

Anti-IgE (omalizumab) inhibits late-phase reactions and inflammatory cells after repeat skin allergen challenge

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Background: Anti-IgE (omalizumab) inhibited early and late asthmatic reactions and infiltration of inflammatory cells in asthmatic bronchial biopsies at baseline. The effect of chronic allergen exposure on these outcomes is unknown. Repeat allergen challenge in human skin represents a suitable model to address this question.

Objective: To study the effect of anti-IgE (omalizumab) on early-phase (EPR) and late-phase (LPR) skin reactions and cellular infiltration by using a repeat skin allergen challenge designed to imitate chronic allergen exposure.

Methods: Twenty-four atopic allergic volunteers received omalizumab or placebo for 12 weeks. Paired intradermal challenges of allergen (30 biological units) and diluent control were administered on 9 occasions at 2-week intervals.

Early-phase and late-phase skin reactions and cellular infiltration in skin biopsies (using immunohistochemistry and in situ hybridization) were measured at intervals.

Results: Compared with placebo, omalizumab-treated patients had a progressive reduction in the LPR that was significantly greater than its effect on the EPR (median, -63% vs -24% respectively; $P = .009$). In addition, significant reduction of the LPR was reached within 2 weeks of commencing treatment, compared with 8 weeks for the EPR. There was a priming effect of repeated allergen challenge on infiltration of eosinophil, neutrophil, T_H2 ($CD3^+/IL-4^+$), and total $Fc\epsilon RI^+$ cells in patients on placebo that was abrogated in those receiving omalizumab.

Conclusion: The more marked effect of omalizumab on the LPR and prevention of the repeat-dose priming effect on several inflammatory cell types support a role for anti-IgE treatment in conditions associated with chronic allergic inflammation. (*J Allergy Clin Immunol* 2005;116:558-64.)

Key words: *FcεRI, eosinophils, allergy, mast cells, T cells, omalizumab*

Abbreviations used

AUC: Area under the curve
BU: Biological units
EPR: Early-phase reaction
HDM: House dust mite
LAR: Late asthmatic reaction
LPR: Late-phase reaction
 P^A : AUC for allergen
 P^{A-D} : AUC for allergen minus diluent
 P^D : AUC for diluent
SPT: Skin prick test

The late-phase reaction (LPR) is often considered a model of chronic allergic inflammation because it is associated with local infiltration by various cell types including eosinophils, basophils, and neutrophils. However, a disadvantage of the procedure is that it involves only a single exposure. Repeat allergen challenge, on the other hand, imitates the chronicity of allergen exposure in a standardized fashion.¹

The classic experiments of Dolovich et al² and Solley et al³ indicated that the cutaneous LPR was IgE-dependent because it could be induced by specific IgE antibody alone or $F(ab')_2$ fragments of an anaphylactogenic anti-IgE. Frew and Kay⁴ observed, however, that the cutaneous LPR and the wheal and flare reaction had different antigen dose thresholds, suggesting that the mechanisms of the 2 reactions may be partially independent.

Fahy et al⁵ showed that anti-IgE (omalizumab; Xolair; Novartis Pharmaceuticals, East Hanover, NJ) given twice weekly for 3 weeks suppressed both the early and late asthmatic reaction (LAR) as well as the numbers of circulating eosinophils. Inhibition of the LAR was more marked than the early asthmatic reaction, suggesting either mast cell activation leading to downstream inflammatory effects or nonmast cell, IgE-dependent events involving IgE (Fc) receptors on antigen-presenting cells.⁶

In the current investigation, we have studied the effect of omalizumab on the early-phase and late-phase skin reaction. Because of the previous observations on the different allergen dose thresholds required to elicit these responses,⁴ we hypothesized that the kinetics of inhibition of the 2 reactions may differ. This provided a further rationale for performing skin allergen challenge at several time points over the 12-week study period. Therefore, the

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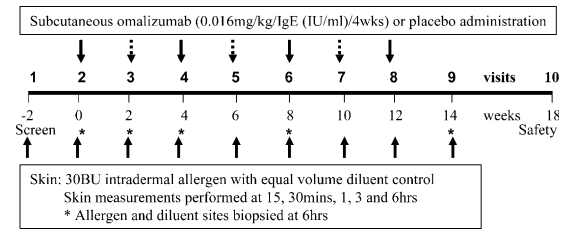


FIG 1. The study design. The times when omalizumab or placebo were administered and the occasions on which skin challenges with allergen and diluent were performed (with or without skin biopsies) are indicated in the flow diagram. The dose of drug administered was 0.016 mg/kg/IgE (IU/mL) every 4 weeks (solid arrows). Individuals requiring doses between 450 and 750 mg every 4 weeks had the monthly dose administered in 2 equal portions at 2-weekly intervals (solid and interrupted arrows) to minimize the number of injections while maintaining adequate drug levels.

current experimental design not only provided a model of chronic exposure but also enabled us to study the time course of inhibition by anti-IgE of both the early-phase reaction (EPR) and the LPR.

In addition we wished to determine whether anti-IgE treatment inhibited the influx into the tissues of inflammatory cells such as eosinophils, neutrophils, basophils, and mononuclear cells. We have previously shown that eosinophils and neutrophils peak at 6 hours and the other cells at late time points.⁷ Because omalizumab was particularly effective in decreasing the magnitude of the LAR⁵ and has been shown to reduce baseline airway inflammatory cells⁸ and to have efficacy in persistent severe asthma,⁹ we hypothesized that it would inhibit the late-phase cutaneous reaction and associated inflammatory cell infiltration in a repeat skin challenge model.

METHODS

Volunteers

Twenty-four atopic volunteers with allergy within an age range of 18 to 50 years were recruited. Volunteers were defined to be allergic by a positive skin prick test (SPT) to 1 or more common aeroallergens with clinical symptoms of rhinitis or asthma. Use of oral corticosteroids or other anti-inflammatory drugs were not permitted during the study or in the preceding 3 months, and antihistamines were prohibited for 5 days before each study visit. The study was approved by the ethics committee of the Royal Brompton and Harefield National Health Service Trust, London, United Kingdom, and all volunteers gave informed consent before participation in the study.

Study design

This was a randomized, double-blind, placebo-controlled, parallel group study involving 10 visits, with the first 9 taking place at 2-week intervals (Fig 1). The final safety visit took place 4 weeks later. At the screening visit, visit 1, SPTs to 5 common aeroallergens (house dust mite, grass and tree pollen, cat and dog dander) and baseline serum total IgE levels were performed. Taking into consideration the SPT results and clinical history, an allergen was selected for each volunteer for use throughout the study that caused nasal symptoms clinically as well as a positive SPT result. The different allergens used

TABLE I. Baseline characteristics of omalizumab-treated and placebo-treated groups*

	Omalizumab (n = 12)	Placebo (n = 12)	P value
Age, y	27.5 (23-47)	28.5 (23-47)	.9
Sex, M:F	8:4	4:8	
Baseline IgE level, IU/mL	70.5 (18-577)	121.5 (30-472)	.4
Baseline EPR size, cm	1.4 (1.15-2.1)	1.5 (1.0-3.2)	.5
Baseline LPR size, cm	5.5 (3.3-7.2)	5.4 (3.1-6.4)	1.0

*Data were nonparametric and expressed as medians (minimum and maximum values).

for intradermal testing in the 2 groups were as follows: omalizumab-treated group, house dust mite (HDM; 6), cat (6); placebo-treated group, HDM (5), cat (4), grass pollen (2; challenged out of season only), dog (1). An intradermal challenge with 30 biological units (BU) of this allergen was administered to ensure an allergen-induced late-phase skin response (at 6 hours) of at least 30 mm mean diameter.

Visits 2 to 9 constituted the assessment and treatment phase of the study. At each visit, 30 BU allergen and a separate diluent control were given intradermally on the dorsal surface of the forearms at least 6 cm from sites of previous challenges. Measurements of the weal were performed at 15 minutes (EPR) and the size of the skin response at 6 hours (LPR), as previously described.⁴ Cutaneous reactions were measured by a single investigator throughout the study, and this investigator was not involved in the administration of omalizumab/placebo to maintain blinding. Four-millimeter skin biopsies were taken of both the allergen and diluent sites at 6 hours on visits 2, 3, 4, 6, and 9. SPTs to the same 5 common aeroallergens and total serum IgE levels were repeated at visits 9 and 10. Free IgE was not measured, but previous studies have shown a decrease in free IgE with increases in total IgE after omalizumab treatment.^{10,11}

Volunteers were randomized to receive subcutaneous injections of either omalizumab or placebo for a total of 12 weeks (visits 2-8 inclusive). On visit 2, all baseline measurements and biopsies were performed before initial drug administration. The dose and dosing intervals were based on the serum half-life of omalizumab, efficacy results from previous studies, and the individual's body weight and baseline serum IgE. This ensured that volunteers received a dose of omalizumab, or placebo, equivalent to 0.016 mg/kg/IgE (IU/mL) per 4 weeks. For individuals requiring 450 to 750 mg of drug, the monthly dose was divided into 2 equal portions administered every 2 weeks to minimize the number of injections at any one time while maintaining adequate drug concentrations.

Tissue processing, immunohistochemistry, and in situ hybridization

Tissue processing, immunohistochemistry, and in situ hybridization were performed as previously described in detail.^{7,12} The alkaline phosphatase–antialkaline phosphatase method was used to enumerate cells binding to mAbs against human mast cells (anti-tryptase; Dako, Cambridge, United Kingdom), T-cell subtypes (CD3, CD4, CD8; Dako), macrophages (CD68; Dako), neutrophils (anti-neutrophil elastase; Dako), α-FcεRI (22E7; gift of Dr Jarema Kochan, Hoffmann-La Roche,¹³ Nutley, NJ), and basophils (BB1; gift of Dr A. Walls, University of Southampton). The rabbit anti-FcεRI antibody (997) was used to double-stain with CD1⁺ dendritic cells (Dako) and BB1⁺ basophils. The cDNA encoding human IL-4 has been used as templates to produce riboprobes for in situ hybridization in our previous studies.^{6,13}

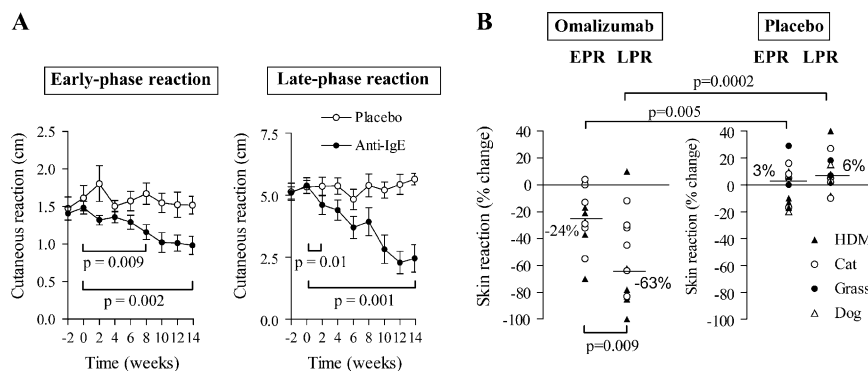


FIG 2. A, Effect of omalizumab or placebo treatment on the allergen-induced early-phase and late-phase skin responses over the treatment period. Values are expressed as the mean diameter in centimeters (means $\pm$ SEMs; $n = 12$ in omalizumab-treated and placebo-treated groups). Significant changes were analyzed by Wilcoxon signed-rank test. **B,** Percentage changes in the size of allergen-induced early-phase and late-phase skin reactions after treatment with omalizumab or placebo ($n = 12$, both groups). The crossbars represent the median values. Significant changes between groups were analyzed by Wilcoxon rank-sum test.

Statistical analysis

Data were analyzed by using nonparametric tests for all results. Within-group changes for total serum IgE, SPT diameter, and early and late cutaneous reactions were analyzed by using a Wilcoxon signed-rank test, and the changes between treatment groups were tested by using a Wilcoxon rank-sum test. For each of diluent, allergen, and difference between allergen and diluent, the area under the curve (AUC) was calculated from 0 to 14 weeks by using the trapezoidal rule for each parameter. The significance of the difference between these variables within each treatment group was tested with a Wilcoxon signed-rank test. Differences between the 2 treatments were tested with a Wilcoxon rank-sum test. A P value of $\leq .05$ was considered significant.

RESULTS

All 24 volunteers completed the study. Both groups were matched for age and the sizes of the EPR and LPR at baseline, although there were more men in the omalizumab-treated group. There was a nonsignificant baseline elevation in total IgE in those receiving placebo (Table I).

Adverse events

The proportion of patients who had at least 1 adverse event was 33% and 67% in the omalizumab-treated and placebo-treated groups, respectively. All adverse events were considered mild and consisted mainly of viral upper respiratory tract infection in both groups. None of the events was considered to be drug-related. In the placebo group, 1 volunteer developed transient atopic dermatitis over the site of intradermal allergen challenge on visits 8 and 9, and another had a single episode of exercise-induced urticaria 12 hours after intradermal allergen challenge on visit 7 that resolved spontaneously within 2 hours.

Total IgE levels and SPTs

There was a significant increase in total IgE concentrations at 14 weeks ($P = .0005$) and 18 weeks ($P = .0005$)

compared with baseline in the omalizumab group. The mean SPT diameter (calculated as size of positive reactions divided by 5, ie, the number of allergens tested) showed a significant reduction at 14 weeks ($P = .0005$) and 18 weeks ($P = .002$) in the omalizumab group, with no change seen in the placebo group.

Allergen-induced early and late-phase cutaneous reactions

In the placebo group, the multiple skin allergen challenge design did not affect the size of the early-phase or late-phase skin reactions (Fig 2, A). Omalizumab produced a progressive reduction in both the allergen-induced early-phase and late-phase skin responses compared with placebo (Fig 2, A). The decrease in the size of the EPR (compared with time 0) was significant by 8 weeks after the start of treatment ($P = .009$) and continued to decrease until the end of the study ($P = .002$). As shown in Fig 2, B, the median reduction from baseline was 24% ($P = .005$). In contrast, the reduction in the size of the allergen-induced LPR in omalizumab-treated subjects was evident by 2 weeks ($P = .01$) and continued to decrease to 14 weeks ($P = .001$; Fig 2, A). There was a median reduction in the size of the LPR of 63% ($P = .0002$; Fig 2, B). There was a significantly greater percentage difference in the LPR compared with the EPR ($P = .009$). Thus, omalizumab had a significantly greater effect on the LPR than the EPR in terms of both the degree and the kinetics of inhibition. These results did not appear to be influenced by the choice of allergen used for priming (Fig 2, B).

Cell counts in skin biopsies

For all cell counts, the between-group differences (omalizumab vs placebo) are expressed as AUC for allergen minus diluent (P^{A-D}), diluent alone (P^D) or allergen alone (P^A ; Table II). Absolute cell counts at baseline (pretreatment) for omalizumab and placebo are available in the Online Repository (see Table E1 in the online version of this article at www.mosby.com/jaci).

TABLE II. AUC data for all inflammatory cells after allergen and diluent challenge from 0 to 14 weeks in both treatment groups*

	Omalizumab			Placebo			P values between groups		
	Allergen	Diluent	P value	Allergen	Diluent	P value	P ^{A-D}	P ^D	P ^A
Eosinophils†	478.2 (231.5-652.0)	10.8 (2.85-39.2)	.0005	970.3 (579.9-1335.7)	27.5 (5.4-77.4)	.0005	.007	.4	.009
Neutrophils†	958.1 (667.9-1323.2)	239.1 (173.6-370.5)	.0005	1426.8 (1036.8-1482.2)	264.8 (157.6-357.2)	.0005	.05	.6	.06
Basophils†	208.3 (131.0-329.8)	88.0 (13.7-173.1)	.001	266.3 (183.4-380.7)	146.1 (92.8-202.5)	.0005	.3	.3	.3
Mast cells‡	267.7 (200.6-331.0)	339.7 (322.0-430.3)	.0005	260.6 (251.9-335.1)	400.9 (385.9-427.4)	.0005	1.0	.6	.8
Macrophages†	135.2 (74.1-267.7)	116.9 (84.4-134.1)	.6	120.5 (105.8-224.7)	154.3 (136.3-180.4)	.6	.4	.009	.7
Dendritic cells†	12.6 (88.8-129.4)	135.9 (97.4-181.2)	.2	143.2 (99.5-174.7)	166.2 (123.2-202.0)	.3	.8	.5	.2
FcεRI ⁺ cells†	84.6 (43.3-107.9)	61.5 (45.9-79.7)	.2	148.2 (130.8-223.3)	131.6 (79.5-144.8)	.01	.1	.008	.001
CD45RO ⁺ cells†	223.4 (118.6-464.4)	162.9 (68.3-247.6)	.2	499.8 (210.2-532.4)	249.4 (126.4-389.6)	.08	.6	.2	.4
CD3 ⁺ cells†	286.6 (139.8-493.5)	223.1 (148.7-263.4)	.09	393.3 (260.3-507.3)	214.9 (153.8-251.7)	.002	.2	.9	.2
CD4 ⁺ cells†	175.9 (148.0-263.0)	161.9 (96.1-215.0)	.09	229.9 (165.9-420.1)	167.8 (131.5-229.1)	.002	.3	.4	.2
CD8 ⁺ cells†	84.2 (67.4-136.9)	72.4 (57.5-135.6)	.7	93.1 (58.5-132.6)	100.5 (97.8-109.6)	.3	.1	.3	1.0

*All values expressed as AUC of cells/mm². P values represent the within-group difference (Wilcoxon signed-rank test) between allergen and diluent and the between-group differences (Wilcoxon rank-sum test). The absolute cell counts (cells/mm²) pretreatment are shown the Online Repository (see Table E1 in the online version of this article at www.mosby.com/jaci).

†Results shown are allergen – diluent values expressed as medians (interquartile range).

‡Results shown are diluent – allergen values expressed as medians (interquartile range).

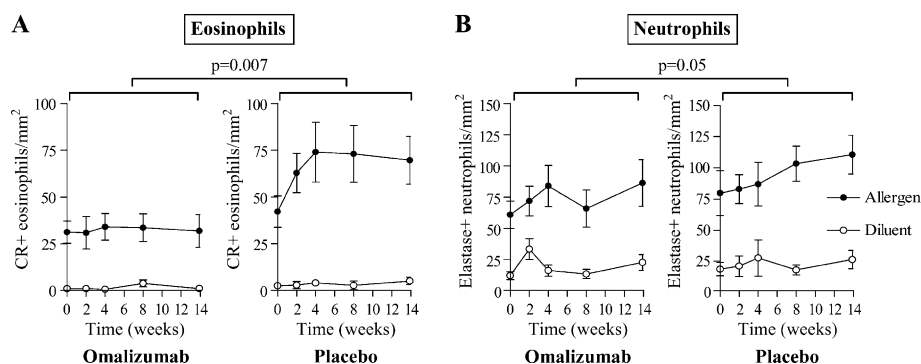


FIG 3. Effect of omalizumab or placebo treatment on (A) Congo red⁺ (CR⁺) eosinophils and (B) elastase⁺ neutrophils enumerated in skin biopsies taken 6 hours after diluent or allergen challenge over the treatment period. The results are expressed as the number of positive cells (means ± SEMs) per square millimeter of skin biopsy (n = 12, omalizumab; n = 12, placebo). The AUC was calculated from 0 to 14 weeks by using the trapezoidal rule for the difference between allergen and diluent (P^{A-D}), and significant differences between groups were analyzed by Wilcoxon rank-sum test.

Eosinophils and neutrophils. There were no significant changes in the number of eosinophils or neutrophils in diluent-treated sites in either the omalizumab-treated or placebo-treated groups. With allergen, eosinophils showed a priming effect in the placebo group as a result of multiple allergen injections that was maximal by 4 weeks. The increases in eosinophils and neutrophils after allergen challenge were significant for both active and placebo (eosinophils placebo, $P = .0005$; omalizumab, $P = .0005$; neutrophils placebo, $P = .0005$; omalizumab, $P = .0005$). However, the priming effect was abrogated in the omalizumab group compared with placebo (P^{A-D} eosinophils, $P = .007$; neutrophils, $P = .05$; P^A eosinophils, $P = .009$; neutrophils, $P = .06$; Fig 3; Table II).

CD3⁺ and CD3⁺/IL-4⁺ cells. The increase in CD3⁺ cells after allergen challenge was significant in the placebo group ($P = .002$) but not for the active group; there were

no between group significances using the AUC analysis (Table II). Double-staining CD3⁺/mRNA IL-4⁺ cells were also counted, but only at week 0 and week 14, as representative of a T_H2/Tc2 population of cells. There were increases in CD3⁺/IL-4⁺ cells in subjects receiving placebo (but not in those receiving omalizumab) in the diluent but not allergen-challenged sites ($P = .03$) with a between-group difference trend ($P = .06$; Fig 4). When week 0 was compared with week 14, there were also significant increases in CD3⁺ cells in the diluent sites ($P = .02$) in the placebo (but not active) group with a between-group difference of $P = .05$.

FcεRI⁺ cells. There were significant allergen-induced increases in FcεRI⁺ cells in the placebo group ($P = .01$) but not in the omalizumab-treated group (Table II). However, there was no between-group significance in P^{A-D} . In the placebo group, there was a clear priming effect of

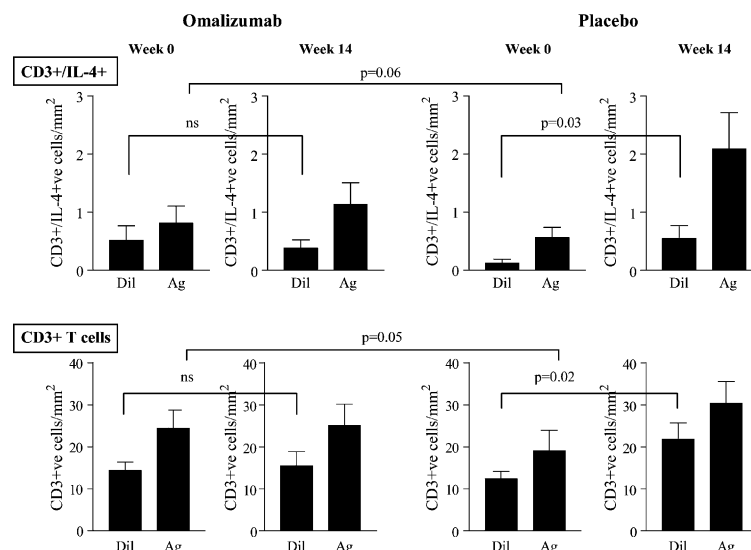


FIG 4. Effect of omalizumab or placebo treatment on (A) CD3⁺/IL-4⁺ T cells and (B) CD3⁺ T cells enumerated in skin biopsies taken 6 hours after diluent (Dil) and allergen (Ag) challenge at baseline and after 14 weeks of treatment. The results are expressed as the number of positive cells (means $\pm$ SEMs) per square millimeter of skin biopsy (n = 12, omalizumab; n = 12, placebo). Statistical differences in the biopsies taken at diluent challenged sites only within each treatment group were analyzed by Wilcoxon signed-rank test, and between-group differences were analyzed by Wilcoxon rank-sum test. Similar analyses for allergen sites alone or allergen minus diluent were both significant.

multiple allergen challenge observed at allergen-injected sites and, to a lesser extent, at diluent-injected sites that was significantly different from that in the omalizumab-treated subjects (P^A , $P = .001$; P^D , $P = .008$; Table II).

Double-staining showed that these FcεRI⁺ cells were composed of BB1⁺ basophils, CD1⁺ dendritic cells, and Congo red⁺ eosinophils. However, the numbers were too small in these 6-hour biopsies for accurate FcεRI⁺ differential cell counts (data not shown).

Other inflammatory cells. There were no between-group significances in the allergen-induced changes (P^{A-D}) in macrophages, CD8⁺ T cells, basophils, dendritic cells, or CD45RO cells. There were significant increases (allergen vs diluent) in placebo-treated ($P = .0005$) and omalizumab-treated ($P = .001$) subjects for basophils. With CD4⁺ cells, the allergen-induced increase with placebo was significant ($P = .002$), but with omalizumab, this was only a trend ($P = .09$; Table II). The P^D for macrophages was also significant ($P = .009$). However, no allergen-induced increases in macrophages were observed in these 6-hour biopsies. With mast cells, there were decreased numbers at allergen-injected sites (compared with diluent sites) in both the omalizumab and placebo subjects (Table II). Thus, the data from the omalizumab-treated and placebo-treated subjects were similar, with no evidence that in this experimental model, omalizumab lowered baseline mast cell counts.

DISCUSSION

In our current study, omalizumab was well tolerated, and there were the expected elevations in total serum

IgE (because of reduced clearance of omalizumab:IgE complexes) and decreases in SPT reactions in the active-treated but not placebo-treated group, indicating appropriate bioavailability of the mAb. The nonsignificant differences in baseline serum IgE were not considered to be clinically relevant, especially as the range values were very similar. The sex imbalance at baseline is also considered to be inconsequential.

Omalizumab had a significantly greater effect on the late-phase, as opposed to the early-phase, allergic skin reaction in terms of both the time course of inhibition and the magnitude of the response. Beck et al¹⁴ reported that in 3 subjects, omalizumab decreased the expression of FcεRI on mast cells at baseline, results in keeping with previous studies showing reductions in FcεRI expression on circulating basophils after treatment with anti-IgE.¹⁵ In both the placebo and active group, the immediate wheal and flare reaction was associated with a decrease in the number of tryptase-positive mast cells in skin biopsies, consistent with degranulation and previously observed in biopsies from skin LPR.¹⁶ We were not able to demonstrate a reduction in mast cell degranulation after treatment with omalizumab in this study. If mast cell mediators are responsible for both the EPR and LPR, then the far more pronounced effect of omalizumab on the LPR may have been a result of a different allergen threshold for LPR as opposed to EPR.

Alternatively, or in addition, LPR, like the LAR, may have a T-cell component. Studies in mice have shown that anti-IgE mAb suppresses T_H2 cytokine production by inhibiting IgE-CD23-facilitated antigen presentation to T cells.⁶ Furthermore, in human beings, allergen injection immunotherapy and T-cell peptides suppress the late-phase

skin response before changes in the early reaction are demonstrable.^{17,18} Because these forms of therapy appear to induce some form of T-cell tolerance or anergy, this implies that T-cell products may directly cause the redness and edema of the skin LPR. The inhibition of this reaction by anti-IgE would again invoke pathways that involve allergen trapping and focusing by IgE bound to either high (FcεRI) or low (CD23) IgE receptors on cutaneous dendritic/Langerhans cells. Of interest is a recent finding that omalizumab reduced FcεRI expression by 83% in the precursor DC1 subset in the blood of human atopic subjects.¹⁹

We use the term *priming* to refer to the increases in cell counts observed in the placebo group at either allergen-injected or diluent-injected sites compared with baseline (pretreatment sites). There have been several reports of the effect of multiple allergen injections in the nose, airways, and skin of human atopic subjects.¹ In general, the results are contradictory with outcomes, depending on the protocol. In some, this leads to tolerance/desensitization, and in others, enhanced inflammatory migration can be observed after several applications of allergen.¹ It is likely that the timing between injections is critical for determining priming or tolerance. This may explain why the primary effect appeared to plateau after 4 weeks.

Although multiple allergen injections did not affect the magnitude of the baseline EPR or the LPR in the placebo group, they had a marked effect on cellular infiltration. These enhanced allergen-induced increases of eosinophils and FcεRI⁺ cells in the skin biopsies were inhibited by omalizumab. Similar, significant, but far less marked findings (ie, a priming effect in the placebo group and inhibition by omalizumab) were observed with neutrophils. Similar results at diluent sites were also observed with CD3⁺/IL4⁺ (representing T_H2/Tc2) cells. The numbers of double-positive cells were small, and the results could not have been satisfactorily achieved with CD4, rather than CD3, as the T-cell marker. We speculate that these changes in cellular infiltration were not simply a result of local mast cell effects because all allergen and diluent challenges were performed at sites that were spatially and temporally separated from sites of previous intradermal injection. However, this finding is consistent with omalizumab having effects on other IgE receptor-bearing cells besides the mast cell—for example, IgE receptor-bearing dendritic cells presenting to T cells. It should also be noted that biopsies were taken 6 hours after allergen challenge. We have previously shown that this time point is optimal for eosinophils and neutrophils, whereas CD3, CD4⁺, and basophils peak later, at 24 hours.⁷

It was particularly interesting to note the increase in the baseline number of skin-homing allergen-specific T cells (as measured in the biopsies from diluent-challenged sites) after multiple allergen challenges in the placebo-treated group. Picker et al,²⁰ using cutaneous blisters overlaying delayed-type hypersensitivity sites, concluded that repeated activation in skin or skin-associated peripheral lymph nodes enhances cutaneous lymphocyte-associated

antigen expression on T cells and focuses their recirculation to the skin. Thus, the increases in eosinophils, neutrophils, and CD3⁺ cells in the placebo-treated group may be explained by the following scenario. Mast cell-derived mediators, particularly CC chemokines such as eotaxin, lead to eosinophil infiltration.²¹ Allergen also interacts with IgE-bearing cutaneous dendritic cells, which migrate to regional lymph nodes.²² Allergen-specific T cells then recirculate to the site of injection, possibly causing a general increase in skin-homing T cells. These allergen-specific T cells may be IL-5⁺ T_H2 cells that induce eosinophil release from the bone marrow.²³ Finally, all of these events are amplified with each successive intradermal injection of allergen.

Our findings support previous studies suggesting that infiltrating inflammatory cells, especially eosinophils, are not directly promoting the redness and swelling characteristic of the cutaneous LPR, because reduction of skin eosinophils in atopic subjects by intravenous infusions of anti-IL-5 (mepolizumab) did not affect the size of the LPR but did downregulate markers of remodeling.²⁴ The current findings are also consistent with previous observations in the airways of patients with asthma, that eosinophils do not influence the LAR²⁵ but may play a downstream role in repair and remodeling events.²⁶

Thus, our studies confirm the original observations of Dolovich et al² and Solley et al³ suggesting that the EPR and LPR are both IgE-dependent (but not necessarily totally mast cell-dependent). The current findings using multiple allergen challenges suggest, but do not prove, that there may be nonmast cell IgE-dependent pathways (ie, cell-mediated eosinophilic hypersensitivity reactions) that are susceptible to treatment with omalizumab. This may be of particular relevance in the asthmatic airways, where, unlike the skin, there is baseline inflammation with the presence of low numbers of already activated T_H2 cells.²⁷ These may expand rapidly after inhalational allergen challenge through IgE-dependent allergen trapping and focusing by dendritic cells. Indeed, isolated LAR can be elicited by allergen-derived T-cell peptide epitopes that do not crosslink IgE but activate airway T cells directly in an MHC-restricted fashion.²⁸

In summary, the data indicate that omalizumab may be acting through other mechanisms besides IgE-dependent mast cell release of inflammatory mediators and that an anti-IgE approach is logical in the treatment of allergic disease associated with chronic allergen exposure.

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