Airway mast cells and eosinophils correlate with clinical severity and airway hyperresponsiveness in corticosteroid-treated asthma

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Background: The relationship between airway inflammation and asthma severity in corticosteroid-treated asthma is unclear.
Objectives: Our purpose was to characterize the inflammatory cell profile of the airway lumen and epithelium in corticosteroid-treated asthma and to relate these findings to clinical and physiologic markers of asthma severity.
Methods: Adults (n = 20) with asthma received standardized high-dose inhaled corticosteroid therapy with beclomethasone 2000 μg per day for 8 weeks. Airway responsiveness to methacholine and hypertonic (4.5%) saline solution was then assessed, followed by sputum induction and, 1 week later, bronchoscopy with bronchoalveolar lavage and bronchial brush biopsy to assess inflammatory cells.
Results: Clinical asthma severity was associated with airway hyperresponsiveness. Metachromatic cells were the main granulocyte present in bronchial brush biopsy specimens and correlated with airway responsiveness to saline solution (r = –0.75), methacholine (r = –0.74), peak flow variability (r = 0.59), and clinical asthma severity (r = 0.57). Eosinophils were the main granulocyte present in sputum and correlated with airway responsiveness to saline solution (r = –0.63) but not with other clinical markers of asthma severity. Bronchoalveolar lavage cell counts were not related to clinical asthma severity.
Conclusions: In asthmatic patients treated with corticosteroids, the dominant inflammatory effector cell in the epithelium is the metachromatic cell, and in sputum it is the eosinophil. These cells correlate with the degree of airway hyperresponsiveness. Clinical asthma severity correlates with airway responsiveness and epithelial metachromatic cells. Induced sputum eosinophils and airway responsiveness to hypertonic saline solution may be useful markers of airway inflammation for clinical practice. (J Allergy Clin Immunol 2000;105:752-9.)

Key words: *Asthma, sputum, eosinophil, mast cell, airway responsiveness, inflammation, corticosteroid*

Abbreviations used
BAL: Bronchoalveolar lavage
BBB: Bronchial brush biopsy
IQR: Interquartile range
NaCl: Sodium chloride
PD_{20}: Provocation dose causing a 20% fall in FEV_1
PEF: Peak expiratory flow

Current asthma management guidelines[1,2] emphasize the importance of understanding asthma as an inflammatory disease characterized by an infiltrate of eosinophils and mast cells,[3] which develops under the control of cytokines from T helper type 2 lymphocytes.[4] Asthma treatment is adjusted according to an assessment of severity based on clinical parameters such as symptoms and lung function because measurement of inflammation is rarely performed in clinical practice. As observed in the International Consensus Report on the diagnosis and management of asthma,[2] these clinical severity classifications differ little among countries that have developed asthma management guidelines. Recently, the concept of asthma severity has been further clarified to distinguish severity of background disease (called activity, or control) from the severity of exacerbations that may occur.[5] These classifications are clinically useful because they permit a stepwise adjustment of anti-inflammatory therapy according to the level of severity.[1,2]

It is important, however, to establish that clinical markers of asthma severity are an adequate reflection of airway inflammation. In patients who are not taking corticosteroid therapy, studies have identified that clinical parameters are correlated with the number and activity of eosinophils in the lamina propria, epithelium, and airway lumen.[6-8] In daily clinical practice clinicians are required to assess asthma severity and modify treatment in patients who are already taking maintenance inhaled corticosteroid therapy. Although the same clinical parameters are assessed as in patients who are not taking corticosteroids, it is not established how inflammatory markers relate to clinical parameters in corticosteroid-treated asthma.[5]

Although corticosteroid treatment both reduces airway eosinophilia and improves asthma severity,[9] the relationship between airway inflammation and asthma severity may be different in patients taking corticosteroid from

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steroid-naive patients. For example, Sont et al[10] could not identify a relationship between airway inflammation and clinical asthma severity in adults treated with inhaled corticosteroids. They used bronchial biopsy specimens and found that inflammatory cells persisted in the lamina propria despite corticosteroid therapy and that this was related to airway responsiveness but not to symptoms. Airway inflammation in asthma involves the epithelium and airway lumen as well as the lamina propria, and the distribution of inflammatory cells can be different in the airway lumen and epithelium from that of the lamina propria.[11] It is currently unclear whether inflammatory cells also persist in these other compartments of the airway mucosa in asthmatic patients treated with corticosteroids, and if so, whether they are related to markers of clinical severity.

The current study had two aims. The first aim was to characterize the inflammatory cell profile of the airway lumen and epithelium in corticosteroid-treated asthma. We used bronchial brush biopsies (BBB) to sample airway epithelium, and bronchoalveolar lavage (BAL) and induced sputum to sample inflammatory cells from the airway lumen. The second aim was to examine the relationship between inflammatory cells and clinical markers of asthma severity in corticosteroid-treated asthma. Both the dose and duration of inhaled corticosteroid therapy were carefully standardized. Subjects were treated for an 8-week period with high-dose inhaled beclomethasone before assessment of airway inflammation and clinical asthma severity. We then related inflammatory markers to clinical severity, which was assessed as recommended in current guidelines, and to several measures of airway responsiveness.

METHODS

Subjects

Twenty nonsmoking adults (10 women) with a wide range of asthma severity participated in this study (Table I). Subjects were recruited from a list of consecutive attendances for asthma to the referral clinics of 2 respiratory physicians (P. G., N. S.). The diagnosis of asthma was based on, first, a clinical history of episodic wheeze, breathlessness, chest tightness, or cough and, second, on documented variable airflow obstruction from a 20% change in FEV_1 either spontaneously after inhalation of β_2-agonist or a PD_{20} to methacholine inhalation challenge testing of <12 μmol.[12] Each subject had clinically stable asthma and no recent (past month) respiratory tract infection or asthma exacerbation. Exclusion criteria were current smoking, prior smoking with a >10 pack-year smoking history, chronic airway obstruction (FEV_1 <70% predicted), current or recent (past 3 months) use of ingested corticosteroids, and contraindications to bronchoscopy. Subjects used β_2-agonist aerosols on an as-needed basis for symptom relief. Inhaled corticosteroids were used by 18 subjects at entry at a mean (SE) dose of 1173 (300) μg per day. Subjects gave written informed consent and the study was approved by the Hunter Area Health Service Research Ethics Committee.

Study design

The study consisted of 4 visits, conducted over an 8-week period. At study visit 1, written informed consent was gained and clinical history was obtained with use of a physician-administered questionnaire. Baseline spirometry, methacholine airway responsiveness, and allergy skin prick tests were performed. Subjects were instructed in the use of metered-dose inhaler through a valved holding chamber (Volumatic, Allen and Hanbury, Melbourne) and asked to complete a symptom, medication, and peak expiratory flow (PEF) diary. Subjects then commenced a standardized treatment program consisting of high-dose inhaled corticosteroids for 8 weeks. Beclomethasone, 2000 μg per day, was prescribed for 8 weeks, taken as 4 inhalations of 250 μg twice daily, through the spacer. Compliance was assessed by weighing the canisters and all subjects had >80% compliance. Subjects were contacted by telephone every 2 weeks to reinforce compliance and assess symptom response.

At study visit 2, after 6 weeks of high-dose corticosteroid therapy, spirometry and methacholine inhalation testing were repeated. The methacholine test was extended to assess maximal airway narrowing and the presence of a methacholine plateau response. One week later (week 7, study visit 3), airway responsiveness to hypertonic saline solution was assessed and sputum was induced for cytologic analysis. Clinical asthma severity was assessed at this visit, on the basis of a review of clinical history, the preceding week's symptom and PEF diary, and spirometry. Bronchoscopy with BAL and BBB was performed within the next 7 days, at study visit 4. Subjects continued inhaled beclomethasone until visit 4.

Allergy skin tests

Allergy skin prick tests were performed with 14 common allergen extracts with histamine and buffered saline solution as positive and negative controls, respectively. After 15 minutes a wheal of >3 mm was considered to be a positive prick test result and subjects with >1 positive test were considered atopic.

Symptom and peak flow assessment

Each day subjects recorded the best of 3 PEF readings before and after 200 μg of salbutamol in the morning and again in the evening, and they also rated their asthma symptoms. Symptoms were scored with use of a 7-point category scale where 1 represented "no symptoms" and 7 represented the "most severe symptoms ever." Clinical asthma severity was rated as mild, moderate, or severe according to the integrated severity score described in the Australian asthma management guidelines,[1] which closely approximate the National Heart, Lung, and Blood Institute guidelines.[2] Measurements of current daytime symptoms, nocturnal and morning symptoms from asthma, bronchodilator use, FEV_1, PEF, and hospitalizations were used to assign the patient a severity rating of either mild, moderate, or severe. The patient was assigned to the most severe grade in which any feature occurred.

Airway responsiveness

Spirometry was performed with use of a Vitalograph Compact electronic spirometer (catalog No. 4200, Vitalograph, Buckingham, UK). Before inhalation challenge testing, subjects withheld inhaled β_2-agonists for 6 hours and theophylline for 48 hours. Methacholine airway responsiveness was assessed with use of the method of Yan et al,[12] with results expressed as the PD_{20}. Maximal airway narrowing and methacholine plateau responses were assessed by extending the challenge until either the FEV_1 fell by 50% from baseline or a cumulative methacholine dose of 134 μmol was delivered. PD_{20} values less than 12 μmol indicate airway hyperresponsiveness.

Airway responsiveness to hypertonic saline solution was assessed with the method of Smith and Anderson[13] with 4.5% sodium chloride (NaCl) aerosol delivered from an ultrasonic nebulizer (Timeter, maximum output 2.6 mL/min) through a Hans Rudolph 2700 2-way nonrebreathing valve box with a rubber mouthpiece and nose clips. Subjects inhaled 4.5% NaCl for doubling time periods (0.5, 1.0, 2.0, 4.0, and 8.0 minutes). FEV_1 was measured twice 1 minute after each inhalation period. The nebulizer transducer assembly and tubing were weighed before and after completion of

TABLE I. Subject characteristics at baseline

Subject No.	Age (y)	Allergy*	Asthma duration (y)	Inhaled steroid (μg/d)	FEV_1 (% predicted)	PD_{20} (μmol)
1	67	2	11	2000†	97	0.10
2	49	11	10	1500†	89	0.08
3	51	4	30	2000†	93	2.31
4	59	0	3	2000	81	0.33
5	37	3	11	2000	84	0.51
6	44	9	15	400	74	0.18
7	41	4	11‡	2000†	84	0.02
8	31	3	21‡	0†	98	0.05
9	39	8	8	1000	73	0.50
10	29	5	13‡	400†	79	0.08
11	36	6	20‡	400†	88	0.12
12	33	0	14‡	250†	89	0.49
13	34	3	1	1000	106	13.70
14	37	4	8‡	2000†	93	2.94
15	50	0	4	2000	81	3.27
16	65	6	2.5	2000	98	5.16
17	19	8	0.5	0	92	0.75
18	38	0	5	700	114	9.06
19	37	0	3	800	102	2.75
20	67	3	9	1000†	110	2.80
Mean (SD)	43 (13)	3.9 (3.3)	10 (7.7)	1173 (776)	91.3 (11.3)	2.26 (3.52)

PD_{20}, Dose of methacholine causing a 20% fall in FEV_1.
*Allergy: number of positive skin prick tests from a panel of 14 allergen extracts.
†Subject had previously used oral corticosteroid for asthma exacerbation.
‡Previously hospitalized for asthma.

the challenge to calculate nebulizer output and the dose of 4.5% NaCl delivered to each subject. PD_{20} values less than 20 mL indicate airway hyperresponsiveness.

Sputum induction

Sputum was induced by encouraged expectoration during the ultrasonic nebulization of hypertonic saline solution.[14] Subjects were encouraged to expectorate into a sterile container after each dose of saline solution. The test was stopped when the FEV_1 had fallen by >20% or 15.5 cumulative minutes of nebulization time had elapsed. If the FEV_1 fell by >20% during the challenge, then salbutamol 200 μg was administered with a pressurized metered-dose inhaler and valved holding chamber (Volumatic). If a satisfactory sputum sample was not obtained at the time the FEV_1 had fallen by >20%, nebulization with 4.5% saline solution continued for 4-minute periods once the FEV_1 had returned to within 10% of baseline.

Bronchoscopy

Fiberoptic bronchoscopy (Pentax FB-ISX) was performed according to the guidelines of the American Thoracic Society for bronchoscopy in asthmatic subjects,[15] as previously described.[16] Each subject was premedicated with 0.6 mg of atropine by subcutaneous injection and 0.2 mg of inhaled salbutamol. BAL was performed with the bronchoscope wedged into a subsegment of the middle lobe bronchus. Two hundred milliliters of prewarmed sterile 0.9% saline solution was instilled in 20-mL aliquots and aspirated during gentle (<100 mm Hg) wall suction. The fluid was collected on ice into sterile polypropylene centrifuge tubes and held at 4°C. The bronchoscope was then placed in the lingula bronchus for the performance of standardized BBB that was directly smeared onto glass slides.[16]

Sample processing

Sputum was processed as described.[17] Briefly, the sputum volume and macroscopic characteristics were recorded, and a 300 μL aliquot of sputum plug was aspirated from the Petri dish with a positive displacement pipette. The aliquot was added to 2700 μL of dithiothreitol 1:10 (Sputolysin, Calbiochem, La Jolla Calif), mixed by rotating for 30 minutes at room temperature, and filtered through 50 μm nylon gauze. A total cell count was performed and cytocentrifuge slides were prepared (Shandon Cytospin II, Sewickey, Pa). The quality of induced sputum samples was assessed on the basis of the number of sputum plugs and squamous contamination, as described.[18] BAL was processed as described.[16] Total cell counts and viability were performed in duplicate on neat samples with use of a Neubauer hemocytometer. Cytocentrifuge preparations were made with 100 μL aliquots of BAL fluid (1×10^6/mL).

Cytochemistry

Air-dried, alcohol-fixed cytocentrifuge preparations (BAL, sputum) and direct smears (BBB) were stained with May-Grunwald Giemsa and a cell differential was performed by counting 400 cells in random fields. Metachromatic cells were stained with 0.5% toluidine blue at pH 0.1 after fixation in Carnoy's fluid.[16] Metachromatic cells in BBB, BAL, and induced sputum have previously been characterized as formalin-sensitive and tryptase-positive mast cells.[16,19] In bronchial brushings, where there was a high percentage of metachromatic cells, 1500 nucleated cells were counted in each of 2 slides. Metachromatic cells in BAL slides were counted as described previously.[16]

Analysis

The FEV_1 response to the challenge tests was expressed as the percentage fall from the mean baseline value and was plotted against log nebulized cumulative dose. The PD_{20} was calculated by linear interpolation of the last 2 points of the dose-response curve and used to characterize the position of the dose-response curves to methacholine and 4.5% saline solution. PD_{20} values were log-transformed for analysis. Methacholine dose-response curves were also characterized by their maximal response. A maximal response plateau was considered to be present if 2 or more data points of the highest doses

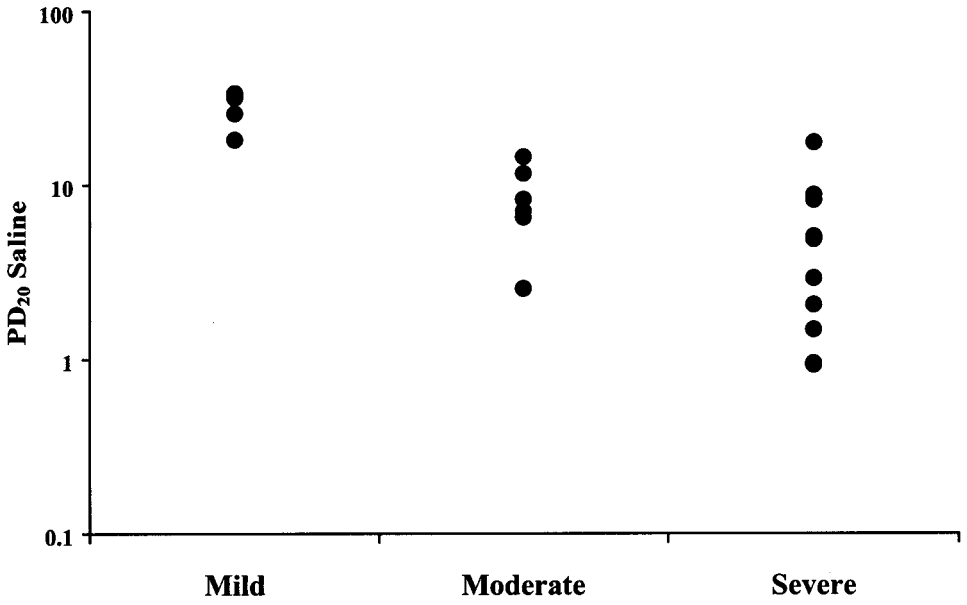

FIG 1. Clinical asthma severity was related to airway responsiveness to hypertonic (4.5%) saline solution (P = .002).

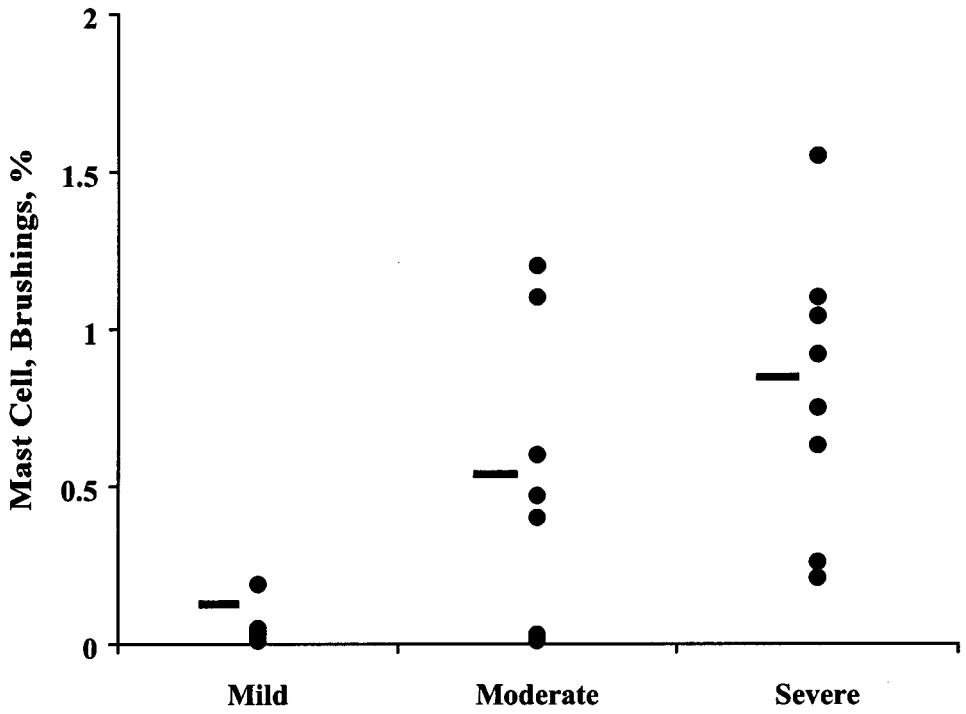

FIG 2. Clinical asthma severity was related to airway mast cells. Intraepithelial metachromatic cells were higher in subjects with more severe asthma (P = .02).

fell within a 5% range. The level of the maximal response was calculated by averaging consecutive data points on the plateau or, in the absence of a plateau, by taking the maximum response itself. Variability of PEF was calculated as the difference between the maximum and the minimum PEF, expressed as a percentage of the maximum PEF. Variability was calculated for each day, and the average of 7-day results between visits 2 and 3 was used for analysis.

Normally distributed continuous variables were summarized as mean with SD in parentheses. Inflammatory cell counts were summarized as the median with interquartile range (IQR). Data were compared with use of the Kruskal-Wallis test or Friedman's test as appropriate, with post hoc analysis of differences between groups done with the Mann-Whitney or Wilcoxon test and Bonferroni-adjusted P values. Categoric variables were compared with the chi-square or Fisher

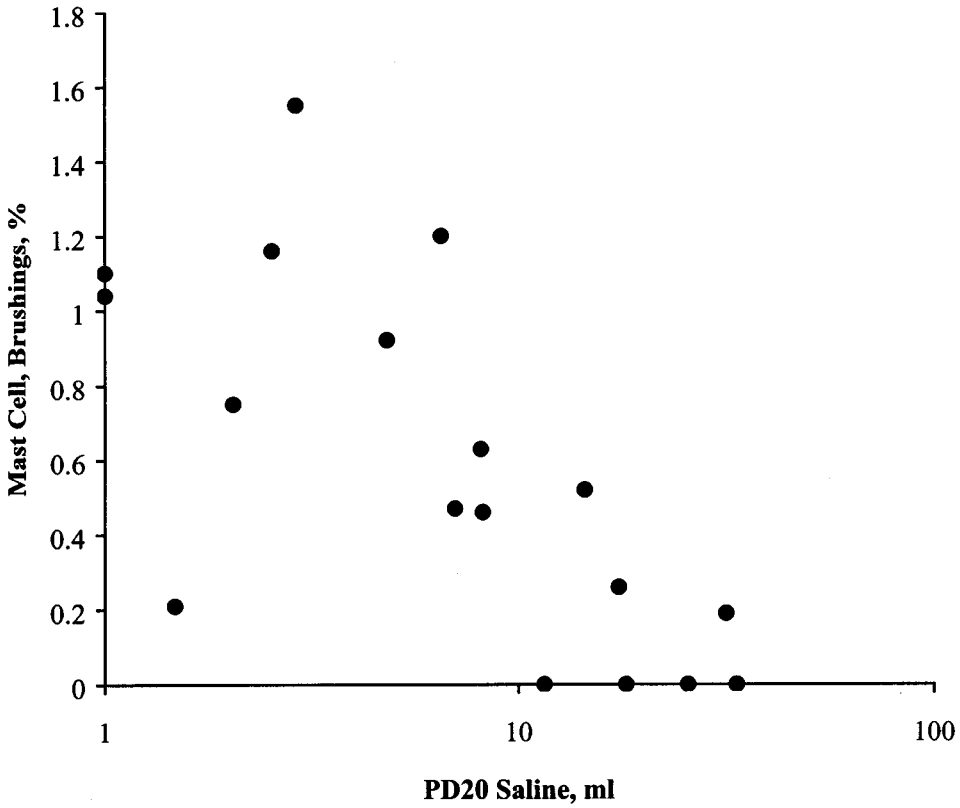

FIG 3. Airway hyperresponsiveness to hypertonic saline solution, expressed as PD_{20} saline, was significantly correlated with intraepithelial mast cells in corticosteroid-treated asthma, $r = -0.71$, $P < .05$.

TABLE II. Asthma severity and airway responsiveness after 6 weeks of high-dose inhaled corticosteroid therapy

Subject No.	Methacholine PD_{20} fold change	Maximal airway narrowing (%)	PD_{20} saline solution (mL)	PEF variability (%)	Mean symptom score	Asthma severity score	β_2-Agonist use (puffs/d)
1	4.0	48.0	2.0	36.6	2.85	Severe	4.57
2	11.3	55.3	8.6	19.5	2.16	Severe	6.33
3	0.8	46.0	4.7	23.2	1.14	Severe	7.42
4	5.4	67.0	2.8	23.3	2.00	Severe	4.43
5	0.9	59.0	6.4	20.0	1.43	Moderate	2.00
6	5.8	62.0	14.4	39.4	2.70	Moderate	2.00
7	1.0	56.5	8.0	27.3	3.88	Severe	5.29
8	4.8	50.0	0.9	20.7	4.50	Severe	28.00
9	1.0	48.0	0.9	14.9	2.00	Severe	9.43
10	6.5	69.0	1.4	20.6	1.00	Severe	6.86
11	1.0	47.0	2.5	12.0	3.00	Moderate	4.00
12	1.0	58.0	8.1	18.4	1.00	Moderate	4.00
13	4.5	24.4*	33.5	2.0	3.00	Mild	4.00
14	2.1	55.0	25.7	7.1	1.00	Mild	2.00
15	5.2	45.0	11.5	15.5	1.00	Moderate	4.00
16	2.3	34.0	31.7	12.3	2.00	Mild	2.00
17	6.8	62.0	17.4	10.1	3.00	Severe	6.30
18	2.6	35.0*	18.2	9.0	1.00	Mild	1.00
19	2.9	39.0*	NA	NA	NA	NA	NA
20	2.8	44.0	7.0	17.4	2.57	Moderate	4.00
Mean (SD)	3.7 (2.7)	50.2 (11.5)	10.9 (10.2)	18.4 (9.3)	2.2 (1.1)		5.7 (5.8)

Fold change, Change in PD_{20} methacholine after 6 weeks of high-dose inhaled corticosteroid therapy; *PEF*, from 7 days' recording between visits 2 and 3; *NA*, data not available.

*Plateau response reached during methacholine provocation challenge.

TABLE III. Cell counts in BAL fluid, BBB specimens, and induced sputum

	BAL	BBB	Induced sputum
Total cell count ($\times 10^6$/mL)	0.21 (0.12)*	NA	3.09 (5.2)
Mast cells (%)	0.15 (0.39)*	0.47 (0.85)†	0.02 (0.07)‡
Eosinophils (%)	0.49 (2.0)	0.0 (0.0)†	1.11 (6.24)‡
Neutrophils (%)	1.5 (5.4)*	0.0 (0.25)†	24.1 (27.01)‡
Macrophages (%)	90.6 (16.8)*	0.05 (1.06)†	64.7 (38.9)‡
Lymphocytes (%)	0.89 (2.1)*	0.0 (0.0)†	0.18 (0.44)‡
Bronchial epithelial cells (%)	2.61 (3.6)	99.1 (1.38)†	3.3 (5.7)‡

Values are medians (IQR). *NA,* Not assessed.
Comparisons between groups (Friedman's test with Bonferroni-adjusted *P* values):
*$P < .05$ BAL versus sputum.
†$P < .05$ BAL versus BBB.
‡Sputum versus BB $P < .05$.

exact tests where appropriate. Associations between variables were examined with the rank correlation coefficient. Two-tailed tests were used and significance was accepted at the 5% level.

RESULTS

At the completion of high-dose inhaled corticosteroid therapy, clinical asthma scores were rated as severe in 9 subjects, moderate in 6 subjects, and mild in 4 subjects (Table II). Many (60%) of the subjects had persisting methacholine airway hyperresponsiveness, with an absent plateau response (80%) and increased variability of peak expiratory flow (Table II). Most subjects (12, 60%) also had persisting hyperresponsiveness to the indirect-acting stimulus hypertonic saline solution. Clinical asthma severity was strongly associated with increasing airway responsiveness to both hypertonic saline solution ($P = .006$, Fig l) and methacholine ($P = .02$).

Inflammatory cell counts (Table III)

BAL recovered 67.5 (30.0%) of the instilled fluid and contained 0.21 (0.12) $\times 10^6$ cells per milliliter. The dominant cell in the BAL fluid was the macrophage. BBBs recovered predominantly epithelial cells (Table III, $P < .001$). The inflammatory cell profile of the airway lumen (BAL, sputum) was different from that of the bronchial epithelium (BBB). Neutrophils and eosinophils were the main granulocytes present in BAL. Although metachromatic cells were present, they were in comparatively small numbers. By contrast, metachromatic cells and neutrophils were the dominant granulocytes within the airway epithelium. Eosinophils were seen in the BBB of one subject only.

Sputum induction yielded 2.5 mL (IQR 1.75) of sputum with a mean (SD) total cell count of 3.09 (5.2) $\times 10^6$/mL. Sputum quality was satisfactory, with a quality score of 3.0 (IQR 1.0). The dominant cell type in sputum was the macrophage, which comprised 64.7% of the cells present. Sputum had a greater percentage of eosinophils than either BAL or BBB and more neutrophils than BAL. There was a significantly lower percentage of mast cells in sputum than in either BAL or BBB. Sputum eosinophils were significantly correlated with BAL eosinophils ($r = 0.65$, $P < .05$).

TABLE IV. Relationship between cell counts (%) in BBB, BAL, and induced sputum and physiologic measures

	PEF variability	PD_{20} methacholine	MAN	PD_{20} saline
Metachromatic cells				
BBB	0.59*	–0.74*	0.39*	–0.75*
BAL	–0.13	–0.38	–0.22	–0.46
Sputum	0.23	–0.35	0.14	–0.20
Eosinophils				
BBB	–0.09	0.29	–0.18	0.13
BAL	0.26	–0.31	0.19	–0.43
Sputum	0.43	–0.29	0.21	–0.63

Values are rank correlation coefficients. *MAN,* Maximal airway narrowing achieved during high-dose methacholine challenge.
*$P < .05$.

Clinical asthma severity and airway inflammation

Intraepithelial metachromatic cells were related to clinical asthma severity, with higher metachromatic cell counts observed in subjects with more severe asthma (Kruskal-Wallis, $P = .02$; rank correlation, 0.57, $P < .05$; Fig 2). Metachromatic cell numbers were also higher in those subjects who had required oral corticosteroids in the past (0.63% vs 0.16%, $P = .02$). Asthma duration was positively correlated with epithelial metachromatic cell counts ($r = 0.50$). Neither BAL nor sputum eosinophils were related to clinical asthma severity ($P > .05$).

Association between airway responsiveness and airway inflammation

Intraepithelial metachromatic cells in BBB correlated significantly with airway responsiveness to hypertonic saline solution (Fig 3, Table IV), airway responsiveness to methacholine, and PEF variability. There were no associations between other inflammatory cell types in BBB and PD_{20} methacholine. Subjects without a plateau response (n = 16, Table II) had greater numbers of metachromatic cells in BBB (0.70% vs 0.09%, $P = .01$). Sputum eosinophils correlated with airway responsiveness to hypertonic saline solution ($r = –0.63$, $P < .05$).

BAL cell counts were not associated with measures of airway responsiveness ($P > .05$, Table IV).

DISCUSSION

This study evaluates the relationship among airway inflammation, airway hyperresponsiveness, and clinical asthma severity in corticosteroid-treated asthma. We assessed airway inflammation in 2 distinct compartments, the epithelium and the lumen, in subjects treated with a standardized optimal course of treatment with an inhaled corticosteroid. We then assessed the clinical severity of asthma by a composite score as recommended in current management guidelines. Our results indicate that in corticosteroid-treated asthma different inflammatory granulocytes predominated in the 2 airway compartments sampled. Metachromatic cells and neutrophils were the main granulocytes within the epithelium, whereas eosinophils and neutrophils predominated in the airway lumen. The dominant cell type was correlated with airway responsiveness, and epithelial metachromatic cells were associated with both clinical asthma severity and asthma duration.

Several sampling methods were used to assess the inflammatory cell infiltrate in the different airway compartments. These methods are complementary to bronchial biopsy, which mainly samples lamina propria. The airway epithelium was sampled with use of BBB, which is reproducible,[16,20,21] and the differential cell counts in healthy and asthmatic subjects are well described.[16,20,22] Cells from the airway lumen were obtained by 3 different methods. Induced sputum was used. This is a well-established technique with good reproducibility that can be performed on multiple occasions for serial monitoring.[18,19,23-25] BAL was also used; however, its relevance as a sampling method for airway diseases is less well established because it is variably mixed with alveolar cells and is seldom associated with other asthma variables. In this study BAL was the least-informative sampling method because there were no relationships between BAL cell counts and clinical asthma severity or airway responsiveness observed. Although the relationships between airway responsiveness and airway inflammation were strongest for BBB, they were also present for induced sputum. In view of the noninvasive nature of this technique, a combination of airway responsiveness and induced sputum cell counts may be the most helpful for day-to-day monitoring of asthma.

Most of the study subjects were using varying doses of inhaled corticosteroid at baseline. The purpose of the treatment phase of the study was to standardize the dose and duration of therapy to ensure that each subject had received optimal and standardized delivery of high-dose inhaled corticosteroid therapy before the assessment of the association between inflammatory cells and clinical variables. Airway inflammation was not assessed at baseline because the primary purpose of this study was not to describe the anti-inflammatory action of inhaled corticosteroids in asthma, because this is now well recognized.[9]

The results of this study emphasize that the nature of the airway inflammatory response may differ within distinct zones of the airway.[11] The airway epithelium in corticosteroid-treated asthma contained predominantly mast cells and very few eosinophils, confirming the results of Riise et al.[22] In contrast, the eosinophil was the dominant granulocyte in the airway lumen. Differing integrin-mediated homing mechanisms may explain the mast cell predominance in the epithelium. Mast cells are known to express the mucosal homing molecules, $\alpha 4\beta 7$ integrin and L-selectin, as well as a mucosal retention complex comprising $\alpha E\beta 7$ integrin, which recognizes E-cadherin on mucosal epithelial cells,[26] and these could facilitate the epithelial accumulation of mast cells. Differing integrin expression on eosinophils may also impair retention within the epithelium because airway eosinophils shed L-selectin when activated.[27] $\alpha E\beta 7$ is not reported to be expressed by eosinophils or myeloid (HL-60) cells.[28] Corticosteroids may also modulate this process, preventing eosinophil adherence to airway epithelium. Eosinophil adherence to bronchial epithelial cells is modulated by intercellular cell adhesion molecule-1[29] and vascular cell adhesion molecule-1, both of which are inhibited by corticosteroid treatment.[30,31] Together, these data support a role for corticosteroid therapy preferentially clearing eosinophils from the superficial epithelial layer, which is consistent with the results of this and another study.[32]

Airway responsiveness was associated with the severity of airway inflammation. This study has identified that in corticosteroid-treated asthma epithelial metachromatic cells were closely associated with airway responsiveness to hypertonic saline solution and methacholine. The lack of an association between epithelial eosinophils and airway responsiveness probably represents the virtually complete clearance of eosinophils from the airway epithelium with corticosteroid therapy. In the airway lumen, where there was a persistence of eosinophils, this was associated with airway responsiveness to hypertonic saline solution. Bronchial biopsy specimens from corticosteroid-treated asthmatic subjects also demonstrate that both eosinophils and mast cells within the lamina propria are correlated with airway responsiveness.[10] Together, these findings indicate that the intensity of airway inflammation in asthma is associated with the degree of airway responsiveness and that this relationship holds true in both steroid-naive[6-8] and steroid-treated[10] asthmatics and also within the distinct compartments of the airway wall.

Asthma severity, when assessed with use of an integrated clinical score,[1] was associated with inflammatory cells in the epithelium and with airway responsiveness. This emphasizes the importance of using several clinical variables to assess asthma severity because it is well documented that reliance on symptoms alone to monitor asthma can result in underestimation of asthma severity and in undertreatment.[33] When a composite clinical score was used, this was related to the 2 key pathophysiologic features of asthma, namely, airway inflammation and airway hyperresponsiveness, and this relationship was still

present despite the use of high-dose inhaled corticosteroid therapy. This is an important observation that validates the assessment of asthma severity as currently recommended in guidelines. Because hypertonic saline solution challenge can be used to assess both airway responsiveness and induced sputum for analysis of inflammatory cells at a single visit,[13] it will be important to evaluate whether the use of this test as a noninvasive inflammatory marker can improve clinical management of patients with asthma.

This study has demonstrated that airway inflammation can persist in the airway lumen and epithelium despite high-dose corticosteroid therapy in asthma. The dominant inflammatory cell in the airway lumen (eosinophil) and the airway epithelium (mast cell) were related to the severity of airway hyperresponsiveness in corticosteroid-treated asthmatic patients. Epithelial mast cells were also related to clinical asthma severity. Saline solution airway responsiveness and induced sputum each have a role as objective markers of the asthmatic process and provide information in addition to symptoms and lung function.

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