

Allergen challenge causes inflammation but not goblet cell degranulation in asthmatic subjects

Steven R. Hays, MD,^{a,b} Prescott G. Woodruff, MD, MPH,^{a,b} Ramin Khashayar, MD,^{a,b}
Ronald E. Ferrando, MS,^b Jane Liu, MS,^b Patricia Fung, BA,^a Chang Qing Zhao, MD, PhD,^a
Hofer H. Wong, BS,^b and John V. Fahy, MD^a San Francisco, Calif

Background: An allergen challenge to the airways of sensitized mice causes eosinophilic airway inflammation and degranulation of goblet cells, which lead to airway obstruction. However, whether allergen challenge causes a similar pattern of airway inflammation and goblet cell degranulation in human beings is unknown.

Objective: The purpose of this study was to determine whether allergen challenge increases airway inflammatory cells and causes goblet cell degranulation in human subjects with asthma.

Methods: In bronchial biopsy specimens taken from 8 asthmatic subjects at 1 and 24 hours after allergen challenge, we measured eosinophil and neutrophil numbers as indicators of inflammation. We also measured goblet cell mucin stores and the amounts of secreted mucin in bronchial lavage as indicators of goblet cell degranulation.

Results: Airway eosinophil numbers at both 1 and 24 hours after allergen challenge were twice as high as those after diluent challenge. Changes in neutrophil numbers were smaller and statistically insignificant. Goblet cell mucin stores measured in tissue stained with alcian blue/periodic acid-Schiff did not decrease significantly from baseline to 1 hour and actually tended to increase at 24 hours. This increase was significant in the subgroup of subjects with normal stored mucin levels at baseline. Mucin-like glycoprotein concentrations in bronchial lavage did not change significantly at either time point.

Conclusion: Although allergen challenge in asthmatic subjects increases airway eosinophil numbers as early as 1 hour after challenge, this inflammatory response does not cause goblet cell degranulation. In fact, in subjects with normal baseline mucin stores, allergen challenge increases goblet cell mucin stores. (*J Allergy Clin Immunol* 2001;108:784-90.)

Key words: Asthma, goblet cells, allergen, mucin, eosinophils, neutrophils

Not only is mucus hypersecretion a common symptom of asthma¹; it is independently associated with an accelerated rate of decline in FEV₁.² Furthermore, fatal asthma

Abbreviations used

CAST: Computer-assisted stereology toolbox

NO: Nitric oxide

PC₂₀: Provocative concentration of methacholine that causes a 20% decrease in FEV₁

ma is nearly always associated with mucus plugs occluding airways.³⁻⁶ Goblet cells, in their ability to degranulate and secrete mucins, are a significant source of mucus in the airway and therefore play an important role in the pathophysiology of asthma.

It is known that high-dose airway allergen challenge in mice causes massive goblet cell degranulation, leading to airway obstruction and asphyxial death.^{7,8} Potential mediators of goblet cell degranulation in airway diseases characterized by allergic airway inflammation include 5-lipoxygenase derivatives of arachidonic acid,⁷ neutrophil elastase,⁹ chymase,¹⁰ and eosinophil cationic protein.¹¹

To our knowledge, there have been no published studies exploring mechanisms of goblet cell degranulation in human asthma. We hypothesized that allergen challenge causes goblet cell degranulation in asthmatic subjects by increasing the number of inflammatory leukocytes and releasing mucin secretagogues. To test this hypothesis, we measured the numbers of eosinophils and neutrophils in bronchial biopsies and bronchial wash fluid as indicators of allergic airway inflammation from asthmatic subjects at baseline and at 1 and 24 hours after allergen challenge. We also measured stored mucin in goblet cells in bronchial biopsy specimens and secreted mucin in bronchial wash fluid as indicators of goblet cell degranulation at these same time points.

METHODS

Subjects

Eight asthmatic subjects were enrolled. Their clinical characteristics are described in Table I. Inclusion criteria were a history of asthma, FEV₁ as a percentage of predicted > 60%, and hyperresponsiveness to methacholine (PC₂₀ < 8 mg/mL). Exclusion criteria were use of inhaled or oral steroids or a history of a respiratory tract infection in the previous 6 weeks. An additional exclusion criterion was tobacco use within the past 10 years or >5 pack-years of total smoking history. All subjects signed a consent form approved by the Committee on Human Research at the University of California, San Francisco.

From ^athe Division of Pulmonary and Critical Care Medicine, Department of Medicine, and ^bthe Cardiovascular Research Institute, University of California, San Francisco.

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Reprint requests: John V. Fahy, MD, Box 0111, University of California, San Francisco, 505 Parnassus Avenue, San Francisco, CA 94143.

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Protocol

Each of the 8 subjects underwent bronchoscopy after an airway challenge on 3 different days, 6 weeks apart. One bronchoscopy was done either 1 hour (4 subjects) or 24 hours (the other 4 subjects) after allergen diluent (control) challenge; the second bronchoscopy was done 1 hour after allergen challenge (all 8 subjects); the third bronchoscopy was done 24 hours after allergen challenge (all 8 subjects). During visit 1, a history and physical examination, spirometry, allergen skin prick testing, and methacholine challenge were used to characterize the subject. During visit 2, the subject underwent allergen titration skin prick testing and then underwent bronchoscopy at either 1 or 24 hours after the allergen diluent (control) challenge. During visits 3 and 4, the subject underwent bronchoscopy 1 and 24 hours after allergen challenge. During visits 2, 3, and 4, the subject underwent spirometry and nitric oxide (NO) measurement before and at 13 time points between 20 minutes and 24 hours after the diluent and allergen challenges. Spirometry and NO were measured to assess the bronchial response to the airway challenge. Goblet cell volume and mucin-like glycoproteins were measured in bronchial biopsies and bronchial wash fluid to assess mucin stores and mucin secretion, respectively. Eosinophils and neutrophils were measured in bronchial biopsies and wash fluid to assess the degree of cellular inflammation in response to airway challenge.

Spirometry and methacholine bronchial challenge test

Bronchodilators were withheld for 8 hours before testing. Spirometry and methacholine challenges were performed through use of methods previously described.¹²

Allergen skin prick tests

Skin prick testing for allergens was performed through use of standardized disposable allergy prick test needles (Morrow Brown, Topeka, Kan). Four common inhalant allergens—*Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, cat pelt, and ryegrass—were used, as were a diluent control and a histamine control. (All of these were obtained from Greer Lab, Inc., Lenoir, NC.) 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.

Allergen challenge

In each subject, the allergen (*D. farinae*, *D. pteronyssinus*, cat, or ryegrass) that elicited the largest wheal on skin prick testing was selected for inhalation allergen challenge. The initial dose of allergen for inhalation was 4 serial dilutions below that predicted to cause a 20% decrease in FEV₁.¹³ The baseline FEV₁ was taken as the highest of 3 values measured 10 minutes after inhalation of allergen diluent. The subject then took 5 breaths (functional residual capacity to total lung capacity) of increasing serial concentrations of allergen at 12-minute intervals. FEV₁ was then measured twice, 10 minutes after each dose step; the higher value was recorded. If the postallergen FEV₁ was >85% of baseline, the next dose step was delivered; if it was 80% to 85% of baseline, then FEV₁ was measured 10 minutes later. If the FEV₁ continued to be 80% to 85% of baseline, the same allergen dose was repeated; however, if it was >85% of baseline, the next dose step was delivered. The challenge was stopped when dosing with allergen caused the FEV₁ to fall to <80% of baseline.

Allergen diluent challenge

The response to allergen diluent was determined through use of methods and equipment identical to those described for allergen challenge, except that 3 concentrations (1:1000, 1:100, and 1:10) of diluent (glycerin in saline solution) were administered by aerosol.

TABLE I. Baseline clinical characteristics of the 8 study subjects

Sex: male/female	1/7
Age (y)	31 ± 6
FEV ₁ (L)	2.8 ± 0.4
FEV ₁ (% predicted)	84 ± 17
PC ₂₀ (mg/mL)	0.39 (0.15 to 8.12)

Values are means ± SDs, except PC₂₀ value, which is reported in terms of geometric mean (range).

Bronchoscopy

Bronchoscopy was performed in accordance with guidelines for research bronchoscopy in asthmatic subjects¹⁴ through use of a specific method described previously.¹⁵ The bronchoscope was introduced orally and wedged in a subsegmental bronchus of the lingula. We performed a bronchial wash by instilling two 50-mL aliquots of sterile saline solution and suctioning back after each aliquot. When the bronchial wash was complete, the bronchoscope was passed to the other lung, where up to 8 endobronchial biopsies were obtained at the minor carina through use of a spiked fenestrated forceps (Pentax KH2411S and Pentax 8228, Pentax Precision Instrument Corporation, Orangeburg, NY).

Processing and analysis of the bronchial biopsy specimens

The biopsy specimens were transferred from the biopsy forceps directly into 10% formalin and incubated for 1 hour at 4°C. After 1 hour, the formalin was replaced with fresh formalin and the specimens were incubated at 4°C for an additional 3 hours. They were then dehydrated in graded alcohol solutions at room temperature, cleared with CitriSolv (Fisher Scientific, Pittsburgh, Pa), and transferred to melted paraffin at 55°C (Paraplast X, Fisher Scientific, Pittsburgh, Pa) for 30 minutes. The paraffin was replaced with fresh paraffin twice for 30 minutes each time and then embedded. Molds were hardened, removed from the mold, and stored at room temperature. Paraffin-embedded sections were cut at 2 µm and stored at room temperature.

To identify mucin in goblet cells in the tissue sections, 2-µm sections were deparaffinized and stained with alcian blue and periodic acid-Schiff reagent (Sigma, St Louis, Mo), as previously described.¹⁶ The volume of stored mucin per volume of epithelium and per surface area of basal lamina was then measured through use of methods of design-based stereology, as previously described.¹⁶ These mucin volumes were measured in 5 high-power fields from 6 bronchial biopsies (for a total of 30 fields). Fewer fields were analyzed in some instances because of epithelial desquamation in the biopsy specimens. We quantified epithelial desquamation in the biopsies at baseline and after allergen challenge using methods previously described.¹⁶

Eosinophils and neutrophils in the bronchial biopsy specimens were identified immunohistochemically through use of specific mAbs and were enumerated through use of methods of design-based stereology. Eosinophils were labeled with an antibody specific for eosinophil cationic protein (EG-2, Pharmacia Upjohn, Kalamazoo, Mich). Neutrophils were labeled with an antibody to neutrophil elastase (NP57, DAKO, Carpinteria, Calif) through use of the following immunohistochemistry protocol: First, the paraffin sections were deparaffinized and incubated in 3% hydrogen peroxide and absolute methanol for 10 minutes to block endogenous peroxidase activity. Next, the sections were rinsed in PBS and preblocked with 1% horse serum/PBS for 15 minutes at room temperature. The excess serum was then blotted off. The sections were then incubated in primary antibody diluted in horse serum (NP57 antibody was diluted at 1:100; EG2 antibody was diluted at 1:1000) overnight at 4°C. Sections were then rinsed in PBS, incu-

TABLE II. Change in FEV₁ from baseline after diluent and allergen challenges

Time after challenge	FEV ₁ (% change)			
	After diluent challenge		After allergen challenge	
	1 h (n = 4)	24 h (n = 4)	1 h (n = 8)	24 h (n = 8)
20 min	0.5 ± 2.4	4.7 ± 4.0	-40.4 ± 14.2	-41.9 ± 24.1
7 h	ND	0.4 ± 6.1	ND	-25.2 ± 18.5
24 h	ND	5.5 ± 8.8	ND	-4.4 ± 11.2

Values are means ± SDs.

ND, Not done.

bated in biotinylated secondary antibody (1:500 dilution, Vector Labs, Burlingame, Calif) for 1 hour at room temperature, rinsed in PBS, and incubated in ABC reagent (Vector Labs) for 1 hour at room temperature. Finally, the sections were rinsed in PBS and incubated in DAB Plus reagent (Zymed Labs, South San Francisco, Calif) for 10 minutes at room temperature.

To measure the numeric density of neutrophils and eosinophils in endobronchial tissue, we used the physical disector method.¹⁷ Cell numbers in bronchial biopsies are usually counted as the numbers of cell profiles seen in 2-dimensional tissue sections. The reference compartment is usually the length of basal lamina or a defined area of submucosa or epithelium. This method of counting is subject to bias, because the cell profile number in a 2-dimensional section is a function not only of the cell number but also of the cell size; larger cells are more likely to appear in the profile and therefore to be counted. The physical disector method avoids this bias by counting the cell number in a pair of serial sections that are a known distance apart (usually 30% of the diameter of the object of interest). Through use of this 3-dimensional counting system, 2 serial sections are inspected simultaneously and a cell is counted only if it is seen in one section (the "reference section") but not in the next (the "look-up" section). This method helps to avoid the problems of counting cells twice and preferentially counting larger cells.

We used the Computer Assisted Stereology Toolbox (CAST) grid system (Olympus Denmark, Albertslund, Denmark) to apply the physical disector to determine cell numbers in bronchial biopsy specimens. The CAST system consists of an integrated set of components, including a microscope (Olympus), video camera (JVC Digital color, JVC A/S, Tatstrup, Denmark), automated microscope stage, computer (IBM 300PL), and CAST Grid software. Three serial sections, each 2 µm thick, were cut. The first and third sections were paired and used as the reference and look-up sections. Thus the total thickness of our physical disector was 4 µm. The reference and look-up sections were then mounted side by side on the microscope stage. Microscopic images of the sections were captured at a final magnification of 20× and projected in real time to the computer monitor. Each reference biopsy section image was then outlined in its entirety through use of the CAST software's delineating function. By means of the CAST software's fix-points function, the reference and look-up sections were aligned so that they could be examined simultaneously. On average, 16 fields within each section were selected randomly, the computer-linked microscopy stage moving at a step length of 350 µm. Simultaneous examination of these sections on a split computer screen allowed unbiased counting of eosinophils and neutrophils. Through use of a point-counting grid generated by the CAST software and superimposed on each image, the points hitting epithelium and submucosa and the number of neutrophils and eosinophils were counted in each field. A toolbox on the computer screen facilitated the point counting and the automatic logging of data into spreadsheet files. In this way, the number

of eosinophils and neutrophils per volume of epithelium and submucosa (N_v) was determined through use of the following formula:

$$N_v = 1/((alf) \times h \times \Sigma Q/\Sigma P,$$

where alf is the area of the field, h is the distance between biopsies, ΣQ is the sum of the cell counts, and ΣP is the sum of points counted in the reference space. Surface area of the basal lamina, volume of epithelium, and volume of submucosa were also measured in the samples.

Analysis of bronchial wash

The bronchial wash sample was placed on ice in the bronchoscopy laboratory and transferred within 5 minutes to the processing laboratory. Total and differential cell counts and concentrations of mucinlike glycoprotein in the wash were measured as described previously.¹⁸

Measurement of exhaled NO

Exhaled NO was measured through use of a Sievers NO Analyzer (Sievers, Boulder, Colo). All measurements were performed with the subject seated and not wearing nose clips. The subject breathed in through his or her mouth to total lung capacity and then, without a breath hold, breathed out to residual volume at a constant pressure of 20 mmHg. The NO measurements were taken at the plateau. The measurement was repeated 2 times after a rest period of at least 30 seconds. The 3 measurements were averaged to yield the final value.

Statistics

All measurements were made on coded samples to obscure the identities of subjects. Data were entered into a computer spreadsheet (Microsoft Excel, Microsoft, Redmond, Wash) and exported to a statistics program (Stata, Stata Corporation, College Station, Tex) for descriptive and comparative statistics. The paired t test was used to compare the changes in mucin volume, mucin protein, neutrophils, and eosinophils. The command *nptrend* was used to test for differences in mucin volume in samples obtained after diluent challenge and at 2 different time points after allergen challenge. *Nptrend* provides an extension of the Wilcoxon rank-sum test; it was developed to handle any situation in which a variable is measured for individuals in 3 or more ordered groups.¹⁹ A P value of less than .05 was considered significant.

RESULTS

Changes in pulmonary function and NO after control and allergen challenges

Challenge with allergen diluent reduced FEV₁ by less than 6% from baseline at 20 minutes, 7 hours, and 24 hours (Table II). In contrast, allergen challenge reduced FEV₁ by 40% at 20 minutes, by 25% at 7 hours, and by

4% at 24 hours (Table II). Five of the 8 subjects had a late asthmatic response, as evidenced by a decrease from baseline in FEV₁ of at least 15% between 2 and 7 hours after allergen challenge (data not shown). Exhaled NO levels decreased below baseline at 1 hour and 7 hours after allergen challenge but significantly increased at 24 hours (Table III).

Changes in cellular inflammation after control and allergen challenges

The number of eosinophils per volume of endobronchial tissue more than doubled at 1 hour and remained elevated at 24 hours after allergen challenge (Fig 1). When determined separately in the epithelial and submucosal compartments of the biopsy tissue, the number of eosinophils was increased in each compartment (Fig 1). Unlike the increase in eosinophils, the number of neutrophils per volume of endobronchial tissue in the biopsies increased by only 20% to 25% at 1 hour and 24 hours; this increase was not significant (Fig 1). When determined separately in the epithelial and submucosal compartments of the biopsy tissue, the number of neutrophils was increased in the submucosal compartment but decreased in the epithelial cell compartment (Fig 1).

The allergen-induced changes in total and differential cell counts in bronchial lavage fluid mirrored the changes in the bronchial biopsies (Table IV). Specifically, the eosinophil percentage in the lavage increased from baseline by 2- to 3-fold at both 1 hour and 24 hours after allergen challenge. The neutrophil percentage increased from baseline by only 20% to 25% in these samples at 1 hour and 24 hours, and these changes were not statistically significant.

Epithelial integrity after control and allergen challenges

The percentage of basement membrane covered by no epithelial cells (completely denuded epithelium) or by a single layer of basal cells (partially denuded epithelium) did not change significantly from baseline at either the 1- or the 24-hour time point after allergen challenge (Table V).

Changes in goblet cell mucin stores after control and allergen challenges

The volume of stored mucin in goblet cells did not decrease from baseline at either 1 hour or 24 hours after allergen challenge (Figs 2 and 3). In fact, there was a small but insignificant increase in the volume of stored mucin in the biopsies analyzed at 24 hours. To determine whether the volume of stored mucin at baseline affected the response to allergen challenge, we examined the effect of allergen challenge on goblet cell mucin stores in subjects with normal and elevated baseline mucin stores. In previous studies, we found that the volume of stored mucin per volume of epithelial cells in healthy subjects is usually <10%.⁹ Accordingly, we divided the 8 subjects into 2 groups: those whose stored mucin baseline levels were greater than 10% and those whose baseline levels were less than 10%. Four subjects had baseline levels in

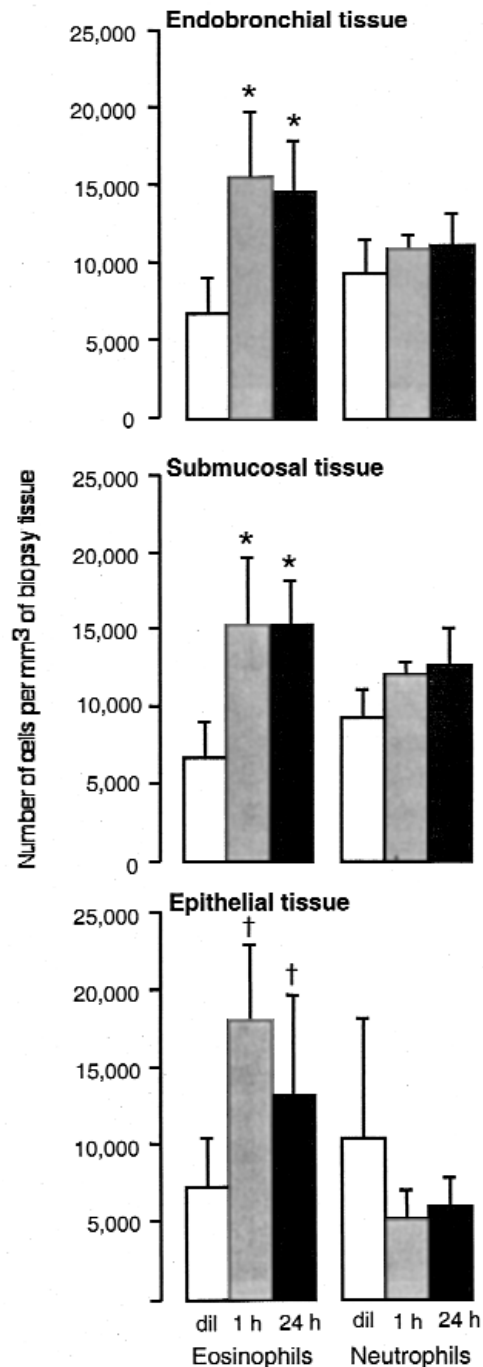


FIG 1. Eosinophil and neutrophil numbers in endobronchial biopsy tissue and in the submucosal and epithelial compartments after diluent and allergen challenges. *dil*, After diluent challenge. * $P < .05$ and † $P < .1$ vs diluent challenge.

the normal range, and these subjects had a 2-fold increase in stored mucin 24 hours after allergen challenge ($P < .04$ by trend test; Fig 3). In contrast, the 4 subjects with high stored mucin levels at baseline had a slight but insignificant decrease in stored mucin at 24 hours (Fig 3).

The changes observed in mucin-like glycoprotein levels in bronchial lavage were consistent with the changes in

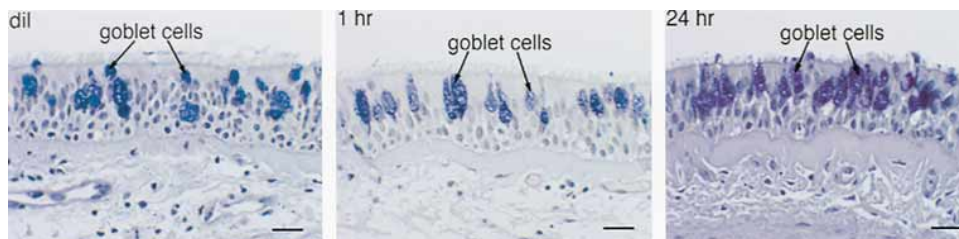


FIG 2. Photomicrograph of a bronchoscopic biopsy from an asthmatic subject shows increased mucin stain 24 hours after allergen challenge. The epithelium was stained with alcian blue/periodic acid-Schiff. *dil*, After diluent challenge. Bar = 20 μ m.

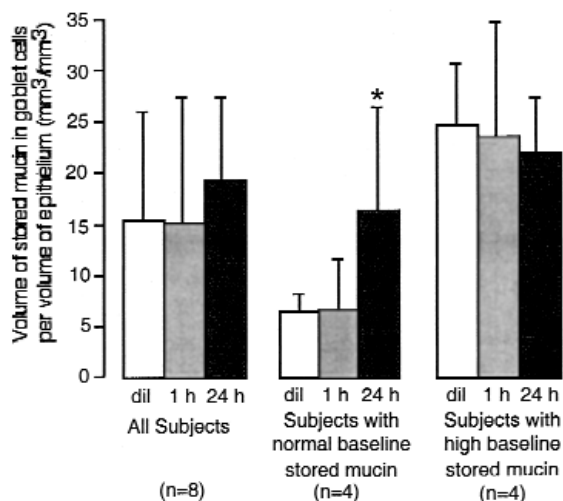


FIG 3. Volume of stored mucin in epithelium after diluent challenge and 1 hour and 24 hours after allergen challenge. *dil*, After diluent challenge. * $P < .05$ vs after diluent challenge by trend test.

mucin stores in the biopsy tissue. Specifically, there was no significant change in mucin-like glycoprotein concentrations in the bronchial wash at either 1 hour or 24 hours after allergen challenge (Table VI). When subgroups with normal and high baseline mucin stores were evaluated separately, there was also no significant change in mucin-like glycoprotein concentrations in the bronchial wash at either 1 or 24 hours after allergen challenge (data not shown).

DISCUSSION

The main finding of this study is that in asthmatic subjects, allergen challenge does not cause goblet cell degranulation, even in the presence of significant allergen-induced airway eosinophilia. In our 8 asthmatic subjects, though airway eosinophils increased after allergen challenge, goblet cell mucin stores were not decreased at 1 hour and were slightly increased at 24 hours. This increase occurred in a subgroup of subjects with normal baseline mucin stores and could represent either a hypertrophic or a hyperplastic response of goblet cells to allergen. We also found that the allergen-induced increase in

airway eosinophils was larger than the increase in neutrophils. This finding is in contrast to results in sensitized animals challenged with allergen (especially sheep, dogs, and guinea pigs),²⁰⁻²³ which have large increases in airway neutrophils after airway allergen exposure. Our data are consistent, however, with most studies in human beings; they show only minor changes in airway neutrophils after allergen challenge.²⁴

In contrast to our findings, studies in mouse and guinea pig models of asthma clearly show that allergen challenge causes goblet cell degranulation.^{7,8,23} However, in our study, despite an allergen challenge protocol that resulted in a mean 40% reduction in FEV₁ and a mean 2.5-fold increase in eosinophil numbers, we did not observe any reduction in goblet cell mucin stores in the airway epithelium or any increase in mucinlike glycoprotein in bronchial wash.

Although our findings are not consistent with animal studies, they are consistent with clinical studies of the effects of β -agonists after allergen challenge. These studies suggest that the principal mechanism of early- and late-phase bronchoconstriction to allergen is contraction of airway smooth muscle²⁵⁻²⁷ and not hypersecretion of mucins. Our study does not rule out the possibility that allergen exposure might cause goblet cell degranulation in other clinical circumstances, such as when an individual has a respiratory tract infection. In addition, our study does not rule out the possibility that allergen-induced goblet cell degranulation could have occurred in small airways that could not be sampled during bronchoscopy. However, the lack of increase in bronchoalveolar lavage mucin concentrations after allergen challenge argues against this possibility.

A surprising finding of our study was that eosinophil numbers increased significantly within 1 hour of allergen challenge in both biopsy tissue and lavage samples. Moreover, the increase at 1 hour was as large as that seen at 24 hours. The rapid accumulation of eosinophils in the airway after allergen challenge suggests that the mechanism of this accumulation of eosinophils does not require *de novo* synthesis and secretion of eosinophil-active cytokines, chemokines, or lipid mediators. Rather, accumulation must be a consequence of mechanisms involving preformed mediators that promote rapid transmigration of eosinophils from the vascular space to the airway mucosa when activated by allergen exposure.

TABLE III. Nitric oxide levels in exhaled air after diluent and allergen challenges

Time after challenge	Nitric oxide (ppm)			
	After diluent challenge		After allergen challenge	
	1 h (n = 4)	24 h (n = 4)	1 h (n = 8)	24 h (n = 8)
Baseline	79.2 ± 29.0	93.1 ± 98.1	65.6 ± 20.7	83.6 ± 27.5
1 h	80.4 ± 33.7	93.8 ± 95.7	ND	69.1 ± 28.1*
7 h	ND	86.9 ± 80.4	ND	75.1 ± 27.7*
24 h	ND	96.6 ± 92.3	ND	109.3 ± 18.6*

Values are means ± SDs.

ND, Not done.

**P* < .05 vs after diluent challenge.

TABLE IV. Total and differential cell count in bronchial wash after diluent and allergen challenges

	After diluent challenge	1 h after allergen challenge	24 h after allergen challenge
Cell count (× 10 ³ /mL)	85.0 ± 25.9	123.8 ± 70.5	112.5 ± 50.8
Epithelial cells (%)	6.6 ± 3.8	7.8 ± 3.1	6.6 ± 4.0
Macrophages (%)	86.2 ± 5.8	80.7 ± 7.1	80.6 ± 11.1
Neutrophils (%)	2.7 ± 2.2	3.0 ± 2.4	3.4 ± 3.7
Eosinophils (%)	0.7 ± 0.7	2.6 ± 1.9*	2.0 ± 1.9*
Lymphocytes (%)	3.8 ± 5.3	5.7 ± 8.5	7.4 ± 6.0†

Values are means ± SDs.

**P* = .02 and †*P* = .038 vs after diluent challenge.

TABLE V. Percentage of basement membrane with denuded, partially denuded, and intact epithelium after diluent and allergen challenges

	After diluent challenge	1 h after allergen challenge	24 h after allergen challenge
Denuded	20.0 ± 8.5	16.3 ± 3.8	22.4 ± 13.0
Partially denuded	53.0 ± 9.4	65.1 ± 4.6	57.8 ± 11.2
Intact epithelium	22.3 ± 10.9	18.6 ± 6.3	19.8 ± 11.0

Values are means ± SDs.

TABLE VI. Mucin-like glycoprotein in bronchial wash after diluent and allergen challenge

	After diluent challenge	1 h after allergen challenge	24 h after allergen challenge
Mucin protein (μg/mL)	2.3 ± 1.1	1.8 ± 0.7	2.3 ± 1.2

Values are means ± SDs. No significant differences were found among these 3 groups.

Rather than decreasing, goblet cell mucin stores increased at 24 hours, especially in the subgroup of subjects who had normal baseline mucin stores in goblet cells. Possible mechanisms include allergen-induced increases in IL-9²⁸ or IL-13,^{29,30} leading to upregulation of mucin gene expression and mucin glycoprotein synthesis. Recent data suggest that the effect of IL-13 on mucin synthesis is mediated by the epidermal growth factor receptor.³¹ Interestingly, we did not observe any increase in epithelial mucin stores in subjects whose mucin stores were already high at baseline. The reason could be that feedback mechanisms operate in the airway to limit goblet cell size or number.

The small but significant decrease in expired NO at 1 and 7 hours is probably explained by the effects of repeated spirometry maneuvers in the first 7 hours after allergen challenge. Through unknown mechanisms, repeated spirometry causes a fall in exhaled NO that is

maximal after 30 minutes and persists for at least 8 hours.³² Our finding that NO levels increased at 24 hours after allergen challenge is consistent with previous work in which allergen-induced increases in exhaled NO peaked between 8 and 21 hours after challenge.³³

We examined changes in the airway mucosa and in bronchial lavage fluid at 2 time points after whole-lung allergen challenge in human subjects. To do this required 3 bronchoscopies in each subject at 6-week intervals, 1 of which was performed within the first hour after allergen challenge. Albuterol administration successfully reversed most or all of this early-phase bronchoconstriction and allowed bronchial biopsies to be sampled during the early phase. Although albuterol administration was necessary before each bronchoscopy, we doubt that its use masked any effect of allergen on goblet cell mucins. Mediator release induced by allergen should have had sufficient time to degranulate goblet cells before albuterol was

administered, and lower stored mucin in the tissue and higher secreted mucin in the lavage should have revealed this. In addition, though β -agonists can improve mucociliary clearance,³⁴ they have only minor effects on mucin glycoprotein release from human airway explants.

Another methodologic aspect of our study protocol worthy of emphasis is that we analyzed the bronchial biopsies using methods of design-based stereology. To date, very few studies have applied stereology as a method of quantitative microscopy in bronchial biopsies. Our study demonstrates the feasibility of using stereology to quantify remodeling (epithelial mucin changes) and inflammation (neutrophil and eosinophil numbers) in biopsy tissue.

In conclusion, we have found that though allergen challenge increases the number of eosinophils in the airway within 1 hour of allergen challenge, this inflammatory response does not cause goblet cell degranulation. Allergen challenge actually increases goblet cell mucin within 24 hours in subjects with normal baseline goblet cell mucin stores. The mechanisms of goblet cell degranulation and goblet cell hyperplasia in asthma remain poorly understood but deserve further investigation because of the importance of mucin hypersecretion from goblet cells as a cause of asthma morbidity and mortality.

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