time periods (30 s, 1 min, 2 min, 4 min) from a DeVilbiss 2000 ultrasonic nebulizer (DeVilbiss Health Care; Somerset, PA) with 23-cm corrugated tubing and a Hans Rudolph 2700, two-way nonrebreathing valve box (Hans Rudolph; Kansas City, MO) with a rubber mouthpiece and nose clips, as described.15 The nebulizer output was 1.8 mL/min and particle size (mass median aerosol diameter) < 5 μm. FEV1 was measured 60 s after each saline solution dose. The test was stopped when the FEV1 had fallen by ≥ 20% or 15 min (cumulative) of nebulization time had elapsed. If FEV1 had fallen ≥ 20% during the challenge, β2-agonist was administered using a metered-dose inhaler, and the challenge continued when FEV1 had recovered to within 10% of baseline. The dose of saline solution delivered to the patient was determined by weighing the nebulizer cup and tubing before and after the challenge.

Sputum Induction

Sputum induction was conducted concurrently with the saline solution challenge, as described.15 Before the hypertonic saline solution challenge was commenced, the sputum-induction procedure was explained to the subject. The technician demonstrated how to cough and clear the throat, in order to propel mucus from the lungs into the mouth, and then to empty these contents into a sterile sputum container. Subjects were asked to rinse their mouth with water before the procedure to help
eliminate squamous cell contamination of the sputum sample. They were asked to cough between each dose of nebulized saline solution to clear their throats and expectorate into the container. This procedure continued until an adequate sample, containing > 0.5 mL visible mucocellular material was obtained. If a satisfactory sputum sample was not obtained at the time the FEV₁ had fallen ≥ 20%, nebulization with 4.5% saline solution was continued for 4-min periods once the FEV₁ had returned to within 10% of baseline.

**Sputum Processing**

Sputum was selected from saliva and processed as described. Briefly, sputum was treated by adding four volumes of 0.1% dithiothreitol (Sputolsyn 10%; Calbiochem; La Jolla, CA) and mixed by rotating for 30 min at 37°C, followed by four volumes of phosphate-buffered saline solution. The suspension was filtered through a 60-µm nylon gauze (Millipore; North Ryde, NSW, Australia) and a total cell count of leucoocytes and viability were determined. The cell suspension was centrifuged at 200g for 10 min, and supernatant was aspirated and stored at −70°C. The cell pellet was resuspended in phosphate-buffered saline solution to attain a concentration of 1 × 10⁶ cells/mL and 70 µL placed into cups of a Shandon III cytocentrifuge (Shandon Cytospin; Sewickey, PA) for slide preparation.

**Cytochemistry**

A differential count was obtained from 400 cells counted on May-Grünwald-Giemsa-stained cytopreps. Eosinophils were enumerated from slides stained with Chromotrope 2R in the same fashion. Cells staining positive for the secreted form of eosinophil cationic protein (ECP) were identified using a monoclonal antibody (EG2; Pharmacia; Cambridge, MA) and detected using the alkaline phosphatase antialkaline phosphatase (APAAP) technique, as described.12 These cells were further differentiated as eosinophils or macrophages based on conventional morphologic criteria, and the proportion of macrophages staining positive for EG2⁻ was evaluated to identify whether enhanced eosinophil apoptosis and macrophage ingestion could explain the paucity of eosinophils in subjects with noneosinophilic asthma.16 IL-8 positive cells were detected using a monoclonal antibody (mouse anti-human IL-8; Pharmingen; San Diego, CA) and detected by the APAAP technique. Positive and antibody (mouse IgG₁) controls were included in each staining run.

**Fluid Phase Measurements**

The concentrations of ECP, myeloperoxidase, and IL-8 were determined in sputum supernatant, by radioimmunoassay (ECP RIA; Kabi Pharmacia Diagnostics AB; Uppsala Sweden) and by enzyme-linked immunosorbent assay (MPO; Oxis International; Portland, OR, and IL-8; R & D Systems; Minneapolis, MN) with standard curves based on dilutions of purified ECP, myeloperoxidase, or recombinant IL-8, respectively. The limits of detection of the fluid phase assays were 2 ng/mL, 1.6 ng/mL, and 32 pg/mL, respectively.

**Analysis**

Airways responsiveness was assessed as the PD₂₀, and was log transformed for analysis. Results are presented as mean/median with SD/SE/interquartile range (IQR) as appropriate. Fluid phase measures and absolute cell counts were log transformed for analysis. Group comparisons were conducted using analysis of variance with Bonferroni post hoc testing for normally distributed variables and Kruskall-Wallis testing for nonparametric data. Associations between variables were examined using Pearson’s or Spearman correlation coefficients as appropriate. Significance was accepted when p < 0.05. A priori, we defined subjects as having sputum eosinophils within the normal range (< 2.5%, noneosinophilic), or as having increased sputum eosinophils (> 2.5%), based on our previously reported sputum normal values.6

**Results**

The 56 subjects with persistent asthma (Table 1) reported a long history of asthma and were receiving oral corticosteroid.31,32

| Table 1—Clinical Characteristics of Subjects With Persistent Asthma and Control Subjects* |
|-----------------------------------------------|---------------|---------------|---------------|---------------|
| Characteristics                          | Mild Persistent | Moderate Persistent | Severe Persistent | Control Subjects |
| Patients, No.                              | 11            | 26            | 19            | 8             |
| Male/female gender, No.                    | 3/8           | 3/23          | 10/9†         | 1/7           |
| Age, yr                                    | 41 (4.6)      | 43 (3.2)      | 56 (3.4)§      | 35 (2.3)      |
| ICS dose, µg/d†                            | 1,573 (184)   | 1,585 (91)    | 1,832 (196)   | 0 (0)         |
| Asthma duration, yr                        | 19 (4.4)      | 24 (3.7)      | 28 (4.1)      |                |
| Past smoking, No. (%)                      | 5 (45)        | 13 (50)       | 11 (57)       | 2 (25)        |
| Atopy, %                                   | 73            | 73            | 58            | 38            |
| OCS courses in past year, No. (%)          |               |               |               |               |
| 0                                          | 7 (64)        | 14 (58)       | 10 (53)       | 0             |
| 1                                          | 3 (27)        | 6 (25)        | 7 (37)        | 0             |
| ≥ 2                                        | 1 (9)         | 4 (17)        | 2 (10)        | 0             |
| FEV₁, % predicted†                         | 96 (3.6)      | 80 (2.8)      | 50 (2.1)| 105 (4.1)   |
| FEV₁/VC, %†                                | 76 (2.8)      | 72 (1.2)      | 59 (2.9)      | 82 (1.1)      |
| PD₂₀, mL‡                                  | 9.2 (2.6)     | 8.0 (1.1)     | 4.1 (0.9)     | NR            |
| BDR, %‡                                    | 29 (3.3)      | 27 (1.5)      | 34 (2.6)      | NR            |

*Data are presented as mean (SEM) unless otherwise indicated. NR = not reactive; OCS = oral corticosteroid.†‡†ICS = inhaled corticosteroid (beclomethasone), where 1 µg beclomethasone = 1 µg budesonide = 0.5 µg fluticasone.

†FEV₁ was recorded prior to bronchodilator treatment.

‡BDR = bronchodilator response to inhaled albuterol (200 µg) as percent baseline FEV₁.

§p < 0.05 among asthma groups.
high-dose inhaled corticosteroids. Eleven subjects (20%) had mild persistent asthma, 26 subjects (46%) had moderate persistent asthma, and 19 subjects (34%) had severe persistent asthma. The subjects with severe persistent asthma were older with more severe airway obstruction and more severe airway hyperresponsiveness \( (p < 0.05) \). The eight control subjects (one male subject) were aged 35 years (mean) with a mean FEV\(_1\) of 105.1% predicted.

**Sputum Cell Counts**

There were two distinct inflammatory cell patterns that were consistent across the range of clinical severity in persistent asthma (Fig 1, Table 2). The first group \((n = 23)\) had typical eosinophilic inflammation with a sputum eosinophil count of 8% and comprised 41% of the subjects. The second group \((n = 33)\) had suppressed eosinophil counts \((median \text{ eosinophils } 1.0\%)\) and comprised 59% of the subjects. The subjects with noneosinophilic asthma had evidence of active disease with current symptoms and AHR similar to those with eosinophilic asthma \((Table 2)\). There were no current smokers studied. The prevalence of past smoking was similar between the groups \((p > 0.05; \text{Table 2})\). Sputum parameters were not related to the type, dose, or duration of corticosteroid used \((p > 0.05)\).

Sputum neutrophils were increased in subjects with noneosinophilic asthma \((Fig 2, \text{Table 3})\). Both the percentage neutrophils \((64\% \text{ vs } 14\%, \ p = 0.0001; \text{Table 3})\) and the absolute neutrophil number were elevated \((283 \times 10^9/\text{mL vs } 41 \times 10^9/\text{mL}, \ p = 0.02; \text{Fig 2})\) in the noneosinophilic group when compared with eosinophilic asthma. Subjects with noneosinophilic asthma also had a significantly elevated absolute neutrophil count compared to control subjects \((49 \times 10^9/\text{mL, } p = 0.006; \text{Fig 2})\). The intensity of sputum cellularity, as reflected by the total cell count, was higher in the noneosinophilic group than the eosinophilic \((p = 0.03)\) and control subjects \((p < 0.05; \text{Table 3})\). Other cell types were similar between the groups. Sputum cell counts including neutrophils were similar between former smokers and never-smokers \((p > 0.05)\). Median sputum neutrophil percentage was 46\% \((\text{IQR, 10.6, 73.9})\) in former smokers and 46\% \((\text{IQR, 16.1, 70.0})\) in never-smokers \((p = 0.05)\).

![Figure 1](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21962/)

**Figure 1.** Sputum eosinophils (chromotrope 2R-positive cells) in mild, moderate, and severe persistent asthma. E+ = eosinophilic asthma; E- = noneosinophilic asthma. Individual data points are shown as open circles. \( p < 0.05, \text{E+ vs E- asthma} \).

![Figure 2](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21962/)

**Figure 2.** Absolute sputum neutrophils (polymorphonuclear neutrophils [pmn], open bars) and fluid-phase IL-8 (median hatched bars) in persistent eosinophilic \((E+)\) and noneosinophilic \((E-)\) asthma patients and healthy control subjects. \( \ast = p < 0.01 \).

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**Table 2—Clinical Characteristics of Eosinophilic and Noneosinophilic Persistent Asthma**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Eosinophilic Asthma</th>
<th>Noneosinophilic Asthma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, No.</td>
<td>23</td>
<td>33</td>
</tr>
<tr>
<td>Male/female gender, No.</td>
<td>4/19</td>
<td>12/21</td>
</tr>
<tr>
<td>Age, yr</td>
<td>47.7 (3.5)</td>
<td>46.6 (2.9)</td>
</tr>
<tr>
<td>Smoking past, No. (%)</td>
<td>14 (61)</td>
<td>15 (45)</td>
</tr>
<tr>
<td>Atyopy, No. (%)</td>
<td>19 (83)(\dagger)</td>
<td>19 (58)</td>
</tr>
<tr>
<td>Asthma severity, %†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild persistent</td>
<td>13</td>
<td>21</td>
</tr>
<tr>
<td>Moderate persistent</td>
<td>52</td>
<td>43</td>
</tr>
<tr>
<td>Severe persistent</td>
<td>35</td>
<td>36</td>
</tr>
<tr>
<td>Lung function</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV(_1), % predicted</td>
<td>71 (4)</td>
<td>74 (4)</td>
</tr>
<tr>
<td>FEV(_1)/VC %</td>
<td>70 (2)</td>
<td>68 (2)</td>
</tr>
<tr>
<td>BDR</td>
<td>29 (2.1)</td>
<td>31 (1.8)</td>
</tr>
<tr>
<td>PD(_{20}), mL</td>
<td>6.5 (1.2)</td>
<td>7.4 (1.1)</td>
</tr>
<tr>
<td>Asthma symptoms‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morning waking</td>
<td>6.5 (1.3)</td>
<td>6.5 (1.0)</td>
</tr>
<tr>
<td>Night waking, nights</td>
<td>2.9 (0.9)</td>
<td>2.2 (0.7)</td>
</tr>
<tr>
<td>(\beta_2)-Agonist use, puffs/d</td>
<td>3.7 (0.7)</td>
<td>4.5 (0.7)</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICS daily dose, (\mu)g/d</td>
<td>1,626 (156)</td>
<td>694 (101)</td>
</tr>
</tbody>
</table>

*Data are presented as mean (SE) unless otherwise indicated. See Table 1 for expansion of abbreviations.
†National Heart, Lung, and Blood Institute asthma guidelines severity classification.
‡Past 2 weeks.
\(\dagger p = 0.04\).
Myeloperoxidase and IL-8 were higher in noneosinophilic asthma patients when compared to control subjects (p < 0.05; Fig 2). Sputum IL-8 levels correlated with the number of neutrophils present (r = 0.72, p < 0.05; Fig 3). This association was present in each group and with all groups combined (Fig 3). In former smokers, sputum IL-8 (18 ng/mL) was similar to never-smokers (16.6; p = 0.37), as was ECP (2,410 ng/mL vs 1,265 ng/mL; p = 0.37). There was a tendency for myeloperoxidase to be higher in former smokers (300 ng/mL vs 207 ng/mL; p = 0.05). IL-8 positive cells were present (mean [SE]) as 11% (5.2%) of cells in eosinophilic asthma, and 25% (5.3%) of cells in noneosinophilic asthma (p = 0.13). Most of the cells staining positive for IL-8 were neutrophils, (97.9%; SE, 1.06%); however, some macrophages and epithelial cells also were positive for IL-8 immunoreactivity.

Sputum supernatant ECP levels were elevated in subjects with eosinophilic asthma (p = 0.04) when compared to control subjects (p < 0.05; Fig 2). ECP was also higher in subjects with noneosinophilic asthma when compared to control subjects (p < 0.05). Sputum ECP was correlated with sputum total cell count (r = 0.69, p < 0.001), sputum eosinophils (r = 0.76; p < 0.001), and sputum IL-8 (r = 0.65, p < 0.001). In eosinophilic asthma, 84 (10.8)% of eosinophils were positive for EG2, which recognizes ECP. In order to test the hypothesis that the reduced level of eosinophils in noneosinophilic asthma was due to enhanced eosinophil apoptosis and macrophage ingestion, we measured macrophages staining positive for EG2. The median (IQR) percentage of macrophages staining positive for EG2 was 15% (3.8, 52.5) in eosinophilic asthma, compared to 0.0% (0.0, 22.5) in noneosinophilic asthma (p = 0.05). The proportion of samples with EG2+ macrophages in noneosinophilic asthma (39%) was significantly less than in eosinophilic asthma (80%, p = 0.03; Fig 4), indicating that excessive macrophage ingestion of apoptotic eosinophils was unlikely to be responsible for the reduced eosinophil count in noneosinophilic asthma.

**Discussion**

This study has confirmed that there is heterogeneity of airway inflammation in persistent asthma and identified evidence of neutrophilic inflammation and IL-8 secretion in noneosinophilic asthma. Using induced sputum, we could identify two distinct patterns of airway inflammation. The first pattern was a typical eosinophilic inflammation, where the percentage of eosinophils was increased and there was eosinophil degranulation with elevated ECP.

![Figure 3](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21962/ on 06/27/2017)
Most subjects, however, had persistent symptoms with suppressed eosinophil counts. In these asthmatics, there was an increase in sputum neutrophils and high levels of ECP and myeloperoxidase. These changes may be mediated by increased levels of the potent chemokine IL-8. The findings demonstrate that induced sputum may be useful in defining the characteristics of airway inflammation in persistent asthma.

Airway inflammation in asthma may be more heterogeneous than previously thought. Wenzel et al.\(^2\) described increased airway neutrophils in patients with severe, refractory prednisone-dependent asthma, where neutrophils were increased in the airway lumen, airway wall, and lung interstitium. These changes occurred in both eosinophilic and noneosinophilic refractory asthma.\(^2\) Pavord et al.\(^4\) reported noneosinophilic asthma in mild asthmatic subjects, and Turner et al.\(^5\) described noneosinophilic asthma during a study of exacerbations of asthma. Neutrophils were not increased in these subjects. Our results confirm the existence of noneosinophilic asthma, and extend prior observations by reporting the comparatively high prevalence of noneosinophilic inflammation in persistent asthma. Future work should evaluate the frequency of noneosinophilic asthma in steroid-naïve asthmatic patients.

We have identified a role for neutrophilic inflammation in noneosinophilic persistent asthma. There were increases in both relative and absolute neutrophil numbers, myeloperoxidase, and the potent neutrophil chemoattractant, IL-8. The role of neutrophils in symptomatic asthma is controversial. In stable asthma, there is no clear indication of increased neutrophils\(^6\)–\(^9\); however, in viral-induced exacerbations, increased neutrophils are seen.\(^10\)–\(^13\)

Neutrophil products can cause airway narrowing, increased mucus secretion,\(^17\) and increased airway smooth-muscle responsiveness.\(^18\)

The sputum neutrophilia in noneosinophilic asthma could represent an effect of the disease or of its treatment.\(^2\)–\(^3\) Corticosteroids do not reduce neutrophilic inflammation in airway disease,\(^19\) and persistent airway neutrophilia could even be maintained by corticosteroid-induced inhibition of neutrophil apoptosis.\(^20\) Alternatively, IL-8 could mediate increased neutrophil influx and reduced apoptosis.\(^20\) It is also possible that structural airway wall changes such as chronic bronchitis, bronchiectasis, or subepithelial fibrosis could be responsible for the airway neutrophilia, since neutrophilic inflammation is characteristic of these diseases.\(^19\)–\(^21\)

Our data support a role for IL-8 in mediating the neutrophil influx in noneosinophilic asthma, since IL-8 levels were increased in noneosinophilic asthma and were correlated with neutrophil numbers. IL-8 is a potent chemokine that acts as a chemoattractant and activating agent for both neutrophils and IL-5 primed eosinophils. IL-8 seems to be the main chemoattractant for neutrophils in the lungs.\(^22\) Although IL-8 is produced by a large number of cells, in this study we found that the neutrophil was the main airway luminal cell containing IL-8, suggesting autocrine secretion of IL-8 in persistent asthma. Levels of IL-8 correlated with ECP and myeloperoxidase in sputum supernatant, which is consistent with a role for IL-8 as a degranulating agent for eosinophils and neutrophils. Recently, Page et al.\(^23\) reported that eosinophil major basic protein could induce IL-8 production by neutrophils. Thus, the correlation of ECP with IL-8 is also consistent with eosinophil degranulation contributing to neutrophil IL-8 release in noneosinophilic asthma. IL-8 levels were high despite the use of inhaled corticosteroids. The data on suppression of IL-8 by corticosteroids are variable, but suggest that there is some, although incomplete suppression. In vivo IL-8 release from epithelium and smooth muscle is reduced by up to 30% with corticosteroid treatment,\(^24\) whereas steroids do not inhibit IL-8 release from a human mast cell line. In vivo, inhaled steroid can reduce sputum and serum IL-8 by 30 to 60% in bronchiectasis and COPD.\(^25\)–\(^26\) The persistence of high levels of sputum IL-8 is consistent with incomplete suppression of IL-8 release by corticosteroids. There is clearly a need to identify alternative treatments to target IL-8 in airway inflammation.

The observation of high levels of sputum ECP in noneosinophilic asthma is interesting. Typically, ECP is associated with eosinophil inflammation, and in this study the highest levels were observed in the group with eosinophilic asthma.\(^27\) However, increased ECP has been seen in the absence of eosinophils in other neutrophil-mediated airway diseases such as bronchiectasis\(^28\) and chronic obstruc-

![Figure 4. Macrophages (median) staining positive for ingested ECP (EC2*, via APAAP) as percent macrophages positive (open bars) and percentage of subjects with positive samples (hatched bars) in eosinophilic and noneosinophilic asthma. * = $p = 0.03$; + = $p = 0.05$.](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21962/)
tive airway disease. This raises the possibility that neutrophils may contain ECP, which has been demonstrated by Sur et al.

Another interesting feature was the persistence of clinically active disease and AHR in the absence of eosinophilia. We investigated the possibility that the known ability of corticosteroids to induce eosinophil apoptosis and consequent macrophage ingestion could have caused rapid clearance of eosinophils from the airway in noneosinophilic asthma. The data do not support this hypothesis, as there were few, and in some cases, no macrophages containing eosinophil proteins in noneosinophilic asthma. In contrast, the eosinophil group had strong evidence of active eosinophil turnover with 15% of macrophages staining positive for ECP. This figure is higher than previously observed in moderate asthma.

In conclusion, this study demonstrates that there is heterogeneity of airway inflammation in persistent asthma. The typical eosinophilic pattern occurs in a minority of subjects. The majority of subjects have noneosinophilic asthma with neutrophil degranulation and a neutrophil influx that may be mediated by IL-8. These data support the use of induced sputum to identify the pattern of airway inflammation in asthma, and suggest opportunities to base therapy on the underlying mechanisms in persistent asthma.

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