

The pathophysiology of rhinitis

V. Sources of protein in allergen-induced nasal secretions

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Allergic rhinitis is characterized by a profuse rhinorrhea in addition to paroxysms of sneezing, nasal congestion, and pruritus. To define better the sources of nasal secretion produced during rhinitis, nasal allergen challenges were performed on nine atopic subjects with seasonal rhinitis. A single dose of allergen was sprayed into one side of the nose, and nasal lavages were collected bilaterally for 7 hours. Nasal lavages were assayed for protein (total protein, albumin, lactoferrin, and lysozyme) and mediator (histamine and prostaglandin D₂) content. Protein concentrations increased and remained elevated above baseline levels in both ipsilateral and contralateral secretions for up to 3 hours after allergen challenge. The proportion of albumin relative to total protein (the albumin percent) increased on the ipsilateral side, whereas the relative proportions of lactoferrin and lysozyme (the lactoferrin percent and lysozyme percent) increased on the contralateral side. Prostaglandin D₂, but not histamine, increased selectively on the ipsilateral side. These data suggest that the ipsilateral protein secretory response is due to allergen-induced mast cell mediator release causing increased vascular permeability, whereas the contralateral protein secretory response is primarily a reflex-induced glandular secretion. (J ALLERGY CLIN IMMUNOL 1991;88:33-42.)

Key words: Allergic rhinitis, nasal challenge, lavage, protein, albumin, lactoferrin, lysozyme

The allergic reaction in the nose involves a complex interaction between allergen and multiple effector cells. Once an inhaled allergen interacts with mast cell-bound IgE, a series of biochemical reactions and cellular responses follows, associated with the recovery of inflammatory mediators in nasal secretions. These mediators are capable of producing varied responses, including increasing vascular permeability, stimulating glandular secretion, attracting inflammatory cells, stimulating irritant nerves and neural pathways, and modulating the release of secretory products (including mediators and cytokines) from other resident cells in the nasal mucosa.

Many nasal allergen provocation studies have focused on the measurement of inflammatory mediators

Abbreviations used

AR:	Allergic rhinitis
Lf, Lf%:	Lactoferrin and lactoferrin percent
Ly, Ly%:	Lysozyme and lysozyme percent
Alb, Alb%:	Albumin and albumin percent
PGD ₂ :	Prostaglandin D ₂
LPR:	Late-phase reaction

released from cells into pooled samples of nasal secretions or lavage fluid.¹⁻²¹ Recognizing that many different cellular mediators may all produce a similar end organ response (i.e., rhinorrhea), we examined a different component of the allergic reaction, the characterization of the sources of allergen-induced secretions. In particular, we determined which protein sources (vascular and/or glandular) participate in the production of nasal secretions after allergen challenge and compared the secretory responses between the two sides of the nose. Specific proteins from the vascular system (Alb)²² and proteins derived specifically from serous cells in the submucosal glands (Lf and Ly²³) were measured in nasal lavages as representative protein markers in nasal secretions.

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MATERIAL AND METHODS

Subjects

Nine adult subjects with AR (four male and five female subjects) between the ages of 26 and 38 years were studied after informed consent was obtained. The allergen selected for each subject (either ragweed or grass allergen) was determined by a history of rhinitis during the appropriate allergy season and by the presence of a positive prick skin test (a wheal 3 mm or greater above the control wheal) to the appropriate allergen (1:20 wt/vol; Holister-Stier Laboratories, Spokane, Wash.). All challenges were performed outside the allergy season, at a time when the subjects were asymptomatic and had not received any allergy medications for at least 1 week. None of the subjects had taken astemizole, and one had been taking topical inhaled steroids or cromolyn within 1 month before challenge. In addition, no subject was studied within 3 weeks of recovery from an upper respiratory tract infection.

Material

Nasal-challenge material included normal saline and glycerinated extracts of ragweed pollen, mixed grass pollen, and meadow fescue grass pollen (1/20 wt/vol; Holister-Stier Laboratories). Allergen-challenge solutions were prepared by diluting these glycerinated extracts tenfold with normal saline, producing identical 1:200 dilutions of allergen.

Challenge methods

The nasal-challenge technique has been described previously.^{22, 23} Nasal catheters were inserted into both nasal cavities and attached to suction, and then four nasal prewashes with saline (4 ml each) were performed on both sides of the nose to reduce preexisting secretions. Ten minutes before allergen challenge, a control challenge of 0.3 ml of saline was sprayed into one nasal cavity (ipsilateral side) and allowed to incubate for 10 minutes, and then bilateral nasal lavages (4 ml each) were collected at time 0 minutes. This baseline saline challenge served as the control for all later challenges. Then, a 0.3 ml allergen challenge was sprayed into the same side of the nose. This gentle spray technique confines the allergen-challenge solution to the ipsilateral side of the nose. After 3 minutes, a 4 ml saline lavage (the allergen rinse) was performed on the allergen-challenge side only (to remove proteins associated with the allergen-challenge solution). Nasal lavages (4 ml each) were then collected bilaterally at 10, 20, 30, 60, 180, 300, and 420 minutes after the allergen challenge. Each lavage was collected during 10-minute intervals (except for the first ipsilateral lavage, which represented only a 7-minute collection because of the allergen rinse). A positive challenge was defined as the development, after allergen challenge, of two or more of the following symptoms and signs: sneezing, itching, rhinorrhea, and nasal congestion. In addition, changes in pulse or blood pressure and the development of facial flushing were recorded throughout the study. Nasal-lavage samples were aliquoted and stored at -70°C for subsequent analysis (as described).

Assays

Total protein. Protein in each sample was measured according to the method of Lowry et al.²⁴ with bovine serum Alb as the standard. It has been demonstrated in preliminary studies that a 4 ml saline wash (the allergen rinse) effectively removes proteins that are exogenously sprayed into the nose, thus allowing for the measurement of endogenous-secreted proteins only.

Alb. Human Alb was measured by a competitive ELISA as described.²⁵ The assay range is between 1 and 100 $\mu\text{g/ml}$.

Lf. Human Lf was measured by a modified noncompetitive ELISA.^{23, 26} One hundred microliters of rabbit anti-human Lf (Dako Corp., Santa Barbara, Calif.), diluted 1:1000 in 0.1 mol/L of carbonate buffer, pH 9.6, was added to each well of polyvinyl chloride microtiter plate (Dynatech Laboratories, Chantilly, Va.) and incubated at 37°C for 90 minutes. The wells were then blocked with 1% goat serum (Gibco Laboratories, Grand Island, N.Y.), diluted in a buffer consisting of phosphate-buffered saline, pH 7.4, and 0.05% Tween 80 (Fisher Scientific Co., Fairlawn, N.J.). Standards of Lf (Sigma Chemical Co., St. Louis, Mo.) or sample (100 μl) were then incubated at 37°C for 90 minutes. The wells were washed, and rabbit antihuman Lf-horseradish-peroxidase-conjugate (Cappel Laboratories, Cochranville, Pa.), diluted 1:1000 in phosphate-buffered saline and 0.05% Tween 80, was added to each well and again incubated at 37°C for 90 minutes. The reaction was developed with an o-phenylenediamine dihydrochloride substrate (Sigma Chemical Co.) and read at 490 nm. The assay range is between 1 to 200 ng/ml.

Ly. Ly activity was measured by a turbidimetric assay based on the enzymatic hydrolysis of bacterial cell walls as described.^{23, 27} Fifty microliters of hen Ly standard (Sigma Chemical Co.) or sample was added to a 1 ml suspension of *Micrococcus lysodeikticus* (Sigma Chemical Co.) (0.25 mg/ml in 0.1 mol/L of PBS, pH 7.2), and the decrease in absorbance at 450 nm/min was recorded on a spectrophotometer (Hewlett-Packard Co., Palo Alto, Calif.). The assay range is between 1 and 100 $\mu\text{g/ml}$.

Alb%, Lf%, and Ly%. The Alb%, Lf%, and Ly% were all calculated by dividing the respective proteins by the total protein and multiplying by 100%.

Histamine. Histamine was measured by a competitive RIA as described²⁸ (AMAC, Inc., Westbrook, Me.). The range of this assay is between 0.06 and 16.7 ng/ml.

PGD₂. PGD₂ was also measured by a competitive RIA, as previously described (Amersham Corp., Arlington Heights, Ill.).²⁹ The assay range is between 30 and 2000 pg/ml.

Statistics

Measurements of secretions were analyzed by a three-way analysis of variance with the subjects ($N = 8$), side of nose (ipsilateral and contralateral), and time ($n = 8$) serving as the three variables. Differences between the sides of the nose were the focus of the statistical analyses. Nasal responses were compared in two ways: for side-to-side re-

sponses that differed significantly from each other *throughout* the entire study and for side-to-side differences that were significant only at specific time points. A side-of-nose time interaction was included in the model, and all remaining interactions were pooled and used as the reference source of variability. If the above interaction (side-of-nose time) was nonsignificant, then a comparison of the two sides of the nose was made across the entire time course, represented in the figure legends by a single p value. If, in contrast, the side-of-nose time interaction was significant, then comparisons were made at each time point, and individual p values (represented by asterisks in the figures) were corrected for multiple comparisons by use of the Bonferroni inequality. These data were logged, and in all instances, the residuals were found to follow a normal distribution when they were analyzed by a test according to the method of Shapiro and Wilk.³⁰

RESULTS

Of the nine subjects who underwent nasal allergen challenge, one subject failed to clinically react to allergen challenge and had no objective changes on nasal lavage analysis, despite a positive clinical history and positive skin test. Therefore, these data for this individual were not included in the analyses below.

Control saline challenges produced neither nasal symptoms nor signs, as previously reported.^{22, 25} As an additional control, a tenfold dilution of glycerinated saline solution was prepared with saline (making a 5% glycerine concentration) and tested as a challenge solution in a few subjects. Since this control challenge did not produce symptoms or signs and nasal lavage measurements were indistinguishable from saline challenges, normal saline was used as the control for subsequent allergen challenges.

Allergen challenge, in contrast, produced paroxysms of sneezing, itching, and/or nasal congestion within the first 3 minutes in all eight atopic subjects, without producing any changes in blood pressure or pulse. In comparison, allergen challenge in nonatopic control subjects (data not presented) failed to produce either symptoms or signs. In atopic subjects, sneezing and itching occurred early, within the first minute of the challenge. By 10 minutes, all eight subjects noted ipsilateral nasal congestion that lasted for at least 20 minutes. Half the subjects developed bilateral watery and/or mucoid rhinorrhea within the first 10 minutes. Between 1 to 3 hours after allergen challenge, four subjects developed nasal irritability, defined by episodic sneezing, pruritus, and/or mild nasal congestion (on the ipsilateral side only). No symptomatic reactions were noted beyond 3 hours after allergen challenge.

Nasal lavages were assayed for the following pro-

teins: total protein, Alb, Lf, and Ly. Total protein levels after unilateral saline challenge were similar on both sides of the nose. Compared to control challenge, allergen challenge produced dramatic bilateral increases in protein secretion for at least 1 hour after challenge (Fig. 1). Ipsilateral protein secretion was significantly greater than contralateral secretion throughout the time course. Although ipsilateral protein secretion reached its peak at 60 minutes, contralateral secretion peaked earlier, at 10 minutes, and remained at that level for 60 minutes. By 3 hours, both ipsilateral and contralateral protein levels were back at baseline levels.

The Alb secretion was distinctly different between the two sides of the nose (Fig. 2). Ipsilateral Alb secretion far exceeded contralateral secretion during the first hour ($p = 0.02$), reaching its peak early (at 20 to 30 minutes), whereas contralateral Alb secretion remained at a low level throughout the study.

Baseline values of Alb% were similar on both sides of the nose after control challenge, even though the ipsilateral value appears slightly (but insignificantly) higher (Fig. 3). After allergen challenge, however, the Alb% values differed significantly for the first 20 minutes of the study. The ipsilateral Alb% increased threefold (up to 45%) at 20 minutes, then fell to around 25% for the remainder of the first hour. In contrast, the contralateral Alb% did not increase significantly from its control value during the same time period. The slight increase in contralateral Alb% during the final hours reflects the unusually elevated values from one of the subjects and is not significant. Thus, allergen challenge disproportionately increased ipsilateral Alb secretion (compared to total protein), increasing the Alb%. The contralateral Alb, in contrast, increased in direct proportion to the total protein without changing the Alb%.

The glandular protein, Lf, increased bilaterally, compared to control values, after allergen challenge. However, there were no significant side-to-side differences (Fig. 4). Although the contralateral Lf secretion reached a peak after 10 to 20 minutes and then decreased during the first hour, the ipsilateral Lf continued to increase for an hour before returning to its control values at 3 hours.

The Lf%, in contrast, differed dramatically between the two sides of the nose (Fig. 5). On the ipsilateral side, Ag challenge produced no significant change in Lf%, whereas the contralateral Lf% increased significantly during the first 20 minutes and then decreased toward baseline by 60 minutes. This finding indicates that Lf secretion increased in parallel with total protein secretion on the ipsilateral side, whereas it increased disproportionately on the contralateral side.

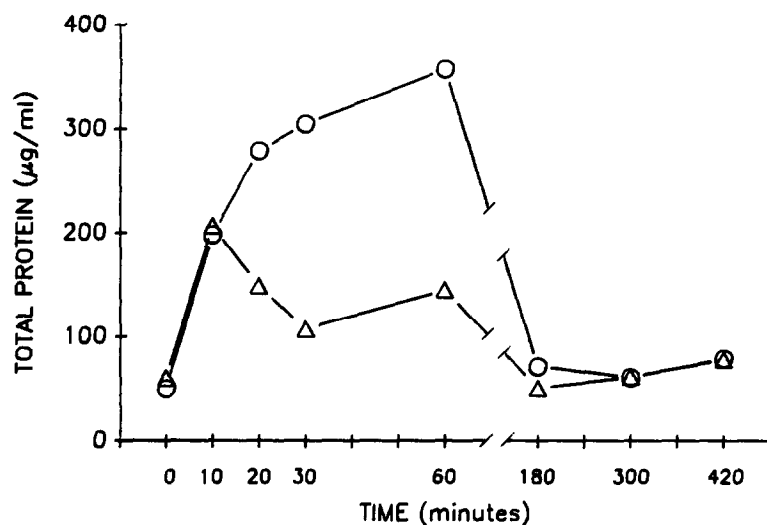


FIG. 1. Total protein in nasal lavages after unilateral nasal allergen challenge ($n = 8$ subjects). Allergen was applied to the ipsilateral side of the nose at time zero, and nasal lavages were collected serially from both sides of the nose for 7 hours. Symbols represent geometric mean values ($n = 8$). Ipsilateral protein secretion ($\circ$) was significantly greater than contralateral secretion (Δ) across the entire time course ($p < 0.001$ for side-to-side comparison by analysis of variance).

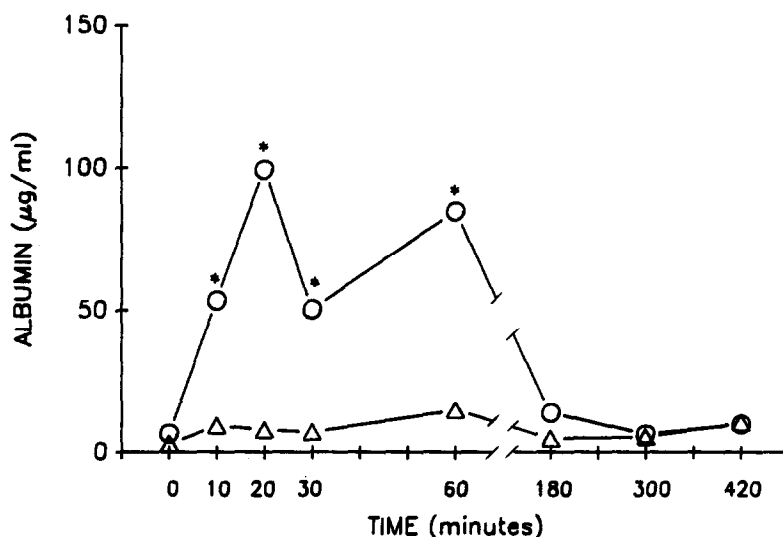


FIG. 2. Alb secretion in nasal lavages after allergen challenge. Ipsilateral ($\circ$) and contralateral (Δ) symbols represent geometric mean values ($n = 8$); the asterisks (*) represent p values ($p = 0.02$) for side-to-side comparisons at specific points.

The secretion of Ly also increased bilaterally after unilateral allergen challenge (Fig. 6). Ly secretion increased abruptly and remained elevated for 60 minutes on both the ipsilateral and contralateral sides (without significant side-to-side differences). These Ly% data are similar to the Lf% data (Fig. 7). Ipsilaterally, Ly% did not change significantly from control levels,

whereas the contralateral Ly% increased significantly and remained elevated above control levels and above ipsilateral levels throughout the entire study.

Mediator secretion

Measurable levels of histamine were found in all nasal lavages after control saline challenge, despite

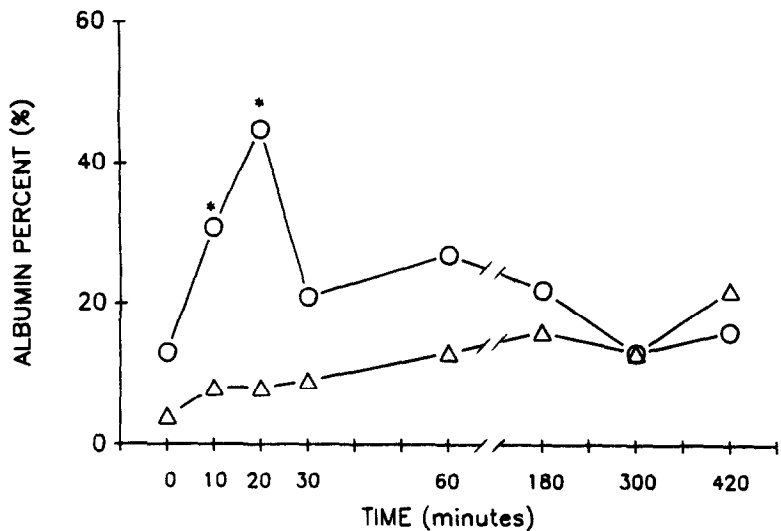


FIG. 3. Alb% values in nasal lavages after allergen challenge. Ipsilateral (○) and contralateral (△) symbols represent geometric mean values ($n = 8$). The asterisks (*) represent p value ($p < 0.05$) for side-to-side comparisons at specific time points.

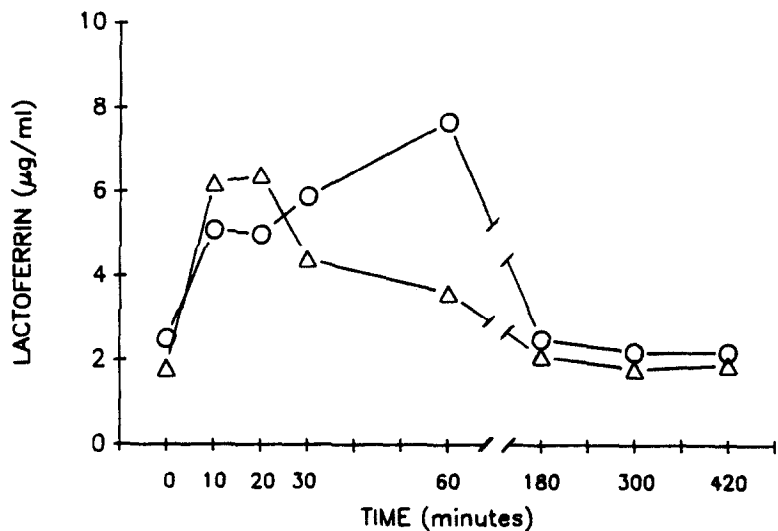


FIG. 4. Lf secretion in nasal lavages after allergen challenge. Ipsilateral (○) and contralateral (△) symbols represent geometric mean values ($n = 8$).

the initial four saline prewashes before the challenge. However, no significant changes in histamine secretion were observed on either side of the nose after allergen challenge, even though the subjects developed pronounced nasal symptoms and signs.

In marked contrast to histamine, allergen challenge produced dramatic ipsilateral increases in PGD_2 secretion, which were significantly greater than the contralateral levels of increase (Fig. 8). The levels in-

creased to a peak within 10 minutes in all eight subjects, remained significantly elevated for 30 minutes, then decreased to baseline values by 3 hours. On the contralateral side, there was a small insignificant increase in PGD_2 at 10 minutes, with a gradual decrease thereafter.

DISCUSSION

This study confirms that a single dose, unilaterally applied nasal allergen-challenge produces a sustained

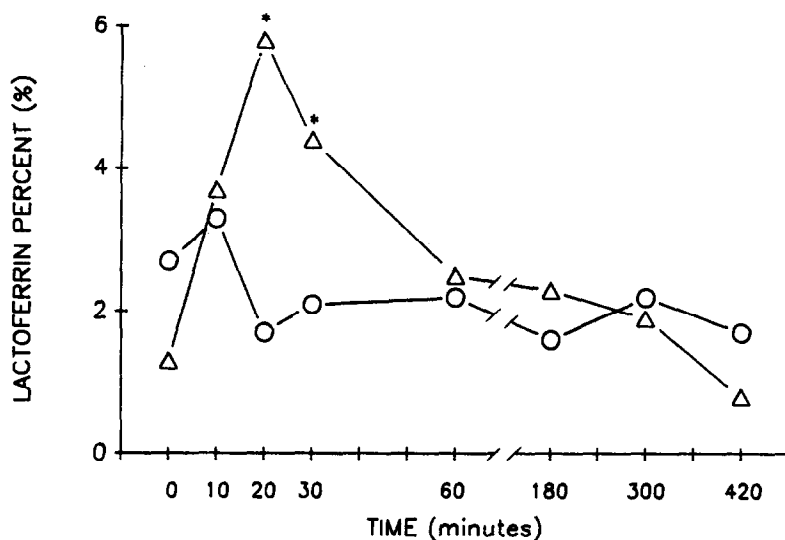


FIG. 5. Lf% values in nasal lavages after allergen challenge. Ipsilateral (°) and contralateral (Δ) symbols represent geometric mean values ($n = 8$). Asterisks (*) represent p values ($p = 0.02$) for side-to-side comparisons at specific time points.

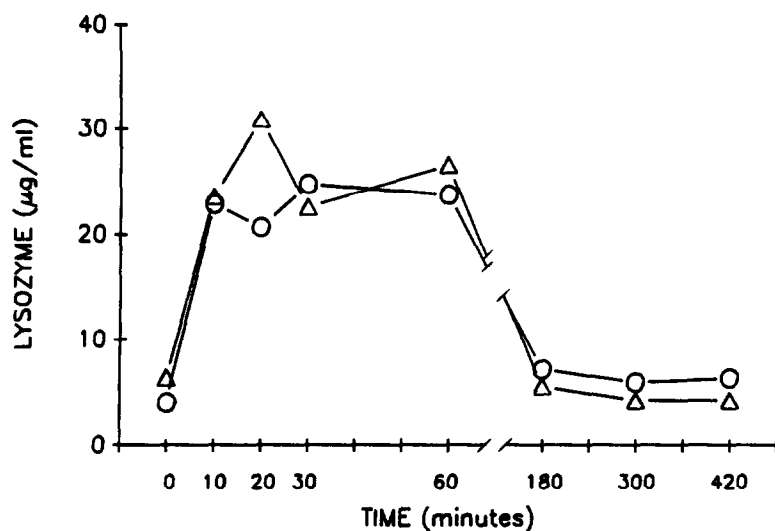


FIG. 6. Ly secretion in nasal lavages after allergen challenge. Ipsilateral (°) and contralateral (Δ) symbols represent geometric mean values ($n = 8$).

bilateral secretory response. The ipsilateral protein secretion primarily represents a localized increase in vascular permeability, whereas the contralateral secretory response reflects relatively pure glandular secretion.

According to the literature, the concept that nasal allergen provocation produces a bilateral secretory response is well established.³¹⁻³⁵ The ipsilateral response is believed to reflect mast cell mediator release, as evidenced by increases in several mast cell mediators in secretions obtained from allergen-challenged sites.^{33, 35}

In contrast, the contralateral response is not believed to depend on local mediator release but, instead, depends on reflex stimulation (the nasonasal reflex) of cholinergic efferent nerves.³¹⁻³⁵ This contralateral secretory response can be significantly inhibited by surgical transection of the Vidian nerve, which contains the major cholinergic nerves supplying the nose.³⁴ Studies that support this conclusion, however, have primarily used subjective symptom scores and gross measurements of recovery of nasal secretion (i.e., measuring "drips per minute" or number of handkerchiefs used). Unfortunately, neither subjective

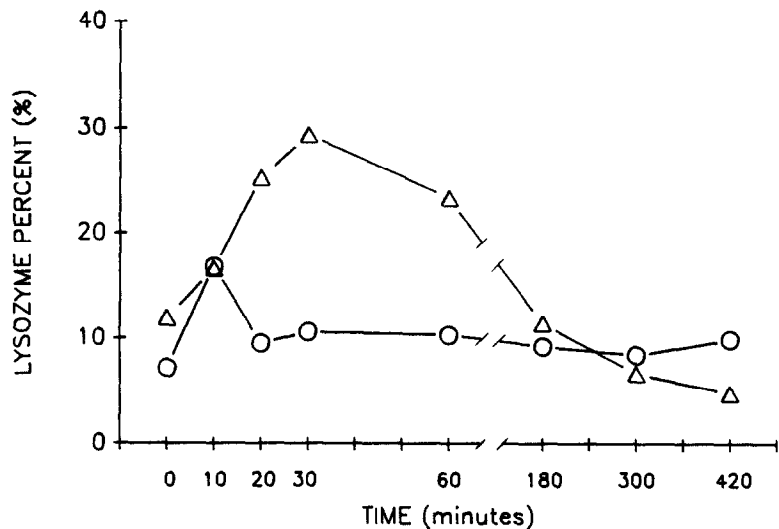


FIG. 7. Ly% values in nasal lavages after allergen challenge. Ipsilateral (°) and contralateral (Δ) symbols represent geometric mean values ($n = 8$). Contralateral values were significantly greater than ipsilateral values across the entire time course ($p = 0.006$).

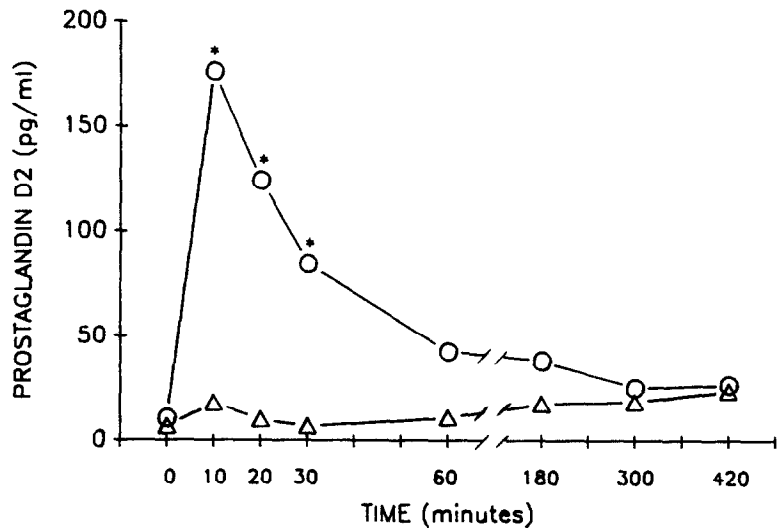


FIG. 8. PGD₂ secretion in nasal lavages after allergen challenge. Ipsilateral (°) and contralateral (Δ) symbols represent geometric mean values ($n = 8$). Asterisks (*) represent p values ($p < 0.05$) for side-to-side comparisons at specific time points.

symptom scores nor crude measurements of recovery of secretions provide significant information about the pathophysiology of AR.

Mediator measurement has been used for assessing the nasal response to both natural allergen exposure³⁶ and to nasal allergen provocation^{1-21, 33, 35, 36} and suggests the involvement of certain activated cells in the allergic reaction. Histamine, prostaglandins, leukotrienes, tryptase, mediators from eosinophils and neutrophils, and bradykinin have all been demonstrated to increase in nasal secretions after allergen challenge.

These studies have presented powerful evidence on which our current understanding of the cells responsible for initiating AR is based.

Relatively little information is known about changes in constituent nasal proteins secreted during AR.²⁰ The objective of this study was to examine allergen-induced nasal protein secretion, determine which secretory sources are involved, and compare the ipsilateral and contralateral secretory responses. The ability to determine the sources of secretion is a clinically relevant concern since it may be possible to

pharmacologically manipulate or inhibit one or more distinct sources of nasal protein secretion, irrespective of the stimulus.

The immediate reaction to unilateral nasal allergen challenge is characterized by nasal and oropharyngeal pruritus, ipsilateral nasal congestion, sneezing, and profound ipsilateral and contralateral nasal secretion. This study demonstrated that the nasal protein secretory response to allergen challenge is prolonged (lasting up to an hour or more) and that distinct differences exist between ipsilateral and contralateral secretions.

The ipsilateral total protein secretion is about three times as great as the contralateral secretion. All the proteins measured in this study increased in concentration in nasal lavages after allergen challenge, regardless of which side of the nose was examined. However, when protein proportions were calculated as percent terms (relative to total protein), several differences between ipsilateral and contralateral secretions became apparent. The ipsilateral Alb% increased rapidly and remained elevated for several hours after allergen challenge, whereas the contralateral Alb% did not change significantly from its baseline level. In previous nasal-challenge studies, increases in both Alb concentration and Alb% reflected increases in vascular permeability, whereas increases in Alb concentration without changes in Alb% suggested an increase in glandular secretion.^{22, 23, 25} Thus, the Alb data from this study suggest that allergen challenge produces a sustained ipsilateral protein secretion caused by increased vascular permeability, whereas the contralateral secretion reflects increased glandular secretion.

Measurements of the antimicrobial proteins, Lf and Ly, both of which are secreted selectively from the serous glands in the nose,²³ support this conclusion. Nasal-lavage levels of both these proteins are far greater than can be accounted for by serum contamination or by neutrophil accumulation in nasal lavages.²³ The absolute concentrations of Lf and Ly increased on both sides of the nose and remained elevated for at least an hour after allergen challenge. However, the ipsilateral versus contralateral responses of Lf% and Ly% differed dramatically (Figs. 5 and 7). The Lf% and Ly% on the ipsilateral side did not change from control values, whereas the contralateral Lf% and Ly% increased significantly for up to an hour after Ag challenge. Previous data from methacholine and histamine nasal challenge experiments suggest that increases in both the concentration and percentage of Lf and Ly represent increased glandular secretion, whereas increases in concentration without a change

in the percentages of these two proteins suggest a concomitant increase in both glandular secretion and vascular permeability.²³

The mediators, histamine and PGD₂, were measured as markers of mast cell degranulation. For histamine, measurable levels of histamine in nasal secretions were detected after control saline challenge, as previously described.^{1, 2, 8, 10} The histamine levels did not rise significantly on either side of the nose after allergen challenge, an observation also noted previously.⁸

This lack of increase in histamine, particularly on the ipsilateral side, is difficult to explain in light of the fact that all subjects experienced typical allergic symptoms after allergen challenge. However, the assay used in this study is quite specific for histamine,²⁸ and it accurately measures histamine added to nasal secretions (data not presented). The assays used in some of the other studies that describe increased histamine in secretions may recognize additional molecules besides histamine.³⁷ It is more likely that the dose of allergen chosen for this study caused dramatic responses but no measurable histamine secretion. This dose represents a typical allergen response in the Washington, D.C., area during the peak of the allergy season. Most other studies use three to 10 times (or more) the dose used in this study, causing a larger reaction than that observed here, associated with measurable histamine release.

In contrast to histamine, PGD₂ levels were essentially unmeasurable after saline challenge, despite the use of a very sensitive assay.²⁹ The PGD₂ level rose rapidly and dramatically on the challenged side of the nose and correlated well with the development and time course of nasal symptoms. This allergen-induced increase in nasal lavage PGD₂ has also been noted previously.^{1-9, 13, 16-18} In contrast, there were no significant changes in PGD₂ levels on the contralateral side of the nose. Since PGD₂ is believed to be predominantly a mast cell marker (it is not generated in significant concentrations by basophils⁴), these data suggest that PGD₂ measurement may be a more sensitive marker of mast cell degranulation than histamine.

Overall, the response to allergen challenge on the ipsilateral side predominantly reflects increased vascular permeability, resulting in a large exudation of Alb (and other vascular proteins) into nasal secretions, dramatically diluting out smaller increases in glandular proteins (Lf and Ly). The contralateral nasal secretion, in contrast, appears to represent a pure glandular response, presumably stimulated by an allergen-induced nasal reflex. The enhanced contralateral se-

cretion of Lf and Ly is not diluted out by other proteins, as observed on the ipsilateral side, since there is no competing source of secretion (i.e., no increased vascular permeability). Thus, the increase in both of these glandular proteins appears more pronounced than on the ipsilateral side.

The duration of the bilateral secretory response was unexpectedly long, since it was anticipated that the allergen response would last about 20 to 30 minutes, similar to the immediate allergic reaction to the skin. We are unaware of previous studies in which nasal secretions have been collected and analyzed for both vascular and glandular proteins at frequent intervals within the first hour after a single allergen challenge. In fact, many published nasal allergen-provocation studies use repetitive allergen challenges, with up to three to four allergen doses administered at 10- to 15-minute intervals. These data presented here suggest that a single dose of allergen produces a dynamic and prolonged nasal secretion. As a consequence, it might be expected that the effects of a single dose of allergen would continue to affect the nasal mucosa at the time a second challenge is administered 10 to 15 minutes later. Therefore, in studies in which frequent and repetitive doses of allergen are administered, caution may be necessary when the responses are interpreted.

Although a few subjects noted mild nasal congestion and irritability within the first 3 hours after allergen challenge, none manifested the typical symptoms and signs of an LPR later than the 3-hour time point. Although LPRs have been produced in some nasal challenge studies, the frequency of the development of LPRs after allergen challenge is not well reported. It should be noted that, in this study, the total dose of allergen was smaller (about 300 PNU) than the total dose used in many other studies (typically, 1000 PNU or larger). In addition, the exposure time of the allergen to the nasal mucosa was short (3 minutes) compared to the 10- to 15-minute exposures used in other studies. Nevertheless, the allergen dose and exposure time used here clearly induced an immediate allergic reaction in the nose.

Thus, these data suggest that the nasal protein secretion produced during nasal allergen provocation reflects a predominance of localized increased vascular permeability, with a much smaller component of reflex glandular secretion. Since several of the mast cell mediators (e.g., histamine, prostaglandins, and leukotrienes) are potent vasoactive agents, it appears that this vascular response may be one of the principal responses occurring in AR. The glandular secretion may be due to reflexes initiated by histamine (or other mediators with irritant properties) and is believed to

involve a muscarinically mediated secretion. Based on these data, the use of agents capable of modulating vascular permeability should prove to be useful in reducing both the secretion and congestion in AR.

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