

Allergen challenge-induced entry of α_2 -macroglobulin and tryptase into human nasal and bronchial airways

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Background: Microvascular-epithelial exudation of bulk plasma may characterize inflammatory airway diseases. This study compares the acute allergen challenge-induced mast cell and exudative responses in nasal and bronchial airways. The focus is on α_2 -macroglobulin as an index of luminal entry of plasma exudates.

Methods: Separate nasal and bronchial allergen challenges were carried out outside the pollen season in eight patients with pollen-induced seasonal allergic rhinitis. The levels of different-sized plasma proteins (albumin molecular weight, 66,000 d and α_2 -macroglobulin molecular weight, 725,000 d) and tryptase were determined in pre- and postchallenge nasal lavage and bronchoalveolar lavage (BAL) fluids. Diluent and increasing doses of allergen were sprayed into the right nasal cavity, and each challenge was followed by a nasal lavage (volume, 15 ml) with a "nasal pool" device (recovery, >80%). Endobronchial allergen challenge (individual doses) and BAL (volume, 2 × 25 ml) were performed in a lobe bronchus through a fiberoptic bronchoscope (recovery, 30%). Saline challenge and BAL were carried out in the contralateral lung as control.

Results: The levels of albumin, α_2 -macroglobulin, and tryptase increased dose-dependently in postchallenge nasal lavage fluids ($p < 0.05$) and correlated to nasal symptoms. In particular, albumin and α_2 -macroglobulin correlated ($r = 0.98$, $p < 0.001$). Both α_2 -macroglobulin and tryptase, but not albumin, were increased in BAL fluids from the allergen-challenged side ($p < 0.05$).

Conclusion: Local allergen challenge causes luminal entry of tryptase and α_2 -macroglobulin in the nose and bronchi of patients with allergy. We suggest that mast cell and plasma exudation responses may be similar in human nasal and bronchial airways and that albumin levels (in BAL fluids) may not well reflect the exudation process in bronchial airways. (*J ALLERGY CLIN IMMUNOL* 1995;96:239-46.)

Key words: Allergic rhinitis, airway inflammation, bronchoalveolar lavage, albumin, α_2 -macroglobulin, tryptase

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Luminal entry of plasma occurs in airway defense and inflammation. With its adhesive proteins, enzymes, oligopeptides, peptide mediators, and cytokines, the plasma exudate is a potentially important mechanism in the lamina propria, across the epithelium, and on the surface of the nasal and the tracheobronchial mucosa.^{1, 2}

Animal data and subsequent observations in human beings have characterized the airway mucosal exudation process as a bulk flux of plasma.³⁻⁵ The small size restriction reflects a dramatic increase in the permeability of both the microvascular wall and the epithelial lining. However, the plasma exudation process has not been associated

Abbreviations used

BAL:	Bronchoalveolar lavage
FEV ₁ :	Forced expiratory volume in 1 second
NAL:	Nasal lavage

with or followed by increased airway absorption ability.⁶⁻⁸ Experimental *in vitro* and *in vivo* data suggest that airway epithelial junctions may yield, in a noninjurious and fully reversible way, to the exudate that moves up between and all around each epithelial cell.⁹⁻¹¹ Valve-like paracellular pathways across the mucosa may thus be created by the exuded plasma itself, having the effect of slightly increasing the hydrostatic pressure load on the basolateral aspects of columnar epithelial cells.^{9,10} Luminal entry of bulk plasma is so swift that it may prevent the formation of airway edema. As further support for an easy luminal escape, it has been demonstrated, in guinea pig airways, that marked exudation of plasma can occur without being associated with increased transport of plasma macromolecules to regional tracheobronchial lymph nodes.¹²

Allergen challenge is well known to promptly increase luminal entry of albumin in nasal airways,¹³⁻¹⁵ and preliminary results suggest, in agreement with animal observations,⁴ that this reflects luminal entry of bulk plasma.¹⁶ The small plasma protein albumin (molecular weight, 66,000 d), which is frequently analyzed in nasal and bronchial lavage fluids,^{13,14,17} may enter the airway lumen by passive diffusion and secretion, as well as by the exudation mechanism. Albumin is also the most common protein component of normal airway and alveolar surface liquids. Hence, high and variable baseline levels of albumin in bronchoalveolar lavage (BAL) fluids may invalidate the use of this protein as a plasma exudation index, particularly if the inflammatory exudation response is a restricted bronchial process that does not involve the peripheral pulmonary tissue. This reasoning should have relevance for asthma, which is an airway and not a pulmonary disease.

This study, which involved patients with allergic rhinitis, used both endonasal and endobronchial allergen challenges in the same subject to assess airway exudation responses. As previously demonstrated by Sedgwick et al.,¹⁸ who examined acute release of mast cell mediators, a response to endobronchial allergen challenge can also be expected in patients with allergic rhinitis. In addition to albumin, we have analyzed α_2 -macroglobulin

and tryptase in nasal lavage (NAL) and BAL fluids. This study tests the hypothesis that nasal and bronchial inflammatory responses may be similar and that large plasma proteins, compared with albumin, may better indicate the occurrence of exudation, particularly in the lower airways.

METHODS

Subjects

Four women and four men, with a mean age of 35 years (range, 21 to 46 years), were enrolled in the study. They had a history of tree or grass pollen-induced rhinitis, recurring dry cough during the pollen season interpreted as slight bronchospasm, a positive skin prick test response to birch or timothy pollen, and increased levels of specific IgE antibodies in the blood as determined by RAST (Table I). Six of the patients (nos. 1, 2, 3, 5, 6, and 8) were free of symptoms and were not taking any medications outside the pollen season, although three of them demonstrated bronchial hyperresponsiveness outside the pollen season (Table I). (These findings are in agreement with previous studies demonstrating bronchial hyperreactivity in some patients with allergic rhinitis,¹⁹ especially during the pollen season.²⁰) None of these six patients used inhalant therapy during the pollen season. Two of the patients (nos. 4 and 7) occasionally used inhalant therapy with a β_2 -adrenergic agonist (therapy was stopped at least 5 days before bronchial and nasal challenges); both of these patients demonstrated abnormally low forced expiratory volume in 1 second (FEV₁) and bronchial hyperreactivity to histamine (Table I). Bronchial histamine challenge and local nasal and bronchial challenges with the appropriate allergen were performed outside the pollen season (January to April and September to November 1992). Bronchial provocations with histamine were performed at least 10 days before the endobronchial allergen challenge, and at least 1 week elapsed between the bronchial and nasal allergen challenges. The subjects were included after informed consent was obtained and both verbal and written information had been provided. The guidelines for fiberoptic bronchoscopy and BAL recommended by the National Institutes of Health, the American College of Chest Physicians, the American Academy of Allergy and Immunology, and the American Thoracic Society were followed.^{21,22} The investigation was approved by the Ethics Committee at Huddinge University Hospital at Karolinska Institute, Stockholm, and was in accordance with the Helsinki Declaration II.

Skin testing

Freeze-dried purified allergenic extracts of birch and timothy grass pollen were used (Aquagen SQ; ALK, Copenhagen, Denmark). A 50% (vol/vol) glycerol solution (Soluprick SQ; ALK, Copenhagen, Denmark) was used for skin prick testing on the volar aspect of the forearm. Histamine dihydrochloride (10 mg/ml) was used as positive control and diluent as negative control. Titration with 10 to

TABLE I. Patient characteristics, atopic status, baseline FEV₁, and doses at skin and lower airway provocations

Patient No.	Age (yr)	Sex	Allergen	Total IgE (kU/L)	RAST class	Skin prick test end-point concentration (SQ/ml)	FEV ₁ (% predicted)	Lower airway provocation	
								Histamine aerosol challenge, end-point dose (μg)	Allergen endobronchial challenge, end-point concentration (SQ/ml)
1	39	F	Timothy	100	3	10.000	123	330	1.000
2	32	F	Timothy	410	3	100.000	118	>1320 (neg)	100.000
3	44	F	Timothy	172	4	10.000	120	>1320 (neg)	100.000
4	37	M	Birch	91	3	100.000	81	33	10.000
5	32	M	Birch	120	3	10.000	99	330	1.000
6	46	F	Birch	26	3	10.000	101	110	1.000
7	27	M	Birch	66	3	10.000	87	660	1.000
8	21	M	Birch	130	1	100.000	102	>1320 (neg)	100.000

100,000 SQ-U/ml was performed, and the histamine wheal equivalent allergen concentration was determined.

Blood test

Total IgE levels and RAST values were determined in venous blood samples (Pharmacia CAP system; Pharmacia Diagnostics, Uppsala, Sweden).

Bronchial responsiveness to histamine

Bronchial histamine provocations were done with a Spira Elektro 2 nebulizer system (Hämeenlinna, Työkeskus, Tavastehus, Finland). Aerosol delivery time of the nebulizer was adjustable to 0.5 second with an onset of nebulization after 100 ml. The volume of each inhalation was approximately 500 ml at a flow rate of 0.5 L/min. After baseline measurements of FEV₁, the subjects inhaled increasing doses (seven dose steps) of histamine: one, three, and six inhalations of 0.16% buffered histamine diphosphate and one, three, six, and 12 inhalations of 1.6% histamine solution, starting from an initial dose of 11 μg and increasing to a maximum dose of 1320 μg. FEV₁ was measured 3 minutes after each dose of histamine. The test was stopped when FEV₁ had decreased by 20% or more from the baseline value. The logarithmic histamine dose was plotted against FEV₁ to determine the provocative dose of histamine causing a 20% fall in FEV₁.

Endobronchial allergen challenge and BAL

Fiberoptic bronchoscopy, endobronchial allergen challenge, and BAL were performed according to methods previously described.²³ In brief, the participants were treated as outpatients and came to the bronchoscopy unit in the morning for a local endobronchial allergen provocation. Subcutaneous premedication with 10 mg of oxicon chloride and 0.4 mg of scopolamine bromide was given 30 minutes before the procedure. The patients were monitored for cardiac arrhythmias

with a single-lead electrocardiogram and for oxygen saturation by finger probe oximetry. A flexible fiberoptic bronchoscope (1T20; Olympus, New Hyde Park, N.Y.) was inserted via the nasal route. Intermittent doses of lidocaine (40 mg/ml) were sprayed on the vocal cords and in the bronchial tree to a total standardized dose of 8.0 ml. The subjects were placed in a supine position to allow best return by gravitational force from the lingular or middle-lobe bronchus, which was assigned randomly for saline and allergen challenges. A plastic catheter was placed immediately proximal to the placebo-assigned bronchus, and 0.5 ml of saline solution (0.9%) was instilled. Three minutes later, a two-channel polyvinyl catheter, equipped with an inflatable latex balloon (PUL 6.8-110-SGA; William Cook Europe, Västra Frölunda, Sweden) was introduced and positioned just distal to the bronchial meatus. The balloon was inflated with air (0.3 to 0.6 ml) until a visible seal was achieved. Then the lavage procedure was performed: 25 ml of saline solution was instilled through the catheter and then aspirated by hand-manuevered suction. This procedure was performed twice, and the returned lavage fluids were collected separately, and the lavages were named control (C)1 and C2. Thereafter, the bronchoscope was positioned in the opposite lung, and the allergen challenge was performed through a plastic catheter. Consecutive challenges with 0.5 ml of different allergen solutions were performed. The first allergen concentration was 1/100 of the previously determined histamine wheal equivalent dose, and 10-fold incremental doses were given thereafter at 3-minute intervals until a positive reaction was observed. A positive reaction was defined as visible blanching, followed by reddening of the mucosa and reduction of bronchial lumen (see Table I for individual allergen challenge doses). Then the balloon-equipped catheter was inserted, and the bronchus was lavaged twice with 25 ml of saline solution as previously described. The lavages were named allergen (A)1 and

A2. The BAL fluids were immediately centrifuged (250 g for 10 minutes), and the supernatants were frozen (-20°C).

Nasal allergen challenge and lavage

Nasal allergen challenges and lavages were performed in a sequence. Only the right nasal cavity was used for challenges and lavages. Allergen challenges were performed with a handheld nasal spray pump delivering 50 μl per actuation. Two actuations (100 μl) were given at each provocation. Freeze-dried purified extracts of birch and timothy grass pollen dissolved in manufacturer's diluent (isotonic solution with human serum albumin 0.03% and phenol 0.5%) were used (Aquagen SQ). Final concentrations of 1000, 10,000, and 100,000 SQ-U/ml were used, delivering total doses of 100, 1000, and 10,000 SQ-U, respectively, to the right nasal cavity. NALs were performed according to a previously described method.²⁴ A compressible plastic container, a "nasal pool" device, filled with 15 ml of isotonic saline solution (0.9%) at room temperature was used. The participants were asked to bend their heads forward (60 degrees from the vertical position) during the lavages. While participants remained in this position, the container was compressed and the right nasal cavity was gently filled with saline solution, which was kept there for 2.5 minutes before it was returned to the container. An initial baseline lavage was immediately followed by two quick lavages (30 seconds each), which were discarded. Ten minutes later, a prechallenge lavage was performed and was followed by a diluent provocation. Thereafter, saline lavages were performed 10 minutes after each nasal provocation. The lavages were named baseline, prechallenge, diluent, 100 SQ-U, 1000 SQ-U, and 10,000 SQ-U. If excessive rhinorrhea occurred after provocation, the patients were told to bend their heads forward and to occlude their right nostrils with the nasal pool adapter to prevent excessive nasal secretions from dripping out or being swallowed before lavage was performed. After delivery, the lavage fluids were immediately centrifuged (250 g for 10 minutes), and the supernatants were frozen (-20°C).

Analytical assays

Albumin. Albumin was measured with a radioimmunoassay sensitive to 6.25 ng/ml.²⁵ The intra- and interassay coefficients of variation were 5% and 10%, respectively.

α_2 -Macroglobulin. α_2 -Macroglobulin was measured with a radioimmunoassay sensitive to 7.81 ng/ml.²⁵ The intra- and interassay coefficients of variation were between 4% to 6% and 3% to 7%, respectively.

Tryptase. Tryptase was measured with a radioimmunoassay (Pharmacia Tryptase RIAC; Pharmacia Diagnostics) sensitive to 2 ng/ml.²⁶ Both the intraassay and interassay coefficients of variation were approximately 2%.²⁶

Statistics

Nonparametric statistics were used. Friedman's test, followed by Wilcoxon signed-rank test, was used for comparison of lavage fluid levels, and Spearman's rank correlation coefficient was used for calculations of putative correlations. For BAL fluid analysis the mean levels of the mediators in the two lavages were calculated and used for statistical comparison: saline-challenged side versus allergen-challenged side (C_{mean} vs A_{mean}). For NAL fluid analysis, comparison was made between lavages after diluent and allergen challenges.

RESULTS

BAL fluid analysis

BAL fluid recovery (mean $\pm$ SEM) was 30.8% $\pm$ 4.1% (C_{mean} = mean of C1 and C2) and 33.7% $\pm$ 4.3% (A_{mean} = mean of A1 and A2). BAL fluid levels (C_{mean} and A_{mean}) of albumin, α_2 -macroglobulin, and tryptase are presented in Fig. 1. All BAL fluid samples were processed for analysis. The levels of albumin and α_2 -macroglobulin were clearly above the detection limits of the assays in all samples. Tryptase was demonstrated in all BAL samples from the allergen-challenged side and in half of the BAL samples from the saline-challenged (control) side. There were no statistically significant changes in recovery rate or lavage fluid levels of albumin between the control and allergen-challenged sides, whereas significantly increased levels of α_2 -macroglobulin ($p < 0.05$) and tryptase ($p < 0.05$) were recorded on the allergen-challenged side.

NAL fluid analysis

NAL fluid levels of albumin, α_2 -macroglobulin, and tryptase are presented in Fig. 2. All NAL fluid samples were processed for analysis. The levels of albumin and α_2 -macroglobulin were clearly above the detection limit of the assays in all samples. Tryptase was detected in all samples after allergen challenge and also in the prechallenge lavage fluids of six patients. The NAL fluid levels of albumin, α_2 -macroglobulin, and tryptase increased dose-dependently on allergen challenge ($p < 0.001$ for all three mediators with Friedman's test. Comparison with Wilcoxon signed-rank test demonstrated no significant increased levels of albumin or α_2 -macroglobulin after 100 SQ-U as compared with diluent challenge, whereas $p < 0.05$ for all other comparisons (diluent vs 100 SQ-U vs 1000 SQ-U vs 10,000 SQ-U) for albumin, α_2 -macroglobulin, and tryptase.

Nasal symptoms

Nasal symptoms are presented in Fig. 2. A total nasal symptom score was calculated from the sum

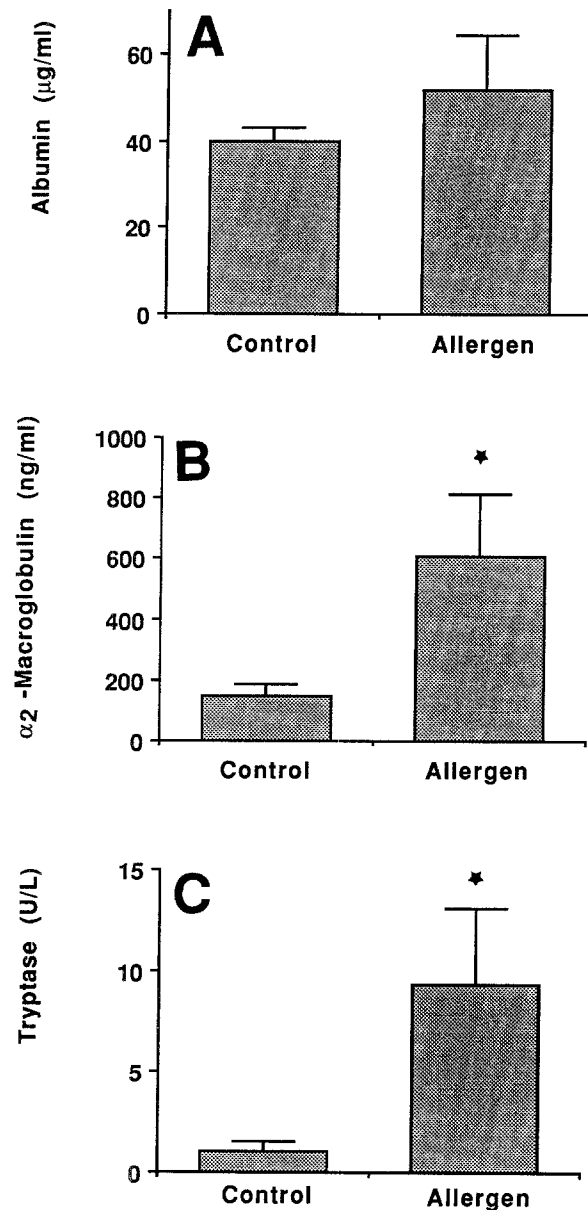


FIG. 1. BAL fluid levels (mean ± SEM) of albumin (A), α₂-macroglobulin (B), and tryptase (C) after endobronchial challenge with saline solution (control side, C_{mean}) and allergen (A_{mean}). * $p < 0.05$; C_{mean} versus A_{mean} (Wilcoxon signed-rank test).

of the different scores (sneezing, secretion, nasal blockage). Sneezing (number of sneezes) was transformed into scores of 0 (0 sneezes), 1 (1 to 4 sneezes), 2 (5 to 9 sneezes), and 3 (10 or more sneezes). Total nasal symptoms score increased dose-dependently on allergen challenge ($p < 0.001$ with Friedman's test; $p < 0.05$ for all comparisons [diluent vs 100 SQ-U vs 1000 SQ-U vs 10,000 SQ-U] with Wilcoxon signed-rank test).

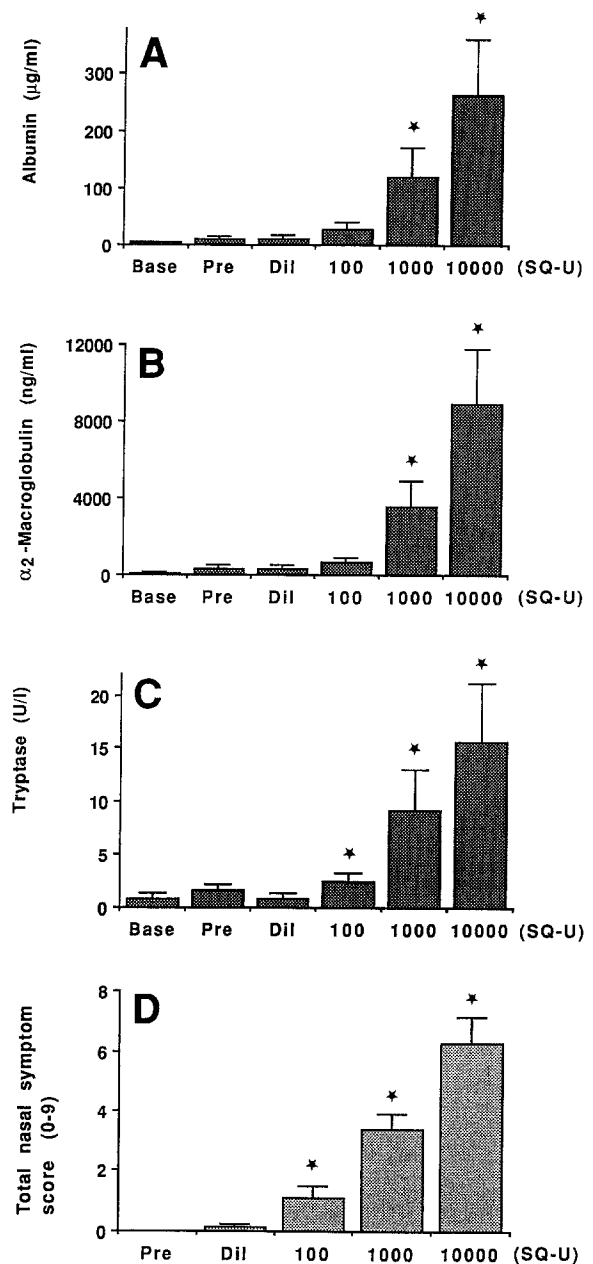


FIG. 2. NAL fluid levels (mean ± SEM) of albumin (A), α₂-macroglobulin (B), and tryptase (C), and total nasal symptoms score (mean ± SEM) (D) after repeated nasal challenges with increasing doses of allergen. Total nasal symptoms score (0-9) was calculated from the sum of nasal blockage score (0-3), secretion score (0-3), and number of sneezes transformed into scores (0-3). Base, Baseline; Pre, prechallenge; and Dil, diluent challenge. * $p < 0.05$; diluent challenge versus 100, 1000, and 10,000 SQ-U, respectively (Wilcoxon signed-rank test).

Correlations

Correlations between levels of mediators were higher in NAL fluids than in BAL fluids (Fig. 3). There was a particularly strong correlation be-

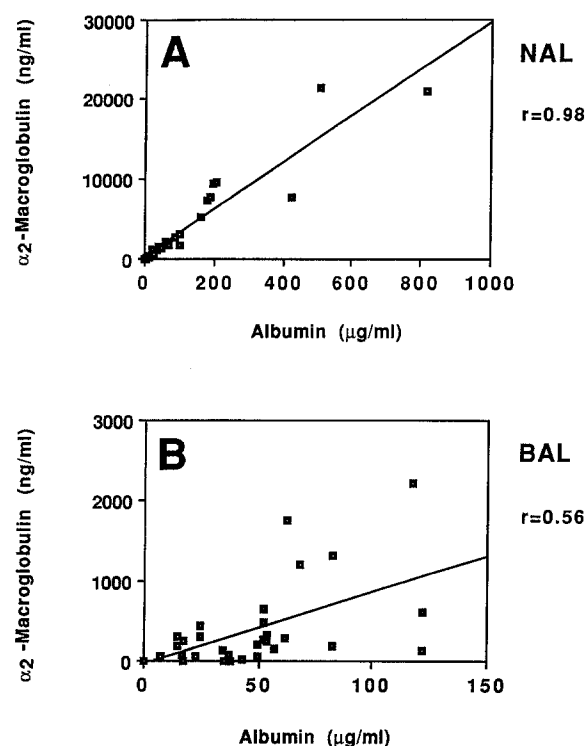


FIG. 3. Correlations between albumin and α_2 -macroglobulin in NAL (prechallenge + diluent + 100 SQ-U + 1000 SQ-U + 10,000 SQ-U) (A) and BAL (C1 + C2 + A1 + A2) (B) fluids (r = Spearman's rank correlation coefficient; NAL: $p < 0.001$, BAL: $p < 0.01$).

tween albumin and α_2 -macroglobulin in NAL fluids (calculations made on prechallenge + diluent + 100 SQ-U + 1000 SQ-U + 10,000 SQ-U) ($r = 0.98$, $p < 0.001$, Spearman's rank correlation coefficient). Other correlations in NAL fluids were also strong ($r = 0.72$ and 0.71 for tryptase to albumin and α_2 -macroglobulin, respectively; $r = 0.81$, 0.81 , and 0.80 for total nasal symptoms to tryptase, albumin, and α_2 -macroglobulin, respectively; $p < 0.001$ for all r values, Spearman's rank correlation coefficient), whereas weak correlations were found in BAL fluids (calculations made on C1 + C2 + A1 + A2) ($r = 0.56$ and 0.54 for α_2 -macroglobulin to albumin and tryptase, respectively; $p = \text{NS}$ for albumin to tryptase; $p < 0.01$, Spearman's rank correlation coefficient).

DISCUSSION

This study, which involved patients with allergy, demonstrates that topical allergen challenge of the airway mucosa of both nasal and bronchial passages produces increased levels of tryptase, a mast cell activation index, and increased levels of α_2 -macroglobulin, which is a marker of mucosal exu-

dation of bulk plasma. The NAL technique revealed highly significant co-exudation of albumin. In sharp contrast, the BAL fluid levels of albumin did not differ between challenge and control conditions. These observations on lavage fluid levels of tryptase and α_2 -macroglobulin support the notion that nasal mucosal mechanisms may mimic the inflammatory responses that occur in the bronchi.²

In the nasal airways of the subjects in this study, a wide range of allergen dosages produced graded increments in plasma exudation indices in the NAL fluids, along with similarly graded increments in both symptoms and tryptase levels. The total nasal symptoms score correlated well with the mucosal output of either tryptase or each of the plasma proteins. These findings are in agreement with the notion that mast cell-derived factors cause some of the acute symptoms at allergen challenge. They further suggest that mast cell-derived factors may be involved in the microvascular permeability response to allergen challenge. A role of histamine has already been established because treatment with antihistaminic drugs reduced both symptoms and nasal mucosal output of albumin at acute allergen challenge in subjects with allergic rhinitis.^{15, 27}

Mucosal challenge-induced plasma exudation responses involve an initial phase during which the subepithelial tissue is flooded with extravasated plasma.^{11, 28} Then the plasma crosses the epithelial basement membrane. It moves up between all epithelial cells in the area of interest¹¹ and starts entering the airway lumen, frequently within a minute after challenge. The exudation process thus constitutes a "lamina propria lavage," which may bring a variety of factors to the mucosal surface in addition to those derived directly from the circulation. The increased tryptase levels recorded in this study may reflect release from superficial mast cells in the nose and the bronchi, but the possibility that some tryptase has also been moved to the surface by the plasma exudation process cannot be excluded.

Because α_2 -macroglobulin belongs to the group of largest plasma solutes, the results of this study suggest that all plasma protein systems are moved to the mucosal surface in the acute inflammatory response of the airways. The plasma-derived agents include bradykinins, fibrinolysis peptides, complement fragments, other peptide mediators, and a number of cytokines including some growth factors. Extravasated plasma also deposits structural and adhesive proteins, such as fibrinogen and fibronectin, in the airways.²⁹ α_2 -Macroglobulin is

an interesting plasma protein because it is known to bind, carry, and target several cytokines.³⁰

Our observation of significantly increased BAL fluid levels of α_2 -macroglobulin in the presence of nonsignificant changes in the levels of albumin supports the hypothesis that the large plasma proteins may be superior BAL fluid markers of bronchial exudation of plasma. α_2 -Macroglobulin may also be a more useful marker of mucosal exudation than fibrinogen,²³ which is more or less trapped in the airway tissue and on the mucosal surface by fibrin formation.⁴

In contrast to the BAL techniques, the NAL technique used in this study is versatile and can be repeated frequently without undue consequences. Indeed, a desired result of this NAL regimen is to create low and reproducible baseline levels of mucosal surface solutes before the challenge-induced changes. The excellent correlation between albumin and α_2 -macroglobulin levels in the NAL fluids in this study was therefore expected. The "nasal pool" device further verifies that a large and reproducible mucosal surface area, specifically belonging to the airways, is gently and efficiently lavaged each time. The recovery with this NAL technique is greater than 80%,²⁴ whereas the BAL recovery was only about 30%. The advantages of the NAL technique appear useful, especially if important bronchial mechanisms can be mimicked and examined in the human nose.²

In conclusion, our data indicate that the acute response to allergen challenge of both the nasal and the bronchial mucosa involves a prompt mast cell response and a prompt luminal entry of bulk plasma. Specifically, this study indicates that α_2 -macroglobulin is a good marker of the acute inflammatory exudation response in both NAL and BAL fluids.

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