Alterations in Airway Inflammation and Lung Function During Corticosteroid Therapy for Atopic Asthma*

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| **Introduction:** Although corticosteroid therapy for asthma improves lung function and reduces airway inflammation, the relation between these two events is unclear. This article investigates associations between changes in bronchial inflammation and lung function during high-dose inhaled corticosteroid therapy for asthma. |
| **Methods:** Nine subjects with atopic asthma received high-dose inhaled fluticasone propionate (FP), 2,000 μg/d for 8 weeks. Fiberoptic bronchoscopy with endobronchial biopsies, spirometry, and histamine provocation challenge were performed on each subject at baseline, after 2 weeks, and again after 8 weeks of therapy. Spearman rank correlation coefficients between changes in parameters of bronchial inflammation and lung function were computed. |
| **Results:** As expected, significant down-regulation of airway inflammation and improvements in lung function were observed after both short-term and long-term therapy with high-dose inhaled FP. During corticosteroid therapy, changes in lymphocyte and macrophage numbers in bronchial biopsy specimens were closely correlated. Changes in EG1+ eosinophils were associated with changes in EG2+ eosinophils after 8 weeks of therapy. Although changes in airway inflammation and changes in lung function were not closely associated after 2 weeks of therapy, changes in eosinophils (EG1) in bronchial biopsy specimens correlated with changes in bronchodilator response ($r = 0.77, p = 0.016$) after 8 weeks of therapy. |
| **Conclusion:** In patients with atopic asthma, changes in bronchial eosinophils and lung function during steroid therapy are closely related but do not occur simultaneously. |

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Key words: airway inflammation; asthma; biopsy; eosinophil; immunopathology

Abbreviations: ANOVA = analysis of variance; FP = fluticasone propionate; MoAb = monoclonal antibody; PC20 = provocative concentration of histamine causing a 20% fall in FEV1

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**C**orticosteroids are the most effective therapy for asthma, and current guidelines emphasize their use in all but mild asthma. Corticosteroid therapy down-regulates airway inflammation and improves lung function. Some studies suggest that steroid-induced changes in bronchial inflammation and lung function are closely associated. Other data suggest that changes in these two processes occur independently. Heterogeneity of asthma within subject groups and variations in dose and duration of therapy could explain this paradox. We hypothesized that repeat measures of airway inflammation (by endobronchial biopsy) and lung function during corticosteroid therapy might provide insights into their kinetics of change. A complete understanding of the relation between changes in airway physiology and lung function during corticosteroid therapy for asthma might allow accurate titration of anti-inflammatory therapy.

Airway inflammation in asthma is characterized by infiltrates of several cell types, including T lymphocytes, macrophages, and eosinophils. Although many cell types have been shown to play an important role in disease activity, asthma may occur in the absence of one or more of these “effector” cells. Moreover, lung function in patients with asthma is
also characterized by several distinct features, including air trapping, airflow limitation, and airway hyperreactivity.1 To date, no single inflammatory or lung function parameter is entirely reliable at predicting disease severity, or response to antiasthma therapy.4 There is a need for more data on the relation between airway inflammation and lung function in asthma.

Flexible fiberoptic bronchoscopy with endobronchial biopsy allows precise quantification of airway inflammation in asthma.5,16 The use of endobronchial biopsy is considered a “gold standard” technique for the quantification of airway inflammation. When numbers of T cells, macrophages, and eosinophils are quantified by immunohistochemistry and computerized image analysis, the measures are highly reproducible at the within-section, within-biopsy, and intraindividual levels.6,17,18 We have previously shown that interindividual variability is greater than intraindividual variability.17 However, our laboratory has previously demonstrated that, because of biological variability, observed changes in airway inflammation are dependent on sampling interval.18 Steroid-induced changes to both lung function and airway inflammation are thought to be dependent on the duration of therapy. For example, spirometry findings generally improve before bronchial reactivity.6,19 Observed changes in measures of asthma severity are dependent on the time interval between measures and the duration of steroid therapy.6,20 We hypothesized that the study of repeat measures of airway inflammation during corticosteroid therapy might provide important data about the relation between airway inflammation and lung function in patients with atopic asthma. This article details the relation between changes in bronchial inflammation (using endobronchial biopsy specimens) and lung function during therapy with high-dose inhaled fluticasone propionate (FP) for atopic asthma.

Materials and Methods

Subjects

Study participants were enrolled in a randomized, double-blind, placebo-controlled, parallel group trial of high-dose inhaled FP for asthma. The results of this study and comparisons between placebo and steroid treatments have been published elsewhere.6 Nine subjects with atopic asthma were assigned (in a double-blind fashion) to receive FP, 2,000 μg/d, via a metered-dose inhaler attached to an aerosol chamber (Volumatic Spacer; Allen and Hanburys, Greenford, UK). Bronchodilator medication and caffeine-containing drinks were withheld for at least 12 h before each study visit, and subjects always attended the laboratory at 9 AM. Compliance with medication and adverse effects were checked and recorded during each visit. One day prior to each fiberoptic bronchoscopy (day 0–1, day 13, and day 55), histamine bronchial provocation challenges were performed according to a standardized technique.21 A complete dose-response curve for inhaled histamine was recorded using doubling concentrations of histamine dissolved in normal saline solution, starting at 0.03 mg/mL and going up to 16 mg/mL. FEV1 was measured 30 s and 90 s after each dose using a Gould 2400 computerized spirometer (Gould Instruments; Cleveland, OH). The test was terminated if the FEV1 decreased by > 20% from the post-saline solution value or if a concentration of 16 mg/mL was reached. PC20 was determined by linear interpolation of the concentra-

Table 1—Baseline Characteristics of the Nine Subjects Who Completed the Protocol

<table>
<thead>
<tr>
<th>Age, yr</th>
<th>Sex</th>
<th>Asthma</th>
<th>Duration, yr</th>
<th>Skin-prick Testing*</th>
</tr>
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<tbody>
<tr>
<td>21</td>
<td>Female</td>
<td>1</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>Female</td>
<td>&gt; 10</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>Female</td>
<td>&gt; 10</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Male</td>
<td>&gt; 10</td>
<td>+ + +</td>
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<tr>
<td>25</td>
<td>Male</td>
<td>1</td>
<td>+</td>
<td></td>
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<tr>
<td>27</td>
<td>Male</td>
<td>&gt; 10</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>Female</td>
<td>6–10</td>
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<td>Male</td>
<td>&gt; 10</td>
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<tr>
<td>45</td>
<td>Male</td>
<td>&gt; 10</td>
<td>+ + + +</td>
<td></td>
</tr>
</tbody>
</table>

*Skin-prick testing was performed using a panel of common antigens including grass and birch pollen, tree pollen, cat, dog, and house dust mite. + = one positive skin-prick test response to a panel of antigens.
tion/FEV\textsubscript{1} response curve. Patients were allowed neither albuterol nor salbutamol for 12 h and 36 h, respectively, prior to bronchial challenge. Bronchodilator responses were studied 4 h prior to bronchoscopy (on days 0, 14, and 56). FEV\textsubscript{1} was measured before and 20 min after the administration of albuterol, 200 μg, via the spacer device. The postbronchodilator FEV\textsubscript{1} and percentage change from baseline (bronchodilator response) were recorded.

**Fiberoptic Bronchoscopy**

Fiberoptic bronchoscopy was performed on three occasions in each subject: at baseline, after 2 weeks, and after 8 weeks (day 0, day 14, and day 56). Subjects were sedated with IV propofol, and continuous oxygen was administered via nasal cannula. Oxygen saturation, BF, and ECG were continuously monitored throughout the procedure. Using a flexible fiberoptic bronchoscope (Olympus BF10, Olympus, Tokyo, Japan), up to three endobronchial biopsy samples were obtained through the bronchoscope with spike and cup forceps from the second-generation right upper lobe bronchus. Biopsy samples were immediately placed on sterile phosphate-buffered saline solution-moistened gauze, embedded in optimum cutting temperature medium, and snap-frozen in isopentane (precooled in liquid nitrogen). The samples were then stored in liquid nitrogen (for < 1 month) until further analysis.

**Immunohistology**

Cryostat sections (6 μm in thickness) of the endobronchial biopsy samples were cut at −25°C and placed onto poly-L-lysine-coated microscope slides. The slides were air dried for 60 min, fixed in chloroform/acetone (1:1), wrapped in cling film, and stored at −20°C until used. Each biopsy sample was sectioned within 1 month of freezing. At least 40 sections were cut and stored at −20°C until use. Representative sections of all samples were stained with 0.1% toluidine blue to reveal morphology and tissue integrity. Immunohistochemistry was employed to identify subsets of inflammatory cells. The indirect immunoperoxidase technique was used to identify T cells, using a cocktail of monoclonal antibodies (MoAbs) [MoAbs CD2, CD3, CD7, and CD8], macrophage/monocytes (MoAb CD68), and eosinophils (MoAbs EG1 and EG2).

**Cell Quantification**

A blinded investigator (L.W.P.) quantified cell numbers. The presence, distribution, and quantity of cells were assessed using a computerized image analysis system (Solitaire; Seescan; Cambridge, UK). Three areas of epithelium and subepithelial connective tissue to a depth of 10 to 12 cells were measured in each section. Total areas of 12 × 10\textsuperscript{4} μm\textsuperscript{2} were quantified on duplicate sections for each cell type in each biopsy specimen. Areas of each high-power field were determined with the image analyzer by drawing frames on the computer screen around the area to be quantified. These frames were designed to avoid damaged regions and areas of muscle and cartilage. The numbers of positive cells (brown by immunoperoxidase staining) within framed areas were point counted and all results were then reduced to cells per unit area by dividing the number of cells in each field by the area of section in squared micrometers calculated by the computer. Only nucleated cells were counted.

**Statistics**

The intraindividual ratios of change after 2 weeks of therapy (measure at 2 weeks divided by measure at baseline) and 8 weeks of therapy (measure at 8 weeks divided by measure at baseline) were calculated for each parameter. Spearman correlation coefficients between ratios of change at both time intervals were calculated using SAS software (SAS Institute; Cary, NC). The significance of changes after 2 weeks and after 8 weeks of inhaled corticosteroid therapy was estimated by analysis of variance (ANOVA).

**Results**

The data presented in this article were obtained during a biopsy-controlled study of the effects of inhaled FP on airway inflammation. Twenty-seven subjects were recruited. Four subjects were withdrawn: two were withdrawn due to noncompliance with medication (placebo [n = 1], FP [n = 1]), and two were withdrawn due to pregnancy (placebo [n = 1], FP [n = 1]). Eighteen atopic asthmatic subjects (placebo [n = 9], FP [n = 9]) completed the protocol. Individual measures of lung function and airway inflammation for the nine atopic subjects who received inhaled FP and completed the protocol are presented in Figures 1–3. Other details of dropouts, adverse events, and changes in lung function and airway inflammation in the placebo-treated subjects have been published previously.6,18

**Correlations Between Changes in Lymphocytes, Macrophages, and Eosinophils**

After a 2-week interval, changes in T cells, CD45Ro lymphocytes, and CD68+ cells were all significantly correlated with each other (Table 2). Changes in eosinophils (EG1 or EG2) were not associated with changes in T cells, CD45Ro lymphocytes, or CD68+ cells (Table 2).

After an 8-week interval, changes in T cells, CD45Ro, and CD68 cells were all significantly correlated with each other (Table 3). Changes in eosinophils (EG1 or EG2) were not associated with changes in T cells, CD45Ro lymphocytes, or CD68+ cells (Table 3). Changes in EG1 were associated with changes in EG2 after 8 weeks of therapy (Table 3). There was no association between changes in EG1 and EG2 after 2 weeks of therapy.

**Correlations Between Changes in Lung Function and Airway Inflammation**

With regard to associations between changes in airway inflammation and changes in lung function, changes in EG1 were associated with changes in bronchodilator response (r = 0.77, p = 0.016) only after a period of 8 weeks (Fig 4). There was no association between the rates of change of inflammatory cells and measures of airway pathophysiology after a 2-week interval (data not shown).
This study examines associations between repeat measures of bronchial immunopathology and airway physiology during corticosteroid therapy for atopic asthma. Changes in bronchial lymphocytes and changes in macrophages are highly correlated over both short-term (2 weeks) and longer-term (8 weeks) intervals. Changes in total (EG1) eosinophils and changes in EG2+ eosinophils are highly correlated over an 8-week interval. Changes in airway eosinophils and changes in bronchodilator responsiveness are correlated after 8 weeks of therapy, but not after 2 weeks of therapy. Therefore, while changes in many of the cellular components of bronchial inflammation appear to be interdependent, only changes in eosinophils appear to be closely related to alterations in lung function. Changes in airway eosinophils and changes in lung function do not occur simultaneously. This dissociation has two important implications. First, when repeat measures are obtained from the same set of subjects, relative changes in bronchial inflammation and changes in lung function

**Figure 1.** Measures of T cells (top, A), CD45Ro primed T lymphocytes (center, B), and CD68+ macrophages (bottom, C) in bronchial biopsy samples in subjects with atopic asthma (n = 9) at baseline, and after 2 weeks and 8 weeks therapy with high-dose inhaled FP, 2.000 μg/d. *p < 0.01, ANOVA; **p < 0.001, ANOVA.

**Figure 2.** Measures of EG1 eosinophils (top, A) and EG2+ eosinophils (bottom, B) in bronchial biopsy samples in subjects with atopic asthma (n = 9) at baseline, and after 2 weeks and 8 weeks of therapy with high-dose inhaled FP, 2,000 μg/d. **p < 0.001, ANOVA.
are dependent on sampling interval. Second, changes in bronchial inflammation and lung function are closely associated but they do not occur simultaneously.

There are at least five alternative hypotheses to explain the coexistence of bronchial inflammation and asthmatic-type physiology: (1) there is no relation between airway inflammation and asthma (i.e., their coexistence in bronchial asthma is a coincidence); (2) bronchial inflammation and airway pathophysiology are causally related; (3) a common factor causes both bronchial inflammation and pathophysiology; (4) bronchial inflammation indirectly causes abnormal physiology (by an intermediate factor); and (5) changes in airway physiology precede and cause airway inflammation. The search for associations between changes in measures of airway inflammation and physiology is made complex by the variability of measures over time, because variability is the hallmark of asthma. The current data suggest that changes in airway inflammation and lung function are closely related, but associations between changes in these two processes may be dependent on the duration of sampling interval.

The demonstration of a close association between longer-term (but not short-term) changes in EG1 and changes in lung function has several implications for our understanding of the kinetics of asthma. This result supports several previous animal and human studies that demonstrate that tissue damage due to eosinophils and the development of airway pathophysiology do not occur simultaneously. In humans, increases in sputum eosinophils have been observed prior to deteriorations in asthma control. Fluctuations in the severity of airway hyperreactivity are thought to result, in part, from prior injury to the bronchial mucosa by inflammatory cells, most notably the eosinophil. Moreover, in sensitized mice,

Table 3—Spearman Rank Correlation Coefficients Between Changes in Bronchial Inflammation After 8 Weeks of Inhaled Corticosteroid Therapy (n = 9)*

<table>
<thead>
<tr>
<th>Variables</th>
<th>EG2</th>
<th>EG1</th>
<th>CD68</th>
<th>CD45Ro</th>
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<tr>
<td>T cells</td>
<td>-0.2 (0.6)</td>
<td>-0.2 (0.6)</td>
<td>0.83 (0.005)</td>
<td>0.83 (0.005)</td>
</tr>
<tr>
<td>CD45Ro</td>
<td>-0.36 (0.33)</td>
<td>-0.47 (0.2)</td>
<td>0.73 (0.02)</td>
<td></td>
</tr>
<tr>
<td>CD68</td>
<td>-0.13 (0.7)</td>
<td>-0.2 (0.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EG1</td>
<td>0.84 (0.01)</td>
<td></td>
<td></td>
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</table>

* p values given in parentheses.
increases in airway eosinophils precede the development of airway hyperreactivity after allergen challenge. \(^{26}\) The cellular components of airway inflammation are dependent on one another for recruitment and survival. This article is, to our knowledge, the first investigation of associations between short-term and longer-term changes in bronchial immunopathology in the same set of asthmatic subjects during inhaled corticosteroid therapy. In this human, \textit{in vivo} model of asthma, the associations between the rates of change of airway macrophages and T cells are consistent with \textit{in vitro} models of macrophage and T-cell biology. \(^{28,29}\) In the asthmatic airway, the cellular components of bronchial inflammation may be dependent on one another for survival and recruitment (both via cell-cell interaction and through the secretion of soluble factors, such as cytokines and chemokines). \(^{24,29–31}\) We find that short-term and longer-term changes in eosinophils are not closely correlated with changes in T cells or macrophages. Although an association might have been observed if repeat measures were obtained at another time point, airway eosinophil recruitment and activation are probably not wholly orchestrated by local airway T cells and macrophages.\(^{29,32–33}\) Our results are consistent with the thinking that other factors (circulating eosinophils, serum IgE, and serum cytokines, for example) may influence bronchial eosinophilic inflammation.\(^{15,34–36}\)

In summary, significant associations exist between longer-term (but not short-term) changes in airway eosinophils and longer-term changes in bronchodilator responsiveness. Although changes in airway eosinophils are associated with changes in lung function,\(^{37}\) these two processes do not occur simultaneously.

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\textbf{References}


