Relationship between histamine and physiological changes during the early response to nasal antigen provocation

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Baroody, Fuad M., Stephen Ford, David Proud, Anne Kagey-Sobotka, Lawrence Lichtenstein, and Robert M. Naclerio. Relationship between histamine and physiological changes during the early response to nasal antigen provocation. J. Appl. Physiol. 86(2): 659–668, 1999.—To investigate the temporal relationships of mediator release and physiological changes during the early response to allergen, we challenged allergic individuals intranasally with antigen and followed their responses. This was done by using small filter paper disks to challenge one nostril and collect secretions from both the challenged and the contralateral nostril, thus enabling us to evaluate the nasonasal reflex. There was a significant increase in sneezing after allergen challenge that peaked within 2 min and returned to baseline. The weights of nasal secretions as well as nasal symptoms increased immediately and remained significantly elevated for 20 min in both nostrils. Nasal airway resistance increased slowly, reaching its peak at ~6 min after challenge on the ipsilateral side, but it did not change on the contralateral side. Histamine levels peaked 30 s after removal of the allergen disk on the side of challenge, whereas albumin levels peaked after those of histamine. Lactoferrin paralleled the increase in secretion weights and occurred in both nostrils. Increasing doses of antigen produced dose-dependent increases in all parameters, whereas control challenges produced no response. These studies describe a human model for the evaluation of the allergic response that is capable of simultaneously measuring mediator release and the physiological response, including the nasonasal reflex. This model should prove useful in studying the mechanism of allergic rhinitis in humans.

ipsilateral; contralateral; lactoferrin; albumin

ALLERGIC RHINITIS affects ~20% of Americans, and some evidence suggests an increasing prevalence of the disease (1, 6). Although there are effective treatments for the disease, many issues remain to be resolved with regard to its pathophysiology that could lead to even more effective therapy.

To study the pathophysiology of allergic rhinitis, we have investigated the relationship between antigen exposure and the physiological changes that lead to the development of symptoms. We focused our efforts on the human nose because it represents the relevant organ in the relevant species. In contrast to ex vivo studies that isolate single organs or cells, our studies challenge the organ in vivo and study the resulting response that represents the net result of interactions of all its individual components such as glands, vessels, and cells. With use of a model of nasal provocation, we can control the dose and timing of allergen administration in relation to the resulting responses. The resultant responses, subjective and objective, are also monitored accurately, thereby allowing us to evaluate the nasal allergic reaction in a controlled setting. The safety of these provocation techniques has been established in numerous published studies (5, 11, 13).

The involvement of inflammatory mediators, such as histamine, in the response to natural and induced allergen exposure has been well documented. We were the first to use nasal lavage combined with nasal provocation to show the appearance of histamine in nasal secretions during the early reaction (11). Our observations combined with studies of nasal provocation with histamine and studies showing the efficacy of H1 antihistamines strongly supported a role for this mediator in the allergic reaction (10, 12).

Despite its tremendous usefulness in describing nasal pathophysiological mechanisms, the technique of nasal lavage has some limitations. Repeated lavages interfere with the reporting of subjective nasal congestion and its objective counterpart, the measurement of nasal airway resistance (NAR). In some experiments, decongestants are used to facilitate lavage, and this further interferes with the measurement of NAR. Nasal lavage also dilutes nasal secretions to an unknown degree and interferes with the subjective sensation of rhinorrhea. Because of the unknown dilution, the exact volume of generated secretions and the concentration of a specific mediator in nasal secretions cannot be measured accurately. Furthermore, because nasal lavage samples both nasal cavities and the nasopharynx, it is difficult to localize the source of mediators with use of this technique. Additionally, one cannot challenge one nostril and monitor responses on the opposite side to evaluate neuronal contributions to the nasal response, such as the nasonasal reflex. To overcome these limitations, we modified an existing localized challenge technique (7) and used it to study the kinetics of histamine release and physiological changes during the early allergic reaction. The results clarify the role of histamine in the early allergic reaction and provide a framework for the evaluation of other mediators.

METHODS

Subjects. A total of 18 volunteers participated in the different studies. The 14 allergic subjects (8 men and 6
informed consent before each experiment. The Joint Committee on Clinical Investigation of the Johns Hopkins Medical Institutions gave approval for the studies, and each patient gave informed consent before each experiment.

Study design. We performed a series of studies. In part I, we studied the kinetics of histamine release and physiological changes in both nostrils of nine allergic subjects outside of their allergy season, in response to challenge of a single nostril with the diluent for the antigen extract: followed by 333 PNU of either grass or ragweed extracts. The following parameters were measured every 2 min for 20 min after antigen challenge: sneezes, weights of generated secretions, and levels of histamine in collected nasal secretions. Secretion weights and histamine levels were measured on both sides of the nasal cavity after challenge of one cavity, to investigate the nasonal reflex. The small volume of secretions collected precluded the simultaneous measurement of multiple mediators in nasal secretions. The same subjects underwent the exact same challenge procedure at least 2 wk later, and NAR was measured in both nasal cavities by using anterior rhinomanometry.

In part II, we used a similar challenge protocol to investigate the release of lactoferrin, a glandular marker, in nasal secretions in seven allergic subjects outside of their allergy season. Parameters were monitored on both the side of challenge and the contralateral nostril, every 2 min for 20 min, after challenge with 333 PNU of allergen extract. We measured sneezes, symptoms, weight of generated secretions, and levels of lactoferrin in the secretions. Three nonallergic subjects were challenged by using the same protocol and served as negative controls.

In part III, on the basis of the kinetics of the single challenge, we developed a dose-response relationship with three increasing doses of antigen, each preceded by a diluent challenge, and measured parameters every 2 min for a total of 10 min in nine allergic subjects outside of their allergy season. In these experiments, we only recorded responses in the challenged nostril. We measured sneezes, secretion weights, symptom scores, NAR, and levels of histamine and albumin in the secretions. We then performed control experiments by using the same protocol to challenge four nonallergic subjects with antigen and three allergic subjects with diluent for the antigen extracts.

Materials. Ragweed and mixed grass extracts (timothy, june, meadow fescue, and orchard) as well as the diluent for the antigen extracts (sterile phenol-buffered saline) were purchased from Greer Laboratories (Lenoir, NC). Antigen extracts were diluted with PBS to make solutions of 66, 666, and 6,666 PNU/ml concentration. Fifty microliters of these solutions were applied to filter paper disks that were used for challenges and for 30 s when generated secretions were collected. These times were selected on the basis of preliminary experiments in which histamine and methacholine were used (3).

In part I, experiments were started by recording baseline sneezes and secretion weights (from both nostrils). On the basis of prior experience with lavage techniques showing that nasal lavages with 10 ml of 0.9% sodium chloride USP from Kendall McGaw Laboratories, Irvine, CA) to bring levels of preexisting mediators to a stable baseline. Five minutes after the lavages, a second baseline measurement of sneezes and bilateral secretion weights was obtained. After another 5-min interval, a sham challenge was performed by applying a disk with 50 µl of the diluent for the antigen extract on the left nasal septum for 1 min. Thirty seconds after removal of the challenge disk, a preweighed collection disk was placed at the same location in both nostrils for 30 s. Sneezes were recorded for 5 min after diluent challenge. A disk with 333 PNU of either grass or ragweed extract was then placed on the left nasal septum for 1 min. Thirty seconds after removal of the challenge disk, secretions were collected from both sides of the nasal septum simultaneously for 30 s by applying filter paper disks on the anterior portion of the nasal septum. Six simultaneous collections were performed at 60-s intervals. After the last collection, the challenge disk was removed, and the collection disk was placed 50 µl of the diluent for the antigen extract on the right nasal septum for 1 min. Thirty seconds after removal of the challenge disk, the collection disk was placed on the right nasal septum for 30 s. After another 5-min interval, a second challenge was performed.

Challenges in part I were started by recording baseline sneezes and secretion weights (from both nostrils). The challenge protocol was identical to the one in part I, except that the allergen extract used for the challenges was 3, 33, and 333 PNU, respectively. Antigen solutions were kept refrigerated at 4°C between challenges, thus allowing the same antigen solutions to be used throughout the same series of experiments. The filter paper disks used for the challenges and the collection of nasal secretions were made from Shandon filter cards (Shandon, Pittsburgh, PA) by using an 8-mm hole puncher. They were 1.2 mm in thickness and held 50 µl of solution. The collection disks were placed in 1.5-ml microtubes, and the disk-tube combination was weighed by using a Mettler AE 240 electronic balance before and after secretion collection. The weight of secretions generated after each challenge could thus be calculated. Each of two microtube-disk combinations was weighed 10 times, yielding a SD of 0.05 mg. NAR was measured by anterior rhinomanometry by using an A240 digital rhinomanometer (Allergopharma).

Challenge protocol. All challenges and collection of nasal secretions were performed by applying filter paper disks to the middle portion of the anterior third of the nasal septum, just posterior to the mucocutaneous junction. This was done under direct vision by using a headlight, nasal speculum, and duckbill forceps. The left nostril was always challenged. The disks were applied for 1 min for challenges and for 30 s when generated secretions were collected. These times were selected on the basis of preliminary experiments in which histamine and methacholine were used (3).

In part II, experiments were started by recording baseline sneezes and secretion weights (from both nostrils). On the basis of prior experience with lavage techniques showing that nasal lavages with 10 ml (5 ml in each nostril) of warm (37°C) saline solution (0.9% sodium chloride USP from Kendall McGaw Laboratories, Irvine, CA) to bring levels of preexisting mediators to a stable baseline. Five minutes after the lavages, a second baseline measurement of sneezes and bilateral secretion weights was obtained. After another 5-min interval, a sham challenge was performed by applying a disk with 50 µl of the diluent for the antigen extract on the left nasal septum for 1 min. Thirty seconds after removal of the challenge disk, a preweighed collection disk was placed at the same location in both nostrils for 30 s. Sneezes were recorded for 5 min after diluent challenge. A disk with 333 PNU of either grass or ragweed extract was then placed on the left nasal septum for 1 min. Thirty seconds after removal of the challenge disk, secretions were collected from both sides of the nasal septum simultaneously for 30 s by applying filter paper disks on the anterior portion of the nasal septum. Six simultaneous collections were performed at 60-s intervals. After the last collection, the challenge disk was removed, and the collection disk was placed on the right nasal septum for 30 s. After another 5-min interval, a second challenge was performed.
lavages, challenge with diluent followed by challenge with 333 PNU of grass or ragweed extract. We measured responses in both nostrils in response to challenge of the left nasal cavity. In addition to sneezes and secretion weights, we eluted secretions for the measurement of lactoferrin and asked the subjects to score their symptoms of runny, stuffy, and itchy nose in each nostril separately by using the following scale: 0 = no symptoms, 1 = mild, 2 = moderate, and 3 = severe. All parameters were recorded at 2-min intervals after allergen challenge except lactoferrin levels, which were measured at 30 s after challenge and then every 4 min thereafter.

In part III, a baseline recording was obtained followed by five saline nasal lavages (5 ml in each nostril) and another baseline measurement after 5 min. Five minutes after the second baseline, 50 µl of the diluent for the allergen extracts were applied to the left nasal septum for 1 min. Thirty seconds after removal, a collection disk was applied to the same location on the left septum to collect nasal secretions for 30 s. Five minutes later, 50 µl containing 3 PNU of allergen extract were applied to a filter paper disk and used to challenge the septum for 1 min. Thirty seconds after removal of the challenge disk, a collection disk was applied to the same location of the nasal septum for 30 s followed by serial disks at 2-min intervals for a total of 10 min. Therefore, six collection points were available for the allergen challenge. Five minutes after the last collection, a second diluent challenge was performed followed by the next higher dose of allergen (33 PNU), and the same sequence of collections was performed. A third diluent challenge was followed by challenge with 333 PNU of allergen extract. The parameters recorded were secretion weights and the secretions were eluted for the measurement of histamine and albumin. After each diluent challenge and at the end of each series of consecutive collections after allergen challenge, the patients were asked to score their symptoms by using the same scoring system detailed above, and NAR was measured by using anterior rhinometry after the subject was asked to blow their noses to clear accumulated secretions. Sneezes were also counted during the 5- and 10-min intervals after diluent and allergen challenges respectively.

Elution of nasal secretions. After the collection disk-microtube combination was weighed and the weight of secretions was calculated, PBS was used for elution of albumin, histamine, and lactoferrin. Secretions were allowed to elute in 300 µl of PBS for 24 h at 4°C for the measurement of histamine and albumin and in 250 µl of PBS for 30 min for the measurement of lactoferrin.

After the concentration of the mediator in the eluate was obtained, and because the volume of eluate and that of generated nasal secretions were known, it was possible to calculate the total amount of the mediator recovered, and these data are reported. For example, if the concentration of histamine (ng/ml) obtained from the radioenzymatic assay is referred to as X and the volume of eluate used in the experiments is referred to as Y, then to convert the value of histamine obtained from the assay to total histamine (Z) during each specific time point we used the following formula: Z (ng) = X (ng/ml) × Y (µl) × secretion weight (mg)/1,000 (µl/ml). To perform these calculations, we assumed that 1 mg of secretions collected is equivalent to 1 µl of secretions, because 95% of mucus is composed of water, which has a specific density of 1 kgl. Recovery of histamine and albumin from filter paper disks was evaluated by adding known amounts of the two substances to filter paper disks and eluting them in 300 µl of buffer for 24 h at 4°C (2). The eluate was then assayed for histamine and albumin and compared with values obtained by adding similar amounts of standard histamine and albumin to eluate without disks. Recovery of histamine from the disks into the eluate ranged from 75 to 97% and that of albumin from 62 to 81%, suggesting good recovery of the mediators from the disks. Recovery experiments with lactoferrin produced recovery rates ranging from 53 to 65%, with the optimal elution time for lactoferrin being 30 min.

Mediator assays. Histamine was measured by using a radioenzymatic assay sensitive to 250 pg/ml (4). Each sample was assayed in triplicate. Levels of histamine below the detection limit of the assay were arbitrarily assigned a value of 0.1 ng/ml. Lactoferrin and albumin were measured with noncompetitive ELISAs that used a modification of a previously published method (15). The lowest limits of detection for both these assays were 1 ng/ml, and levels of albumin or lactoferrin in nasal secretions that were below these limits were arbitrarily assigned values of 0.5 ng/ml. All samples from the same individual during one experiment were run in the same assay to limit interassay variability.

Statistics. Because the data was not normally distributed, nonparametric statistics were used. The data are depicted as medians with 25th and 75th percentiles or as individual data to provide an appreciation of the variability between subjects. Each set of repeated measurements was analyzed by Friedman one-way ANOVA. If overall significance was found, individual points within the same curve were analyzed by using the Wilcoxon matched-pairs signed-ranks test. When comparing allergic subjects challenged with allergen to the control experiments (allergic subjects challenged with diluent or nonallergic subjects challenged with allergen), we computed the total change from diluent challenge provoked by the active challenge by summing the changes from diluent challenge for each of the time points after the active challenge. Comparison of total change for allergen vs. control challenges was then undertaken by using Wilcoxon matched-pairs signed-ranks test for paired data and Mann-Whitney U-test for nonpaired observations. If more than two groups were compared (e.g., allergic subjects after allergen challenge, allergic subjects after diluent challenge, and nonallergic subjects after allergen challenge), ANOVA was first performed (Friedman for paired data and Kruskall-Wallis for nonpaired data). The statistical tests were performed by using a Macintosh computer (Apple Computer, Cupertino, CA) and Statview II statistical software (Abacus Concepts, Berkeley, CA).

RESULTS

After having determined that histamine could be eluted from the disks, we selected nine allergic individuals and challenged them with 333 PNU of either ragweed or grass antigen (part I). There was an immediate increase in sneezes in the 30-s period that followed removal of the challenge antigen (P = 0.03; Fig 1). Secretion weights were measured on both sides of challenge. Compared with diluent, there was a significant increase in ipsilateral secretion weights at the first collection time point and for several time points after challenge (P < 0.05; Fig 1). Contralateral secretion weights were also increased significantly at all time points after allergen challenge compared with diluent (P < 0.05; Fig 1). The secretions generated on the contralateral side of challenge were less than those obtained at the site of allergen challenge, with significant differences between the two sides after diluent and at 30 s and 2, 4, 10, 12, 14, 18, and 20 min after allergen challenge.
Ipsilateral histamine levels increased significantly compared with diluent at 30 s and 2 min after allergen challenge ($P \leq 0.01$) and were not significantly different from levels after diluent challenge at the rest of the sampled time points (Fig. 1). Contralateral levels of histamine did not show any statistically significant changes over the entire sampling period (ANOVA, $P = 0.1$). When levels of histamine were compared between the ipsilateral and contralateral sides of challenge, there were significant differences at the 30 s and 2-, 4-, 6-, 12-, and 20-min time points, with the ipsilateral levels being higher than the contralateral levels ($P \leq 0.05$). NAR increased significantly in the challenged nostril at all time points starting at 2 min ($P \leq 0.01$ vs. diluent), with mean peak nasal congestion occurring 6 min after challenge (Fig. 1). There were no significant increases in NAR in the contralateral nostril (ANOVA, $P = 0.4$) and no significant differences between the NAR of the ipsilateral and contralateral nostrils.

When examining the timing of occurrence of the peak physiological responses in the individual subjects, we found that histamine levels almost always peaked within the first 2 min after provocation (Table 1), which suggests immediate release of this preformed mediator after allergen deposition on the nasal mucosa. The sneezing and ipsilateral secretory responses also seemed to occur relatively early after allergen provocation, but the increase in NAR on the side of challenge occurred consistently at later time points ranging from 6 to 20 min after provocation.

In part II, as can be seen from Fig. 2, allergic subjects had a significant increase in the number of sneezes compared with diluent 30 s after allergen challenge ($P < 0.05$) with a return to baseline at the other time points. Total symptom score represents the sum of scores for runny, stuffy, and itchy nose in either nostril, and this showed a significant increase after allergen on both sides of challenge at all recording time points compared with the diluent score ($P < 0.05$). Symptoms on the challenged nostril were higher than those on the contralateral side, with significant differences between the two nostrils 30 s and 2 min after challenge ($P < 0.05$). The weight of generated secretions was increased significantly compared with diluent at almost all time points after challenge in both nostrils ($P < 0.05$) with no significant differences between the two. There were significant increases over diluent in bilateral lactoferrin levels at 30 s and 4 min after allergen challenge ($P < 0.05$) and in contralateral lactoferrin levels at the

![Fig. 1. Time course of ipsilateral and contralateral changes in the minutes after ipsilateral nasal provocation with 333 protein nitrogen units (PNU) of allergen. Data are medians with 25th and 75th percentiles of 9 subjects, and parameter depicted is specified at top of each graph. Protocol is shown on x-axis. B1 and B2, baselines; Dil, response after diluent challenge; 0.5–20, time in minutes after removal of allergen challenge disk from nasal septum. *$P \leq 0.05$ vs. diluent. †$P \leq 0.05$ contralateral vs. ipsilateral. **$P \leq 0.01$ vs. diluent.

(P $\leq 0.05$). Consistent with previous observations, there were measurable levels of histamine in eluted secretions at the first baseline in both nostrils. The series of five 10-ml saline lavages significantly reduced these levels of histamine bilaterally ($P < 0.05$; Fig. 1).

Table 1. Respective time points at which each of the response parameters first peaked after allergen challenge in each of the subjects studied

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
<th>Patient 5</th>
<th>Patient 6</th>
<th>Patient 7</th>
<th>Patient 8</th>
<th>Patient 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histamine</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>2</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>2</td>
</tr>
<tr>
<td>Sneezes</td>
<td>0.5</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>18</td>
<td>0.5</td>
<td>6</td>
<td>0.5</td>
<td>2</td>
</tr>
<tr>
<td>Ipsi NAR</td>
<td>14</td>
<td>6</td>
<td>6</td>
<td>14</td>
<td>12</td>
<td>14</td>
<td>6</td>
<td>20</td>
<td>14</td>
</tr>
<tr>
<td>Ipsi SW</td>
<td>2</td>
<td>4</td>
<td>0.5</td>
<td>2</td>
<td>2</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Contra SW</td>
<td>0.5</td>
<td>6</td>
<td>18</td>
<td>2</td>
<td>0.5</td>
<td>6</td>
<td>0.5</td>
<td>0.5</td>
<td>4</td>
</tr>
</tbody>
</table>

Values are in minutes. Ipsi, ipsilateral to challenge; Contra, contralateral to challenge; SW, secretion weights; NAR, nasal airway resistance. Patient 8 did not have any sneezes after allergen challenge.
20-min time point. There were no significant differences between ipsilateral and contralateral lactoferrin levels.

Total change over diluent challenge was then compared for all response parameters between allergic \((n = 7)\) and nonallergic \((n = 3)\) subjects challenged with allergen. There were more sneezes in the allergic subjects challenged with allergen than in the nonallergic subjects \((P = 0.07)\). The same held true for ipsilateral total symptom scores \((P = 0.07; \text{Fig. 3})\). When comparing ipsilateral secretion weights and lactoferrin levels as well as contralateral symptoms, secretion weights, and lactoferrin levels, we found that allergic subjects had a significantly higher response than did nonallergic subjects \((P < 0.02)\) (Fig. 3).

In part III, as can be seen in Fig. 4, there were no sneezes at baseline or after the diluent challenges, but a dose-dependent increase occurred in the number of sneezes 30 s after challenge with 33 and 333 PNU of allergen \((\text{ANOVA, } P = 0.0001 \text{ and } P < 0.03 \text{ vs. respective diluents})\). Levels of histamine at the 30-s time point after challenge with 3 and 33 PNU of allergen increased significantly compared with the respective diluents \((P < 0.05)\) and were significantly increased compared with the third diluent challenge at the 30-s and 2-, 4-, 6-, and 10-min time points \((P < 0.05; \text{Fig. 4})\). When the three diluents were compared, there was a significant difference \((P = 0.003)\), with the third diluent being significantly lower than the first and second diluents \((P \leq 0.03)\). When levels of albumin were analyzed, there were significant overall differences between measurements at the different time points \((\text{ANOVA, } P = 0.0001)\) as well as significant differences between levels of albumin after the different diluent challenges \((\text{ANOVA, } P = 0.04)\), with the level in response to the diluent that preceded the 333-PNU challenge being higher than the level that preceded the 33-PNU challenge \((P = 0.01)\). There were significant increases in albumin levels after all antigen challenges at most time points compared with diluent as seen in Fig. 4 \((P < 0.04)\). Significantly increased levels of albumin were sustained for longer time periods than were levels of histamine and tended to peak at the 2- and 4-min time points with some lag compared, with peaks of histamine after challenge. There were significant increases in secretion weights after the allergen challenges compared with diluent with the higher doses of allergen leading to the more sustained temporal increases in secretions as seen in Fig. 4 \((\text{ANOVA, } P = 0.0001 \text{ and } P < 0.05 \text{ vs. respective diluents})\). Total symptom scores were measured at the end of each series of challenges and were very low at baseline with significant dose-dependent increases after all doses of allergen compared with the respective diluents \((\text{ANOVA: } P = 0.0001 \text{ and } P < 0.05 \text{ vs. respective diluents})\). There was also a dose-dependent increase in NAR after the allergen challenges compared with respective diluents with the highest resistance after 333 PNU of allergen \((\text{ANOVA, } P = 0.0001 \text{ and } P < 0.04 \text{ vs. respective diluents})\). When the total change over diluent challenge was calculated for all three antigen doses used, there was a dose-dependent increase in all response parameters, with most reaching statistical significance \((\text{Table 2})\). The responses at the higher doses of antigen \((33 \text{ and } 333 \text{ PNU})\) were significantly higher than those at the lower dose \((3 \text{ PNU})\) \((\text{Table 2})\).

As control, we used the same protocol to challenge three allergic subjects with diluent and four nonallergic subjects with antigen. There were no significant changes in any of the parameters measured in these experiments. Total change from diluent challenge at each of the three antigen doses was compared among the three groups. As can be seen from Fig. 5, total change from diluent was negligible or even negative (responses at the time points after challenge were lower than the diluent responses) for most of the parameters in the control challenges, and, in most instances, the total
change for the parameters after allergen challenge in the allergic subjects was significantly higher than in the other two groups ($P < 0.05$). Of note are total symptoms after diluent challenge in allergic subjects, which seemed elevated, but these changes did not reach statistical significance compared with respective diluents.

**DISCUSSION**

Cross-linking of IgE antibodies by antigen in allergic subjects causes mast cells to degranulate, releasing histamine into the surrounding tissue. Our model characterizes the relationship between histamine release and changes in other response parameters during the early reaction to nasal provocation with antigen. Histamine can activate nerves to initiate sneezing and reflex parasympathetic stimulation of glands (3). Our model shows a temporal association between histamine release and glandular stimulation as indicated by secretion weights and changes in the level of lactoferrin, a product of serous cells within the submucus glands. Histamine also increases vascular permeability by its action on H1 receptors on blood vessels. The finding that the peak of histamine release preceded the peak of albumin, a marker of increased vascular permeability, demonstrates the power of our model to resolve physiological events while simultaneously measuring mediator release. In similar kinetic experiments, we have shown the release of PGD2 at the site of allergen provocation, with peak levels occurring at the 2-min time point (16). This again shows the ability of our model to temporally separate physiological responses to allergen challenge because the peak of release of the preformed mediator, histamine, preceded the appearance of the newly synthesized mediator, PGD2. Although histamine provocation can cause an increase in
NAR, our finding of a lack of temporal association between histamine release and NAR is consistent with the lack of efficacy of H1 antihistamines in the treatment of nasal congestion and suggests a role for other mediators in this important physiological response to allergen provocation. Thus, whereas treatment with 60 mg of terfenadine, an H1 antihistamine, reduced sneezing and secretion weights, it had no effect on increases in NAR after nasal provocation with antigen (17). The ipsilateral increase in histamine observed in our experiments concurs with the findings of Malmberg et al. (9) and supports the release of this mediator by mast cells at the site of antigen stimulation only.

Because of the ease of repeated sampling, our model allowed us to extend previously described localized challenge techniques by recording the kinetics of the early response to allergen. Despite its tremendous usefulness, this technique has limitations. In contrast to nasal lavage, the small volume of secretions collected limits the number of mediators that can be assayed at each time point. Consequently successive experiments need to be performed to measure multiple mediators. In future experiments, knowing the kinetics of release may permit the use of different disks for the measurement of different inflammatory products during the same challenge. Furthermore, extremely sensitive assays need to be employed to accurately measure the levels of mediators after their elution from the disks. The technique also requires preliminary experiments to prove the reliability of recovering mediators from the disks.

There are currently three nasal challenge techniques that are most commonly used by investigators to study nasal physiology and pathophysiology. The nasal lavage technique as described by Naclerio and colleagues (11) uses lavage of the nasal cavities with known amounts of physiological solutions to monitor responses after different challenges. The advantages of this technique are that it allows the simultaneous measurement of multiple mediators as well as the sampling of the cellular constituents of nasal secretions. Its disadvantages are the inability to obtain an accurate estimate of the weight of nasal secretions produced in response to challenge due to dilution of these secretions by lavage; the inability to measure NAR after challenge secondary to the need for decongestion before challenge; the fact that repeated lavages interfere with NAR measurements; and the inability to investigate the nasonasal reflex because nasal lavage samples both nasal cavities and the nasopharynx simultaneously. Another nasal challenge technique is that described by Raphael and colleagues (14, 15) that utilizes an aspiration-nasal lavage technique during which a catheter is inserted into the nasal cavity and used to aspirate lavage fluid that is administered by aerosol spray. The advantages of this technique are

<table>
<thead>
<tr>
<th>Parameter/Dose</th>
<th>3 PNU</th>
<th>33 PNU</th>
<th>333 PNU</th>
<th>P Value (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sneezes, no.</td>
<td>0 (0–0)</td>
<td>2 (1–3)*</td>
<td>6 (4–8)*</td>
<td>0.0004</td>
</tr>
<tr>
<td>Histamine, ng</td>
<td>0 (–0.7–0.6)</td>
<td>0.7 (0.2–2.2)†</td>
<td>2.8 (1.2–3.6)*</td>
<td>0.002</td>
</tr>
<tr>
<td>Albumin, µg</td>
<td>132.6 (52.6–203.7)</td>
<td>272.0 (210.4–415.7)†</td>
<td>330.7 (227.2–1,112.2)*</td>
<td>0.02</td>
</tr>
<tr>
<td>SW, mg</td>
<td>31.5 (–7.3–82.4)</td>
<td>85 (70.5–99.7)</td>
<td>88 (61.6–94.9)</td>
<td>0.17</td>
</tr>
<tr>
<td>Symptoms</td>
<td>1 (0–2)</td>
<td>2 (1–5)</td>
<td>6 (3.9–7)‡</td>
<td>0.01</td>
</tr>
<tr>
<td>NAR, kPa·l·s⁻¹</td>
<td>0.07 (0.06–0.2)</td>
<td>0.24 (0.1–0.5)</td>
<td>0.4 (0.2–1.4)</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Values are medians, with 25th–75th percentiles in parentheses. PNU, protein nitrogen units. Symptoms: 0 = no symptoms, 1 = mild, 2 = moderate, 3 = severe. *P < 0.02 vs. 3 PNU. †P ≤ 0.05 vs. 3 PNU. ‡P < 0.05 vs. 33 PNU.
similar to those of nasal lavage, with the additional ability to sample each nostril separately and to use a recently described modification that uses urea in nasal lavages as a marker of nasal epithelial lining fluid volume (8). Inability to measure NAR changes because of the repeated lavages and the catheters in the nostrils remains a disadvantage of this technique. The third technique is described in this paper, and its advantages are the ability to study the nasonasal reflex by sampling nasal secretions from each individual nasal cavity, the ability to use smaller doses of allergen to provoke a response, objective measurement of the volume of nasal secretions produced after provocation, and the ability to measure NAR. Disadvantages include the inability to sample cells from nasal secretions after challenge, although nasal biopsies of the localized challenge site have enabled the study of mucosal cellular changes, and the inability to measure multiple mediators simultaneously. Although no nasal challenge system addresses all questions regarding the pathophysiology of rhinitis, the availability of multiple techniques increases our ability to gain further understanding of this extremely prevalent condition.

As mentioned, this technique allowed us to challenge one nasal cavity and monitor the response in both cavities, providing the opportunity to study the nasonasal reflex. Histamine is only released on the side of challenge and did not show significant increases in contralateral nasal secretions, suggesting that mast cell degranulation occurs only at the site of antigen application. On the other hand, secretion weights, symptoms, and lactoferrin increased in both nostrils after unilateral stimulation with allergen. This contralateral response is mediated by a nasonasal reflex with a parasympathetic efferent arm. We and others have previously shown secretion weights and symptom scores increasing significantly on the side contralateral to challenge (2, 9, 14). In other experiments, we showed that bilateral atropine treatment almost completely inhibited the volume of nasal secretions bilaterally.

![Fig. 5. Comparison of responses of allergic subjects challenged with antigen (n = 9; ■), allergic subjects challenged with diluent (n = 3; ●) and nonallergic subjects challenged with antigen (n = 4; ○). Data are medians with 25th and 75th percentiles of total change over respective diluent challenges for the different parameters. *P < 0.05 vs. response of allergic subjects challenged with allergen.](image-url)
after unilateral nasal challenge with antigen, strongly supporting the role of parasympathetic stimulation of glands in both the ipsilateral and contralateral secretory responses (2). The production of lactoferrin, a glandular marker, on both the side of stimulation and the contralateral nostril in the present studies further supports this conclusion. The presence of albumin in ipsilateral nasal secretions suggests a possible contribution of vascular permeability in the production of nasal secretions on the side of provocation.

Nasal secretions are derived from several sources, which include parasympathetically innervated submucosal seromucous glands, anterior serous glands, goblet cells, lacrimal glands, epithelial transport of ions, and transudation from blood vessels. From the kinetic and dose-response experiments, it seems clear that nasal secretion weight is one of the more sensitive objective parameters for monitoring the response to allergen challenge. The increase in secretion weights occurred immediately after provocation and was the most consistently increased parameter at the lower dose of allergen when multiple doses were used. There was also a dose-dependent increase in these secretions after allergen challenge, although this did not reach statistical significance, as seen in Table 2. This, however, can be explained by the limited capacity of the disks in absorbing secretions and the fact that the capacity of these disks was overwhelmed by the large amount of secretions produced at the higher doses of allergen. Raphael and colleagues (14) examined the protein content of ipsilateral and reflex secretions after unilateral antigen challenge and concluded that the predominant source of ipsilateral secretions was increased vascular permeability, whereas the virtually exclusive source of contralateral secretions was nasal glands. Their challenge model utilizing nasal lavages of separate nostrils made it impossible to quantitate the volume of nasal secretions generated. Our previous study examining the effect of atropine on a similar allergen challenge (2) and our present results illustrate the importance of the manner in which data are interpreted. The source of nasal secretions appears to be almost exclusively glandular on the contralateral side, but it is a mixture of vascular and glandular components on the ipsilateral side. The volume of secretions originating from the nasal glands on the ipsilateral side is mixed with a minimal volume originating from plasma due to vascular leakage. Because the concentration of albumin in plasma is high, the small amount of this fluid that admixes with glandular secretions leads to the marked elevations in albumin observed. This, however, does not necessarily imply that the primary source of ipsilateral secretion volume after antigen challenge is caused by increased plasma influx due to vascular permeability. On the contrary, it seems to be secondary to glandular secretion, as evidenced by marked inhibition by atropine. Along the same lines, it is unlikely that the ipsilateral increase in histamine after challenge was the result of increased secretions, because previous inhibition of secretions with atropine still resulted in significant increases in ipsilateral total levels as well as in the concentration of histamine per unit of nasal secretions (2).

In summary, we have developed a localized-challenge technique and, by using it, we were able to study the kinetics of mediator release as well as the occurrence of physiological changes in allergic subjects challenged with allergen. We have also used this technique to further establish the existence of a nasal reflex and to delineate the important components of this reflex, namely increased secretions and lactoferrin on the contralateral side of stimulation. This localized-challenge technique should help us investigate several other aspects of the pathophysiology of allergic rhinitis, such as the role of neuropeptides in the reflex response, the effects of provocation with other substances, such as capsaicin, and the kinetics of the late-phase reaction.

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