

Nasal challenge with allergen leads to maxillary sinus inflammation

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Background: Allergic rhinitis and chronic rhinosinusitis are both characterized by chronic inflammation.

Objective: We sought to investigate the effect of nasal allergen challenge on the maxillary sinus and study the effect of premedication with loratadine.

Methods: We performed a double blind, crossover, randomized, placebo-controlled study in 20 allergic subjects out of season. After treatment with either placebo or loratadine (10 mg PO daily) for 1 week, a catheter was inserted into one maxillary sinus and used to lavage the cavity. The subjects then underwent nasal challenge with diluent for the allergen extract, followed by 3 concentrations of grass or ragweed. Nasal and ipsilateral sinus lavages were performed after each challenge and then hourly for 8 hours. Sneezes and symptoms were recorded, and the lavage specimens were evaluated for eosinophils and levels of eosinophil cationic protein, albumin, and histamine. Eleven of the subjects underwent a similar challenge with lactated Ringer's solution.

Results: Compared with the lactated Ringer's solution challenge, allergen challenge resulted in significant increases in most early- and late-phase nasal parameters. Allergen challenge of the nose also led to a significant increase compared with control values in maxillary sinus eosinophils and the levels of albumin, eosinophil cationic protein, and histamine during the late response. Loratadine resulted in significant inhibition of the nasal early response compared with that seen with placebo ($P < .05$).

Conclusion: These findings suggest that a neural reflex or systemic allergic inflammation is responsible for the sinus inflammatory response and that this inflammatory response

might play a role in the development of rhinosinusitis in allergic subjects. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Allergic rhinitis, chronic rhinosinusitis, allergen challenge, eosinophils, maxillary sinus, reflex

Chronic rhinosinusitis is a common disease that affects millions of Americans and results in a large health care expenditure.¹ Furthermore, this disease has a significant negative effect on the quality of life of affected adults and children.^{2,3} Recent studies have focused on the role of mucosal inflammation rather than infection in chronic rhinosinusitis, with the prominent presence of eosinophils and T_H lymphocytes in sinus tissues.^{4,5}

A relationship between allergic rhinitis and chronic rhinosinusitis has long been suggested. This relationship has been documented through retrospective clinical reviews that show that at least half of patients presenting with rhinosinusitis also have positive skin test responses.^{6,7} Furthermore, studies also show a high prevalence of sinus disease in patients with allergic rhinitis.^{8,9} By using computed tomographic scans, sinusitis has been demonstrated in allergic patients in some studies^{10,11} but not others.¹² Although these studies are limited by referral-pattern biases and retrospective data collection, they nevertheless suggest a significant clinical association between allergic rhinitis and chronic rhinosinusitis. A mouse model of acute sinusitis and allergic rhinitis has also supported this relationship.¹³

The purpose of this study was to investigate whether allergen-induced nasal inflammation was accompanied by inflammation within the ipsilateral maxillary sinus and the effect of premedication with an H₁ antihistamine, loratadine, on this inflammation.

METHODS

Study design

Twenty healthy subjects with seasonal allergic rhinitis out of their season participated in a double-blind, randomized, placebo-controlled, crossover study to evaluate the effect of 1 week's treatment with loratadine on the nasal and sinus responses after unilateral nasal allergen challenge. After an initial screening visit, the subjects received 10 mg of oral loratadine or placebo once daily in the morning for 1 week and then presented to the laboratory and underwent nasal challenge with allergen and monitoring of the early-phase response (EPR) and late-phase response (LPR). The subjects then had a washout period of 2 weeks and were crossed over to the alternate treatment. Eleven of these 20 subjects returned for a third visit at least 2 weeks after the end of the study described above, and an identical protocol was followed with serial challenges with lactated Ringer's (LR) solution, which served as a control for the effect of the manipulations. The protocol was approved by the Institutional Review Board of the University of Chicago, and all participants read and signed the informed consent form before their participation in the study. The study was completed before mandated registration on clinicaltrials.gov.

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Abbreviations used

ECP: Eosinophil cationic protein
 EPR: Early-phase response
 LPR: Late-phase response
 LR: Lactated Ringer's

Subjects

There were 15 male and 5 female subjects with a median age of 26 years and an age range of 19 to 41 years. All subjects had symptoms of seasonal allergy for at least the previous 2 years and a positive skin prick test response to grass allergen, ragweed allergen, or both. Eleven subjects were allergic to both grass and ragweed, 7 were allergic to ragweed only, and 2 were allergic to grass only. We challenged 13 subjects with ragweed and 7 with grass extracts. When allergic to both, the challenge allergen was chosen based on the usually more symptomatic season. Subjects were otherwise healthy except for possible mild asthma controlled with as-needed bronchodilators. Women of childbearing age were using an acceptable contraceptive method and had to have a negative pregnancy test result before enrollment.

Experimental protocol

Subjects presented to the nasal laboratory and were allowed to rest for 15 minutes to allow equilibration of the nasal mucosa with the local environment. The maxillary sinus catheter was then introduced, and the sinus cavity underwent lavage with four 10-mL aliquots of warm (37°C) LR solution (Fig 1). The subjects were then observed for 4 hours to allow the body to seal the site of puncture. The nasal and maxillary sinus cavities then underwent lavage with four 10-mL aliquots of LR solution, with the first lavage specimen saved for further analysis. This sample was considered baseline. The purpose of lavage was to bring the level of mediators in both the nose and maxillary sinus to a stable baseline. Next a nasal diluent challenge was performed by spraying 2 puffs (0.17 mL) of the diluent for the allergen extracts into the nasal cavity from a metered-dose inhaler. After 10 minutes, the challenged side of the nose and the ipsilateral maxillary sinus both were lavaged with 5 mL of LR solution, and the lavage specimens were saved. The subject was then asked to record the number of sneezes and a nasal symptom score. This was followed by 3 nasal challenges with allergen, referred to as A1, A2, and A3. The concentrations of allergen used for provocation were 100, 300, and 1000 AU of grass extract or 1:2000, 1:200, and 1:20 wt/vol of ragweed extract (Hollister-Stier Laboratories LLO, Spokane, Wash). After the third antigen challenge, the subject waited in the laboratory for 1 hour. The nose and the maxillary sinus were then lavaged with 5 mL of LR solution, and nasal symptoms and the number of sneezes were recorded. This process was repeated each hour for the next 7 hours. After the last lavage specimen was recovered, the maxillary sinus catheter was removed, and the challenge ended. After the challenge ended, the subjects had blood drawn for eosinophil count.

Control experiments

To investigate the possibility that allergen sprayed into the nostril entered the ipsilateral sinus cavity, we performed control experiments, as detailed in the [Online Repository](http://www.jacionline.org) at www.jacionline.org.

Maxillary sinus catheter insertion

The maxillary sinus catheter insertion procedure has been previously described and was performed after achievement of local anesthesia.¹⁴

Nasal symptoms

The number of sneezes was counted after each challenge and for each of the hourly intervals during the LPR. Symptoms of runny nose, stuffy nose, and itchy nose were scored on a scale from 0 to 3 (0, no symptoms; 1, mild; 2, moderate; and 3, severe).

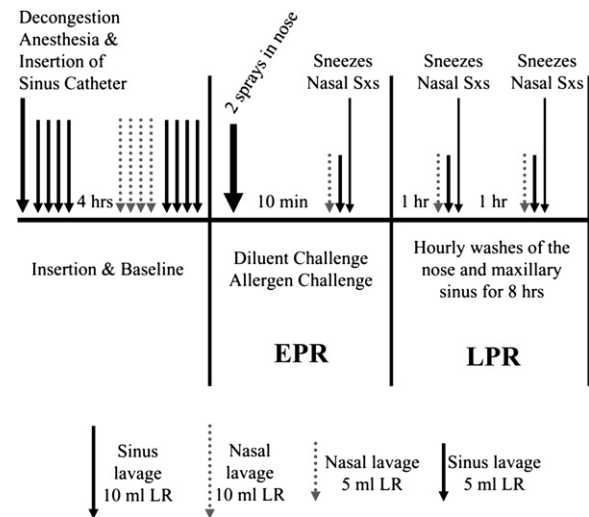


FIG 1. Challenge protocol for subjects, depicting time points of catheter insertion, sneezing and nasal symptom (Sxs) recording, and nasal and sinus lavage.

Nasal lavage

Nasal lavages were performed with warm LR solution, as previously described.¹⁵ The lavage specimens were transferred to plastic tubes and placed on ice until processing.

Eosinophil counts

Lavage specimens were processed for eosinophil identification, as detailed in the [Online Repository](http://www.jacionline.org).

Assays

Human serum albumin was measured by using an ELISA sensitive to 1 ng/mL albumin, as previously described.¹⁶ Details of the other assays are shown in the [Online Repository](http://www.jacionline.org).

Blood count

A complete blood count was performed on samples collected from 19 of 20 subjects after allergen challenge to determine the percentage of circulating eosinophils (the University of Chicago Medical Laboratories).

Statistical analysis

Friedman ANOVA was first performed within each challenge to analyze for significant differences between time points. If statistical significance was established, a *post-hoc* analysis was performed comparing the results of diluent challenge with the results of each of the antigen challenges, as well as each time point of the LPR, by using the Wilcoxon signed-rank test. To compare allergen with control challenge results and placebo with loratadine treatment effects, we calculated the net change over the diluent challenge for the EPR and LPR. For the EPR, this was calculated by subtracting the diluent response from each antigen response and then summing the resultant values. For the LPR, this was calculated by subtracting the diluent response from the responses of hours 1 through 8 and then summing the resultant values. These net changes for the EPR and LPR were then compared between allergen and control challenges by using the Mann-Whitney *U* test. A similar comparison was made between placebo and loratadine treatments by using the Wilcoxon signed-rank test. The percentage of eosinophils between blood and the nasal cavity and maxillary sinus were compared by using a Wilcoxon signed-rank test. A *P* value of less than .05 was considered significant. All statistical tests were performed with Systat 12 statistical software (Systat Software, Inc, San Jose, Calif).

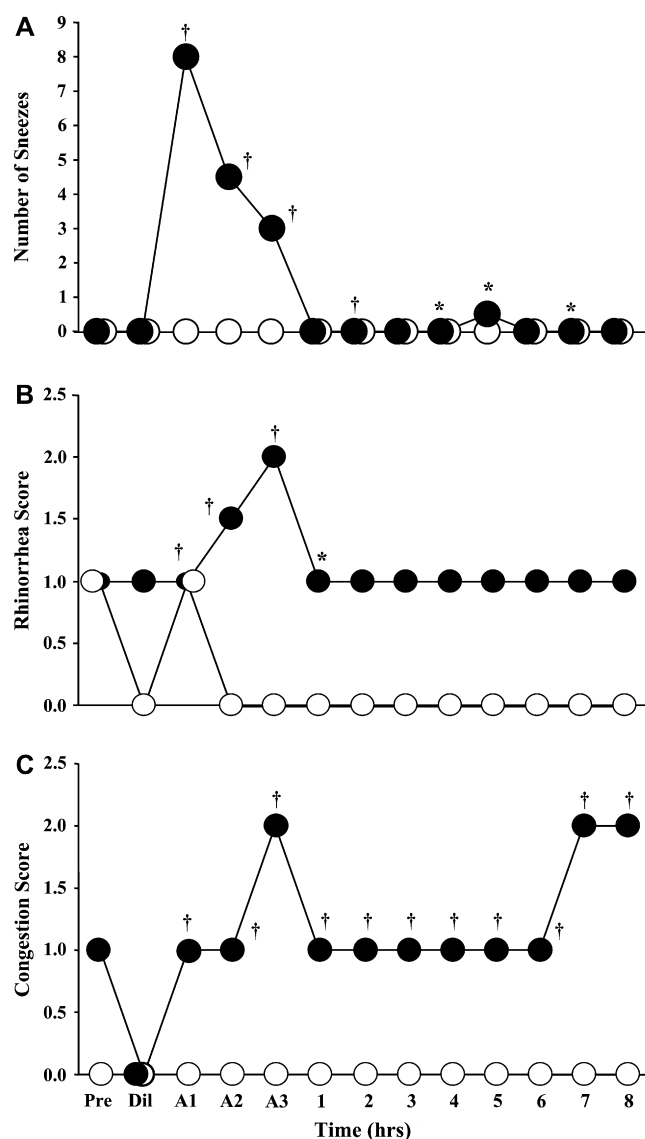


FIG 2. Median sneezes and rhinorrhea and congestion scores for allergen (solid circles, $n = 20$) and control (open circles, $n = 11$) challenges: **A**, number of sneezes; **B**, rhinorrhea score; **C**, congestion score. The protocol is on the x-axis. Pre, Baseline prewash; Dil, diluent for the allergen extract, A, allergen challenge. * $P \leq .05$ and † $P < .01$ versus respective diluent challenges.

RESULTS

Control experiments for antigen entry into the maxillary sinus

These experiments showed that allergen extract at the concentrations sprayed into the nostrils was measurable by the assay and that allergen could be detected only in nasal lavage specimens, but not in maxillary sinus lavage specimens, after administration to the nasal cavity (see the text section and Tables E1 and E2 in the Online Repository at www.jacionline.org).

Allergen versus sham challenges

Nasal symptoms. We compared the responses after placebo treatment with those after sham challenge with the LR solution control. In the control group there were no significant changes in nasal symptoms ($P > .05$, ANOVA). In contrast, there were

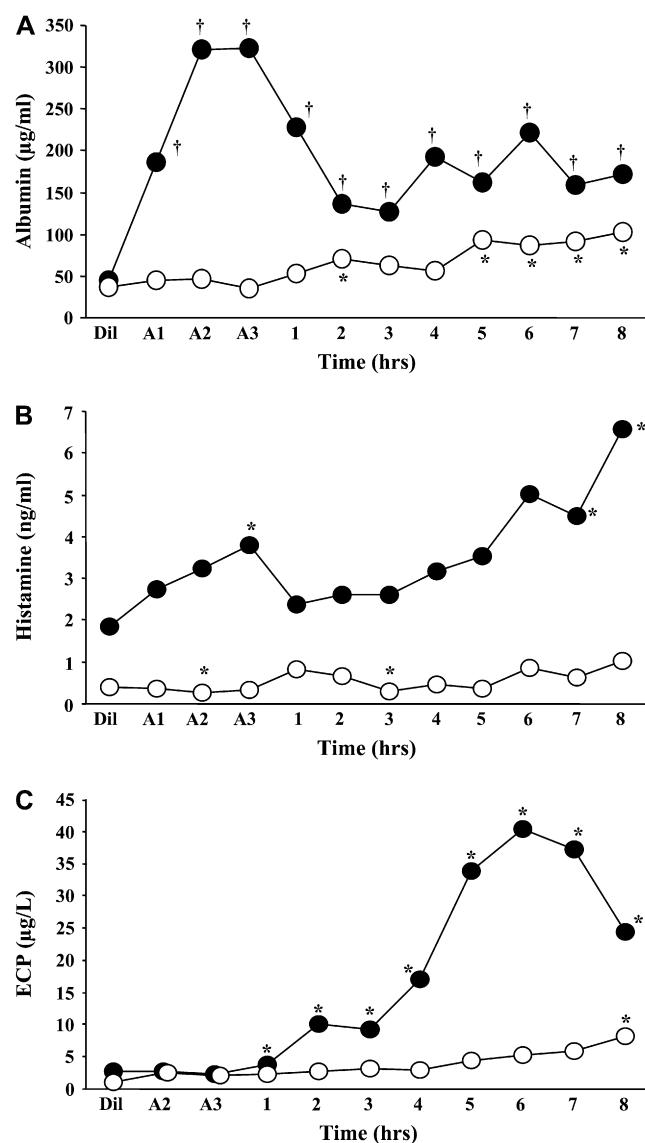


FIG 3. Median levels of mediators in the nasal cavity after allergen (solid circles, $n = 20$) and control (open circles, $n = 11$) challenges: **A**, albumin; **B**, histamine; **C**, ECP. The protocol is on the x-axis, and the labels are as in Fig 2. * $P \leq .05$ and † $P < .01$ versus respective diluent challenges.

significant overall differences in the number of sneezes and rhinorrhea scores, nasal congestion, and itching (data not shown) after the allergen challenges ($P = .0001$, ANOVA), with several time points being significantly increased over diluent response values ($P < .05$, Fig 2). We then calculated the total change from the diluent challenges for the EPR and the LPR separately and compared the control with the allergen challenges. The responses were statistically significantly higher after the allergen challenges for the EPR for all 4 nasal symptoms and for the LPR for all symptoms except for nasal itching ($P < .02$, see Table E3 in the Online Repository at www.jacionline.org).

Nasal biomarkers. After challenge with LR solution, there were significant changes in the levels of albumin, histamine, and eosinophil cationic protein (ECP) in the nasal cavity ($P \leq .02$, ANOVA), with some increases compared with levels seen after diluent challenge (Fig 3). There were also significant changes in the levels of nasal biomarkers when subjects were challenged

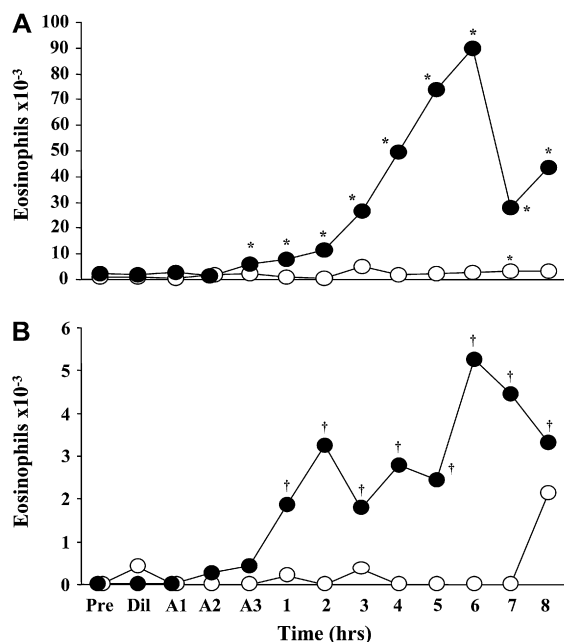


FIG 4. Median number of eosinophils in the nasal cavity and maxillary sinus after allergen (solid circles, $n = 20$) and control (open circles, $n = 11$) challenges: **A**, nasal cavity; **B**, maxillary sinus. The protocol is on the x-axis, and the labels are as in Fig 2. * $P \leq .05$ and † $P < .01$ versus respective diluent challenges.

with allergen ($P = .0001$, ANOVA), with increases at many time points during both the EPR (histamine and albumin) and the LPR (ECP, histamine, and albumin) compared with values after diluent challenge ($P \leq .05$, Fig 3). When comparing control with allergen challenges, subjects challenged with allergen had significantly higher albumin and histamine levels during the EPR ($P < .01$) and significantly higher albumin and ECP levels during the LPR ($P < .01$, see Table E4 in the Online Repository at www.jacionline.org).

Nasal eosinophils. There were significant changes in nasal eosinophil counts after both control challenge with LR solution ($P = .04$, ANOVA) and allergen challenge ($P = .0001$, ANOVA). Compared with the number of eosinophils after diluent challenge, control challenge showed a significant increase only at the 7-hour time point ($P = .009$). However, subjects challenged with allergen had significant increases during the EPR ($P \leq 0.04$) and throughout the LPR ($P \leq 0.003$, Fig 4). When comparing the total change from diluent challenge, subjects challenged with allergen had significantly higher numbers of nasal eosinophils during the LPR ($P = .001$) but not during the EPR (Table I).

Sinus biomarkers. After challenge with LR solution, there were significant changes in the levels of albumin ($P = .0001$, ANOVA) and histamine ($P = .01$, ANOVA), but not ECP, in the maxillary sinus. Compared with levels after diluent challenge, significant increases were found in the levels of albumin in the EPR and LPR, as shown in Fig 5. After allergen challenge, there were significant changes in maxillary sinus levels of albumin, ECP ($P = .0001$, ANOVA), and histamine ($P = .003$, ANOVA). Compared with diluent challenge, there were significant increases in the EPR ($P = .04$) and LPR ($P < .001$) for albumin levels, as well as significant increases during the LPR for histamine and ECP levels ($P \leq .05$, Fig 5). When comparing allergen and control challenge for the EPR and LPR, subjects challenged with allergen

had significantly higher albumin levels in the EPR ($P = .008$) and significantly higher albumin and histamine levels in the LPR ($P \leq .05$, see Table E5 in the Online Repository at www.jacionline.org). Although ECP levels were higher during the LPR in the allergen-challenged group, the changes did not reach statistical significance.

Sinus eosinophils. After control challenge, there was no significant change in eosinophil counts in the maxillary sinus ($P > .05$, ANOVA). In contrast, after nasal challenge with allergen, there was a significant change in maxillary sinus eosinophil counts ($P < .0001$, ANOVA), with significant increases in eosinophil counts in the maxillary sinus at all time points during the LPR compared with levels after diluent challenge ($P \leq .01$, Fig 4). When comparing the allergen and control groups for the EPR and LPR, subjects challenged with allergen had a significantly higher number of maxillary sinus eosinophils during the LPR ($P < .05$, Table I). Please note that the magnitude of the sinus cellular and biomarker response was almost 10-fold lower than that seen in the nasal cavity.

Circulating eosinophils. Complete blood counts were performed on 19 of 20 subjects after allergen challenge. The peak percentage of eosinophils recorded during the LPR was 49.5% for the nasal cavity and 31.2% for the maxillary sinus, both of which were significantly higher than the median percentage of circulating eosinophils (4%; $P = .0001$ and $.0006$, respectively; Fig 6). Also, there was a statistically significantly higher percentage of eosinophils in the nasal cavity when compared with that in the maxillary sinus ($P = .01$).

Effect of loratadine treatment

Nasal responses. When comparing the total change from diluent for the EPR, treatment with loratadine resulted in a significant reduction of sneezing ($P < .001$) and levels of albumin in nasal lavage specimens ($P = .004$) but had no significant effects on other symptoms, mediators, or eosinophils (see Table E6 in the Online Repository at www.jacionline.org). Loratadine had no significant effects on any LPR parameter (see Table E6).

Sinus responses. When the total changes from the diluent response for the EPR were compared between loratadine and placebo, there were no significant effects of loratadine on albumin or histamine levels or total eosinophil counts (see Table E6). Treatment with loratadine led to a significant increase in the levels of ECP during the EPR in the sinus ($P = .03$, see Table E6). Loratadine had no significant effects on any of the sinus LPR parameters (see Table E6).

DISCUSSION

Our data show that in addition to the previously reported early and late inflammatory changes that occur in the nose after allergen provocation, there was an increase in levels of histamine and albumin and numbers of eosinophils in lavage specimens of the ipsilateral maxillary sinus. The magnitude of the maxillary sinus response was smaller than that in the nasal cavity but was nonetheless significantly greater than the response obtained after the diluent challenge and the non-allergen (LR solution)-challenged controls. Furthermore, the percentage of eosinophils found in the maxillary sinus was significantly greater than that found in the circulation. Although there is a small amount of bleeding initiated by the insertion of the maxillary sinus catheter,

TABLE I. Eosinophils ($\times 10^{-3}$) in nasal and sinus cavities

	EPR Allergen	EPR Control	LPR Allergen	LPR Control
Nasal	1.2 (−27.9 to 147.9)	2.6 (−2.8 to 183.3)	702.5 (19.9 to 3673)*	15.4 (02.1 to 707.0)
Sinus	1.2 (−13.1 to 111.7)	0 (−77.1 to 40.5)	24.9 (−1.9 to 973.8)*	1.8 (−176.6 to 163.2)

Values represent the total change over diluent challenge for either EPR or LPR after either antigen or control challenges. Data are depicted as medians (ranges) for 20 patients for antigen challenge and 11 patients for control challenge.

* $P \leq .02$ versus respective control LR solution challenge.

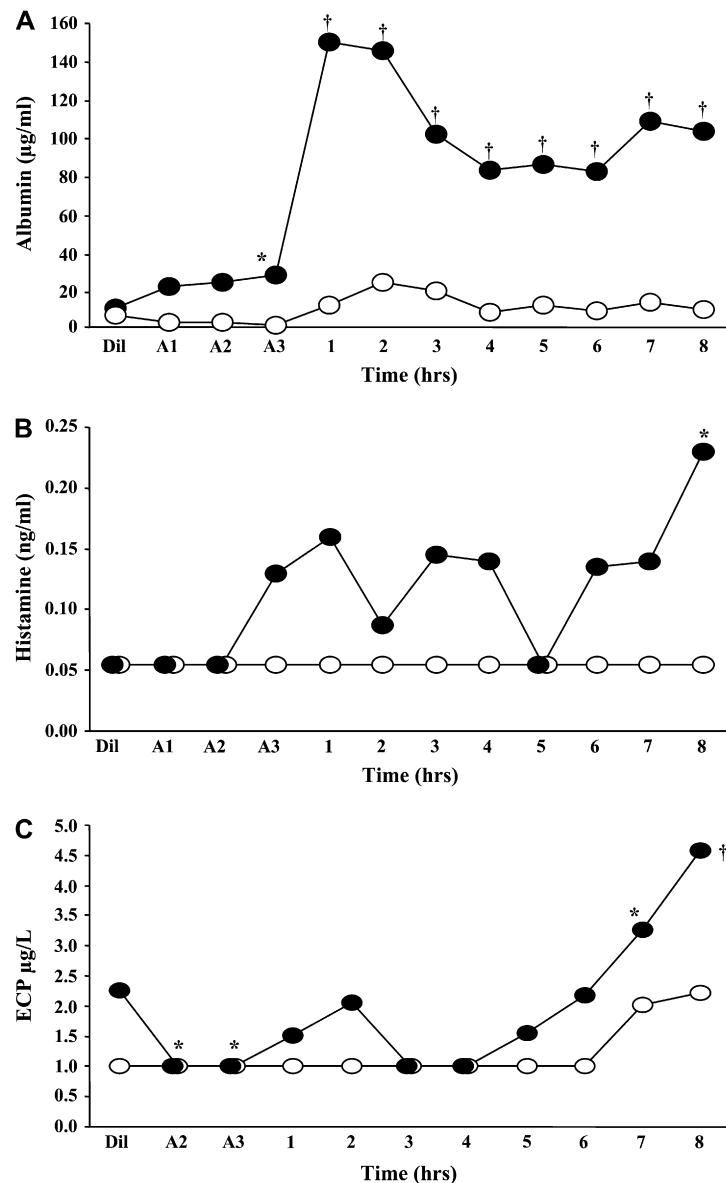


FIG 5. Median levels of mediators in the maxillary sinus after allergen (solid circles, $n = 20$) and control (open circles, $n = 11$) challenges: **A**, albumin; **B**, histamine; **C**, ECP. The protocol is on the x-axis, and the labels are as in Fig 2. * $P \leq .05$ and † $P \leq .01$ versus respective diluent challenges.

this usually ceased by the time the challenges were initiated and was washed out by the preliminary lavages. Thus we do not believe that bleeding into the sinus accounted for the level of eosinophils recovered. Our data suggest a selective recruitment

of eosinophils to the maxillary sinus and nasal cavity after nasal allergen challenge. Although caution should be used when interpreting challenge data because the amount of allergen used is larger than during natural exposure, our results suggest

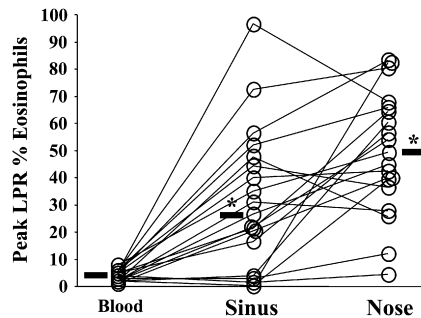


FIG 6. Percentage of eosinophils in blood, the maxillary sinus, and the nasal cavity after allergen challenge depicted as individual values with median bars. The maxillary sinus and nasal cavity data are presented as the median value for the peak LPR percentage of eosinophils for each subject. * $P \leq .01$ versus blood value.

that nasal allergic inflammation causes an inflammatory response within the ipsilateral maxillary sinus, as evidenced by increased vascular permeability and an eosinophil influx.

Eosinophils are thought to play a key role in the inflammatory process in both allergic rhinitis and rhinosinusitis.^{4,17} Allergic subjects with chronic rhinosinusitis have also been shown to have an increased expression of mRNA for T_H2 cytokines.^{5,18}

Multiple theories exist to explain the relationship between allergic rhinitis and sinusitis. One possible mechanism is that allergic rhinitis leads to edema of the nasal passages and interferes with adequate drainage from the sinus cavities. This mechanism would be favored to explain acute rhinosinusitis but does not really explain the persistence of sinus mucosal thickening and inflammation after resolution of anatomic blockages after endoscopic sinus surgery. Another mechanism would be the penetration of allergen into the sinus cavities of allergic subjects and the generation of direct allergic sinus inflammation. This could have led to our observations. However, a major problem with this hypothesis is that the sinus ostia are connected to the nasal cavity by narrow, convoluted bony channels, making it very unlikely for pollen to penetrate the maxillary sinus. Adkins et al¹⁹ performed nasal allergen challenges in nonatopic subjects with Tc99m-labeled ragweed pollen and found intense activity in the nasal cavity but no activity in the paranasal sinuses. Studies of IgE levels and gas composition support the limited exchange between the nasal cavity and the sinuses.^{20,21} Also, mucociliary transport carries mucus out of the sinuses, and thus allergens near the sinus ostium would be carried away from the sinus cavities. All these observations would make the above mechanism unlikely in explaining our findings.

On the other hand, there is evidence that posterior nasal secretions can be propelled into the paranasal sinuses. Gwaltney et al²² measured intranasal pressures during nose blowing, coughing, and sneezing (with nostrils open) in 4 healthy adults and found pressures during nose blowing to be 10-fold greater than during coughing or sneezing. Limited coronal computed tomographic scans were then performed on the subjects after contrast was applied to the nasopharynx or to the floor of the nasal cavity, and contrast was seen in the osteomeatal unit, as well as in the ethmoid and sphenoid sinuses, of all 4 subjects only after nose blowing. The maxillary and frontal sinuses showed contrast in 2 of the 4 subjects, suggesting a lower chance of penetration into these 2 pairs of sinuses.

The results from our dust mite experiment do not support the hypothesis that allergen penetrating into the sinus led to the observed sinus inflammatory response. Subjects were asked to gently blow their noses to help expel the nasal lavage fluid after each dust mite challenge during the control experiment, which is similar to the nose blowing used during the actual experiments. No allergen was detected in the maxillary sinus lavage specimens, whereas increasing levels of dust mite allergen were detected in recovered nasal lavage specimens. Furthermore, as the allergen challenge progressed, the nasal cavity became congested. This would lead to swelling of the mucosa that surrounds the maxillary sinus ostium, making it even more unlikely to get penetration of allergen or nasal lavage fluids into that cavity.

Furthermore, inhibition of the sneezing response by loratadine had no effect on the sinus eosinophilic response.

Another potential explanation for our findings is that allergen challenge causes priming of circulating leukocytes with upregulation of adhesion molecules, making these leukocytes more likely to migrate to other body sites, such as the paranasal sinuses. There is evidence supporting the concept of a distant reaction in response to localized allergen challenge, suggesting potential systemic effects of allergen provocation.^{23,24} These experiments suggest that provocation at one site of the allergic airway (eg, the nose) could lead to an inflammatory response at distant airway sites (eg, the maxillary sinuses) and could very well explain our findings.

A final potential explanation is that nasal allergic inflammation will trigger nasal sensory afferent nerves, which will lead to neurogenic inflammation within the sinuses. Neuropeptides, such as vasoactive intestinal peptide and substance P, are found in epithelial, glandular, and vascular tissues in the nasal cavity and have a variety of proinflammatory and prosecretory effects.²⁵⁻²⁹ Evidence that these neuropeptides are relevant in allergic rhinitis has been provided by Mosimann et al.³⁰ Taken together, these data support the role of substance P and vasoactive intestinal peptide in modulating and enhancing eosinophilic inflammation in allergic disease. The existence of neurogenic reflexes in the upper airway has been well documented by studies of the nasosinal reflex.³¹⁻³⁴ The inhibitory effect of atropine on this response suggests that the nasosinal reflex is mediated, at least in part, by efferent cholinergic stimulation of the contralateral nasal glands.^{33,34}

To explain the response seen within the maxillary sinus, we speculate that there might be a nasosinal reflex. This would be mediated through afferent fibers stimulated on nasal allergen exposure that synapse in the trigeminal ganglion. The response would then travel through efferent fibers back to the nasal cavity, as well as to the paranasal sinuses (in this case the maxillary sinus), resulting in a neural reflex. This seems plausible because it is known that parasympathetic fibers from the sphenopalatine ganglion supply mucous glands of the maxillary sinus.³⁵ Neuropeptides released through this mechanism would enhance the inflammatory response in the maxillary sinus, as seen in this study. Challenging one nasal cavity and sampling both maxillary sinuses might help shed light on this mechanism and the relative contribution of central versus axonal reflexes to this response. The reverse reflex between the sinus and the nasal cavity does not seem to exist because we have previously failed to demonstrate an increase in secretions in the contralateral nasal cavity in response to histamine challenges of the maxillary sinus.¹⁴

In summary, our findings suggest the existence of inflammatory changes within the maxillary sinus cavity in response to nasal provocation with allergen. The changes are small but consistent

and significantly increased over appropriate control values. We hypothesize that these changes are related either to systemic allergic inflammation or to a neurogenic reflex between the nose and sinus cavities. A combination of these 2 mechanisms could also be responsible for our findings. Further experiments will be needed to elucidate the responsible mechanisms. However, these findings are important in that they demonstrate, for the first time, the induction of an inflammatory response in the sinus cavity during nasal allergic inflammation and might help explain the close relationship between allergic rhinitis and sinusitis, 2 very common diseases.

Key messages:

- Allergic rhinitis and rhinosinusitis frequently coexist.
- Allergen challenge of the nose leads to an influx of eosinophils into the adjacent maxillary sinus.
- A reflex between the nose and the sinus or systemic allergic inflammation could explain this observation.

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CONTROL EXPERIMENTS

Methods

To investigate the possibility that allergen sprayed into the nostril entered the ipsilateral sinus cavity, we performed 2 experiments in 2 subjects with dust mite allergy. We chose to perform these experiments with dust mite extracts because accurate determination of the antigen could be performed by means of ELISA. We inserted maxillary sinus catheters in these subjects and 4 hours later performed challenges with diluent for the dust mite extract, followed by increasing concentrations of dust mite (100, 300, and 1000 AU). After each challenge, we lavaged the nose and ipsilateral sinus cavities in identical fashion, as performed in the study protocol. In the first experiment we lavaged the nose and sinus cavities 10 minutes after the challenges and recovered very low allergen levels in the nasal wash specimens. We assumed that the allergen was removed from the nasal cavity by means of mucociliary transport because we waited for 10 minutes between the challenge and the collection of the lavage specimen. To maximize recovery of allergen in the wash specimens, we performed the second experiment in identical fashion but performed the lavages 3 minutes after the challenges. As a control, we also placed specific amounts of allergen in LR solution (the solution used for the lavages) and assayed those separately. The level of detection of the assay was 2.0 ng/mL Der p 1.

Results

In the first experiment we diluted solutions of dust mite extracts to 680, 2040, and 6800 AU/mL. Two sprays from a metered-dose inhaler of these solutions (as would be placed into the nasal cavity during the challenges) contained 100, 300, and 1000 AU of dust mite extracts, respectively. We then sent these solutions to the RAST laboratory for assay in blinded and unblinded fashion, and we also included a solution of LR unspiked with extract. We then mixed 0.2 mL of each of the extracts in 5 mL of LR solution (the equivalent of the lavage volume) and assayed those also. Finally, we challenged the subjects with diluent followed by 3 doses of dust mite (total delivered to the nostril: 100, 300, and 1000 AU) and lavaged the nasal and maxillary sinus cavities either 10 minutes or 3 minutes after the challenge. The data are depicted in [Tables E1 and E2](#). As can be seen in [Table E1](#), the results for the blinded and unblinded samples correlated well and show that the extract at the concentrations sprayed into the nostril was measurable by assay. In [Table E2](#) the number of sneezes after each challenge suggests that the subjects responded well to the challenge. It is also clear that no allergen was detected by the assay in the maxillary sinus lavage specimens. Finally, in the nasal cavity no allergen was detected in the wash specimens after either diluent challenge or the 1-hour postallergen time point. In contrast, there

were increasing levels of allergen detected in the nasal wash specimens after challenge with increasing doses of allergen. More allergen was recovered when the lavages were performed after 3 minutes compared with 10 minutes after the actual challenge. These experiments support the notion that allergen sprayed into the nasal cavity does not reach the maxillary sinus, at least at the detection limit of the assay used. Whether values of less than 2 ng/mL Der p 1 reached the sinus cavity cannot be assessed by these experiments.

METHODS

Eosinophil counts

Lavage specimens collected from subjects were initially centrifuged at 5000 rpm for 15 minutes, and the supernatant was decanted and stored at -20°C for mediator measurements. The cell pellets were then suspended in 9 mL of distilled water to lyse red blood cells, and then 1 mL of $10\times$ PBS was added to bring the samples back to an isotonic solution. Total cells were then counted with a hemocytometer. The sample was again centrifuged, and the supernatant was discarded. The cell pellet was resuspended in LR solution and cytospun on slides. Pilot experiments showed that the percentage of recovered cells was not affected by this processing technique. These slides were evaluated for eosinophil numbers by an observer blinded to treatment protocols and study visits. Two hundred cells were evaluated on slides (when possible), and we enumerated eosinophils, neutrophils, and mononuclear cells and calculated their percentages. Because the total number of cells was known, the total number of eosinophils was calculated. Low cells in the cytospun slides occurred because of low total cell counts or technical difficulties during the preparation process. From our previous experience using this technique, a total number of cells evaluated in the slide of less than 50 leads to a large sampling error. Because of the paucity of the cells in some of the specimens, we made some assumptions relating to eosinophil number. The lowest total number of eosinophils obtained from slides with good cells counted and good total cell count was 25. This number was assigned to specimens in the following situations: (1) when the number of cells evaluated in the smear was less than 50 and (2) when the number of eosinophils and thus the percentage of eosinophils obtained from examining the differential count was zero. Exceptions to this assumption were patients who had a large number of total cells ($>60,000$) but for whom the resultant smears had fewer than 50 cells to evaluate. These samples were considered technically inadequate and are not included in data analysis.

Assays

Human serum albumin was measured with an ELISA sensitive to 1 ng/mL albumin. Any sample that had concentrations lower than 1 ng/mL was assigned a value of 0.5 ng/mL. ECP, a marker of eosinophil secretion, was measured by using a commercially available double-antibody RIA (Pharmacia AB, Uppsala, Sweden). The assay has a sensitivity detection limit of 2 $\mu\text{g/L}$, and levels of less than this limit were assigned a value of 1 $\mu\text{g/L}$. Histamine levels were determined by using an RIA sensitive to 0.11 ng/mL (Immunotech, Marseille, France). Levels of less than the detection limit were assigned values of 0.055 ng/mL. All samples from the same patient were run in the same assay to eliminate interassay variability.

TABLE E1. Dilutions of dust mite extracts

Antigen solution	Unblinded	Blinded	LR + Ag (0.2 mL/5 mL)
1 mL of 680 AU/mL	261	250.7	6.6
1 mL of 2040 AU/mL	5897	6006.6	87.3
1 mL of 6800 AU/mL	22,416	21,171	617.2
LR		<2.0	

Values are in nanograms per milliliter of Der p 1.

TABLE E2. Levels of dust mite allergen extract in the nose and maxillary sinus after nasal challenge

Challenge	10 min			3 min		
	Snz	Nose	Sinus	Snz	Nose	Sinus
Dil	0	<2.0	<2.0	0	<2.0	<2.0
100 AU	4	2.6	<2.0	5	25.9	<2.0
300 AU	7	2.3	<2.0	2	103.5	<2.0
1000 AU	0	9.2	<2.0	3	311.6	<2.0
1 hr post	0	<2.0	<2.0	0	<2.0	<2.0

Snz, Number of sneezes after each challenge; *Dil*, diluent for the allergen extract.

TABLE E3. Symptom scores after nasal challenge

Symptom	EPR		LPR	
	Allergen	Control	Allergen	Control
Sneezes	15.5 (−7 to 65)*	0 (−5 to 2)	4.0 (−20 to 38)*	0 (−12 to 7)
Rhinorrhea	3.0 (−1 to 5)*	0 (−3 to 0)	1.0 (−10 to 9)†	0 (−14 to 1)
Congestion	2.5 (0 to 6)*	0 (−3 to 0)	6.0 (0 to 16)*	0 (−8 to 2)
Itching	3.5 (0 to 8)*	0 (0 to 1)	0.5 (−14 to 20)	0 (−8 to 1)

Values represent the total change from diluent challenge for either EPRs or LPRs after either antigen or control challenges. Data are depicted as medians (ranges) for 20 patients for antigen and 11 patients for control challenges.

* $P < .01$ and † $P < .02$ versus respective control LR solution challenges.

TABLE E4. Levels of biomarkers in nasal lavage specimens

Mediator	EPR		LPR	
	Allergen	Control	Allergen	Control
Albumin (μg/mL)	885 (−2 to 3766)*	1 (−115 to 557)	1195 (82 to 4300)*	246 (−168 to 807)
Histamine (ng/mL)	6 (−435 to 76)*	−0.3 (−41 to 2)	14 (−2571 to 163)	0.1 (−260 to 5)
ECP (μg/L)	0.2 (−45 to 40)	1 (−121 to 37)	195 (14 to 1297)*	18 (−566 to 206)

Values represent the total change from diluent challenge for either EPRs or LPRs after either antigen or control challenges. Data are depicted as medians (ranges) for 20 patients for antigen and 11 patients for control challenges.

* $P \leq .01$ versus respective control LR solution challenges.

TABLE E5. Levels of biomarkers in sinus lavage specimens

Mediator	EPR		LPR	
	Allergen	Control	Allergen	Control
Albumin (μg/mL)	13 (−121 to 6082)*	−4 (−1049 to 4.3)	989 (−558 to 10712)*	35 (−278 to 1269)
Histamine (ng/mL)	0 (−9.7 to 2.7)	0 (−0.5 to 0)	1.0 (−56 to 22.8)†	0.06 (−0.8 to 1.0)
ECP (μg/L)	−2.2 (−19.7 to 0)	0 (−33 to 2.8)	6.9 (−50 to 177)	0 (−121 to 24)

Values represent the total change from diluent challenge for either EPRs or LPRs after either antigen or control challenges. Data are depicted as medians (ranges) for 20 patients for antigen and 11 patients for control challenges.

* $P < .01$ and † $P < 0.05$ versus respective control LR solution challenges.

TABLE E6. Effect of treatment with loratadine on nasal and sinus responses

Parameter	TOD EPR		TOD LPR	
	Placebo	Loratadine	Placebo	Loratadine
Nasal				
Sneezes	15.5 (−7 to 65)	5.5 (0 to 27)*	4.0 (−20 to 38)	3.0 (−6 to 63)
Rhinorrhea	3.0 (−1 to 5)	2.0 (−3 to 6)	1.0 (−10 to 9)	0 (−16 to 15)
Congestion	2.5 (0 to 6)	2.5 (−1 to 5)	6.0 (0 to 16)	4.0 (−1 to 19)
Itching	3.5 (0 to 8)	2.5 (0 to 5)	0.5 (−14 to 20)	0 (−5 to 14)
Histamine (ng/mL)	5.5 (−434.9 to 75.8)	4.3 (−308.2 to 104.7)	13.8 (−2571.2 to 162.9)	30.1 (−374.2 to 1900.7)
Albumin (μg/mL)	885.3 (−1.9 to 3765.8)	122.3 (−414.5 to 1556.6)†	1194.8 (82.4 to 4299.7)	790.6 (−776.7 to 5198.8)
ECP (μg/L)	0.2 (−44.8 to 40.2)	1.2 (−18.9 to 32.1)	194.6 (14.1 to 1296.8)	199.6 (17.1 to 1000.7)
Eosinophils ($\times 10^{-3}$)	1.2 (−27.9 to 147.9)	0.2 (−34.2 to 166.7)	702.5 (19.9 to 3672.9)	418.2 (−2.6 to 1997.8)
Sinus				
Histamine (ng/mL)	0 (−9.7 to 2.7)	0 (−2.6 to 3.1)	1.0 (−56.0 to 22.8)	0.2 (−8.2 to 12.2)
Albumin (μg/mL)	12.8 (−121.0 to 6082.1)	1.7 (−550.2 to 986.9)	988.8 (−558.6 to 10,711.8)	676.0 (−393.5 to 13,700.1)
ECP (μg/L)	−2.1 (−19.7 to 3.7)	0 (−5.8 to 2.9)‡	6.9 (−50.0 to 177.4)	9.4 (−9.2 to 437.2)
Eosinophils ($\times 10^{-3}$)	1.2 (−13.1 to 111.7)	0 (−12.6 to 10.1)	24.9 (−1.9 to 973.8)	26.0 (−0.9 to 267.3)

Values represent the total change from diluent challenge (TOD) for either EPRs or LPRs after pretreatment with either placebo or loratadine. Data are depicted as medians (ranges) for 20 patients.

* $P < .001$, † $P = .004$, and ‡ $P = .03$ versus respective placebo responses.