

Analysis of induced sputum to examine the effects of prednisone on airway inflammation in asthmatic subjects

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To determine whether induced sputum samples might provide a useful means for evaluating the effects of therapy on airway mucosal inflammation, we examined induced sputum samples obtained before and after 6 days of treatment with prednisone (0.5 mg/kg/day) or placebo in a randomized, double-blind study of 24 asthmatic subjects. Induced sputum was analyzed for total and differential cell counts and for concentrations of eosinophil cationic protein, albumin, and mucin-like glycoprotein. We found that the mean ($\pm$ SEM) percentage of eosinophils in sputum samples from the prednisone-treated group fell from $14.1\% \pm 5.0\%$ at baseline to $1.8\% \pm 0.8\%$ after treatment, a decrease significantly greater than in the placebo-treated group (from $10.3\% \pm 4.9\%$ to $11.1\% \pm 4.0\%$; $p = 0.002$). The absolute number of eosinophils also decreased significantly more in the prednisone-treated group than in the placebo-treated group ($p = 0.04$). In addition, eosinophil cationic protein levels in induced sputum fell more in the prednisone-treated group than in the placebo-treated group (from 324 ± 131 ng/ml to 144 ± 84 ng/ml vs 173 ± 50 ng/ml to 188 ± 47 ng/ml; $p = 0.002$). Furthermore, prednisone treatment was associated with a significant increase in peak expiratory flow, an effect that was significantly correlated with the decrease in eosinophil percentage in induced sputum ($r_s = 0.64$, $p = 0.04$). Prednisone treatment was not associated with any significant change in the concentrations of albumin or mucin-like glycoprotein. We conclude that analysis of induced sputum is a useful noninvasive method for studying the effects of asthma therapy on airway eosinophilic inflammation. (J ALLERGY CLIN IMMUNOL 1994;94:861-9.)

Key words: Asthma, induced sputum, corticosteroids, eosinophil, eosinophil cationic protein, albumin, mucin-like glycoprotein

Although airway inflammation is now established as central to the pathophysiology of asthma,¹ there are relatively few controlled studies on the effects of antiinflammatory therapy on markers of airway inflammation.^{2, 3} This is at least partly explained by the difficulty in obtaining samples of airway mucosa or of airway mucosal secretions from asthmatic subjects. Bronchoscopy is an invasive procedure that requires special

Abbreviations used

ECP:	Eosinophil cationic protein
FEV ₁ :	Forced expired volume in 1 second
MLG:	Mucin-like glycoprotein
PC ₂₀ FEV ₁	Provocative concentration of
[methacholine]:	methacholine causing a 20% fall in FEV ₁

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expertise and subjects who are willing to undergo it, and it is impractical for repeated sampling of the airways. In addition, the technique of bronchoalveolar lavage primarily samples the alveolar compartment of the lungs, greatly reducing the airway secretion component of the fluid recovered. Collecting spontaneously expectorated sputum is less invasive than bronchoscopy and yields a sample more concentrated in airway secretions, but many subjects are unable to expectorate spu-

tum consistently, especially when they are in clinical remission.

Recent reports indicate that collecting sputum expectorated by subjects after they inhale hypertonic saline solution (induced sputum) represents a safe noninvasive method for obtaining airway secretions from asthmatic and healthy subjects for research purposes.⁴⁻⁷ We have shown that it is feasible to analyze induced sputum for both cellular and biochemical markers of inflammation,^{5, 6} and we have found that when standardized methods for inducing and processing sputum are used, the samples from asthmatic subjects contain significantly higher percentages of eosinophils and significantly higher levels of eosinophil cationic protein (ECP), albumin, fibrinogen, and a marker of mucin secretion (mucin-like glycoprotein [MLG]) than induced sputum samples from healthy subjects. In a separate study we also found that induced sputum samples obtained from asthmatic subjects after aerosolized allergen challenge show significantly higher percentages of eosinophils and higher levels of ECP at 4 and 24 hours after challenge than at baseline.⁷

Thus cellular and biochemical analyses of induced sputum samples show significant differences between asthmatic and healthy subjects in the baseline state and increases in markers of eosinophilic inflammation after antigen challenge. Together these data show the feasibility of measuring not only cell counts and differentials in induced sputum samples but also of measuring chemicals in the fluid phase of induced sputum. In addition, they demonstrate the possibility of detecting changes in airway inflammation provoked by an external stimulus. A logical extension of these findings is to determine whether analysis of induced sputum can be used as a method for evaluating the effects of therapy on airway mucosal inflammation. Therefore in this study we investigated whether analyses of induced sputum samples before and after antiinflammatory treatment reveal reductions in markers of inflammation. We chose to treat patients with orally administered prednisone because corticosteroids have potent antiinflammatory effects in the airways,⁸ effects that occur rapidly when prednisone is given orally. We conducted a randomized, double-blind, placebo-controlled clinical study of 24 asthmatic subjects. Induced sputum was collected before and after 6 days of treatment with prednisone or placebo and was analyzed for total and differential cell counts and for concentrations of ECP, albumin, and MLG. In addition, blood

was collected and analyzed for cell differential and ECP concentration.

METHODS

Subjects

Twenty-four asthmatic subjects were studied (Table I). Inclusion criteria were a history of symptoms of asthma and bronchial hyperreactivity in response to methacholine. The provocative concentration of methacholine causing a 20% fall in forced expiratory volume in 1 second ($PC_{20}FEV_1$ [methacholine]) was less than or equal to 8 mg/ml. Exclusion criteria were inhaled or oral steroid use in the previous 6 weeks, upper respiratory tract infection in the previous 6 weeks, tobacco use within the past year or smoking history of greater than 10 pack years (total), and a history of hypertension, diabetes, peptic ulcer disease, cataracts, glaucoma, or bipolar disorder (information abstracted from an asthma questionnaire we developed for this purpose and filled out by the subjects). All subjects signed consent forms approved by the Committee on Human Research at the University of California, San Francisco.

Protocol

The study involved three visits to the laboratory. The first visit was for consent, spirometry, allergen skin testing, and methacholine challenge. The second visit (at least 48 hours after visit 1) was for spirometry, sputum induction, venipuncture, and double-blind randomization to either the prednisone group (0.5 mg/kg/day) or the placebo group for 6 days. The subjects took the study medication (either prednisone or placebo capsules) each day for 6 days and returned on the sixth day (at the same time of day) for the third visit. The third visit was for repeat spirometry, sputum induction, blood tests, and a pill count to verify compliance with treatment.

Blood sampling

At the beginning of each visit a 5 ml sample of venous blood was drawn into a serum separator tube and stored on ice until it could be processed.

Spirometry

Bronchodilators were withheld for 8 hours before testing. Spirometry was performed with a rolling seal Ohio 840 spirometer (Ohmeda, Murray Hill, N.J.) according to American Thoracic Society criteria.⁹ Flow volume loops were generated by acquiring and digitizing the electrical volume signal versus time produced by a rolling seal spirometer and then scaling these digitized computer counts to liters by application of a calibration factor. Flow was derived from the change in volume over the change in time (in real time), and flow versus volume was displayed on a graphic cathode ray screen, as well as printed on hard copy. All spirometric values including peak expiratory flow were calculated from the raw data (i.e., volume vs time), and the values

TABLE 1. Clinical characteristics of the study subjects

Subject	Age (yr)	Sex	Atopy*	FEV ₁ (L)	FEV ₁ (% predicted)	PC ₂₀ FEV ₁ (M)† (mg/ml)
Placebo-treated group						
1	49	F	+	1.1	50	0.06
2	35	F	+	2.5	86	0.10
3	29	M	+	3.3	85	0.25
4	30	F	+	2.9	94	0.25
5	30	M	+	4.0	98	0.10
6	30	M	+	3.3	77	0.09
7	30	M	+	3.5	90	2.65
8	34	F	+	1.2	33	0.05
9	29	M	+	3.0	75	0.38
10	22	M	+	4.2	93	0.06
11	22	M	+	2.1	51	0.03
12	37	F	+	1.3	50	0.03
Prednisone-treated group						
1	21	F	+	2.4	73	0.06
2	22	M	+	5.0	109	1.03
3	25	F	+	3.6	103	0.13
4	34	F	+	3.1	94	0.17
5	29	M	+	3.5	103	5.65
6	25	M	+	4.5	94	0.50
7	42	F	+	1.9	61	0.72
8	36	F	+	2.7	87	0.50
9	26	M	+	4.4	129	0.04
10	25	F	+	1.8	60	0.16
11	50	M	+	3.2	82	0.09
12	26	M	+	3.3	73	2.80

*Skin test results positive to one or more of 11 common aeroallergens.

†Denotes the concentration of methacholine causing a 20% decrease in FEV₁.

reported are the best results of three separate maneuvers.

Methacholine challenge

Methacholine challenge was performed with a method modified from that of Chai et al.¹⁰ Methacholine was diluted in calcium- and magnesium-free phosphate-buffered saline and delivered from a hand-held nebulizer (DeVilbiss no. 646; DeVilbiss Health Care, Inc., Somerset, Pa.) connected to a dose-metering device (driven by compressed oxygen at 20 psi) and equipped with a critical orifice, 7 mm in diameter, at the inflow end to allow a maximum inspiratory flow of 500 ml/min. Inspiration by the subject triggered a solenoid valve in the dosimeter to open for 2.1 seconds and deliver an average of 15.1 μ l of methacholine from a 2 ml reservoir. Each subject took 5 inhalations of aerosol, breathing from functional residual capacity to total lung capacity each time. Single forced expiratory volume in 1 second (FEV₁) maneuvers were recorded 1 and 3 minutes after the subject inhaled phosphate-buffered saline diluent, and the best result of these maneuvers was used as the baseline FEV₁. The initial concentration of methacholine administered was 0.03

mg/ml, and a dose-response curve was constructed by administering serial doubling concentrations of methacholine at 5-minute intervals until the lowest FEV₁ maneuver recorded 1 or 3 minutes after methacholine inhalation was less than or equal to 80% of the baseline FEV₁. The PC₂₀ FEV₁ [methacholine] was calculated by linear interpolation between the last two points on the dose-response curve.

Allergen skin testing

Skin prick testing was performed with standardized disposable allergy prick test needles (Morrow Brown, Topeka, Kan.). Eleven common inhalant allergens were used, six of which (house dust, *Alternaria* spp., cat hair, dog hair, *Dermatophagoides pteronyssinus*, *D. farinae*, and diluent control) were obtained from Miles Laboratories, (Spokane, Wash.) and five of which (house dust, mixed grass pollen, *Hormodendrum* spp., mixed trees, mixed weeds, and histamine control) were obtained from Berkeley Biological (Berkeley, Calif.). If the skin wheal diameter caused by the allergen was 3 mm larger than that caused by the diluent control, then the skin test result was considered positive.

TABLE II. Eosinophil percentage of nonsquamous cells and ECP levels in induced sputum from asthmatic subjects before and after treatment

	Eosinophils (%)		ECP (ng/ml)	
	Before	After	Before	After
Placebo-treated group				
1	3.9	4.2	131	246
2	13.1	28.4	270	350
3	0.2	0	141	60
4	0	0.2	24	60
5	2.5	3.0	78	38
6	39.7	8.7	122	87
7	0.5	4.8	106	175
8	30.5	30.5	606	494
9	2.3	20.5	24	57
10	*	*	14	6
11	24.5	31	368	393
12	8.4	9.5	189	291
Mean	9.2	11.1	173	188
SEM	3.8	3.5	50	47
Prednisone-treated group				
1	38.9	0.8	1684	1047
2	0.8	0	30	57
3	0.4	0.3	21	7
4	3.9	0.2	387	38
5	0.7	0	46	23
6	*	*	91	9
7	42.9	7.8	324	115
8	20	1.3	416	27
9	31	6.5	256	54
10	15	0.8	365	242
11	1	1.3	38	17
12	0.8	0.4	236	94
Mean	14.1	1.8	325	144
SEM	5.0	0.8	131	84

*One subject in each group had greater than 95% squamous cells, and we could not count 200 nonsquamous cells in order to perform a differential.

Sputum induction

Sputum induction was performed as previously described.⁵ Briefly, all subjects were pretreated with 180 µg albuterol administered by metered-dose inhaler, and then with inhaled nebulized sterile 3% saline solution for 20 minutes from a DeVilbiss 65 ultrasonic nebulizer, the reservoir of which was filled with 3% saline solution (100 ml). (This nebulizer generates particles with a mean mass median diameter of 4.5 µm and has an output of 2.4 ml/min.) Subjects were encouraged to cough throughout the procedure, and then regularly interrupted inhalation of hypertonic saline solution to expectorate all secretions, including both sputum and saliva, into a clean plastic container.

Prednisone randomization

Subjects were randomized to prednisone or placebo treatment according to a sequence determined from a

table of random numbers. Identical capsules containing either prednisone (10 mg) or lactose (250 mg) were prepared by the University of California, San Francisco Drug Product Services Laboratory.

Sputum processing

The volume of the induced sputum sample was determined and overlaid with an equal volume of dithiothreitol 0.1% (Sputalysin 10%; Behring Diagnostics Inc., Somerville, N.J.). The sample was then mixed gently by vortex mixer and placed in a shaking water bath at 37° C for 15 minutes to ensure complete homogenization. The sample was removed from the water bath periodically for further brief gentle vortex mixing. Ten microliters of the homogenized sputum was used to determine the total cell count with a standard hemocytometer. Two hundred fifty microliters of homogenized sputum (diluted in saline solution to

TABLE III. Mean $\pm$ SEM induced sputum volume, cell differentials, and albumin and MLG levels before and after treatment

	Placebo-treated group		Prednisone-treated group	
	Before	After	Before	After
Sputum volume (ml)	15.8 $\pm$ 3.0	17.9 $\pm$ 3.8	16.7 $\pm$ 3.2	14.7 $\pm$ 2.1
Total cell count ($\times 10^6$)	4.2 $\pm$ 1.6	1.9 $\pm$ 0.3	2.9 $\pm$ 0.7	3.2 $\pm$ 0.8
Squamous cells (%)	27.3 $\pm$ 7.4	25.0 $\pm$ 7.5	33.2 $\pm$ 7.8	41.3 $\pm$ 9.5
Epithelial cells (%)*	10.9 $\pm$ 4.1	4.3 $\pm$ 1.1	3.1 $\pm$ 1.7	3.2 $\pm$ 1.5
Macrophages (%)*	52.8 $\pm$ 6.3	54.2 $\pm$ 7.0	36.7 $\pm$ 6.3	45.3 $\pm$ 7.6
Neutrophils (%)*	24.1 $\pm$ 6.1	29.2 $\pm$ 5.5	44.5 $\pm$ 7.0	48.3 $\pm$ 8.3
Eosinophils (%)*	10.3 $\pm$ 4.9	11.1 $\pm$ 4.0	14.1 $\pm$ 5.0	1.8 $\pm$ 0.8
Lymphocytes (%)*	2.3 $\pm$ 0.9	1.8 $\pm$ 0.6	1.4 $\pm$ 0.4	1.4 $\pm$ 0.5
Albumin (μ g/ml)	364 $\pm$ 122	296 $\pm$ 69	587 $\pm$ 325	463 $\pm$ 280
MLG (mg/ml)	6387 $\pm$ 1875	4453 $\pm$ 1379	6459 $\pm$ 1787	7378 $\pm$ 2206

*Calculated as a percentage of nonsquamous cells.

prevent cell crowding on the slide) was spun in a cytocentrifuge (model 7 cytopsin; Shandon Scientific, Sewickley, Pa.) and stained with Diff-Quik stain (Baxter Scientific Products, Miami, Fla.). At least 200 nonsquamous cells on each sputum slide were read by two investigators blinded to the treatment code. Averaged cell differentials of the two investigators are reported. The remainder of the homogenized sputum was centrifuged at 1037g for 5 minutes. The supernatant was aspirated and frozen at -70°C for later analysis.

Biochemical assays

ECP and albumin concentrations in induced sputum supernatant samples were determined with sensitive radioimmunoassays (Pharmacia Diagnostics Inc., Fairfield, N.J.). We calculated albumin values in our samples based on the basis of radioimmunoassay results obtained for serial dilutions of human albumin (Sigma Chemical Co., St Louis, Mo.) in saline solution. MLG levels were determined by ELISA as previously described.⁶

Blood samples

Blood was collected, and samples were centrifuged at 220g. Serum was stored at -70°C for later determination of ECP. Cell counts were done in the clinical laboratory by flow cytometry techniques.

Statistics

Data are presented as means $\pm$ SEM. Within-group changes in outcome variables in the prednisone and placebo groups were each compared with Wilcoxon's signed-rank test. Between-group changes in outcome variables were compared with the Mann-Whitney U test. Spearman's rank order test was used to determine correlations between data. A probability value of less than 0.05, with a two-tailed test, was considered significant.

RESULTS

Overall, the baseline induced sputum values for the prednisone and placebo groups were not significantly different for total and differential cell counts and concentrations of ECP, albumin, and MLG (Tables I to III). The induced sputum samples obtained after prednisone treatment showed significant decreases in mean eosinophil percentage and in the concentration of ECP. These decreases were significantly greater than those found in these markers in induced sputum from the placebo-treated group (Fig. 1, Table III). The induced sputum samples revealed no significant effects of prednisone on sputum volume, total cell number, mean percentage of polymorphonuclear leukocytes, squamous cells, lymphocytes, or epithelial cells or on concentrations of albumin and MLG (Table III).

Total eosinophil numbers in the induced sputum samples in the prednisone-treated group fell from $[2.6 \pm 1.2] \times 10^6$ at baseline to $[0.27 \pm 0.1] \times 10^6$ after 6 days of treatment ($p = 0.007$). Total eosinophil numbers in the induced sputum samples in the placebo-treated group was $[3.7 \pm 1.8] \times 10^6$ at baseline and decreased insignificantly to $[2.6 \pm 0.9] \times 10^6$ after 6 days of treatment ($p = 0.93$). This treatment-induced decrease in the prednisone-treated group was significantly greater than the change seen in the placebo-treated group ($p = 0.04$).

Two subjects (one in each treatment group) produced sputum in which the majority of cells were squamous cells, and it was not possible to generate cell differentials on at least 200 nonsquamous cells. Data from these two subjects

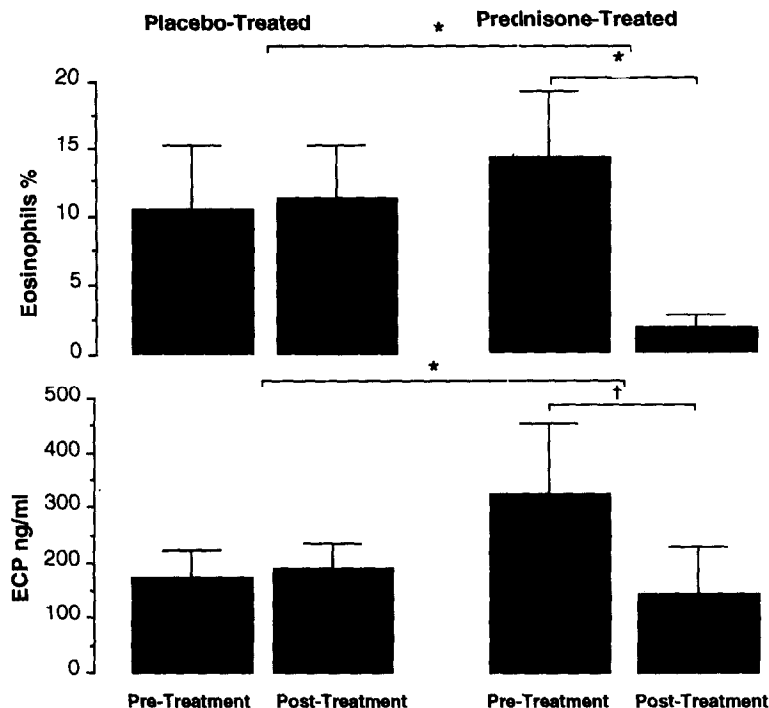


FIG. 1. Top panel, Mean $\pm$ SEM eosinophils as a percentage of nonsquamous cells in induced sputum from prednisone-treated asthmatic ($n = 11$) and placebo-treated ($n = 11$) subjects. **Lower panel,** Mean $\pm$ SEM levels of ECP in induced sputum from prednisone-treated asthmatic ($n = 12$) and placebo-treated asthmatic ($n = 12$) subjects. *Significantly different from change in placebo-treated group ($p = 0.002$). †Significantly different from change in placebo-treated group ($p = 0.003$).

have not been included in the analysis of cell counts and differentials, but we included protein measurements made from the fluid phase of the induced sputum from these two subjects in our analysis of ECP, albumin, and MLG.

The percentage of eosinophils in the blood samples in the prednisone-treated group fell significantly more than in the placebo-treated group (from $7.5\% \pm 1.0\%$ at baseline to $0.9\% \pm 0.3\%$ vs $6.0\% \pm 0.7\%$ to $7.0\% \pm 0.7\%$; $p = 0.0001$). Serum ECP concentrations in the prednisone-treated group similarly fell significantly more than in the placebo-treated group (from 35.4 ± 13.3 ng/ml at baseline to 10.0 ± 1.8 ng/ml vs 21.2 ± 2.9 ng/ml to 18.1 ± 3.0 ng/ml; $p = 0.03$).

The baseline sputum eosinophil percentage correlated significantly with the baseline blood eosinophil percentage ($r_s = 0.49$, $p = 0.03$ [$n = 23$]), and the sputum ECP correlated significantly with the baseline blood ECP level ($r_s = 0.47$, $p = 0.03$ [$n = 24$]). There was also a significant positive correlation between baseline

sputum eosinophil percentage and sputum ECP levels ($r_s = 0.73$, $p = 0.0009$ [$n = 23$]). In addition, the changes in sputum eosinophil percentage and ECP levels correlated significantly with the treatment-induced (prednisone or placebo) changes in blood eosinophils and ECP levels ($r_s = 0.71$, $p = 0.02$; $r_s = 0.47$, $p = 0.03$, respectively). The $PC_{20}FEV_1$ [methacholine] in the 24 subjects correlated significantly with the baseline sputum eosinophil percentage ($r_s = -0.65$, $p = 0.003$) but not with sputum ECP ($r_s = -0.31$, $p = 0.14$), blood eosinophils ($r_s = -0.05$, $p = 0.81$), or blood ECP ($r_s = -0.15$, $p = 0.5$).

Peak expiratory flow increased significantly more in subjects treated with prednisone than in those treated with placebo, although changes in FEV_1 ; forced expiratory flow, mid-expiratory phase; and forced vital capacity were not significantly different between the two groups (Table IV). This improvement in peak flow correlated with the decrease in the percentage of eosinophils in induced sputum from the prednisone-treated group ($r_s = -0.64$, $p = 0.04$).

TABLE IV. Pulmonary function measurements in prednisone- and placebo-treated groups before and after treatment

	Prednisone-treated group		Placebo-treated group	
	Before	After	Before	After
FEV ₁ (L)	3.28 ± 0.29	3.38 ± 0.27	2.70 ± 0.31	2.63 ± 0.33
% Predicted	88 ± 5.2	91 ± 4.87	74 ± 6.3	72 ± 7.0
FVC (L)	4.54 ± 0.28	4.62 ± 0.28	4.11 ± 0.38	4.05 ± 0.38
% Predicted	101 ± 3.6	102 ± 3.7	92 ± 5.0	91 ± 4.7
FEF ₂₅₋₇₅ (L/sec)	2.57 ± 0.40	2.60 ± 0.32	1.78 ± 0.30	1.76 ± 0.34
% Predicted	61 ± 7.6	63 ± 6.1	43 ± 6.7	43 ± 7.4
PEF (L/sec)	7.37 ± 0.58	8.29 ± 0.63*	6.42 ± 0.83	6.39 ± 0.82
% Predicted	77 ± 4.0	87 ± 4.5	65 ± 7.0	65 ± 6.6

*Increase significantly greater than baseline ($p = 0.003$) and compared with the placebo-treated group ($p = 0.01$).

DISCUSSION

In this study we demonstrate that analysis of induced sputum from asthmatic subjects is a feasible means of evaluating the effects of antiinflammatory treatment on airway eosinophilic inflammation. Specifically, we found that brief treatment with orally administered prednisone is associated with a significant decrease in the total number and percentage of eosinophils and in the levels of ECP in induced sputum (Fig. 1).

The decrease in eosinophilic markers in induced sputum described here is generally in agreement with the reported findings for the effects of prednisone or inhaled glucocorticosteroid on markers of eosinophilic inflammation in spontaneously expectorated sputum,¹¹⁻¹³ bronchoalveolar lavage,^{2, 14, 15} and bronchial biopsy specimens.^{3, 16} These similarities in results do not render our study redundant but rather serve to validate sputum induction as a method for collecting airway secretions. The major implication of our findings is that analysis of induced sputum for markers of eosinophilic inflammation can be repeated over time to monitor the effects of antiinflammatory treatment. Because of the obvious advantages of sputum induction over reliance on sputum that is spontaneously expectorated and over reliance on bronchoscopy, we believe that analysis of induced sputum will become a useful noninvasive research tool for studying the effects of asthma treatments on airway eosinophilic inflammation.

The most likely reason for prednisone-associated decrease in eosinophil percentages and ECP levels in the induced sputum samples is that prednisone, by decreasing circulating eosinophil num-

bers and by inhibiting chemoattraction and trafficking of available eosinophils to the airway,^{6, 17-19} caused decreased airway eosinophil inflammation. The effects of glucocorticoids on eosinophil number and activity are confirmed in this study by the significant reduction in blood eosinophil percentages and in serum ECP levels in the prednisone-treated group, reductions that were significantly correlated with the reductions seen in sputum eosinophil percentages and ECP levels.

The lack of a significant decrease in albumin and MLG levels in the prednisone-treated group is surprising but is perhaps explained by the short length of time that prednisone was administered. Further studies with longer treatment periods must be carried out before it can be concluded that steroids do not decrease these markers of inflammation in airway secretions.

Most of the subjects included in this study had mild asthma as reflected by their nearly normal pulmonary function tests at baseline (Table I). Unfortunately, the subjects with the worst pulmonary function were by chance randomized to the placebo group (Table IV). It is not surprising therefore that the improvements in pulmonary function seen in the prednisone-treated group were modest and only reached statistical significance for the peak flows generated during the flow volume loop maneuver. We nevertheless found a significant correlation between the decline in the percentage of eosinophils and the improvement in peak flow ($r_s = 0.64$, $p = 0.04$). We also found that the bronchial responsiveness to methacholine in these subjects was significantly correlated with the baseline eosinophil percentages in their sputum but not in their blood. These

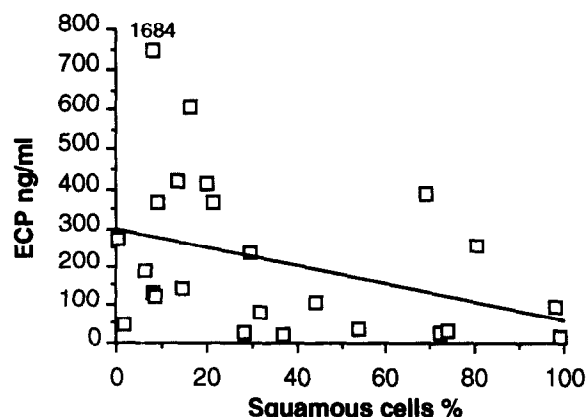


FIG. 2. Correlation between ECP levels and percentage of squamous cells in induced sputum from asthmatic subjects at baseline ($n = 24$). The inverse correlation was significant ($r_s = 0.55$, $p = 0.008$).

findings support the hypothesis that eosinophils and their inflammatory products directly contribute to airway hyperreactivity and airflow obstruction in asthma.

Our approach has been to collect all secretions expectorated during the sputum induction procedure and analyze them without trying to set minimum standards for the adequacy or acceptability of an induced sputum sample. Although this approach is different from that of others who prefer to extract mucus plugs from expectorated sputum in an effort to ensure the subglottic origin of the analyzed sample,⁴ we have found that analyzing the whole sample is quicker and allows analysis of cell count and differential and of the fluid phase of the sample for inflammatory mediators. We have previously reported that the presence of saliva in induced sputum does not invalidate this approach.^{5, 6} In this study we were unable to perform differential cell counts on nonsquamous cells from two subjects (one in each group) because there were too few nonsquamous cells on the cytospin-prepared sputum slides. The presence of a high percentage of squamous cells in induced sputum samples suggests that a large portion of the sputum is comprised of saliva. We confirmed this by finding a significant inverse correlation between percentage of squamous cells and ECP levels in induced sputum from the induced sputum samples collected at baseline from the 24 subjects (Fig. 2). It is also apparent from Fig. 2 that the percentage of squamous cells is not the only determinant of ECP levels in induced sputum. For example, some samples with a low percentage of squamous cells had relatively

low ECP levels, and some sputum samples with high percentages of squamous cells had relatively high ECP levels (Fig. 2). Thus we elected to include data on protein measurements from the fluid phase of the two subjects in whom we could not determine the cell differential of nonsquamous cells. For the present, we believe it best to accept all induced sputum samples as adequate rather than set arbitrary standards for adequacy. We acknowledge that the significant association between the percent of squamous cells and the level of ECP in induced sputum means that between- or within-group comparisons of inflammatory markers in induced sputum require that mean squamous cell percentages in induced sputum samples, collected from groups before and after an intervention or between separate groups, be similar (as they were in this study).

In summary, we demonstrate that treatment of asthmatic subjects with orally administered prednisone significantly decreases the percentage of eosinophils and the levels of ECP in induced sputum, and we suggest that analysis of induced sputum is a useful noninvasive method for studying the effects of asthma therapy on airway eosinophilic inflammation.

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