Airway Tissue Mast Cells in Persistent Asthma

Predictor of Treatment Failure When Patients Discontinue Inhaled Corticosteroids

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Study objectives: To determine if persistent airway tissue mast cells are associated with treatment failure when patients discontinue inhaled corticosteroids (ICS).

Design: Double-blind, randomized, placebo-controlled trial.

Setting: Multicenter, tertiary referral centers.

Patients or participants: Forty-five subjects with asthma recruited from six medical centers in the United States.

Interventions: The Asthma Clinical Research Network undertook a 28-week, randomized, multicenter, double-blind, placebo-controlled trial of 164 subjects with clinically stable, persistent asthma. A subset of subjects (n = 45) underwent bronchoscopy with endobronchial biopsy and BAL at the end of a 6-week run-in period, during which all subjects received triamcinolone acetonide (TAA), 400 µg bid. Airway tissue mast cells, eosinophils, neutrophils, macrophages, and T cells were quantified morphometrically along with determination of BAL tryptase. At the end of the run-in period, subjects were then randomized to receive salmeterol (42 µg bid), placebo, or continue TAA for 16 weeks followed by a second bronchoscopy.

Measurements and results: Outcome variables included airway tissue mast cells, eosinophils, neutrophils, macrophages, and T cells that were quantified morphometrically and BAL tryptase. Thirty-five subjects completed the treatment phase; an additional 10 subjects, who were randomized to either salmeterol or placebo after the run-in, had treatment failure. When the bronchoscopy results performed at the end of the run-in, prior to randomization, were analyzed, the treatment failure group demonstrated significantly more tissue mast cells as compared to the nontreatment failure group despite 6 weeks of therapy with TAA (p = 0.04). BAL tryptase was also significantly higher in the treatment failure group (p < 0.0001). Of those subjects who completed the study, tissue mast cells and BAL tryptase did not change significantly within any of the treatment groups during the treatment phase (p > 0.05).

Conclusions: Persistent elevations in airway tissue mast cells and BAL tryptase after treatment with TAA predict treatment failure in patients for whom discontinuation of ICS is being considered.

Key words: asthma; bronchoscopy; mast cell

Abbreviations: ACRN = Asthma Clinical Research Network; ICS = inhaled corticosteroids; IL = interleukin; IQR = interquartile range; NAEPP = National Asthma Education and Prevention Program; PC20 = provocative concentration of methacholine resulting in a 20% fall in FEV1; PEF = peak expiratory flow; SOCS = Salmeterol or Corticosteroids; TAA = triamcinolone acetonide

Asthma is a disease characterized by airway inflammation, specifically, the accumulation of eosinophils, T cells, and mast cells.1 Given these histopathologic characteristics, current asthma management guidelines emphasize the importance of anti-inflammatory therapy.2 Inhaled corticosteroids (ICS), first-line therapy for asthma, reduce airway inflammation and asthma severity3; however, inflammation may persist despite ICS treatment.4 Gibson et al4 demonstrated that airway mast cells persisted following 8 weeks of high-dose beclomethasone therapy (2,000 µg/d), a finding that correlated with bronchial hyperresponsiveness. Despite the well-known finding that corticosteroid therapy reduces airway eosinophilia and improves asthma severity,5 bronchial biopsy specimens from corticosteroid-
treated asthmatic patients demonstrated that both eosinophils and mast cells persisted within the lamina propria and their numbers correlated with airway hyperresponsiveness.3

The mast cell, potentially armed with antigen-specific IgE antibody, is localized at the interface of the internal and external environment within the lung where it can interact with aeroallergen.6 Through non-IgE-dependent mechanisms, mast cells can respond to nonimmunologic mediators including neuropeptides, complement fragments, and eosinophil mediators.6 Finally, they have also been shown to participate in the pathogenesis of the early and late-phase asthmatic responses,7 and their mediators and cytokines contribute to the persistent inflammation of chronic asthma.8

Although inhaled corticosteroids (ICS), because of their anti-inflammatory effect, are generally accepted as the “gold standard” for treatment of persistent asthma, patients and clinicians often stop ICS therapy when symptoms improve or switch to a nonsteroid alternative, because of concerns about the potential for adverse effects. The Asthma Clinical Research Network (ACRN) of the National Heart, Lung, and Blood Institute previously reported that patients with persistent asthma that are accepted as the “gold standard” for treatment of persistent asthma, patients and clinicians often stop ICS therapy when symptoms improve or switch to a nonsteroid alternative, because of concerns about the potential for adverse effects. The Asthma Clinical Research Network (ACRN) of the National Heart, Lung, and Blood Institute previously reported that patients with persistent asthma that are well controlled by low doses of triamcinolone acetonide (TAA) cannot discontinue the TAA without risk of clinically significant loss of asthma control.9 When TAA was discontinued in subjects whose asthma was considered mild, but under good control, 30% experienced treatment failure within 16 weeks. Given the established role of mast cells in allergic respiratory responses within the lung, and their participation in altering airway physiology in asthma, we examined a subset of subjects in the ACRN Salmeterol or Corticosteroids (SOCS) trial to evaluate whether mast-cell numbers, or mast-cell mediator release, were also markers that could be associated with treatment failure after cessation of ICS.

Materials and Methods

Subjects

Subjects with asthma, as defined by the American Thoracic Society,3 who met recommended criteria for treatment with ICS were recruited. Exclusion criteria included a > 5 pack-year cigarette smoking history or any cigarette use in the last year, use of any other medications except oral contraceptives and nasal beclomethasone, and/or an upper respiratory infection within 6 weeks of study.

Study Design

The SOCS trial was conducted by the ACRN between February 1997 and December 1998.10 This trial was a 28-week, randomized, double-blind, double-dummy, placebo-controlled, prospective, multicenter trial in subjects with persistent asthma comparing the efficacy of ICS (TAA, 400 µg bid), an inhaled long-acting β-adrenergic agonist (salmeterol xinafoate, 42 µg bid) and placebo, during 16 weeks of therapy, and for 6 weeks following cessation of therapy. Subjects entered a 6-week run-in during which they received TAA, 400 µg or four puffs bid. At the end of the run-in, those subjects whose FEV₁ was > 80% predicted, whose average peak expiratory flow (PEF) variability was < 20%, and who demonstrated > 85% compliance with twice-daily PEF measurements were randomized. Compliance was determined by evaluating diary cards and use of the Airwatch device (Enact; Palo Alto, CA). A subject was considered compliant if the TAA four puffs bid was logged correctly in the diary cards during the run-in and the Airwatch device was used prior to TAA administration. If TAA was recorded less than twice daily on > 12 days, the subjects were not randomized. Those subjects who met the above criteria were randomized to receive either TAA (400 µg bid or four puffs bid plus two puffs bid salmeterol placebo), salmeterol (42 µg bid or two puffs bid plus four puffs bid TAA placebo) or placebo (two puffs bid of salmeterol placebo and four puffs bid of TAA placebo) for the next 16 weeks, using a double-dummy, blinding technique (Fig 1). All subjects were given albuterol inhalers to use for rescue treatment throughout the study. Subjects underwent skin testing to a standard aeroallergen skin test panel to evaluate atopy.

A randomly chosen subset (n = 45) of the 164 subjects enrolled in the SOCS trial underwent bronchoscopy at the end of the run-in and at the end of treatment phase. Subjects also underwent methacholine challenge testing4 at entry, at the end of the run-in, and at several points during the treatment phase and run-out. This report will focus on findings regarding the first or baseline bronchoscopy and physiology at the end of the run-in period prior to randomization. Subjects whose asthma deteriorated after discontinuation of ICS treatment did not undergo a second bronchoscopy due to safety concerns and confounding by prednisone therapy. All subjects gave informed consent for this institutional review board-approved protocol.

The SOCS trial examined the effect of discontinuing ICS or substituting salmeterol in subjects well controlled on TAA. Because we anticipated that some subjects’ asthma control would worsen with ICS withdrawal, we established specific criteria to define “asthma exacerbation” and “treatment failure,” and we provided specific treatment algorithms for each of these events. Asthma exacerbations and treatment failure criteria are outlined in Table 1. Physician judgment regarding patient safety was also included as a criterion for treatment failure, in addition to other criteria, to allow the investigators some flexibility in case the defined criteria were not exactly met and there were issues of patient safety. Subjects who met treatment failure criteria were...
treated with a short burst of prednisone and/or open-label TAA (400 μg/4 bid) for the remainder of the study. Study inhalers of salmeterol or placebo were continued. The term treatment failure, as we have defined it, grew out of our desire to establish a “safety net” that would ensure that no subject suffered an adverse event as a result of reduction of ICS therapy. Prior to its use in this trial, a similar set of criteria was validated in another ACRN initiated study evaluating ICS reduction in which treatment failure was used as the main outcome measure.

**Bronchoscopy**

A subset of the 164 subjects (n = 45) underwent bronchoscopy with endobronchial biopsy and BAL at the end of the run-in and prior to randomization as previously described. The site of biopsy was randomized to either the right or left lower lobes, followed by BAL in the opposite lung, either the right middle lobe or lingula. Prior to the bronchoscopy, subjects underwent spirometry before and after 0.18 mg of albuterol from a metered-dose inhaler. Atropine, 0.4 mg, was administered IV. Lidocaine (4%) was used to anesthetize the upper airway, and xylocaine (1%) was applied to the laryngeal area, trachea, and orifice of the right lower or left lower lobe bronchi via the bronchoscope. A maximum of 600 mg or 9 mg/kg was used, as this dose range is associated with a low rate of toxicity. Four to six endobronchial biopsies were obtained from the third to fourth generation of the right or left lower lobes, and BAL was performed in the opposite lung using four 60-mL aliquots of sterile normal saline solution at 37°C. Lavage fluid was obtained by immediate gentle hand suction applied to each instilling syringe. Supplemental oxygen was administered throughout the procedure along with monitoring of heart rate and oxygen saturation. Vital signs were monitored in each clinical research area for at least 2 h after the procedure.

All testing was performed at each site with standardized equipment and procedures. Network staff were trained and tested to ensure proficiency and uniformity in all procedures. All spirometric testing, including that for methacholine challenge, was overread by a single member of the network for quality control. To standardize the procedure across all centers prior to start of the study, a training session for bronchoscopy also took place that entailed viewing of a procedural videorecording, as well as hands-on assistance with bronchoscopy and specimen processing. Research coordinators were required to pass practical and written tests prior to certification to assist with bronchoscopy. Finally, a distributed data-entry system allowed each clinical center to submit data electronically to the data coordinating center. The data coordinating center entered the data a second time for verification.

**Table 1—Criteria for Asthma Exacerbation and Treatment Failure**

<table>
<thead>
<tr>
<th>Exacerbations</th>
<th>Criteria for Treatment Failure Status during the Randomized Treatment Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased cough, chest tightness, and wheezing with one or more of the following:</td>
<td>Requirements for systemic corticosteroids</td>
</tr>
<tr>
<td>&gt; 8 puffs of rescue albuterol in a 24-h period</td>
<td>&gt; 1 course of prednisone for treatment of asthma exacerbations</td>
</tr>
<tr>
<td>&gt; 16 puffs rescue albuterol in a 48-h period</td>
<td>&gt; 1 emergency department or urgent care visit for treatment of asthma exacerbation</td>
</tr>
<tr>
<td>Peak flow expiratory flow rate &lt; 65% of reference value (the average prebronchodilator value measured during the last 2 weeks of the run-in period)</td>
<td>Hospitalization for the treatment of asthma</td>
</tr>
<tr>
<td>Symptoms despite 60 min of treatment</td>
<td>Physician clinical judgment for safety</td>
</tr>
</tbody>
</table>

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**Figure 1. Schematic of the SOCS protocol.**
**Tissue Analysis**

Tissue samples were fixed in acetone containing protease inhibitors (iodoacetamide, 20 mM; phenyl sulfonyl fluoride, 2 mM) at −20°C overnight and processed into glycol methacrylate resin as previously described. Sections were cut using a random start and stained immunohistochemically using primary mouse anti-human monoclonal antibodies and the avidin biotin peroxidase detection system. The primary antibodies put to use in this study were directed against mast cells (AA1; Dako, Carpenteria, CA), T cells (CD3; Becton Dickinson; San Jose, CA), macrophages (CD68; Dako), neutrophils (neutrophil elastase; Dako), and eosinophils (EG2; Pharmacia; Upsala, Sweden). Sections 2 μm thick were stained, and the sections were imaged to a computer screen using the Image program (National Institutes of Health; Bethesda, MD). The density of inflammatory cells within the tissue was determined by counting the number of cells that intersect a grid placed over the microscopic field. Smooth muscle, submucosal glands, and blood vessels were excluded. Optimal selection of the numbers of sections, fields, and density of the test grid were made according to the general rules for efficient sampling. Within each block, we utilized the general rules of cascade sampling. This method uses the lowest reasonable magnification to increase sample size for measurements and uses the same magnification to measure major compartments and their subcompartments whenever possible. Using a 40 × objective on a light microscope, we divided the total area of the slide into five strata and took one to two equidistant fields per strata. A grid was oriented along the vertical axis, and points counts were made. All of the positive cells within the grid area were counted, and the number of positive cells per square millimeter were calculated.

**BAL Analysis**

β-Tryptase levels in BAL were measured by a sandwich enzyme-linked immunosorbent assay using the B12 monoclonal antibody for capture and biotinylated G5 monoclonal antibody for detection. Plates were coated with 0.01 mol/L Tris, pH 8.5, containing 0.15 mol/L NaCl and 0.05% sodium azide overnight at 4°C. Plates were then washed with 0.01 mol/L Tris, pH 8.5, containing 1 mol/L NaCl, 0.1% bovine serum antigen and 0.05% sodium azide. The density of inflammatory cells within the tissue was determined by counting the number of cells that intersect a grid placed over the microscopic field. Smooth muscle, submucosal glands, and blood vessels were excluded. Optimal selection of the numbers of sections, fields, and density of the test grid were made according to the general rules for efficient sampling. Within each block, we utilized the general rules of cascade sampling. This method uses the lowest reasonable magnification to increase sample size for measurements and uses the same magnification to measure major compartments and their subcompartments whenever possible. Using a 40 × objective on a light microscope, we divided the total area of the slide into five strata and took one to two equidistant fields per strata. A grid was oriented along the vertical axis, and points counts were made. All of the positive cells within the grid area were counted, and the number of positive cells per square millimeter were calculated.

**Results**

### Subject Characteristics

Forty-five subjects completed the initial bronchoscopy; 35 subjects then completed the treatment phase without treatment failure, and 10 subjects in the bronchoscopy group experienced treatment failure after randomization. Subject characteristics in the treatment failure and nontreatment failure groups (separated by treatment) are shown in Table 2.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Placebo (n = 10)</th>
<th>Salmeterol (n = 9)</th>
<th>Triamcinolone (n = 16)</th>
<th>Salmeterol (n = 7), Placebo (n = 3)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>34.4 ± 2.8</td>
<td>30.1 ± 3.6</td>
<td>31.1 ± 2.3</td>
<td>30.1 ± 2.4</td>
<td>0.54</td>
</tr>
<tr>
<td>Male/female gender, No.</td>
<td>5/5</td>
<td>5/4</td>
<td>9/7</td>
<td>1/9</td>
<td>0.02</td>
</tr>
<tr>
<td>FEV1 baseline, L</td>
<td>2.9 (2.3–3.7)</td>
<td>2.4 (2.0–3.3)</td>
<td>2.8 (2.4–3.6)</td>
<td>2.9 (2.4–3.1)</td>
<td>0.71</td>
</tr>
<tr>
<td>FEV1 % predicted baseline</td>
<td>83 (74–95)</td>
<td>82 (74–91)</td>
<td>87 (79–93)</td>
<td>89 (76–101)</td>
<td>0.57</td>
</tr>
<tr>
<td>FVC baseline</td>
<td>4.3 (3.7–4.9)</td>
<td>4.1 (3.6–5.0)</td>
<td>4.5 (3.4–5.1)</td>
<td>3.8 (3.4–4.0)</td>
<td>0.72</td>
</tr>
<tr>
<td>FEV1/FVC baseline</td>
<td>70 (64–74)</td>
<td>64 (56–73)</td>
<td>70 (62–78)</td>
<td>73 (62–81)</td>
<td>0.37</td>
</tr>
<tr>
<td>PC20 baseline, mg/mL</td>
<td>0.4 (0.2–3.0)</td>
<td>0.2 (0.03–0.06)</td>
<td>0.3 (0.3–1.7)</td>
<td>0.43 (0.17–1.2)</td>
<td>0.05</td>
</tr>
<tr>
<td>FEV1 after run-in</td>
<td>3.3 (2.7–3.7)</td>
<td>3.1 (2.3–3.8)</td>
<td>3.2 (2.7–3.7)</td>
<td>2.9 (2.6–3.4)</td>
<td>0.55</td>
</tr>
<tr>
<td>FEV1 % predicted after run-in</td>
<td>92 (83–100)</td>
<td>89 (85–95)</td>
<td>85 (90–97)</td>
<td>93 (88–110)</td>
<td>0.61</td>
</tr>
<tr>
<td>FVC after run-in</td>
<td>4.2 (3.5–5.1)</td>
<td>4.7 (3.5–5.3)</td>
<td>4.4 (3.6–5.6)</td>
<td>3.9 (3.5–4.4)</td>
<td>0.42</td>
</tr>
<tr>
<td>FEV1/FVC after run-in</td>
<td>74 (67–78)</td>
<td>71 (58–79)</td>
<td>69 (63–81)</td>
<td>78 (69–84)</td>
<td>0.30</td>
</tr>
<tr>
<td>PC20 after run-in, mg/mL</td>
<td>0.50 (0.18–0.60)</td>
<td>0.27 (0.17–1.66)</td>
<td>0.98 (0.33–1.58)</td>
<td>0.55 (0.21–3.5)</td>
<td></td>
</tr>
</tbody>
</table>

*Data are presented as mean ± SEM or median (range) unless otherwise indicated.
†Data are presented as median (range).
The mean number of puffs per of ICS used prior to the run-in was 5.8 ± 0.5 puffs in the nontreatment failure group, and 4.0 ± 0.6 puffs in the treatment failure group, and these differences trended toward significance at p = 0.08. Table 3 lists the ICS used prior to the run-in in the treatment failure and nontreatment failure groups.

The subjects with treatment failure had been randomized to either salmeterol (n = 7) or placebo (n = 3). None of the subjects randomized to TAA experienced treatment failure. Within the placebo group, the mean time to treatment failure was 54.3 ± 15 days; within the salmeterol group, the mean time to treatment failure was 38.7 ± 9.8 days (p = 0.41). When the subjects who experienced treatment failure in the salmeterol and placebo groups combined were compared to those subjects without treatment failure in the salmeterol and placebo groups, there were no differences in baseline spirometry, PC_{20} at baseline or after the run-in, and β_{2}-agonist use at randomization. However, the treatment failure group used slightly more inhaled β_{2}-agonist (0.44 puffs per day vs 1.1 puffs per day), but this was not statistically significant (p = 0.44). When the groups were analyzed separately, i.e., those subjects in the salmeterol group who experienced treatment failure were compared to those subjects who did not, and the subjects in the placebo group who experienced treatment failure were compared to those subjects who did not, there were no differences in the above-mentioned parameters. Again, the subjects who experienced treatment failure in the salmeterol group used slightly more puffs of short-acting, inhaled β_{2}-agonists (0.44 puffs per day vs 1.33 puffs per day), but this was not statistically significant (p = 0.51).

Tissue and BAL Analysis

The tissue mast cell results from the baseline bronchoscopy performed after the run-in and prior to randomization in the treatment failure group, and each of the three nontreatment failure groups are shown in Figure 2. The treatment failure group demonstrated significantly more AA1-positive mast cells as compared to the nontreatment failure groups.

<table>
<thead>
<tr>
<th>ICS</th>
<th>Nontreatment Failures</th>
<th>Treatment Failures</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flunisolide</td>
<td>4 (n = 1)</td>
<td>2 ± 1.3 (n = 2)</td>
<td>ND</td>
</tr>
<tr>
<td>Fluticasone</td>
<td>3.6 ± 1.3 (n = 5)</td>
<td>4 ± 1.3 (n = 2)</td>
<td>0.57</td>
</tr>
<tr>
<td>Triamcinolone</td>
<td>6.6 ± 0.7 (n = 18)</td>
<td>4.8 ± 0.5 (n = 5)</td>
<td>0.15</td>
</tr>
<tr>
<td>Beclomethasone</td>
<td>5.6 ± 1.2 (n = 8)</td>
<td>6 (n = 1)</td>
<td>ND</td>
</tr>
</tbody>
</table>

*Data are presented as mean ± SEM puffs per day.
†Data from three subjects are missing.
‡Statistical analysis not done (ND) between groups due to low sample size (n = 1 in one of the groups).

**Table 3—ICS Used by Subjects at Study Entry Prior to Run-in**

**Figure 2.** Airway tissue expression of AA1-positive cells per square millimeter in treatment (Rx) failure and the three nontreatment (Non-rx) failure groups: placebo, salmeterol (Salm), and TAA. Values represent medians and IQRs. Horizontal lines represent median values.
Of the subjects who completed the trial, there were no significant changes in airway tissue mast cell numbers between the first and second bronchoscopies, regardless of treatment group. There were no differences between treatment failure and nontreatment failure groups in regard to tissue eosinophils (median cell count, 0/mm² [IQR, 0 to 12.7] vs 0/mm² [IQR, 0 to 3.8], p = 0.19), neutrophils (median, 26.8/mm² [IQR, 4.8 to 51.2] vs 12.9/mm² [IQR, 4.6 to 92.5], p = 0.98), or T cells (median, 0/mm² [IQR, 0 to 11.3] vs 0/mm² [IQ, 0 to 48.7], p = 0.97). Macrophages were higher in the nontreatment failure group, and this trended toward significance (7.4/mm² [IQR, 0 to 40] vs 29.4/mm² [IQR, 13.1 to 48.5] in the treatment failure and nontreatment failure groups, respectively, p = 0.09).

BAL tryptase was also significantly higher in the treatment failure group than in the nontreatment failure group (Fig 3). Similar to airway tissue mast cells, the BAL tryptase did not change significantly between the first and second bronchoscopy in the subjects who completed the trial, regardless of treatment group.

**Discussion**

We have shown that the persistence of mast cells in airway tissue and increased BAL tryptase after ICS treatment are associated with treatment failure when patients discontinue ICS. In this particular analysis, the median number of tissue mast cells in the treatment failure group of 28.3/mm² appeared to be a “threshold,” as only 2 of 35 nontreatment failure patients exhibited mast-cell numbers above this value, compared with the 7 of 10 treatment failure subjects exhibiting at least this value. The presence of significantly elevated mast-cell tryptase in the treatment failure subjects (fivefold higher than the other groups) provides additional confirmation that mast cells in these subjects were activated despite ICS therapy. The numbers of tissue mast cells and levels of BAL tryptase were significantly lower in the subjects who were able to complete the treatment phase without an exacerbation, and did not vary significantly before and after treatment. However, these values are significantly higher, particularly in the treatment failure group, than tissue mast cells and BAL tryptase reported in nonasthmatic control subjects. These observations also support the hypothesis that increased activated mast cells while receiving ICS are associated with treatment failure when patients discontinue ICS.

Mechanisms underlying the persistence of mast cells in airway tissue despite corticosteroid therapy may relate to differential regulation of mast-cell mucosal homing as compared to other inflammatory cells such as eosinophils. The human mast cell is derived from a CD34+ bone marrow progenitor that is recognized by the surface expression of the receptor tyrosine kinase c-kit, the receptor for stem-cell...
factor.6 Their local terminal differentiation is under the influence of several T-cell cytokines such as interleukin (IL)-3, IL-4, IL-9, and stem-cell factor.8 In addition, the mast cell and macrophage are known to express the αEβ7 integrin and L-selectin, which promote mast cell retention in airway epithelium.21 Mast cells also express αEβ7 integrin, which recognizes E cadherin on mucosal epithelial cells, enhancing binding.22 Corticosteroids may modulate this process by preventing adherence of eosinophils to airway epithelium, as they inhibit intercellular adhesion molecule-1 and vascular cell adhesion molecule-1, which modulate eosinophil adherence.23 Therefore, corticosteroids may impair eosinophil retention but, in some cases, may favor mast-cell retention in airway tissue.

Not only are the numbers of mast cells increased, but their activation may also be increased in the treatment failure subjects, as demonstrated by the increased BAL mast-cell tryptase. Tryptase is the principal enzyme accounting for trypsin-like activity of human mast cells, and mast cells containing only tryptase are found in lung tissue and intestinal mucosa.8 Tryptase has several functions in vitro that may be important in the airway inflammation, stimulation of eosinophil chemotaxis, up-regulation of intercellular adhesion molecule-1 expression, activation of tissue matrix metalloproteinases, and stimulation of fibroblast proliferation and collagen synthesis.24,25 Other mast-cell types in lung, skin and bowel, as well as eosinophils, neutrophils, monocytes and lymphocytes from peripheral blood, have no detectable tryptase.26 The measurement of tryptase release in vitro allows a precise assessment of mast-cell activation. Beyond the effects of tryptase, mast-cell activation is significant, as mast cells have been shown to release other mediators involved in airway inflammation such as leukotriene C4 and T-helper type 2 cytokines IL-4, IL-5, and IL-13.27,28 In our previous report, sputum tryptase also increased in the placebo and salmeterol groups, but was not directly associated with treatment failure.29 Therefore, BAL tryptase appears to be a more sensitive measure of treatment failure.

We also observed that in those subjects who completed both bronchoscopies, there was no significant change in airway tissue mast cells or BAL tryptase when the airway tissue was compared at the end of the run-in period (after TAA and prior to randomization) and after 16 weeks of treatment with either TAA (n = 16), salmeterol (n = 9), or placebo (n = 10). It is possible that by recruiting additional subjects to undergo bronchoscopy in order to reach our desired sample size, we may have created a “survivor effect.” These 35 subjects, where 19 subjects subsequently randomized to placebo, salmeterol, and TAA,9 certainly one can interpret these findings as suggesting a “failure to treat” instead of treatment failure. The main SOCS study9 of which this was a part, was designed to test whether subjects who met the National Asthma Education and Prevention Program (NAEPP) criteria for treatment with ICS could be switched to a long-acting β-agonist, salmeterol, without loss of asthma control. Although the NAEPP and other guidelines did not recommend salmeterol as monotherapy, this was based on expert opinion, not data. Subjects initially met criteria for ICS use, and thus were recruited for study; however, at the time this study was initiated, there were no published data on the use of salmeterol as monotherapy, and no specific recommendations against this, as the study was initiated prior to the release of the NAEPP expert panel report 2 guidelines.29 In fact, even the 1997 revised NAEPP guidelines (expert panel report 2) suggest that reduction in ICS may be appropriate for patients whose clinical improvement is sustained for several months.20 Furthermore, the use of salmeterol as monotherapy was becoming prevalent in clinical practice, presumably because patients and clinicians continued to have concerns about the potential for steroid toxicity. Thus, the SOCS study was designed to rigorously test the possibility that subjects who did well on ICS might switch successfully to monotherapy with salmeterol. Although our bias, based on expert opinion, was that ICS were the preferred

V}-agonist, salmeterol, and thus were recruited for study; however, at the time this study was initiated, there were no published data on the use of salmeterol as monotherapy, and no specific recommendations against this, as the study was initiated prior to the release of the NAEPP expert panel report 2 guidelines.29 In fact, even the 1997 revised NAEPP guidelines (expert panel report 2) suggest that reduction in ICS may be appropriate for patients whose clinical improvement is sustained for several months.20 Furthermore, the use of salmeterol as monotherapy was becoming prevalent in clinical practice, presumably because patients and clinicians continued to have concerns about the potential for steroid toxicity. Thus, the SOCS study was designed to rigorously test the possibility that subjects who did well on ICS might switch successfully to monotherapy with salmeterol. Although our bias, based on expert opinion, was that ICS were the preferred
treatment, we did not know what proportion of subjects would deteriorate after cessation of steroid therapy, nor did we know the time course over which deterioration might occur. We therefore designed the study with an elaborate safety net to ensure that subjects whose ICS were withdrawn were followed up closely. As part of this safety net, we established specific criteria to define asthma exacerbation and treatment failure. As defined a priori in this study, at a time when there were no studies documenting that salmeterol monotherapy was not efficacious, loss of asthma control while receiving salmeterol therapy was a treatment failure. Cessation of ICS therapy can be considered failure to treat, but that presumes knowledge that salmeterol monotherapy is not efficacious treatment—which was not the case prior to the SOCS study.9

The SOCS study9 examined whether salmeterol monotherapy might substitute for ICS, and we found that it was associated with an unacceptable rate of loss of asthma control; however, it is interesting to note that while approximately 30% of these subjects failed, 70% did not. We suggest that mast cells may be a cause for treatment failure in those subjects where ICS are removed, as those who did not exacerbate off ICS (the salmeterol and placebo groups) demonstrated lower tissue mast cells. To illustrate this point, Figures 2, 3 include tissue mast cells and BAL tryptase in the treatment failure group and the subjects in the three treatment groups who completed the study. We feel the BAL tryptase levels in particular, which are indicative of activated mast cells, were significantly higher in the treatment failure group than the other groups (Fig 3). The mast-cell number was also higher in the treatment failure group, but there is some overlap (Fig 2).

Although the fact that treatment failure can occur with discontinuation of ICS is not new information, a possible mechanism may be persistent inflammation, in particular persistent airway tissue mast cells. There were no clinical parameters that distinguished the treatment failure subjects from the nontreatment failure subjects despite reports where increased airway responsiveness was identified as a risk factor for treatment failure.12,30,31 However, this observation may provide physicians with a possible mechanism as to why some patients experience an exacerbation of their asthma when ICS are discontinued. As leukotriene C₄ is a known mast-cell mediator, perhaps these patients may be candidates for leukotriene receptor antagonist therapy. This observation may also be useful for physicians contemplating substitution of ICS with other anti-inflammatory agents, as those patients who demonstrate a reduction in their tissue mast cells with ICS do not appear to exacerbate when ICS are discontinued. However, we do not know if patients with increased airway tissue mast cells will continue to do well on long-term ICS; we know that they do well for at least 16 weeks.

In conclusion, persistent airway tissue mast cells appear to be associated with treatment failure when ICS are discontinued. Airway tissue and BAL analyses via bronchoscopy have revealed a possible pathophysiologic mechanism for these treatment failures not revealed by evaluating sputum tryptase. Further study is required to determine why mast cells in the airways of these patients persist despite adequate treatment with corticosteroids.

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APPENDIX

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REFERENCES


12 Fish JE, Peters SP, Chambers CV, et al. An evaluation of colchicine as an alternative to inhaled corticosteroids in moderate asthma. Am J Respir Crit Care Med 1997; 156:1165–1171


22 Haisel T, Paavonen T, Renkonen R, aEβ7 and a4β7 integrins associated with intraepithelial and mucosal homing are expressed on macrophages. Eur J Immunol 1995; 25:411–417


