
The effects of the nasal antihistamines olopatadine and azelastine in nasal allergen provocation

Patrik Pipkorn*; Candy Costantini*; Curt Reynolds*; Michael Wall†; Margaret Drake‡; Alvin Sanico*; David Proud‡; and Alkis Togias, MD*§

Background: Olopatadine, an antihistamine used in allergic conjunctivitis, is under development as a nasal preparation for the treatment of allergic rhinitis.

Objectives: To evaluate the efficacy of olopatadine in suppressing symptoms and biomarkers of the immediate reaction induced by nasal allergen provocation and to compare olopatadine with azelastine in the same model.

Methods: The study was approved by the Johns Hopkins University institutional review board, and all subjects gave written consent. We studied 20 asymptomatic subjects with seasonal allergic rhinitis. The study had 2 randomized, double-blind, placebo-controlled, crossover phases that evaluated 2 concentrations of olopatadine, 0.1% and 0.2%. In a third exploratory phase, olopatadine, 0.1%, was compared with topical azelastine, 0.1%, in a patient-masked design. Efficacy variables were the allergen-induced sneezes, other clinical symptoms, and the levels of histamine, tryptase, albumin, lysozyme, and cysteinyl-leukotrienes (third study only) in nasal lavage fluids.

Results: Both concentrations of olopatadine produced significant inhibition of all nasal symptoms, compared with placebo. Olopatadine, 0.1%, inhibited lysozyme levels, but olopatadine, 0.2%, inhibited histamine, albumin, and lysozyme. The effects of olopatadine, 0.1%, were comparable to those of azelastine, 0.1%.

Conclusions: Olopatadine, at 0.1% and 0.2% concentrations, was effective in suppressing allergen-induced nasal symptoms. At 0.2%, olopatadine provided evidence suggestive of inhibition of mast cell degranulation.

Ann Allergy Asthma Immunol. 2008;101:82–89.

INTRODUCTION

Olopatadine hydrochloride, (Z)-11-[3-(dimethylamino)propylidene]-6 to 11-dihydrodibenz [b,e] oxepine-2-acetic acid hydrochloride, a tricyclic compound that is approved in the United States and in Europe as an ophthalmic solution for the treatment of the signs and symptoms of allergic conjunctivitis, is being developed as a nasal spray for the treatment of seasonal allergic rhinitis.^{1,2} Olopatadine is a relatively selective H₁-receptor antagonist and has demonstrated mast cell-stabilizing properties.^{3–5}

Antihistamines inhibit several aspects of immediate allergic reactions in various organs. In the nose, this has been consistently shown using the experimental model of nasal allergen provocation.^{6–8} Particularly relevant are studies^{9–11} in which antihistamines were delivered locally to the nasal

cavities, showing impressive inhibitory effects against allergic reactions. Therefore, to put efficacy in perspective, it was important for a topical antihistamine under development to be tested in the nasal allergen challenge model in addition to the conventional clinical trials required by regulatory authorities. This study, which involved 3 phases, was designed with the intention to test the efficacy of 2 nasal preparations of olopatadine hydrochloride, 0.1% and 0.2%, in the nasal allergen provocation model. In the first 2 phases, each olopatadine concentration was tested against placebo. In the third exploratory phase, olopatadine, 0.1%, was compared with azelastine, 0.1%, in a randomized design, which did not include a placebo arm.

METHODS

Study Design

We designed and conducted a study that included 3 phases: 2 double-blind, crossover, randomized phases compared 2 nasal preparations of olopatadine, 0.1% and 0.2%, with placebo in the context of the immediate response to nasal allergen (ragweed or Timothy grass) challenge. The third phase used the same subjects as the first 2 phases but had a subject-masked, crossover, randomized design and compared olopatadine, 0.1%, with azelastine, 0.1% (Astelin). The final study phase was not blinded for the study coordinator (C.C.) who was performing the allergen challenge, because azelastine was obtained in commercially available bottles. How-

Affiliations: * Division of Allergy and Clinical Immunology, Department of Medicine, Johns Hopkins University, Baltimore, Maryland; † Alcon Research, Ltd, Fort Worth, Texas; ‡ Institute of Infection, Immunity, and Inflammation and Department of Physiology and Biophysics, University of Calgary, Calgary, Alberta, Canada; § Division of Pulmonary and Critical Care Medicine, Department of Medicine, Johns Hopkins University, Baltimore, Maryland.

Disclosures: Mr Wall and Ms Drake are employees of Alcon Research, Ltd, the company that sponsored the study; and Dr Togias has previously served as a consultant to Alcon Research, Ltd, and to Medpointe, the makers of Astelin.

Received for publication September 28, 2007; Received in revised form December 15, 2007; Accepted for publication January 10, 2008.

ever, all other members of the study team (C.R., A.S., D.P., and A.T.) were blinded. The 0.1% concentration of olopatadine was chosen as the comparator to azelastine for the third study phase based on an interim analysis performed after completion of the first 2 phases that showed no significant difference between the 2 olopatadine concentrations in terms of symptom scores. Each phase of the study was independently randomized.

In each arm of each phase, an allergen challenge was performed 25 minutes after the study drug had been administered. A minimum washout period of 2 weeks was observed between each study arm and between study phases. All study phases were conducted outside the ragweed or grass pollen season.

Subjects

A total of 20 subjects, 7 men and 13 women, ranging in age from 20 to 64 years (median, 41 years) were enrolled into the study. At the time of the study, subjects had to be free of rhinitis symptoms, in good physical condition, taking no medications, and with well-documented symptoms of seasonal allergic rhinitis confirmed by positive epicutaneous skin test results to ragweed or Timothy grass (average wheal diameter, 3 mm longer than that of the diluent control). The study protocol was approved by the investigational review board of the Johns Hopkins University, and each volunteer gave informed written consent after the nature of the project was thoroughly described.

Nasal Challenge

Nasal challenges were performed on 6 occasions (2 in each of the 3 phases of the study) with the use of an identical protocol. After baseline symptom scores had been obtained, 5 initial nasal lavages with isotonic sodium chloride solution heated to body temperature were performed. The volume of the first lavage was 2.5 mL per nostril. The returned fluids from this lavage were assayed for albumin, a marker of plasma transudation¹²; lysozyme, a marker of serous gland activation¹³; and histamine and tryptase, markers of mast cell activation.¹⁴ The purpose for the measurements in the first lavage was to examine whether the subjects' baseline nasal status was stable throughout the 3 phases (6 arms). The 4 subsequent lavages used 5 mL of isotonic sodium chloride solution per nostril to reduce the preexisting levels of mediators to a new stable baseline.¹⁵ The fluids from these lavages were discarded. Oxymetazoline hydrochloride, 0.05%, an α -adrenergic agonist decongestant, was then administered to ensure nasal patency so that the postallergen lavages could be performed with ease. Oxymetazoline has been previously shown not to affect histamine release or nasal symptoms other than nasal congestion^{15–17} during the early allergic response. Ten minutes after oxymetazoline was administered, another 2.5 mL per nostril nasal lavage was performed. The returned fluids from this lavage were analyzed for the same markers as for the first lavage. The postoxymetazoline symptoms and lavage data were defined as the baseline for the

allergen challenge. Ten minutes after the postoxymetazoline lavage, the test article was administered with 2 sprays in each nostril. Twenty-five minutes after test article administration, the allergen challenge was initiated. The allergen used was chosen based on skin test results and clinical allergy history. The allergen extract was delivered in 3 consecutive log incremental doses, 1 dose every 10 minutes, via metered nasal spray, in both nostrils. For ragweed, the doses were 10, 100, and 1,000 protein nitrogen units because standardized material was not available. For Timothy grass, 25, 250, and 2,500 biologic allergy units were used. At the beginning of each visit, and before the beginning of allergen challenge and 5 minutes after each allergen dose, individual symptom scores for rhinorrhea, nasal pruritus, nasal congestion, and posterior nasal drainage were recorded by the subject with the use of a 100-mm-long visual analog scale¹⁸ marked with "none" at its start and "worst imaginable" at its end. Sneezes were counted during the 10 minutes after each allergen dose. Ten minutes after each challenge step, a 2.5 mL per nostril lavage was performed. The returned fluids from the postallergen lavages were assayed for the previously specified markers. In the third phase of the study, lavage fluids were also analyzed for immunoreactive cysteinyl (cys)-leukotrienes.

Sample Processing and Mediator Assay

The returned nasal lavage sample was placed on ice and was centrifuged at 2,500g, at 4°C, for 15 minutes. The supernatant was transferred to polypropylene tubes, processed for each mediator, and stored at –20°C until analyzed. Aliquots to be assayed for tryptase, albumin, and lysozyme were frozen, with no pretreatment. For those to be assayed for histamine, perchloric acid was added to a final concentration of 2% and the sample was recentrifuged before storing.

Samples for histamine determination were measured as previously described¹⁹ with an automated spectrofluorometric assay sensitive to 1.0 ng/mL (Astoria-Pacific, Inc, Clackamas, Oregon). Tryptase was measured with a previously described enzyme-linked immunosorbent assay (ELISA) method using rabbit polyclonal antibody to human tryptase.^{20,21} The assay has a sensitivity of 1.7 ng/mL. Human serum albumin was measured using a previously described in-house ELISA method, with a sensitivity of 1 ng/mL.²² Lysozyme was also measured using ELISA, with a sensitivity of 1 ng/mL.²³ In the third study, immunoreactive cys-leukotrienes were measured by an in-house carbon-based radioimmunoassay, using a monoclonal anti-leukotriene C₄ (LTC₄) antibody that has greater than 80% cross-reactivity with leukotriene D₄ (LTD₄) and leukotriene E₄ (LTE₄) (Sigma Immunochemicals, St Louis, Missouri). This assay is sensitive to 150 pg/mL.^{24,25}

Statistical Analysis

Each phase of this study was designed and analyzed as an independent trial. Primary efficacy variables were the number of sneezes recorded after each allergen dose and the levels of

mast cell tryptase, albumin, and lysozyme in the nasal lavage fluids. Secondary efficacy variables were the visual analog scale–based scores for rhinorrhea, nasal pruritus, nasal congestion, and posterior nasal drainage and the levels of histamine in nasal lavage fluids. In the third study phase, immunoreactive cys-leukotrienes were also analyzed as a secondary variable.

Nonparametric methods were used in our analysis in accordance with previous work using the allergen challenge model.^{9,26–28} The Friedman analysis of variance was first used to determine whether our efficacy variables were increased over baseline in the 2 placebo arms after allergen challenge. Next, within each of the 3 study phases, we compared the outcomes of the test article with the placebo arm or, for the third phase, between olopatadine and azelastine, using Wilcoxon matched pair signed rank tests. Specifically, within each study arm, the postoxymetazoline baseline values were subtracted from the value obtained after each of the 3 allergen challenge steps. Then, the 3 adjusted (net change from baseline) postallergen values were added to yield a single value representing the total allergen challenge effect. For allergen-induced symptoms, in addition to analyzing each symptom separately, we created a compound variable by adding all individual symptom visual analog scale scores (total symptom score). The total symptom score did not include sneezing counts, because they were not measured with visual analog scales. Statistical significance was accepted at 2-tailed $P \leq .05$.

Based on previous work with the allergen challenge model, we determined that, for the first 2 phases of the study and for each of the primary outcome variables, 15 subjects with completed results would provide us with greater than 80% power to detect differences between placebo and active drug at the previously mentioned level of statistical significance. Being exploratory, the third phase of the study was not supported by formal power analysis.

RESULTS

Twenty subjects met the inclusion criteria to be enrolled into the 3-phase study. No serious adverse events were recorded. With allergen challenge, there were statistically significant increases of all clinical variables (sneezes and visual analog scale scores) in the 2 placebo arms. This was accompanied by significant increases in albumin, lysozyme, and histamine in nasal lavages. However, in the placebo arms, we failed to record statistically significant increases over baseline in tryptase levels with allergen challenge. Therefore, tryptase results were not further analyzed.

In determining the comparability of the 2 visits within each phase, and the overall comparability between the 3 phases, analysis of variance examining the level of symptoms at the beginning of each study visit showed no differences among the 6 visits (3 studies times 2 visits each [data not shown]). Similar analysis was conducted for the biochemical outcomes in the first nasal lavage of each visit. Again, in this analysis, no differences were found except for lysozyme, the

levels of which were lower in the 2 visits of the third phase. However, the levels of lysozyme in these 2 visits did not differ from each other. Furthermore, we conducted the same analysis for all symptoms and lavage outcomes that were recorded after the administration of oxymetazoline, the time point that was chosen as baseline in all 3 phases. In none of these outcomes did we find any differences among the 6 visits (data not shown).

Effects of Olopatadine, 0.1%, and Olopatadine, 0.2%, vs Placebo

Sixteen subjects were evaluable for the first phase of the study (olopatadine, 0.1%, vs placebo). Four subjects did not complete this phase because of respiratory tract infections. These were not judged as related to the study drug. Pretreatment with olopatadine, 0.1%, significantly reduced sneezing (Fig 1A), and all other recorded clinical symptoms, compared with placebo (rhinorrhea, $P < .001$; pruritus, $P < .001$; congestion, $P = .002$; and posterior nasal drip, $P = .03$). The results from the total symptom visual analog scale score are shown in Figure 2A. Among nasal lavage outcomes, there was a significant reduction in lysozyme (Fig 3A). However, there was no significant reduction in albumin (Fig 3C) or histamine (Fig 3E) levels. With the exception of the post-nasal drip outcome, all statistically significant differences remained significant when corrections for multiple comparison effects were applied.

Nineteen subjects were evaluable for the second phase (olopatadine, 0.2%, vs placebo): 1 subject dropped out of the study because of adverse events not related to study drug (headache and rhinitis symptoms). Pretreatment with olopatadine, 0.2%, had an effect on clinical symptoms that seemed equally strong to the effect of olopatadine, 0.1%. Comparison of the net changes from baseline for olopatadine, 0.2%, and placebo demonstrated significant inhibition for sneezing (Fig 1B), rhinorrhea ($P = .048$), pruritus ($P = .01$), congestion ($P = .01$), and posterior nasal drip ($P = .005$). The total symptom score was also significantly inhibited (Fig 2B). In this phase, significant inhibition by olopatadine, 0.2%, was found for all nasal lavage outcomes (Fig 3B, lysozyme; Fig 3D, albumin; and Fig 3F, histamine). With the exception of rhinorrhea and lysozyme, all statistically significant differences remained significant when corrections for multiple comparison effects were applied.

Effects of Olopatadine, 0.1%, vs Azelastine, 0.1%

Eighteen subjects were evaluable for the third phase (olopatadine, 0.1%, vs azelastine, 0.1%). As previously mentioned, 1 subject dropped out of the study in phase 2 because of adverse events not related to study drug (headache and rhinitis). Another subject dropped out of the study for personal reasons. In this phase of the study, olopatadine, 0.1%, was chosen as the concentration to be compared with azelastine, 0.1%, based on an interim analysis that showed no significant difference between the 2 olopatadine concentrations in terms of allergen-induced symptoms (nasal lavage outcomes had

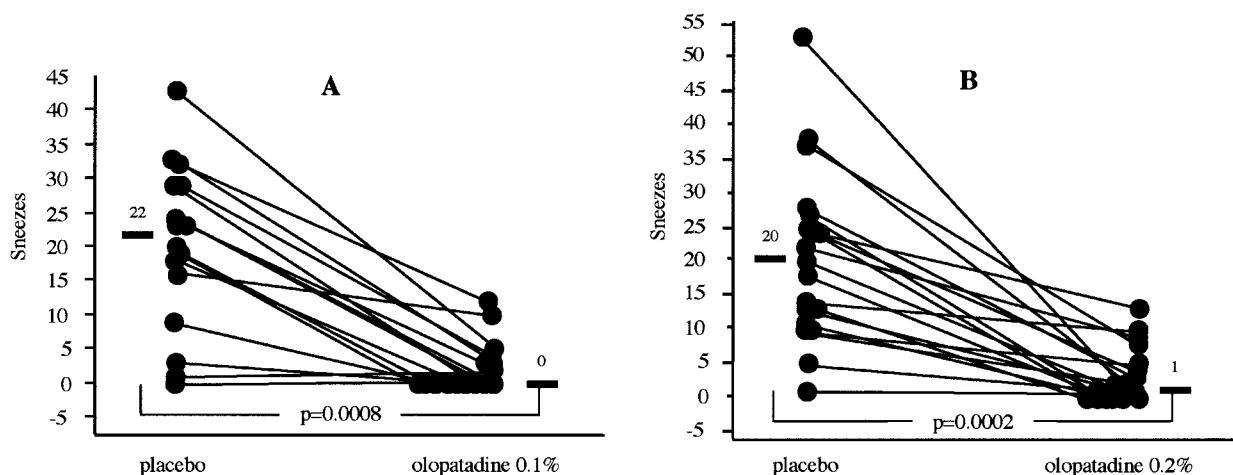


Figure 1. Individual subject data depicting the effect of olopatadine, 0.1% (A) and 0.2% (B), on allergen-induced sneezing. Sneezes were counted during a 10-minute period after each dose of allergen. The net change from baseline after treatment with olopatadine, 0.1% or 0.2%, or each study's placebo is presented. The same subjects participated in both trials. Horizontal bars with numerical values represent the medians for each study arm.

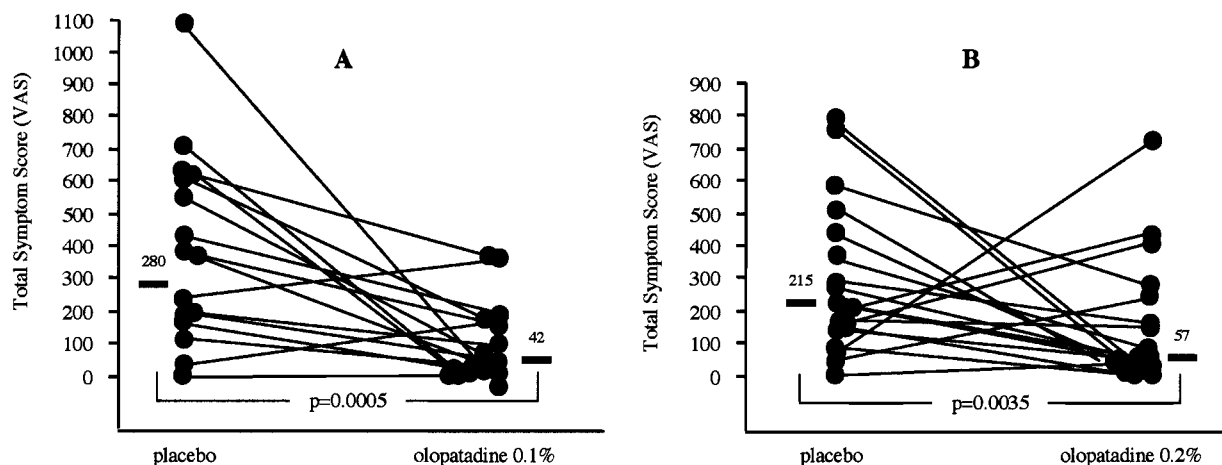


Figure 2. Individual subject data depicting the effect of olopatadine, 0.1% (A) and 0.2% (B), on allergen-induced nasal symptoms. Symptoms were recorded on a 100-mm visual analog scale (VAS). The total symptom score was calculated by adding the VAS score from each recorded symptom (runny nose, stuffy nose, itchy nose, and postnasal drip). For each symptom and for each challenge, the VAS score was generated by adding the net change from baseline for each of the 3 doses of allergen. The same subjects participated in both trials. Horizontal bars with numerical values represent the medians for each study arm. Negative values indicate that the postallergen VAS scores were lower than those at baseline.

not been measured at the interim analysis). Table 1 presents the results from this phase. Comparison of olopatadine, 0.1%, with azelastine, 0.1%, in terms of the total net change from baseline in symptoms showed no significant differences in sneezes, rhinorrhea, pruritus, congestion, or posterior nasal drip. Also, no significant difference was detected in total symptom scores. Similarly, we found no differences between the 2 drugs in the levels of albumin, histamine, or lysozyme. A lower level of immunoreactive cys-leukotrienes was observed in the group treated with olopatadine compared with azelastine (Table 1), but correction for multiple comparisons eliminated the statistical significance of this difference.

DISCUSSION

The results presented in this report demonstrate that single-dose topical treatment with olopatadine reduces all nasal symptoms (including nasal congestion) produced by allergen challenge. Symptom reduction was significant with both preparations of olopatadine (0.1% and 0.2%). On the other hand, olopatadine, 0.2%, resulted in significant reductions in histamine and albumin in nasal lavage fluids, whereas olopatadine, 0.1%, only inhibited the allergen-induced increase in lysozyme levels. Although this project was not designed to directly compare the effects of the lower (0.1%) with the higher (0.2%) concentrations of olopatadine, the nasal lavage

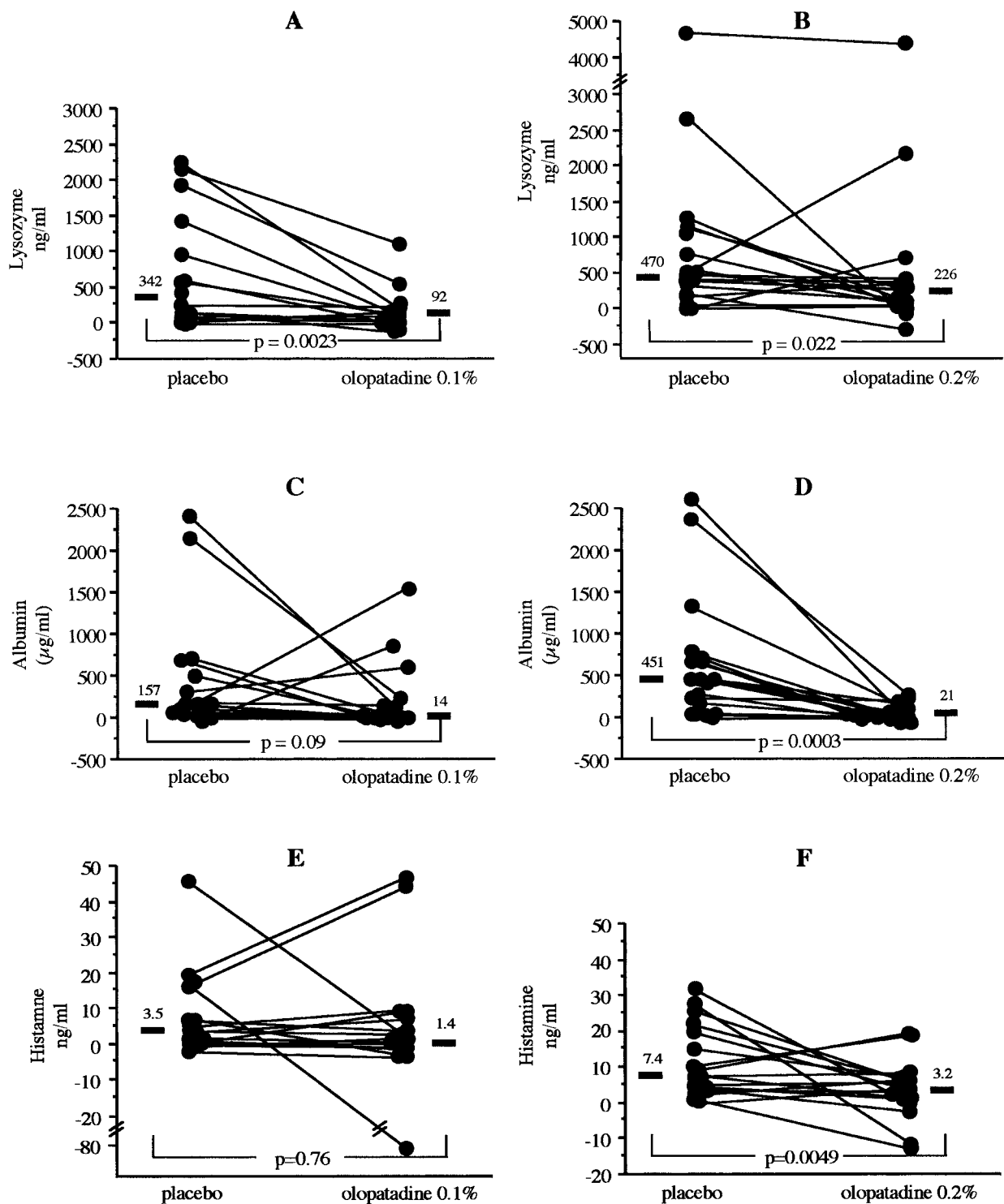


Figure 3. Individual subject data depicting the effect of olopatadine on allergen-induced biomarkers (lysozyme [A and B], albumin [C and D], and histamine [E and F]) in nasal lavage fluids. For each biomarker, the plotted value was generated by adding the net change from baseline induced by each of the 3 doses of allergen. Data are presented from the first phase of the study (oloapatadine, 0.1%, vs placebo) (A, C, and E) and the second phase of the study (oloapatadine, 0.2%, vs placebo) (B, D, and F). The same subjects participated in both trials. Horizontal bars with numerical values represent the medians for each study arm. Negative values indicate that the postallergen levels were lower than those at baseline.

Table 1. Comparison of Olopatadine, 0.1%, With Azelastine, 0.1%, With Respect to Allergen-Induced Nasal Symptoms and Biomarkers in Nasal Lavage Fluids^a

Variable	Olopatadine, 0.1%	Azelastine, 0.1%	P value
Sneezes	4.0 (1.0–10.0)	2.0 (0–9.0)	.33
Rhinorrhea	28.5 (3.0–41.0)	14.0 (6.0–33.0)	.12
Itching	27.0 (3.0–39.0)	5.5 (0–43.0)	.37
Congestion	9.5 (0–27.0)	9.5 (0–27.0)	.98
Postnasal drip	8.5 (–3.0–39.0)	6.5 (0–27.0)	.98
Total symptoms	90.5 (17.0–184.0)	71.0 (18.0–123.0)	.41
Albumin, $\mu\text{g/mL}$	32.8 (5.3–85.6)	24.5 (–4.9–69.9)	.88
Histamine, ng/mL	2.4 (–0.2–5.1)	2.6 (0–9.1)	.83
Lysozyme, ng/mL	31.0 (13.5–63.0)	27.5 (8.5–47.0)	.12
Leukotrienes, pg/mL	1710 (500–4590)	3260 (370–5955)	.04

^a Data are given as median (25th–75th percentile). Values represent the sum of the net changes from baseline induced by the 3 doses of allergen administered during each challenge.

data suggest that olopatadine, 0.2%, may have additional effects.

In an exploratory protocol, olopatadine, 0.1%, was compared with azelastine, 0.1%, a commercially available preparation. In this comparison, no differences in allergen-induced symptoms or in nasal lavage outcomes were found. This phase of the study was not powered to detect statistical significance for the small differences between olopatadine and azelastine that were observed in various outcomes. However, in this experimental model, olopatadine, even at the lowest concentration we used, appeared as effective as azelastine. Azelastine has been shown to have strong clinical efficacy compared with oral antihistamines. For example, azelastine was efficacious when given to individuals in whom therapy with loratadine and fexofenadine had previously failed^{29,30} and has proved more efficacious than cetirizine in at least 1 clinical trial.³¹ Similar studies have not been conducted with olopatadine.

The immediate reaction to nasal allergen provocation is driven by mediators released by mast cells.³² These include histamine and the cys-leukotrienes; antihistamines have been particularly effective in reducing the symptoms of the immediate allergic reaction in the nose,^{6–8} and some evidence for the role of 5-lipoxygenase pathway products has also been generated.^{33,34} With natural allergen exposure, the pathophysiological features of rhinitis are more complex than those of the immediate allergic reaction. Allergic rhinitis is characterized by chronic nasal inflammation, which is a late consequence of allergen exposure. This leads to nasal airway hyperresponsiveness and priming, phenomena that create a background that allows much lower doses of allergen to trigger acute nasal symptoms.³⁵ In addition, immediate symptoms can be caused by irritants that activate the up-regulated sensorineural apparatus.³⁶ Finally, symptoms induced by exposure to allergen may be present for long periods as a result of late-phase reactions.³⁵ Despite the difference in complexity between the experimental challenge model and natural dis-

ease, the immediate reaction to allergen challenge has had a good predictive value with respect to the efficacy of pharmacologic modalities in allergic rhinitis. This is probably because the symptoms produced by immediate allergic reactions constitute a significant element of the disease. On the other hand, and because of the previously mentioned reasons, the effects of antihistamines on the immediate reaction of the nasal allergen challenge model seem to be stronger than those on natural allergic rhinitis; olopatadine, for example, has been shown in clinical studies^{1,2} to have an optimal concentration of 0.6% in reducing symptoms of seasonal allergic rhinitis.

Drug administration directly onto the nasal mucosa probably achieves concentrations higher than those reached through oral administration. Under such concentrations, it is theoretically possible that some antihistamines may exhibit mild anticholinergic effects. In a study by Saengpanich and colleagues,³⁷ nasal azelastine produced a small reduction in the secretory response to methacholine, but this did not reach statistical significance. Such data are not available for olopatadine. In vitro binding of either olopatadine or azelastine to muscarinic receptors is negligible.^{3,38}

In addition to their primary pharmacologic action, many antihistamines possess “antiallergic” properties that may further contribute to their effectiveness. For example, terfenadine, given orally at a 5-fold higher than the approved dose, has been shown to reduce the amount of histamine released in nasal secretions during nasal allergen challenge in a protocol almost identical to ours.⁷ This finding was reproduced in a localized allergen challenge model.³⁹ Other agents, such as cetirizine, did not have an effect on histamine but reduced the levels of cys-leukotrienes in the same model.⁶ During the immediate allergic reaction, histamine and the cys-leukotrienes probably derive from mast cells; therefore, suppression of these mediators implies an inhibitory effect on IgE receptor cross-linking activation of these cells. Indeed, antihistamines of various classes, at high concentrations, have been shown to block in vitro human basophil^{40,41} and mast cell^{9,42} activation by allergen. Interestingly, the inhibitory effect on cys-leukotriene production is stronger than that on histamine release.⁹ Olopatadine inhibits antigen-induced histamine release in sensitized rat basophilic leukemia cells and in human conjunctival mast cells.^{4,5} In a human trial⁴³ using a conjunctival allergen challenge model, olopatadine led to a significant reduction in histamine, in tears, compared with placebo. In the series of experiments presented herein, we have generated further evidence that olopatadine may have a mast cell-stabilizing effect. Suppression of the allergen-induced histamine release in nasal secretions was demonstrated at the higher concentration (0.2%), and a reduction in cys-leukotrienes was also suggested by the findings of the third study; a placebo arm was not included in that study, but the fact that the levels of immunoreactive cys-leukotrienes in nasal secretions were lower with olopatadine, compared with azelastine, indicates that olopatadine had an inhibitory effect on the production of these mediators. Notably, oral azelastine was previously found to suppress cys-leukotriene levels in the

same experimental model,⁸ but nasal azelastine, 0.1%, had no effect on the release of histamine or tryptase after allergen challenge, after 13 days of treatment.³⁷ On the other hand, a single dose of nasal azelastine reduces eosinophilia and neutrophilia at 30 minutes and at 6 hours, respectively, after nasal allergen challenge⁴⁴; one interpretation of this finding could be that it indirectly reflects an in vivo inhibition of mast cell mediator release, because mast cell activation can be the initiator of the cascade leading to inflammatory cell infiltrate. In vitro data with animal and human mast cells and basophils support the concept of azelastine-induced inhibition of cys-leukotriene and histamine release.^{45–48} Our studies aimed at providing even more compelling evidence that olopatadine can inhibit mast cell activation in vivo by measuring mast cell tryptase in nasal lavage fluids. The validity of our tryptase assay in this system was previously demonstrated.²⁰ Unfortunately, for reasons that are unclear to us, we failed to obtain increased tryptase levels in the postallergen challenge lavages in the placebo arms of the first and second phases of the study, compared with baseline. This variable was, therefore, not further analyzed.

The significance of inhibition of mast cell mediator release by antihistamines is not known.⁴⁹ Theoretically, such pharmacologic effect could affect the magnitude of the immediate reaction to allergen because fewer mediators would be released. Also, the development of late inflammatory consequences could be suppressed because some well-established human mast cell products (eg, tumor necrosis factor α ⁵⁰ and cys-leukotrienes⁵¹) are implicated in the development of inflammation and several other inflammatory products have been reported from cultured human mast cells or mast cell lines.⁵² Yet, controversy exists as to the ability of oral antihistamines to suppress inflammatory phenomena, such as the late-phase reaction.^{53,54} Olopatadine has not been tested in this respect, but because this agent most consistently shows evidence of in vivo mast cell inhibition, it would be interesting to examine whether it can suppress late allergic events.

In conclusion, olopatadine, at concentrations of 0.1% or 0.2%, is effective in suppressing the clinical outcomes of the immediate allergic nasal reaction. Its effect in this model is comparable to that of azelastine, 0.1%, the commercially available preparation of this agent. Our work also suggests that olopatadine, at the highest dose used (0.2%), may inhibit in vivo mast cell activation by allergen.

REFERENCES

- Meltzer E, Hampel F, Ratner P, et al. Safety and efficacy of olopatadine hydrochloride nasal spray for the treatment of seasonal allergic rhinitis. *Ann Allergy Asthma Immunol*. 2005;95:600–606.
- Ratner P, Hampel F, Amar N, et al. Safety and efficacy of olopatadine hydrochloride nasal spray for the treatment of seasonal allergic rhinitis to mountain cedar. *Ann Allergy Asthma Immunol*. 2005;95:474–479.
- Sharif NA, Xu SX, Miller ST, Gamache DA, Yanni JM. Characterization of the ocular antiallergic and antihistaminic effects of olopatadine (AL-4943A), a novel drug for treating ocular allergic diseases. *J Pharmacol Exp Ther*. 1996;278:1252–1261.
- Yanni JM, Miller ST, Gamache DA, Spellman JM, Xu S, Sharif NA. Comparative effects of topical ocular anti-allergy drugs on human conjunctival mast cells. *Ann Allergy Asthma Immunol*. 1997;79:541–545.
- Yanni J, Weimer L, Sharif N, Xu S, Gamache D, Spellman J. Inhibition of histamine-induced human conjunctival epithelial cell responses by ocular allergy drugs. *Arch Ophthalmol*. 1999;117:643–647.
- Naclerio R, Proud D, Kagey-Sobotka A, Friedhoff L, Norman P, Lichtenstein L. The effect of cetirizine on the early allergic response. *Laryngoscope*. 1989;99:596–599.
- Naclerio RM, Kagey-Sobotka A, Lichtenstein LM, Friedhoff L, Proud D. Terfenadine, an H1 antihistamine, inhibits histamine release in vivo in the human. *Am Rev Respir Dis*. 1990;142:167–171.
- Shin M, Baroody F, Proud D, Kagey-Sobotka A, Lichtenstein L, Naclerio R. The effect of azelastine on the early allergic response. *Clin Exp Allergy*. 1992;22:289–295.
- Togias A, Naclerio R, Warner J, et al. Demonstration of inhibition of mediator release from human mast cells by azatadine base: in vivo and in vitro evaluation. *JAMA*. 1986;255:225–229.
- Bachert C, Wagenmann M, Vossen-Holzenkamp S. Intranasal levocabastine provides fast and effective protection from nasal allergen challenge. *Rhinology*. 1996;34:140–143.
- Corren J, Rachelefsky G, Spector S, et al. Onset and duration of action of levocabastine nasal spray in atopic patients under nasal challenge conditions. *J Allergy Clin Immunol*. 1999;103:574–580.
- Raphael G, Igrashi Y, White M, Kaliner M. The pathophysiology of rhinitis V: sources of protein in allergen-induced nasal secretions. *J Allergy Clin Immunol*. 1991;88:33–42.
- Raphael G, Jeney E, Baraniuk J, Kim I, Meredith S, Kaliner M. Pathophysiology of rhinitis: lactoferrin and lysozyme in nasal secretions. *J Clin Invest*. 1989;84:1528–1535.
- Metcalfe D, Baram D, Mekori Y. Mast cells. *Physiol Rev*. 1997;77:1033–1079.
- Naclerio R, Meier H, Kagey-Sobotka A, et al. Mediator release after nasal airway challenge with allergen. *Am Rev Respir Dis*. 1983;128:597–602.
- Watase T, Okuda M. The effects of autonomicotropic drugs on allergic nasal mucosa. *Rhinology*. 1986;24:181–186.
- Majchel A, Baroody F, Kagey-Sobotka A, Lichtenstein L, Naclerio R. Effect of oxymetazoline on the early response to nasal challenge with antigen. *J Allergy Clin Immunol*. 1993;92:767–770.
- Linder A. Symptom scores as measures of the severity of rhinitis. *Clin Allergy*. 1988;18:29–37.
- Siraganian R. An automated continuous flow system for the extraction and fluorometric analysis of histamine. *Anal Biochem*. 1974;57:283–294.
- Proud D, Bailey G, Naclerio R, et al. Tryptase and histamine as markers to evaluate mast cell activation during the responses to nasal challenge with allergen, cold, dry air, and hyperosmolar solutions. *J Allergy Clin Immunol*. 1992;89:1098–1110.
- Proud D, Reynolds C, Lichtenstein L, Kagey-Sobotka A, Togias A. Intranasal salmeterol inhibits allergen-induced vascular permeability but not mast cell activation or cellular infiltration. *Clin Exp Allergy*. 1998;28:868–875.
- Riccio M, Proud D. Evidence that enhanced nasal reactivity to bradykinin in patients with symptomatic allergy is mediated by neural reflexes. *J Allergy Clin Immunol*. 1996;97:1252–1263.
- Riccio M, Reynolds C, Hay D, Proud D. Effects of intranasal administration of endothelin-1 to allergic and nonallergic individuals. *Am J Respir Crit Care Med*. 1995;152:1757–1764.
- Hayes E, Lombardo D, Girard Y, et al. Measuring leukotrienes of slow reacting substance of anaphylaxis: development of a specific radioimmunoassay. *J Immunol*. 1983;131:429–433.
- Philip G, Baroody F, Proud D, Naclerio R, Togias A. The human nasal response to capsaicin. *J Allergy Clin Immunol*. 1994;94:1035–1045.
- Naclerio R, Proud D, Togias A, et al. Inflammatory mediators in late antigen-induced rhinitis. *N Engl J Med*. 1985;313:65–70.
- Pipkorn U, Proud D, Lichtenstein L, Kagey-Sobotka A, Norman P, Naclerio R. Inhibition of mediator release in allergic rhinitis by pretreatment with topical glucocorticoids. *N Engl J Med*. 1987;316:1506–1510.
- Pipkorn U, Proud D, Lichtenstein L, et al. Effect of short-term systemic

- glucocorticoid treatment on human nasal mediator release after antigen challenge. *J Clin Invest.* 1987;80:957–961.
29. Berger W, White M. Efficacy of azelastine nasal spray in patients with an unsatisfactory response to loratadine. *Ann Allergy Asthma Immunol.* 2003;91:205–211.
 30. LaForce C, Corren J, Wheeler W, Berger W. Efficacy of azelastine nasal spray in seasonal allergic rhinitis patients who remain symptomatic after treatment with fexofenadine. *Ann Allergy Asthma Immunol.* 2004;93:154–159.
 31. Corren J, Storms W, Bernstein J, Berger W, Nayak A, Sacks H. Effectiveness of azelastine nasal spray compared with oral cetirizine in patients with seasonal allergic rhinitis. *Clin Ther.* 2005;27:1–11.
 32. Togias A, Naclerio R, Proud D, et al. Mediator release during nasal provocation: a model to investigate the pathophysiology of rhinitis. *Am J Med.* 1986;79(suppl 6A):26–33.
 33. Knapp HR. Reduced allergen-induced nasal congestion and leukotriene synthesis with an orally active 5-lipoxygenase inhibitor. *N Engl J Med.* 1990;323:1745–1748.
 34. Lee D, Jackson C, Soutar P, Fardon T, Lipworth B. Effects of single or combined histamine H1-receptor and leukotriene CysLT1-receptor antagonism on nasal adenosine monophosphate challenge in persistent allergic rhinitis. *Br J Clin Pharmacol.* 2004;57:714–719.
 35. Naclerio R. Allergic rhinitis. *N Engl J Med.* 1991;325:860–869.
 36. Togias A. Unique mechanistic features of allergic rhinitis. *J Allergy Clin Immunol.* 2000;105(pt 2):S599–S604.
 37. Saengpanich S, Assanasen P, deTineo M, Haney L, Naclerio RM, Baroody FM. Effects of intranasal azelastine on the response to nasal allergen challenge. *Laryngoscope.* 2002;112:47–52.
 38. Casale TB. The interaction of azelastine with human lung histamine H₁, beta, and muscarinic receptor-binding sites. *J Allergy Clin Immunol.* 1989;83:771–776.
 39. Wagenmann M, Baroody F, Kagey-Sobotka A, Lichtenstein L, Naclerio R. The effect of terfenadine on unilateral nasal challenge with allergen. *J Allergy Clin Immunol.* 1994;93:594–605.
 40. Lichtenstein L, Gillespie E. The effects of the H1 and the H2 antihistamines on “allergic” histamine release and its inhibition by histamine. *J Pharmacol Exp Ther.* 1975;192:441–449.
 41. Schroeder J, Schleimer R, Lichtenstein L, Kreutner W. Inhibition of cytokine generation and mediator release by human basophils treated with desloratadine. *Clin Exp Allergy.* 2001;31:1369–1377.
 42. Genovese A, Patella V, De Crescenzo G, De Paulis A, Spadaro G, Marone G. Loratadine and desethoxycarbonyl-loratadine inhibit the immunological release of mediators from human FcεRI+ cells. *Clin Exp Allergy.* 1997;27:559–567.
 43. Leonardi A, Abelson MB. Double-masked, randomized, placebo-controlled clinical study of the mast cell-stabilizing effects of treatment with olopatadine in the conjunctival allergen challenge model in humans. *Clin Ther.* 2003;25:2539–2552.
 44. Ciprandi G, Pronzato C, Passalacqua G, et al. Topical azelastine reduces eosinophil activation and intercellular adhesion molecule-1 expression on nasal epithelial cells: an antiallergic activity. *J Allergy Clin Immunol.* 1996;98(pt 1):1088–1096.
 45. Achterrath-Tuckermann U, Simmet T, Luck W, Szelenyi I, Peskar BA. Inhibition of cysteinyl-leukotriene production by azelastine and its biological significance. *Agents Actions.* 1988;24:217–223.
 46. Chand N, Pillar J, Nolan K, Diamantis W, Sofia RD. Inhibition of allergic and nonallergic leukotriene C4 formation and histamine secretion by azelastine: implication for its mechanism of action. *Int Arch Allergy Appl Immunol.* 1989;90:67–70.
 47. Hamasaki Y, Shafiegh M, Yamamoto S, et al. Inhibition of leukotriene synthesis by azelastine. *Ann Allergy Asthma Immunol.* 1996;76:469–475.
 48. Little M, Casale T. Azelastine inhibits IgE-mediated human basophil histamine release. *J Allergy Clin Immunol.* 1989;83:862–865.
 49. Togias A. H1-receptors: localization and role in airway physiology and in immune functions. *J Allergy Clin Immunol.* 2003;112(suppl):S60–S68.
 50. Coward WR, Okayama Y, Sagara H, Wilson SJ, Holgate ST, Church MK. NF-κB and TNF-α: a positive autocrine loop in human lung mast cells? *J Immunol.* 2002;169:5287–5293.
 51. MacGlashan D, Schleimer R, Peters S, et al. Generation of leukotrienes by purified human lung mast cells. *J Clin Invest.* 1982;70:747–751.
 52. Zhao W, Oskeritzian C, Pozez A, Schwartz L. Cytokine production by skin-derived mast cells: endogenous proteases are responsible for degradation of cytokines. *J Immunol.* 2005;175:2635–2642.
 53. Charlesworth E, Kagey-Sobotka A, Norman P, Lichtenstein L. Effect of cetirizine on mast-cell mediator release and cellular traffic during the cutaneous late-phase reaction. *J Allergy Clin Immunol.* 1989;83:905–912.
 54. Zweiman B, Atkins P, Moskovitz A, von Allmen C, Ciliberti M, Grossman S. Cellular inflammatory responses during immediate, developing, and established late-phase allergic cutaneous reactions: effects of cetirizine. *J Allergy Clin Immunol.* 1997;100:341–347.

Requests for reprints should be addressed to:
 Alkis Togias, MD
 Division of Allergy, Immunology, and Transplantation
 National Institute of Allergy and Infectious Diseases
 National Institutes of Health
 6610 Rockledge Dr, Room 3065
 Bethesda, MD 20892
 E-mail: togiasa@niaid.nih.gov