

Analysis of cellular and biochemical constituents of induced sputum after allergen challenge: A method for studying allergic airway inflammation

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To determine whether analysis of the constituents of induced sputum permits detection of changes provoked by aerosolized antigen challenge, we performed sputum induction (20-minute inhalation of aerosolized 3% saline solution) before and after aerosolized allergen challenge in eight subjects with asthma. Total cell counts and cell differentials of nonsquamous cells in induced sputum samples were determined after the samples were homogenized in dithiothreitol. Centrifugation of the entire homogenized sputum sample yielded supernatant that could be analyzed for biochemical constituents. We found that the median percentage of eosinophils and neutrophils in induced sputum samples was significantly higher 4 hours after allergen challenge than at baseline (12% vs 0.5%, $p < 0.05$; 30.5% vs 7.5%, $p < 0.05$) and remained high 24 hours after challenge. Median levels of eosinophil cationic protein and histamine in induced sputum supernatants were significantly higher 4 hours after challenge than at baseline (151.3 vs 39.8 ng/ml, $p < 0.05$; 19.4 vs 8.8 μg , $p < 0.05$) and remained significantly higher 24 hours after challenge. Tryptase was detectable in sputum from seven of the subjects, and in these subjects, we found a trend toward an increase in median tryptase levels 4 hours after allergen challenge (4.4 vs 2.2 U/L, $p = 0.09$). We conclude that analysis of induced sputum after aerosolized allergen challenge reveals changes in inflammatory cells and markers similar to those reported in bronchoalveolar lavage fluid and that sputum induction is a useful noninvasive method for studying allergic airway inflammation in asthma. (J ALLERGY CLIN IMMUNOL 1994;93:1031-9.)

Key words: Asthma, allergen, induced sputum, eosinophils, neutrophils, eosinophil cationic protein, histamine, tryptase, mucin-like glycoprotein

Bronchoscopy has been successfully and safely used to sample airway secretions before and after allergen challenge in subjects with allergic asthma and has revealed allergen-induced increases in eosinophils, neutrophils, lymphocytes, mast cell mediators, and eosinophil products in airway lavage samples.¹⁻⁹ In addition, evidence from animal

Abbreviations used

ECP: Eosinophil cationic protein
PBS: Phosphate buffered saline
Raw: Airway resistance
SRaw: Specific airway resistance

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studies and from in vitro studies in which human airway tissue was used suggests that allergen exposure stimulates increased secretion of mucus from the airways.¹⁰⁻¹²

Sampling airway secretions by means of bronchoscopy has a number of disadvantages. Bronchoscopy is an invasive procedure that many subjects with asthma are unwilling to undergo, and multiple bronchoscopic procedures in the same subjects cannot easily be performed over a short period. It is also a time-consuming and expensive procedure that requires highly trained personnel. In contrast, induction of sputum by inhalation of

TABLE I. Anthropometric and clinical data of study subjects

Subject	Sex	Age (yr)	Height (cm)	Weight (kg)	FEV ₁ (L)	FVC (L)	Medications
A	M	30	184	69	4.4	5.5	BA
B	M	28	174	72	3.9	5.4	BA
C	F	33	174	89	2.8	4.4	BA
D	M	27	181	71	4.5	6.6	BA
E	F	28	172	62	3.9	4.8	BA
F	M	29	174	72	3.9	5.8	BA
G	M	30	182	74	4.7	5.8	BA
H	F	24	162	66	3.4	4.5	BA + IS

FEV₁, Forced expiratory volume in 1 second; FVC, forced vital capacity; BA, β -agonist; IS, inhaled steroid (triamcinolone acetate 400 μ g/day in divided doses).

TABLE II. Bronchial challenge data

Subject	PC ₁₀₀ SRaw (M) mg/ml	Allergen delivered	PD ₁₀₀ SRaw (A)	% Fall FEV ₁ after allergen challenge*
A	0.25	MGP	1:3500	29
B	0.5	<i>D. pter</i>	1:180	14
C	0.03	<i>D. far</i>	1:6000	38
D	0.05	MGP	1:650	40
E	0.11	<i>Alternaria</i>	1:240	23
F	0.19	<i>D. far</i>	1:220	23
G	0.85	<i>D. pter</i>	1:350	33
H	0.05	MGP	1:32	20

PC₁₀₀ SRaw (M), Concentration of methacholine required to produce a 100% increase in specific airway resistance (Airway resistance \times Thoracic gas volume) (SRaw); PD₁₀₀ SRaw (A), dilution of allergen required to produce a 100% increase in SRaw; MGP, mixed grass pollen. *D. pter*, *Dermatophytes pteronissinus*; *D. far*, *Dermatophytes farinae*.

*The change from baseline (as a percentage) in the FEV₁ measured 10 minutes after delivery of the most concentrated allergen solution.

aerosolized hypertonic saline solution is a less invasive means of obtaining a sample of airway secretions. We have reported previously that cellular analysis of induced sputum from subjects with asthma reveals increased percentages of eosinophils,¹³ and Pin et al.^{14,15} have shown that the percentage of eosinophils in mucus plugs isolated from induced sputum is increased in subjects with asthma and increases further after aerosolized allergen challenge. We have extended the study of induced sputum to the analysis of some biochemical constituents.^{13,16} By analyzing supernatant obtained by centrifugation of the entire expectorate obtained during sputum induction, we have shown that levels of eosinophil cationic protein (ECP), albumin, fibrinogen, DNA, and a mucin-like glycoprotein are higher in induced sputum from subjects with asthma than from healthy subjects.^{13,16}

To date, our experience with sputum induction has encouraged us that sputum induction is a useful noninvasive method for obtaining samples of airway secretions. In this study we examined whether we could detect changes in the cellular

and biochemical constituents of induced sputum from subjects with asthma after an aerosolized allergen challenge. We reasoned that if allergen-induced changes in induced sputum were similar to those reported in bronchoalveolar lavage fluid (increases in percentages of eosinophils,^{2,6} neutrophils,^{2,6,8} ECP,⁴ histamine,^{5,9} and tryptase⁹), then this study would further validate sputum induction as a useful means of investigating airway inflammation in airway diseases.

METHODS

Subjects

Five men and three women (mean age, 29 years; range, 24 to 33 years) with mild allergic asthma were studied (Table I). All subjects had positive skin prick test results to at least one of 11 common inhalant allergens, and all were hyperreactive to inhaled methacholine (Table II). One of the subjects smoked cigarettes occasionally, and none of the subjects had a history of a respiratory infection in the 6 weeks preceding the study. All subjects signed consent forms approved by the Committee on Human Research at the University of California, San Francisco.

TABLE III. Cell counts and cell differentials in induced sputum before and after allergen challenge

Variable	Baseline I median (range)	Baseline II median (range)	4 Hours after challenge median (range)	24 Hours after challenge median (range)
Induced sputum volume and % squamous cells in induced sputum				
Sputum volume (ml)	20 (6-45)	21 (8-33)	20 (5-37)	18 (11-34)
Median % squamous cells	55 (11-82)	57 (29-88)	47 (17-85)	49 (14-60)
Cell counts and differentials of nonsquamous cells in induced sputum				
Total cells ($\times 10^3/\text{ml}$)	426 (1-2362)	371 (4-1413)	389 (2-1097)	472 (199-3741)
Macrophages (%)	81 (28-97)	78 (16-99)	43 (10-94)	39 (20-86)
Epithelial cells (%)	5.5 (0-16)	1 (0-4)	1 (0-5)	1 (0-2)
Lymphocytes (%)	0 (0-2)	0 (0-2)	0 (0-3)	0 (0-3)
Eosinophils (%)	0.5 (0-34)	2.5 (0-29)	12 (5-52)*	21.5 (4-56)*
Neutrophils (%)	7.5 (0.5-35)	8 (1-83)	30.5 (1-70)*	24.5 (7-63)*

*Significantly greater than baseline I, $p < 0.05$.

Protocol

All subjects came to the laboratory on at least five occasions. The first visit, for characterization, included allergen skin prick tests, spirometry, and methacholine challenge. The second visit (at least 48 hours after the first visit) was for a baseline sputum induction (baseline I). The third visit (20 hours after visit 2) was for a second baseline sputum induction (baseline II). The fourth visit (1 week later) was for aerosolized allergen challenge, followed 4 hours later by a third sputum induction. The fifth visit (20 hours after the third sputum induction) was for a fourth sputum induction. The second baseline sputum induction was done to allow comparison of the effects of a previous sputum induction on another induction performed 20 hours later (the period separating the two sputum inductions after allergen challenge). After each sputum induction, a sample of saliva was obtained.

Allergen skin testing

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

Intradermal skin testing was done only for the allergen that elicited the largest response on skin prick testing. Serial 10-fold dilutions of allergen were applied intradermally, allowing for determination of the threshold concentration of allergen that elicited at least a 3 mm wheal response.

Bronchial challenge procedures

Methacholine challenge was performed by having subjects inhale 10 breaths of methacholine aerosol in concentrations ranging from 0.06 to 8 mg/ml. The aerosol was generated by a hand-held nebulizer (model 646; DeVilbiss Co., Somerset, Pa.), which contained a volume of 2 ml of phosphate-buffered saline (PBS) or methacholine and was equipped with a dose-metering device driven by compressed oxygen at 20 psi. This nebulizer delivers 11 μl of solution per breath. The nebulizer was also equipped with a critical orifice, 4 mm in diameter, at the inflow end to allow a maximum inspiratory flow of 500 ml/min. The subject was instructed to inhale the methacholine aerosol from relaxed end-tidal volume for 3 seconds and then to hold the breath for 3 seconds. Specific airway resistance (SRAW) was calculated for each subject 5 minutes after each dose of methacholine by measuring airway resistance (Raw) and thoracic gas volume in a constant-volume variable pressure whole-body plethysmograph (Warren E. Collins Inc., Braintree, Mass.) every 30 seconds for 2.5 minutes.¹⁷ The mean of the five Raw and thoracic gas volume measurements was then used to calculate the mean SRAW. Baseline measurements of SRAW were made before and after inhalation of PBS, and then inhalations of methacholine were continued until SRAW had increased by at least 100%. The concentration of methacholine that caused a 100% increase in SRAW (PC_{100} SRAW [M]) from the post-PBS value was calculated by linear interpolation between the last two points on the dose-response curve.

The allergen selected for inhalation allergen challenge was the allergen that elicited the largest wheal on skin prick testing, and challenges were performed with the same equipment as described for methacholine challenge. Initial concentration of allergen for inhalation was the threshold concentration that elicited a skin wheal response. Because threshold allergen concentration was often very dilute (usually $<10^{-8}$ mol/L), we selected

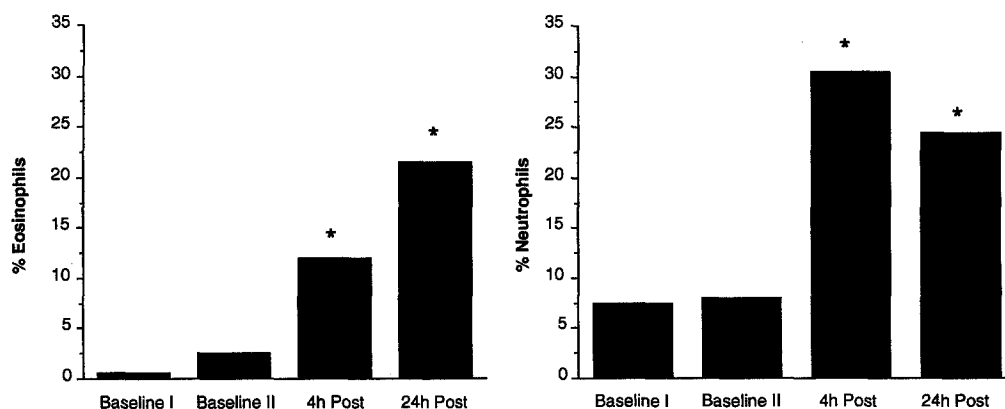


FIG. 1. *Left panel:* Median percentage of eosinophils in induced sputum before and after allergen challenge (asterisk, $p < 0.05$). *Right panel:* Median percentage of neutrophils in induced sputum before and after allergen challenge (asterisk, $p < 0.05$). The range of values is presented in Table III.

10^{-5} mol/L as the second concentration of allergen to be delivered to all subjects, followed by 10-fold serial dilutions. As with the methacholine challenge, subjects were instructed to inhale 10 breaths of each allergen aerosol concentration from relaxed end-tidal volume for 3 seconds and to hold the breath for 3 seconds each time. SRaw was measured 10 minutes after inhalation of each concentration, and serial challenge was continued until SRaw increased by at least 100%. This procedure allowed calculation of the dilution of allergen required to cause a 100% increase in SRaw (Table II). In addition, we performed spirometry (using a rolling seal Ohio 840 spirometer [Ohio Medical Products, Houston, Texas], according to American Thoracic Society standards¹⁸) at the beginning and end of the allergen challenge (but not between individual doses). The percentage decrease from baseline in forced expiratory volume in 1 second 10 minutes after inhalation of the highest concentration of allergen is presented in Table II.

Sputum induction

Immediately before the sputum induction procedure was begun, all subjects were pretreated with 180 μ g albuterol, administered by means of a metered dose inhaler. Subjects then 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 of a mean mass median diameter of 4.5 μ m and has an output of 2.4 ml/min. Subjects were encouraged to cough throughout the procedure, and they regularly interrupted inhalation of hypertonic saline solution to expectorate sputum into a clean plastic container.

Sputum and saliva processing

The volumes of the induced sputum sample and the samples of saliva were determined, and an equal volume of dithiothreitol 10% (Sputolysin; Behring Diag-

nostics Inc., Somerville, N.J.) was added. The samples were then mixed gently by vortex mixer and placed in a shaking water bath at 37° C for 15 minutes to ensure complete homogenization. The samples were removed from the water bath periodically for further brief gentle vortex mixing. Ten microliters of the homogenized sputum and saliva sample was used to determine the total cell count of the samples with the use of a standard hemocytometer. The remainder of the homogenized sputum and saliva was centrifuged at 2000 rpm for 5 minutes. The supernatants were aspirated and frozen at -70° C for later analysis. The cell pellets were resuspended in saline solution, spun in a cytocentrifuge (model 7 cyto spin; Shandon Scientific, Sewickley, Pa.), and stained (Diff-Quik stain; Baxter Scientific Products, Miami, Fla.). At least 200 nonsquamous cells on each sputum slide (in some cases this meant counting up to 1200 cells) and at least 400 cells on each saliva slide were read by an investigator blinded as to the time point of collection of the samples.

Biochemical assays

ECP, tryptase, and albumin concentrations in induced sputum and saliva supernatant samples were determined by means of sensitive radioimmunoassays (Pharmacia Diagnostics Inc., Fairfield, N.J.). We calculated albumin values in our samples on the basis of radioimmunoassay results obtained for serial dilutions of human albumin (Sigma, St. Louis, Mo.) in saline solution. Histamine concentrations were also measured by means of radioimmunoassay (AMAC, Westbrook, Minn.).

Fibrinogen, lactoferrin, and mucin-like glycoprotein levels were determined by means of ELISA, as previously described.^{12, 15}

Statistics

Most of the data were not normally distributed and therefore are presented as the median and range of the

TABLE IV. Biochemical analysis of induced sputum before and after allergen challenge

Variable	Baseline I median (range)	Baseline II median (range)	4 Hours after challenge median (range)	24 Hours after challenge median (range)
Sputum histamine	8.8 (1-19)	9.5 (2-44)	19.4 (3-118)*	19.7 (5-65)*
Saliva Histamine	12.6 (2-60)	12 (1-38)	15.3 (5-202)	9.9 (6-177)
Sputum tryptase (U/L)	1.1 (0-59)	0.9 (0-45)	3.9 (0-112)†	3.5 (0-42)
Saliva tryptase (U/L)	ND	ND	ND	ND
Sputum ECP (ng/ml)	39.8 (18-432)	58.2 (17-262)	151.3 (59-2022)*	186.7 (51-853)*
Saliva ECP (ng/ml)	16.3 (0-40)	5.2 (1-14)	12.9 (2-25)	7.2 (2-20)
Sputum MLG (mg/ml)	1.6 (0.4-4.8)	1.6 (0.6-4.0)	1.9 (0.1-4.3)	1.6 (0.3-4.2)
Saliva MLG (ng/ml)	0.03 (0.006-0.2)	0.01 (0.003-0.06)	0.009 (0.002-0.2)	0.002 (0.003-0.4)
Sputum lactoferrin (μg/ml)	49.5 (8-146)	41.5 (18-107)	43 (5-102)	40.5 (8-71)
Saliva lactoferrin (μg/ml)	1.1 (0.6-6)	2.2 (0.5-11)	4.4 (0.5-13)	3.6 (1-7)
Sputum albumin (μg/ml)	180.4 (92-887)	192 (88-554)	207 (44-661)	274.4 (67-814)
Saliva albumin (μg/ml)	73.4 (21-340)	55.4 (18-94)	56.1 (37-86)	65 (20-109)
Sputum fibrinogen (μg/ml)	49.5 (7-162)	62 (17-90)	31 (8-49)	29 (6-174)
Saliva fibrinogen (μg/ml)	2.2 (0-6)	2 (0-5)	1 (0-4)	2 (0-5) +

ND, Not detected; MLG, mucin-like glycoprotein.

*Significantly greater than baseline I, $p < 0.05$.

†Increase from baseline I not statistically significant, $p = 0.09$.

values. Data from analysis of the baseline II induced sputum samples and the two induced sputum samples collected from each subject after allergen challenge were compared with data from the baseline I induced sputum sample, with Wilcoxon's signed rank test. A probability value of less than 0.05, with a two-tailed test, was considered significant.

RESULTS

Volume, cell counts, and cell differentials of sputum and saliva

We found no significant difference in the median volume of sputum produced during the standardized 20-minute induction procedure and no difference in the median percentage of squamous cells in induced sputum samples from the eight subjects on the 4 study days (Table III). Because we believe that squamous cells in the induced sputum samples represented salivary contamination, we determined total cell counts and cell differentials of the nonsquamous cells in the sputum samples. We did not find significant differences in total cell counts and cell differentials of baseline II sputum samples compared with baseline I sputum samples (Fig. 1, Table III).

We found that the median eosinophil and neutrophil percentages in induced sputum were significantly higher at 4 and 24 hours after allergen challenge than at baseline (Fig. 1). There was no significant increase in any of the other cell differentials after allergen challenge (Table III). Squamous cells accounted for greater than 99.5%

of cells in saliva from all subjects on all study days.

Protein and mediator levels in sputum and saliva supernatants

We did not find significant differences in protein and mediator levels between baseline II sputum and baseline I sputum samples (Table IV, Fig. 2), but we did find significant differences when protein and mediator levels in the sputum samples produced after allergen challenge were compared with the baseline samples. Thus we found that the median ECP and histamine levels in induced sputum were significantly higher at 4 and 24 hours after allergen challenge than at baseline (Table IV, Fig. 2). We also found that tryptase levels were detectable in sputum from seven of the eight subjects and that in these seven subjects there was a trend toward a significant increase in median tryptase levels in the samples collected 4 hours after allergen challenge compared with baseline (Table IV). We did not find significant changes in the levels of albumin, fibrinogen, mucin-like glycoprotein, and lactoferrin in induced sputum after allergen challenge (Table IV).

We found no significant changes in protein and mediator levels in saliva samples collected before and after allergen challenge (Table IV, Fig. 2). We were surprised to find that median baseline histamine levels in saliva were higher than median histamine levels in the paired baseline sputum

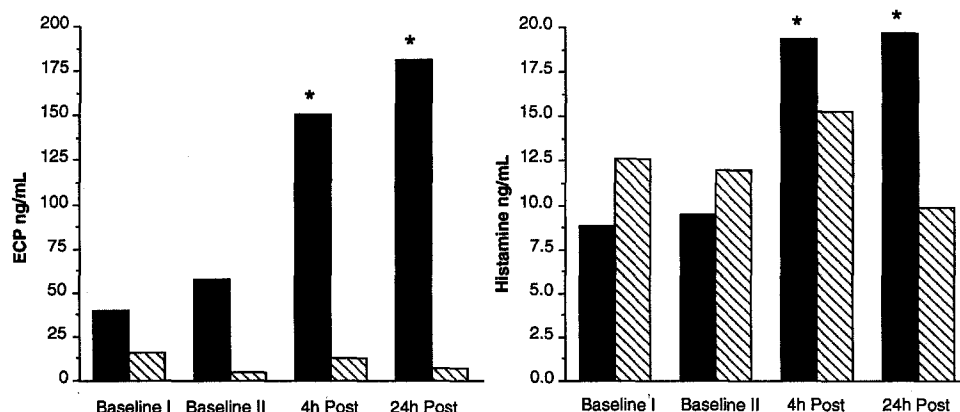


FIG. 2. *Left panel:* Median eosinophil cationic protein (ECP) levels in induced sputum (black bars) and saliva (hatched bars) before and after allergen challenge (asterisk, $p < 0.05$). *Right panel:* Median histamine levels in induced sputum (black bars) and saliva (hatched bars) before and after allergen challenge (asterisk, $p < 0.05$). The range of values is presented in Table IV.

samples I and II (Table IV, Fig. 2). However, because we found that the median salivary histamine levels did not increase significantly from baseline after allergen challenge, we do not think that the significant postchallenge increase in induced sputum histamine can be accounted for by increases in salivary histamine.

DISCUSSION

In this study we found that induced sputum collected 4 and 24 hours after allergen challenge has significantly higher percentages of eosinophils and neutrophils and higher levels of ECP and histamine than induced sputum collected at baseline. These changes are similar to those reported for allergen-induced changes in bronchoalveolar lavage,¹⁻⁹ and we propose that analysis of the cellular and biochemical constituents of induced sputum is a useful, noninvasive means of studying allergen-induced airway inflammation in asthma.

Pin et al.¹⁴ have reported previously that percentages of eosinophils and metachromatic cells in induced sputum from subjects with asthma increase after allergen challenge. However, our approach to the analysis of induced sputum differs significantly from that of Pin et al.^{14,15} They analyzed cells in mucus plugs selected from induced sputum samples, whereas we analyzed cells in the entire induced sputum sample and also measured biochemical constituents in the fluid phase. This approach to the analysis of cells and chemicals in induced sputum requires explanation. Induced sputum is a mixture of secretions derived from the mouth (saliva) and the subglottic airways. We do not try to separate saliva from

subglottic secretions but analyze the entire induced sputum sample and, for cellular analysis, report the cell differential of the nonsquamous cells. We believe that the cell differential of the nonsquamous cells represents the cell differential of the portion of the induced sputum derived from the subglottic airways. This belief rests on the assumption that nearly all cells contributed by the salivary component of the induced sputum sample are squamous cells—an assumption supported by our findings for cellular analysis of saliva in this study and in a previous study,¹³ in which we showed that more than 99% of cells in saliva were squamous cells. We acknowledge that we ignore the possibility that some of the cells from the subglottic contribution to the induced sputum sample may be squamous cells. Although squamous metaplasia has been reported in the epithelium of asthmatic airways, we think any resulting error is unlikely to be important, for we are unaware of studies reporting more than 1% squamous cells in airway lavage samples from subjects with asthma. Thus we report the cell counts and cell differential for the nonsquamous cells in the induced sputum samples, and we submit that these are the cell counts and differentials for the airway secretions in the induced sputum derived from the subglottic airways. We acknowledge that nonsquamous cell counts in induced sputum are diluted to an extent that depends on the amount of saliva in the sputum and that these counts are thus semiquantitative at best. Similar problems with dilution confound the determination of accurate cell counts in bronchoalveolar lavage fluid.

Our analysis of nonsquamous cells in induced sputum before and after aerosolized allergen challenge revealed significant increases from baseline in the percentages of eosinophils and neutrophils at both 4 and 24 hours after challenge. These findings agree with previously published studies of the cellular composition of bronchoalveolar lavage samples collected after aerosolized allergen challenge of subjects with asthma,^{2, 4, 6, 8, 15} although the increase in eosinophils at 4 hours is earlier than has been reported in other studies. In contrast, we found that the percentages of eosinophils and neutrophils in saliva did not change at 4 and 24 hours after allergen challenge. Thus the increase in the percentages of eosinophils and neutrophils in induced sputum must be the result of increases that occurred in the portion of the induced sputum derived from the subglottic airways. We believe that these findings validate using measurement of cell differentials of nonsquamous cells in entire induced sputum samples as a noninvasive, semi-quantitative approach to analyzing changes in cell populations in airway secretions.

Our approach to the analysis and interpretation of chemicals in the fluid phase of induced sputum is illustrated by our findings for ECP levels in induced sputum before and after allergen challenge. We found that ECP levels in induced sputum increased significantly 4 hours after allergen challenge and remained significantly elevated 24 hours after challenge, a finding that is in agreement with previously published observations about allergen-induced changes in ECP levels in bronchoalveolar lavage from subjects with asthma.⁴ In contrast, we found that ECP levels in saliva did not change significantly from baseline at 4 and 24 hours after challenge. We interpret this difference in findings for ECP levels in saliva compared with induced sputum as evidence that the increase in ECP in induced sputum is the result of an increase in ECP in the portion of induced sputum derived from the subglottic airways. Thus for chemical analysis of the fluid phase of induced sputum, we accept that saliva, as a constituent of induced sputum, will in most instances contribute partially to the levels of chemicals measured in induced sputum and will in all cases dilute the levels of chemicals measured. As for cellular analysis, for chemical analysis we do not try to separate saliva from the remainder of the induced sputum. We have found previously,^{13, 16} and again here, that the salivary levels of ECP, albumin, fibrinogen, and mucin-like gly-

coprotein are relatively low, and therefore that the contribution of chemicals in saliva to overall levels in induced sputum is likely to be relatively minor. Most important, as we show here, any dilutional effect of saliva does not preclude detection of antigen-induced changes in ECP levels in induced sputum or, as we have shown previously,^{13, 16} detection of significantly higher levels of ECP, albumin, fibrinogen, mucin-like glycoprotein, or DNA in induced sputum from subjects with asthma compared with healthy subjects. We believe that the data presented here indicate that analysis of ECP levels in induced sputum before and after allergen challenge is a valid method for studying allergen-induced changes in eosinophilic inflammation in airway secretions from subjects with asthma. ECP is a cytotoxic protein derived from eosinophils, but our methods do not allow us to infer whether the higher ECP levels in induced sputum collected after allergen challenge reflect increased eosinophil numbers or increased eosinophil activation or degranulation.

Histamine levels in the induced sputum samples were higher at 4 and 24 hours after antigen challenge than in the baseline samples, and there was a trend toward a significant increase in tryptase levels 4 hours after challenge. Analysis of bronchoalveolar lavage fluid after allergen challenge has also shown increases in histamine and tryptase.⁹ Surprisingly, in our study we found that the levels of histamine in saliva were sometimes higher than in the paired sputum samples (Fig. 2). In a previous study we found no significant correlation between histamine and tryptase levels in saliva and concluded that histamine detected in saliva is unlikely to be derived from mast cells.¹³ One possibility is that the histamine found in saliva results from bacterial synthesis,¹⁹ rather than from mast cell or basophil activation and secretion. Although histamine levels in saliva are high relative to levels in induced sputum, the interpretation of our findings for histamine in induced sputum before and after allergen challenge is similar to our interpretation of other chemicals, such as ECP, in which salivary levels are relatively low. Because histamine levels in saliva did not increase after allergen challenge, whereas histamine levels in induced sputum did, we again conclude that the increase from baseline in histamine levels in induced sputum after allergen challenge must be the result of increases in the portion of the induced sputum derived from the subglottic airways. Furthermore, when taken with the trend toward an increase in median

tryptase levels 4 hours after allergen challenge, we infer that the histamine detected in induced sputum after allergen challenge was probably derived from activated mast cells.

Albumin and fibrinogen levels, plasma-derived proteins that serve as markers of change in vascular permeability, were not significantly different after allergen challenge than at baseline. Measurements of albumin in bronchoalveolar lavage before and after allergen challenge have yielded conflicting results. Some studies show allergen-induced increases in albumin,¹ and others show no significant change.^{2, 20, 21} Salomonsson et al.²¹ also showed that fibrinogen levels increased in bronchial lavage samples immediately after allergen challenge. We did not find differences in fibrinogen or in albumin levels in induced sputum sampled after allergen challenge, perhaps because of differences between our study and those of others in the timing of airway secretion sampling after allergen challenge or because of the possibility that hypertonic saline solution increases bronchovascular permeability (as has been reported in rodent and canine models of airway vascular permeability^{22, 23}). If hypertonic saline solution increases bronchovascular permeability in subjects with asthma, then elevated albumin and fibrinogen levels in baseline induced sputum samples would obscure further allergen-induced increases.

We have shown previously that markers of mucus secretion (mucin-like glycoprotein and lactoferrin) are higher in induced sputum from subjects with asthma than from healthy subjects.¹⁶ We are unaware of any studies of mucus secretion in bronchoalveolar lavage fluid after allergen challenge, but because mucin glycoprotein and lysozyme airway secretion increases in animal models of allergen exposure,⁸⁻¹⁰ and because in vitro experiments on sensitized human bronchi also document glycoprotein secretion induced by allergen exposure,¹¹ we compared mucin-like glycoprotein and lactoferrin levels in induced sputum samples collected before and after allergen challenge and found no difference. Further investigation of the important area of mucus hypersecretion in allergic airway inflammation is warranted.

In summary, we found that aerosolized allergen challenge in subjects with asthma results in increases in eosinophils, ECP, and histamine in induced sputum 4 and 24 hours after challenge. These changes are in close agreement with previously described allergen-induced changes in airway bronchoalveolar lavage fluid. We conclude

that analysis of the cellular and biochemical constituents of induced sputum is a useful means of studying acute changes in allergic inflammation in asthma.

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Possible dual role of anti-idiotypic antibodies in combined passive and active immunotherapy in honeybee sting allergy

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Background: Passive infusion of beekeepers' plasma was shown to protect patients against systemic reactions occurring during active immunotherapy by mechanisms still to be clarified. It is tempting to speculate that anti-idiotypic antibodies could play a role because they are found in beekeepers' plasma and are involved in the regulation of IgE synthesis.

Methods: In this report we studied the effects of passive infusion of a beekeeper's plasma rich in anti-idiotypic antibodies to a patient who experienced systemic reactions to honeybee venom.

Results: We reported, during the days after the infusion, a decrease of clinical sensitivity to the honeybee venom. Indeed, the patient tolerated a cumulative dose of 280 µg of venom without adverse reactions. We also observed decreases in skin mast cell and in basophil sensitivity. After the plasma infusion, a modified rush immunotherapy with honeybee venom was initiated in our patient. In the following 76 weeks, increased levels of anti-idiotypic antibodies in the serum of the patient were associated with a diminution of specific antibodies (IgG and IgE) to honeybee venom.

Conclusion: These results suggest a dual role of anti-id in our combined protocol of passive and active immunotherapy: an immediate action on clinical sensitivity along with a decrease of skin mast cell and basophil sensitivity and an immunoregulatory role on specific antibody production. (*J ALLERGY CLIN IMMUNOL* 1994;93:1039-46.)

Key words: Honeybee, venom, immunotherapy, anti-idiotypic, immunoglobulins

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Allergic reactions to Hymenoptera stings range from large local skin lesions (urticaria, pruritus, and angioedema) to systemic reactions involving more than one system (skin, upper and lower respiratory tracts, gastrointestinal tract), which can lead to circulatory collapse (hypotension and shock). Systemic reactions occur in 0.4% to 4% of the population¹⁻³ and result in at least 40 deaths per year in the United States.⁴ In the last two decades, immunotherapy with venom has become available, and it has been shown that immunotherapy at a dose of 100 µg of venom per month