The Relationship between Airways Inflammation and Asthma Severity

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In order to investigate the relationship between airways inflammation and disease severity, and improve the understanding of persistent asthma, 74 asthmatics, with disease severity ranging from intermittent, to mild to moderate and severe persistent (classified according to the Global Initiative for Asthma [GINA] guidelines), and 22 nonatopic control subjects were studied using the method of induced sputum. Sputum was analyzed for total and differential cell counts concentrations of albumin, and levels of eosinophil cationic protein (ECP), myeloperoxidase (MPO), and tryptase, inflammatory mediators reflecting eosinophil, neutrophil, and mast cell activation. Asthma severity (assessed by FEV_1, peak expiratory flow [PEF] variability, and daily symptom scores) and methacholine airways responsiveness were related to sputum eosinophilia and ECP. In addition, sputum neutrophilia and MPO levels correlated, albeit weakly, with PEF variability and symptom scores, respectively. Tryptase concentrations were raised in mild to moderate asthmatics. Albumin concentrations were significantly raised across the spectrum of asthma severity and correlated with those of tryptase and ECP. Despite treatment with either high doses of inhaled corticosteroids or oral corticosteroids, prominent eosinophilic inflammation with raised ECP was noted. This study points to persistent, disease severity–related airways inflammation in asthma, involving eosinophils, mast cells, and neutrophils, which is evident despite treatment with corticosteroids. Louis R, Lau LCK, Bron AO, Roldaan AC, Radermecker M, Djukanović R. The relationship between airways inflammation and asthma severity.

AM J RESPIR CRIT CARE MED 2000;161:9–16.

Airways inflammation, involving activated eosinophils, mast cells, and T lymphocytes is an established feature of asthma, and has been the key target for treatment. Despite limited knowledge of what determines asthma severity and the nature of inflammation in more severe disease, a series of guidelines have been drawn, recommending stepwise increments in anti-inflammatory and bronchodilator medication to control increasing disease activity (1).

Among the propylactic drugs corticosteroids have been the mainstay of asthma treatment. By consensus, the use of inhaled corticosteroids (ICS) has been advocated in all forms of persistent asthma, starting with mild disease requiring daily use of bronchodilators (1), and reserving oral corticosteroids (OCS) for exacerbations and severe, chronic disease. However, a number of important issues regarding the efficacy of corticosteroids in asthma remain unresolved, the main being the failure to control symptoms in patients on high doses of ICS and OCS. Further important issues include the potential for systemic side effects with OCS and high-dose ICS, and the uncertainty about the dose-dependency of their effects (2).

In this study we have used the technique of sputum induction, a validated research tool (3, 4), to improve the understanding of the inflammatory basis for moderate and severe asthma by identifying cellular factors which may be responsible for persistent symptoms in patients treated with corticosteroids. The safety and feasibility of this method have enabled us to study more than 70 asthmatics from across the spectrum of asthma severity, which would have been considerably more difficult if we had employed bronchoalveolar lavage (BAL) and bronchoscopic biopsy.

Our primary aim was to establish an association between airways inflammation and disease activity. We have, therefore, used frequency and intensity of symptoms and lung function impairment, irrespective of treatment, to classify patients as intermittent, mild to moderate persistent, and severe persistent using the criteria of the National Heart, Lung, and Blood Institute/World Health Organization (NHLBI/WHO) Workshop on the Global Strategy for Asthma (1). As indices of airways inflammation in sputum we have studied validated variables, including cytology, and concentrations of eosinophil cationic protein (ECP), myeloperoxidase (MPO), and tryptase to assess the degree of activation of eosinophils, neutrophils, and mast cells, respectively. To provide pathophysiologic correlates for the inflammatory cells and mediators, concentrations of albumin were determined as an indicator of plasma

(*Received in original form February 11, 1998 and in revised form December 15, 1998*)

Supported by the Medical Research Council of the United Kingdom.

Dr. Renaud Louis was a recipient of a scholarship from the Fondation Leon Frederic, University of Liege, Belgium and the UCB Institute of Allergy, Brussels, Belgium and grant No. 3453697 from the Fond de Recherche Scientifique Medicale (FRSM).

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Am J Respir Crit Care Med Vol 161. pp 9–16, 2000
Internet address: www.atsjournals.org

exudation and airway responsiveness to methacholine was measured. To account for possible confounding effects of corticosteroid usage, further analyses were conducted after subdividing the patients on the basis of treatment with ICS and OCS.

METHODS

Subjects

Seventy-four nonsmoking asthmatics and 22 healthy nonatopic control subjects were studied (Table 1). The majority of asthmatics were atopic, as judged by positive skin prick tests to at least one common aeroallergen (wheal diameter 3 mm greater than saline control) and had airways hyperresponsiveness, as assessed by measuring the provocative concentration of methacholine causing a 20% fall in FEV_1 (PC_{20}) (5). Measurement of PC_{20} methacholine was not possible in the severe and in some moderate asthmatics because of poor lung function ($FEV_1 < 60\%$ of predicted) and/or inability to refrain from bronchodilators. None of the asthmatics had a history of factors that might aggravate asthma, such as chronic sinusitis and gastroesophageal reflux, and there was nothing in the history to indicate hyperventilation and intolerance of nonsteroidal anti-inflammatory drugs. All the control subjects had nonreactive airways to the top concentration of 32 mg/ml and had negative skin tests to a panel of aeroallergens.

Assessment of Disease Activity

Upon enrollment, all the asthmatics, except for those with poor lung function, underwent methacholine challenge and were instructed to record accurately for 2 wk their asthma symptoms, treatment, and twice-daily peak expiratory flow (PEF), and to comply with their usual prescribed medication. No attempt was made to modify their treatment which remained unchanged for 6 wk before sputum induction. Two of 19 patients with intermittent, 25 of 38 with mild to moderate, and 15 of 17 with severe asthma were receiving regular ICS (beclomethasone diproprionate, budesonide, or fluticasone, ranging from 200 to 3,000 μg/d). Four moderate and seven severe asthmatics were receiving between 4 and 45 mg/d of regular oral prednisolone. Thirteen mild to moderate and 14 severe asthmatics received regular long-acting β_2-agonists.

None of the asthmatics had experienced an exacerbation or a respiratory infection for at least 4 wk as indicated by increased symptoms and/or bronchodilator medication. There were no differences in lung function (FEV_1 and PEF) between Days 1 and 14 of the period during which disease activity was assessed (Table 1). Four asthma symptoms (daytime breathlessness, daytime wheeze, daytime cough, and nighttime asthma) were scored by the patients every day using a subjective 0–3 score system where 0 = none, 1 = mild, 2 = moderate, and 3 = severe. The symptoms were added for each day and a mean daily score calculated for the period of assessment. Diurnal PEF variation was calculated by the formula: (evening − morning PEF/ the mean of the evening and morning PEF) × 100 and the results shown as the mean daily variation. FEV_1 was measured at the beginning of the assessment period and before sputum induction, and the latter was used for correlation with sputum parameters.

Classification According to Asthma Severity

We used the recommendations for classification of asthma severity of the NHLBI/WHO Workshop on the Global Strategy for Asthma (1) and modified them to classify the patients into three categories: intermittent, mild to moderate persistent, and severe persistent asthma. Mild and moderate asthmatics were merged into one group because of the possibility that effective treatment of otherwise moderately severe asthmatics with long-acting bronchodilators could improve symptom control to the extent that they no longer met the criteria for moderate disease. Because the primary aim was to seek an association between clinical activity and indices of airways inflammation, only the criteria of disease activity of the guidelines were used for classification.

All but four intermittent asthmatics (n = 19) had increased airway hyperresponsiveness, as defined by a $PC_{20} < 8$ mg/ml (Table 1), episodic symptoms only (< 1 per week), and used < 1 dose of short-acting inhaled β_2-agonist/week. None had nighttime asthma. Their FEV_1 was > 80% of predicted, with PEF diurnal variation < 20%.

Mild to moderate persistent asthmatics (n = 38) had daytime symptoms at least once a week to several times daily, nighttime asthma > 2 times a month, and used short-acting β_2-agonists at least once a week. Their FEV_1 was > 60% of predicted and PEF diurnal variation was < 30%.

All the severe asthmatics (n = 17) had frequent symptoms limiting their activity, with attacks every night and frequent exacerbations. They all had either an $FEV_1 < 60\%$ or predicted or PEF diurnal variation > 30%.

TABLE 1

DEMOGRAPHIC CHARACTERISTICS OF THE ASTHMATICS AND CONTROL SUBJECTS*

		Asthmatics		
	Control Subjects	Intermittent	Mild to Moderate Persistent	Severe Persistent
n	22	19	38	17
Age, yr	30 ± 11	30 ± 10	37 ± 16	40 ± 15
Sex, M/F	11/11	11/8	18/20	5/12
Atopic	0	18	35	14
FEV_1 % pred, Day 1	96 ± 11	92 ± 14	85 ± 14	51 ± 12
FEV_1 % pred, Day 14	98 ± 11	94 ± 15	81 ± 13	53 ± 12
PEF, L/min, Day 1	525 ± 73	532 ± 75	486 ± 100	340 ± 91
PEF, L/min, Day 14	531 ± 75	539 ± 72	477 ± 99	345 ± 90
PEF variability†	ND	2.3 ± 1.7	6.7 ± 4.1	19.9 ± 11.5
PC_{20}, mg/ml‡	> 32	2.96 (0.15–32)	0.61 (0.03–32)	ND
Daily asthma symptom score§	0	0 (0–0.5)	1.69 (0.2–6.8)	9.34 (4–11.7)
On nebulized bronchodilators	0	0	15	14
On ICS‖/OCS¶	0	2/0	25/4	15/7
On theophylline	0	0	2	1
On long-acting β_2-agonists	0	0	13	14

Definition of abbreviation: ND = not measured.

* Age and percentage of predicted (% pred) FEV_1 and PEF values (on the first and fourteenth day of the assessment period), are expressed as mean ± SD.

† Mean daily PEF variability calculated from twice-daily measurements during 2 wk (*see* METHODS).

‡ PC_{20} methacholine values are expressed as geometric mean (range).

§ Symptoms scores are shown as the median (range) daily scores of daytime breathlessness, daytime wheeze, daytime cough, and nighttime asthma (each scored from 0–3; *see* METHODS) calculated over a period of 2 wk prior to sputum induction.

‖ Receiving inhaled corticosteroids.

¶ Receiving oral corticosteroids.

TABLE 2

CELL COUNTS IN CONTROL SUBJECTS AND ASTHMATICS IN INDUCED SPUTUM*

		Asthmatics			
	Control Sujects	Intermittent	Mild to Moderate Persistent	Severe Persistent	p Value†
Total nonsquamous cells, $\times 10^6$/g	1.39 (0.66–3.75)	1.18 (0.27–2.97)	1.27 (0.45–46.4)	1.74 (0.50–18)	0.23
Eosinophils, $\times 10^3$/g	9 (0–135)	45 (4–220)‡	199 (4–2,227)‖	305 (12–10,800)‖**	< 0.0001
% of nonsquamous cells	0.3 (0–3.6)	5 (0.5–14.7)‡	8.8 (0.3–74.4)‖	28.7 (1.7–89)‖¶	< 0.0001
Neutrophils, $\times 10^3$/g	228 (54–2,130)	109 (10–826)	327 (19–38,048)	355 (27–10,178)¶	0.02
% of nonsquamous cells	19.8 (4.5–56.8)	20 (3.5–43.4)	27.3 (1.5–82)	33.6 (1.5–83.2)	0.25
Macrophages, $\times 10^3$/g	928 (335–2,790)	466 (186–1,900)	495 (129–12,922)	328 (72–2,238)§	0.01
% of nonsquamous cells	72.8 (30.2–92)	65.5 (43–85)	47.2 (11–86)§	14.4 (4.69)‖††‡‡	< 0.0001
Lymphocytes, $\times 10^3$/g	24 (12–119)	22 (4–119)	28 (3–464)	15 (0–144)	0.10
% of nonsquamous cells	1.8 (0.6–4.5)	1.7 (0.8–7.5)	1.8 (0–6.3)	1 (0–4)	0.08
Epithelial cells, $\times 10^3$/g	30 (6–300)	32 (6–299)	55 (6–928)	51 (0–679)	0.34
% of nonsquamous cells	2.3 (0.3–8)	2.3 (0.5–15.7)	2.8 (0.3–16.3)	2.6 (0–22.3)	0.32

* Median values with ranges.
† Calculated by the Kruskal-Wallis test.
Comparisons between asthmatic groups and the control group: ‡$p < 0.05$, §$p < 0.01$, ‖$p < 0.001$.
Comparisons with intermittent asthma: ¶$p < 0.05$, **$p < 0.01$, ††$p < 0.001$.
Comparison with mild to moderate asthma: ‡‡$p < 0.01$.

Analysis for the Effects of Corticosteroids

In a subanalysis performed to account for corticosteroid use, patients with mild to moderate asthma treated with ICS (n = 25) were divided according to the use of low-dose (≤ 800 μg of budesonide or beclomethasone dipropionate or 500 μg fluticasone, n = 10) and high-dose ICS (n = 15). Severe asthmatics were subdivided according to the use of OCS (n = 10 on OCS).

Sputum Induction and Analysis

Sputum was induced by inhalation of 4.5% hypertonic saline after premedication with 400 μg of albuterol and processed with dithioerythritol (DTE) as previously reported (6). Samples containing more than 30% squamous cells were excluded from analysis. Cytospins were stained with May-Grünwald-Giemsa and 600 cells (excluding squamous cells) counted. Absolute cell counts were determined from total and differential cell counts and the results expressed as numbers of cells/g of sputum. Mediators in sputum were analyzed using a fluorometric enzyme immunoassay for ECP (Phamacia, Uppsala, Sweden), and a radioimmunoassay (Pharmacia) for MPO and tryptase (6). Albumin was detected by rocket immunoelectrophoresis (6). Levels of mediators and albumin were expressed as weight per volume of sputum.

Statistical Analysis

Comparisons of cell counts, and mediator and albumin levels between all the groups were first made using the Kruskal-Wallis test. In case of significant difference, this was followed by the Dunn's test for comparison between groups. The latter is a variation of the Bonferroni test for not normally distributed data and together with the Kruskal-Wallis test is a conservative way to account for multiple comparisons. Associations between FEV_1, PC_{20} of methacholine, PEF variability, symptom scores, and indices of inflammation were sought by Spearman's coefficient of correlation using data from asthmatics only. The results of rank correlation and p values are shown as determined by the statistical program. To account for the multiple correlations the p values can be multiplied by 3, i.e., the number of correlations for each parameter. The program used for the analyses was InStat (GraphPad Software, Inc., San Diego, CA).

RESULTS

Airway Inflammatory Cells, Mediators, and Albumin

The inflammatory cells counts in sputum were shown as both absolute counts (cells/g of sputum) and percentages of total cell counts. All three groups of asthmatics had a higher sputum eosinophilia than control subjects, with a progressive increase which was related to asthma severity (Table 2 and Figure 1). Absolute neutrophil counts were significantly raised in severe asthmatics compared with intermittent asthmatics (Table 2). The absolute numbers of macrophages were significantly ($p < 0.01$) lower in severe asthmatics compared with

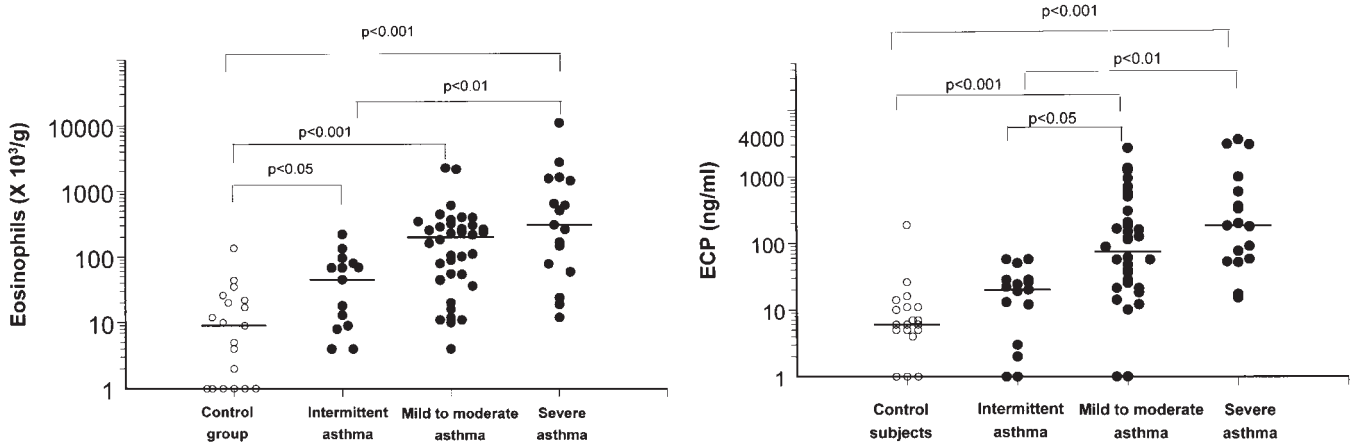

Figure 1. Eosinophil counts and concentrations of ECP in induced sputum of asthmatic and healthy nonatopic control subjects.

TABLE 3

INFLAMMATORY MEDIATORS AND ALBUMIN IN INDUCED SPUTUM*

	Control Subjects	Asthmatics			p Values†
		Intermittent	Mild to Moderate Persistent	Severe Persistent	
ECP, ng/ml	6 (0–187)	20 (0–57)‡	73 (0–2,640)‖¶	180 (17–3,500)‖**	< 0.0001
Tryptase, ng/ml	0 (0–6.7)	2.2 (0–8.7)	2.6 (0–100)§	2 (0–18.5)	0.003
MPO, ng/ml	305 (45–439)	215 (32–1,000)	195 (32–1,000)	500 (169–956)	0.4937
Albumin, μg/ml	112 (22–187)	201 (19–576)	268 (15–2,639)‖	346 (26–1,438)§	0.0006

* Median values with ranges.
† Calculated by Kruskal-Wallis test.
Comparisons between asthmatic groups and the control group: ‡$p < 0.05$, §$p < 0.01$, ‖$p < 0.001$.
Comparisons with intermittent asthma: ¶$p < 0.05$, **$p < 0.01$.

control subjects (Table 2), and the relative macrophage counts were significantly lower in both the severe and mild to moderate asthmatics compared with control subjects ($p < 0.001$ and $p < 0.01$, respectively). Furthermore, there were significant differences in relative macrophage counts between severe asthmatics and both intermittent and mild to moderate asthmatics ($p < 0.001$ and $p < 0.01$). In contrast, there were no differences between the subject groups in lymphocyte numbers (Table 2).

Significant between-group differences were detected for ECP and tryptase, but not MPO concentrations, with a progressive increase in ECP concentrations that was related to asthma severity (Figure 1 and Table 3). Compared with control subjects, tryptase concentrations were significantly ($p < 0.01$) higher only the in mild to moderate asthmatics.

Albumin concentrations were significantly higher in both mild to moderate and severe asthmatics compared with control subjects ($p < 0.001$ and $p < 0.01$, respectively) (Table 3 and Figure 2).

Associations between Inflammatory and Physiologic Indices in Asthmatics

In the intermittent and mild to moderate asthmatics PC_{20} was inversely correlated with ECP concentrations ($r_s = -0.67$, $p < 0.0001$), absolute eosinophil counts ($r_s = -0.55$, $p < 0.001$), and less strongly with absolute neutrophil counts ($r_s = -0.46$, $p < 0.01$) and MPO levels ($r_s = -0.34$, $p < 0.05$). There was a weak, albeit significant, correlation between FEV_1, PEF variability, and daily symptom scores and absolute eosinophil counts and ECP concentrations, between absolute neutrophil counts and PEF variability, and between MPO concentrations and daily symptom scores (Table 4). These correlations remain significant even after correcting for multiple comparisons by multiplying the p values of the number of correlation tests performed for a given inflammatory index (i.e., 3). There was also a weak, but significant, positive correlation between the concentrations of albumin and those of tryptase ($r_s = 0.42$, $p = 0.001$) and ECP ($r_s = 0.36$, $p < 0.01$).

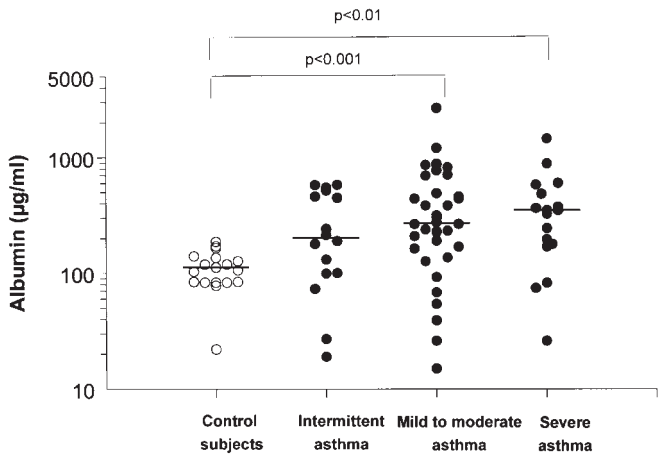

Figure 2. Albumin concentrations in induced sputum of asthmatic and healthy control subjects.

Subanalysis of Mild to Moderate Asthmatics Treated with ICS

There was no significant difference between the mild to moderate asthmatics on low- and high-dose ICS in any of the inflammatory indices in sputum (Tables 5 and 6 and Figure 3). By comparison with control subjects, both those on low-dose ICS and those on high-dose ICS had markedly higher absolute eosinophil counts and concentrations of ECP and albumin (Tables 5 and 6 and Figure 3), but only the asthmatics receiving high-dose ICS had significantly ($p < 0.05$) higher neutrophil counts and raised levels of tryptase ($p < 0.0001$) (Tables 5 and 6 and Figure 3).

Subanalysis of Severe Asthmatics

Severe asthmatics treated with OCS had fewer numbers of neutrophils ($p < 0.05$) and lower albumin levels ($p < 0.05$) in sputum compared with severe asthmatics who were not receiving OCS, with no significant difference in lymphocyte, eosinophil, and macrophage counts, and concentrations of ECP and tryptase (Tables 5 and 6, Figure 3). Compared with control subjects patients receiving OCS had 19-fold higher median eosinophil counts ($p < 0.001$) and 15-fold higher median concentrations of ECP ($p < 0.01$), without an overall increase in tryptase and albumin, and reduced macrophage counts ($p < 0.001$). Severe asthmatics not treated with OCS and 4-fold higher median neutrophil counts ($p < 0.01$), 44-fold higher me-

TABLE 4

ASSOCIATIONS BETWEEN INFLAMMATORY INDICES IN INDUCED SPUTUM AND CLINICAL AND LUNG FUNCTION PARAMETERS*

	FEV_1		PEF Variability		Daily Symptom Score	
	r_s	p Value	r_s	p Value	r_s	p Value
Eosinophils (absolute counts)	−0.43	< 0.001	0.49	< 0.0001	0.43	< 0.001
ECP concentrations	−0.36	< 0.01	0.51	< 0.0001	0.52	< 0.0001
Neutrophils (absolute counts)	−0.20	0.1	0.31	< 0.01	0.23	0.06
MPO concentrations	−0.25	0.07	0.22	> 0.05	0.38	< 0.01

* Correlations were conducted using the Spearman's rank correlation test.

TABLE 5

INFLAMMATORY CELLS IN SPUTUM IN CONTROL SUBJECTS AND PERSISTENT ASTHMATICS GROUPED ACCORDING TO USE OF LOW- AND HIGH-DOSE ICS AND OCS*

		Mild to Moderate Asthmatics		Severe Asthmatics	
	Control Subjects	Low-dose ICS (*n* = 10)	High-dose ICS (*n* = 15)	Not on OCS (*n* = 10)	On OCS (*n* = 7)
Neutrophils, $\times 10^3$/g	228 (54–2,130)	344 (22–1,138)	833 (124–38,040)†	949 (164–10,179)‡	163 (27–1,967)
Eosinophils, $\times 10^3$/g	9 (0–135)	216 (4–603)‡	172 (11–2,227)‖	403 (12–10,800)‖	168 (19–1,610)§
Macrophages, $\times 10^3$/g	928 (335–2,790)	464 (153–2,125)	1,367 (387–12,922)	347 (72–2,288)†	193 (78–431)§
Lymphocytes, $\times 10^3$/g	24 (12–119)	19 (0–121)	42 (10–464)	21 (0–144)	6 (0–22)‡

* Median values with ranges.
Comparisons between asthmatics and control subjects: †$p < 0.05$, ‡$p < 0.01$, §$p < 0.001$, ‖$p < 0.0001$.

dian eosinophil counts ($p < 0.0001$), 56-fold higher median ECP concentrations ($p < 0.001$), 6-fold higher median tryptase concentrations ($p < 0.05$), and 4-fold higher median albumin concentrations ($p < 0.001$) compared with control subjects.

DISCUSSION

In a cross-sectional study of asthmatics from across the entire spectrum of asthma severity we have shown that clinical activity, airways hyperresponsiveness, and lung function are related to eosinophilic and, to a lesser extent, neutrophilic inflammation. The fact that eosinophilic inflammation was evident despite treatment with high doses of ICS and OCS points to the relentless nature of chronic asthma which responds poorly to corticosteroids.

Following our initial observations (6), we provide further evidence that airways eosinophilia is a major determinant of clinical activity regardless of treatment. Our study also identifies ongoing mast cell degranulation with tryptase release as a feature of moderate, persistent asthma which appears to be controlled only by the use of OCS, but does not respond to high doses of ICS. In keeping with the proinflammatory actions of mast cells and eosinophils, there was evidence of persistent microvascular leakage, as supported by raised albumin levels, which correlated with tryptase and ECP concentrations and could also be controlled only by OCS. The neutral mast cell protease, tryptase, has been a focus of interest recently because of its ability to cause edema (7) and attract and activate eosinophils (8). Our findings suggest that tryptase plays an important role in chronic asthma except, perhaps, in severe disease when patients are treated with OCS.

There was significant overlap in values of various sputum inflammatory indices between patients with asthma, including those with severe disease and control subjects, consistent with the ability of treatment to suppress the individual mediators to normal levels in some patients. This highlights the complexity and heterogeneity of cellular mechanisms in asthma of varying disease severity, which are likely to involve other factors, such as airways restructuring, neural mechanisms, and, suggested in a preliminary report (9), persistent infection.

An increasing number of studies are focusing on elucidating the mechanisms that determine asthma severity (10–23). Some of these have been conducted on asthmatics not treated with anti-inflammatory drugs (11–15) and have found a relationship between airways hyperresponsiveness and eosinophilia detected either in BAL, bronchial biopsies, or sputum. A minority of studies has failed to confirm this relationship (17, 18). Emerging evidence suggest that the correlation between airway eosinophilia and hyperresponsiveness persists despite treatment with corticosteroids (16), even though the strength of the correlation is not strong. However, only a few studies to date have investigated the relationship between inflammation and clinical activity as measured by symptoms and treatment. One of these (22), involving fewer subjects than the present study, none of whom were receiving OCS, confirms our observation in finding a severity-related increase in mucosal eosinophil counts in bronchial biopsies of asthmatics classified according to treatment requirements and lung function. A further study of moderate to severe asthmatics classified using the Aas score (23) found a positive, although weak, relationship between this index of clinical severity and sputum eosinophilia shown as a percentage of total cell counts. In contrast to our study, this study did not show a relationship between eosinophilia and either FEV_1 or PC_{20} histamine. Possible reasons for the stronger relationship in our study are the inclusion of more severe patients and a different means of expressing eosinophil counts (absolute as opposed to relative counts) in correlation tests. Furthermore, the methods used to classify patients were different, with the Aas score being based on events during 1 yr as opposed to our own method which

TABLE 6

MEDIATORS AND ALBUMIN IN SPUTUM IN CONTROL SUBJECTS AND PERSISTENT ASTHMATICS GROUPED ACCORDING TO USE OF LOW- AND HIGH-DOSE ICS AND OCS*

		Mild to Moderate Asthmatics		Severe Asthmatics	
	Control Subjects	Low-dose ICS (*n* = 10)	High-dose ICS (*n* = 15)	Not on OCS (*n* = 10)	On OCS (*n* = 7)
ECP, ng/ml	6 (0–187)	113 (0–649)‡	124 (0–2,640)§	337 (51–3,500)§	89 (15–2,960)‡
Tryptase, ng/ml	0 (0–6.7)	0.8 (0–12.9)	2.9 (1–100)§	3.2 (0–18.5)†	0 (0–14.9)
MPO, ng/ml	305 (45–439)	534 (158–835)	169 (32–1,000)	639 (179–956)	ND
Albumin, μg/ml	112 (22–187)	437 (15–859)†	218 (26–815)†	424 (168–1,438)§‖	177 (26–361)

* Median values with ranges.
Comparisons between the asthmatic groups and control subjects: †$p < 0.05$, ‡$p < 0.01$, §$p < 0.001$.
Comparisons between severe asthmatics with and without oral corticosteroids: ‖$p < 0.05$.

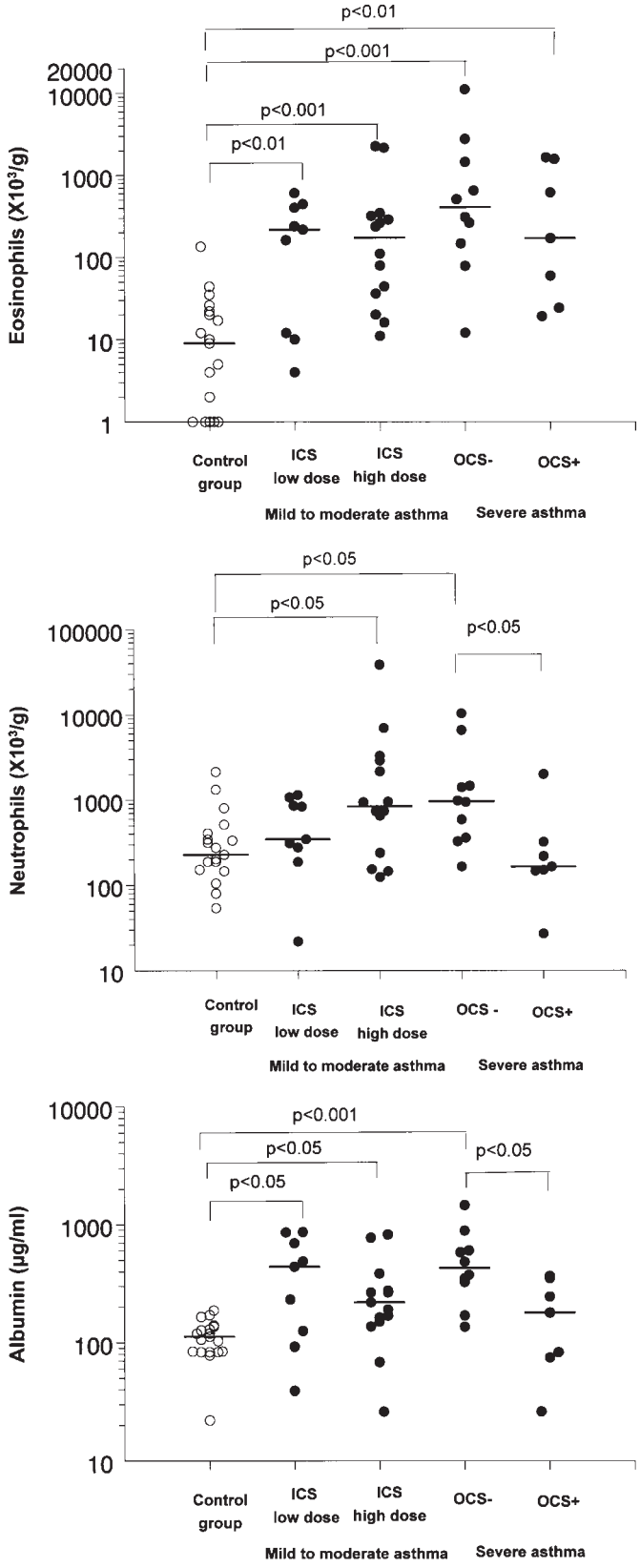

Figure 3. Eosinophil (*top panel*) and neutrophil (*middle panel*) counts, and albumin concentrations (*bottom panel*) in induced sputum in control subjects and asthmatics classified according to use of inhaled (ICS) and oral (OCS) corticosteroids.

took into consideration disease activity immediately prior to sputum induction.

In contrast to studies of mild to moderate asthma, two bronchoscopic studies of severe asthmatics treated with OCS (19, 21) have found little evidence of eosinophilia in the mucosal tissue. This apparent discrepancy suggests that mucosal eosinophilia may be suppressed by OCS whereas luminal eosinophilia is more resistant, perhaps owing to insufficient penetration of the drugs into the superficial layers of the mucosa and lumen. Alternatively, the mechanisms regulating cell counts in different compartments may be different. A number of studies have shown differences in differential cell counts between the luminal and mucosal compartments (24, 25) even though there may be correlations between the mucosa and lumen for individual cell types. Thus, T cells are the most numerous population in bronchial biopsies (26), but are a minority in the lumen as seen in the present and other studies of induced sputum.

Neutrophils have been viewed as playing a minor role in chronic asthma, with a greater contribution to disease exacerbations (27) and asthma deaths (28). In this study we show that asthma severity is associated with sputum neutrophilia. High sputum neutrophil counts were particularly evident in asthmatics who remained symptomatic despite treatment with inhaled steroids. Despite the fact that MPO levels were not raised in the present study, this marker of neutrophil activity correlated, albeit weakly, with methacholine responsiveness and daily symptom scores. Our findings support those from a study by Wenzel and coworkers (21) showing a consistent increase in neutrophils in BAL, and both bronchial and transbronchial biopsies of patients with corticosteroid-dependent severe asthma. The authors of this study could not exclude the possibility of a treatment effect. However, the finding of reduced neutrophilia in severe asthmatics treated with OCS in the present study does not support that explanation.

One conclusion from studies conducted so far is that eosinophilic inflammation is a characteristic feature of intermittent and mild asthma whereas the association of eosinophilia and neutrophilia characterizes more severe disease. It is recognized that chemokines such as RANTES or eotaxin are selectively active on eosinophils whereas platelet-activating factor (PAF) and leukotriene B_4 (LTB_4) are chemotactic for both eosinophils and neutrophils (29). Whether the change in granulocytic infiltration is related to a change in chemoattractants remains to be elucidated, but a recent study showing a disease-related increase in LTB_4 concentrations in BAL (21) suggests that this may well be the case.

We recognize the confounding effects of treatment when interpreting associations between disease activity and mucosal inflammation. To account for the use of corticosteroids, we have conducted a subanalysis of the data. This shows worrying evidence that eosinophil accumulation and activation persist even with treatment with high doses of ICS and OCS. This observation is in line with previous reports (3, 4, 6, 17, 23, 30), although none of the studies to date have analyzed the relationship between sputum indices, and asthma activity and treatment in a systematic manner. Whether and to what extent this contributes to long-term morbidity and increased risk of sudden death remains to be determined. A prospective study involving more than 1,000 asthmatics has shown that peripheral blood eosinophilia greater than 0.45×10^9 per liter increased the relative risk of death from asthma more than sevenfold (31). Sudden-onset fatal asthma (less than 1 h after onset of the attack) may be a different entity which appears to be related to airways neutrophilia (28), whereas eosinophil accumulation seems to play a more prominent role as the duration of the exacerbation incases (32). In support of our observa-

tions, one study of asthma deaths has shown extensive bronchial mucosal eosinophilia despite the fact that approximately half of the patients had been receiving continuous OCS (33).

A number of studies have demonstrated the effectiveness of corticosteroids at reducing airways inflammation (5, 15, 26, 34–36). The clinical relevance of most of these studies is limited by the inclusion of asthmatics with relatively mild disease who are likely to respond to low doses of ICS. Evidence from clinical trials has cast doubt as to whether ICS have a dose-dependent effect in asthma (37). Thus, the use of 2,000 μg of fluticasone has failed to significantly decrease BAL eosinophilia in patients previously treated with 400 μg of beclomethasone dipropionate (38). Our study was not designed to look for the dose effects of corticosteroids. However, the lack of differences in eosinophil counts between asthmatics treated with high- or low-dose ICS and between those patients with severe asthma treated with OCS and those on high-dose ICS only suggests that there may well be a plateau in the anti-inflammatory action of corticosteroids.

Although there is some discrepancy between studies regarding an increase in numbers of T cells in asthmatic airways (14, 24), there is consistent evidence of increased T-cell activation in both mild and severe asthma (13, 19, 32) and sustained production of T helper cell, type 2 (Th2) cytokines (20, 35). The phenomenon of true corticosteroid resistance in patients with asthma is well described, although rare (20, 39). Persistent inflammation could result from overwhelming stimulation by allergens, exposure to environmental factors such as viruses and pollution, poor penetration of available drugs into the distal airways, or other unknown mechanisms. Alternatively, treatment may be ineffective because of a limited ability of corticosteroids to inhibit the recruitment, activation, and prolonged survival of inflammatory cells in these patients. If that is correct, the relative degree of unresponsiveness to corticosteroids would determine disease severity.

Finally, we have observed a relative decrease in the numbers of macrophages in corticosteroid-treated asthmatics. The fact that this has also been observed in endobronchial biopsies and BAL of severe asthmatics (21) suggests that this is not a simple reflection of reduced recovery of cells from the distal airways. While the macrophage is able to produce anti-inflammatory mediators, several studies point to a suppressive effect on T cells (40). Whether and how this determines asthma severity remains to be elucidated.

In conclusion, this study shows that persistent granulocytic, in particular, eosinophilic inflammation is an important factor which determines clinical asthma severity and points to an inability of currently available corticosteroids to fully control the inflammatory processes. The study emphasizes the need for further research into the dose-dependency of corticosteroid effects and inflammatory mechanisms that are poorly responsive to corticosteroids as targets for new anti-asthma compounds.

***Acknowledgment*:** The authors would like to acknowledge the excellent technical assistance of Kate Roberts during the preparation of the manuscript.

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