

Omalizumab rapidly decreases nasal allergic response and FcεRI on basophils

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Background: Omalizumab is a monoclonal anti-IgE antibody that is effective for the treatment of allergic respiratory disorders; however, its onset of action is unknown.

Objective: This study was designed to determine the onset of action of omalizumab through the use of a challenge model to determine time-dependent inhibition of ragweed-induced changes in nasal volume as well as correlate the kinetics of omalizumab-induced decreases in serum free IgE and FcεRI receptors on basophils.

Methods: We conducted a 6-week, randomized, double-blind, placebo-controlled study of 24 rhinitic patients with ragweed allergy. After PD₃₀ ragweed nasal allergen challenge, patients received either omalizumab, ~0.016 mg/kg per IgE (IU/mL), or placebo at days 0 and 28 and were rechallenged with ragweed PD₃₀ dose biweekly. FcεRI expression on blood basophils was determined by flow cytometry at baseline and 7, 14, 28, and 42 days after treatment. IgE levels were measured at baseline and on days 3, 28, and 42.

Results: Mean IgE levels decreased by 96% ($P < .001$) from baseline within 3 days in the omalizumab group. Baseline 30% ragweed-induced nasal volume response was decreased to 20.4% at 7 to 14 days ($P < .001$) and 12.2% at 35 to 42 days ($P < .001$) for the omalizumab group. There was a median decrease in basophil FcεRI expression of 73% ($P < .001$) in the omalizumab group, with maximum inhibition occurring within 14 days of treatment. No significant changes in IgE levels, nasal allergen challenge responses, or basophil FcεRI expression were observed throughout the study in the placebo group. **Conclusions:** Our study showed that the onset of action by omalizumab in blunting ragweed-induced nasal responses is within 2 weeks, and this response was associated with 2 putative mechanisms of action: decreased serum free IgE and decreased FcεRI receptor expression on immune effector cells. (*J Allergy Clin Immunol* 2004;113:297-302.)

Key words: Allergic rhinitis, anti-IgE treatment, omalizumab, FcεRI receptor, basophils

Omalizumab (Xolair, Genentech, South San Francisco, Calif) is a recombinant humanized monoclonal anti-IgE antibody that binds to circulating IgE at the same site

Abbreviations used

MFI: Mean fluorescence intensity

SAR: Seasonal allergic rhinitis

on the Fc domain as the high-affinity IgE receptor, FcεRI. This action effectively blocks the interaction between IgE and mast cells and basophils, thereby preventing the release of inflammatory mediators that cause allergic signs and symptoms. Concomitant with the reduction in serum free IgE levels, omalizumab markedly reduced the density of FcεRI receptors on human basophils measured at 90 days.¹

In addition to its clinical activity in allergic asthma,²⁻⁴ omalizumab has proved to be effective for the treatment of seasonal allergic rhinitis (SAR). The use of omalizumab in ragweed-induced allergic rhinitis was first assessed in a dose-ranging study in 1994. The study concluded that omalizumab was safe and well tolerated.⁵ A follow-up study on ragweed-induced SAR demonstrated that omalizumab was well tolerated and most effective in preventing ragweed-induced symptoms when administered at doses as high as 300 mg SC every 3 or 4 weeks.⁶ A birch pollen allergy rhinitis study confirmed that omalizumab given at 300 mg and administered on a schedule based on baseline total serum IgE levels was effective in controlling symptoms related to SAR.⁷ Omalizumab caused a dose-dependent reduction in serum free IgE levels that was associated with reduced symptoms, rescue medication use, and increased quality of life.^{6,7} The results of these initial studies demonstrated that omalizumab was a safe and important agent for controlling SAR.⁶⁻⁹

Although the effectiveness of omalizumab has been studied extensively in allergic rhinitis and asthma, its onset of action is not known. There are several ways to measure the onset of action. One way is to use a challenge model. Another way is to dose the drug immediately at the start of a pollen season and then compare the daily symptom scores with those for placebo. In previous allergic rhinitis studies with omalizumab, the dosing took place any time from 4 weeks before to a few days after the start of the pollen season, and thus the onset of action was not evaluated. Past studies of omalizumab in asthma have been performed during concomitant inhaled corticosteroid treatment, thus confounding the determination of onset of action. A better understanding of the onset of action of omalizumab is necessary to effectively use the drug in the treatment of allergic diseases.

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TABLE I. Study design

	Screening	Day 0	Day 3	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Drug	—	A/B	—	—	—	—	A/B	—	—
Nasal challenge	A/B	—	—	A	B	A	B	A	B
IgE level	A/B	—	A/B	—	—	—	A/B	—	A/B
FcεRI	—	A/B	—	A/B	A/B	—	A/B	—	A/B

Twenty-four subjects were involved in the study, 16 in the active group and 8 in the placebo group, evenly divided into groups A and B.

To determine the onset of action of omalizumab, we used a challenge model to determine time-dependent inhibition of ragweed-induced changes in nasal volume as measured by acoustic rhinometry. We also measured the kinetics of omalizumab-induced decreases in serum free IgE and basophil FcεRI. In this work, we demonstrate the relation between omalizumab's clinical onset of action and changes in immunologic parameters of immediate hypersensitivity. We found that omalizumab has a rapid onset of action in inhibiting clinical response and decreasing IgE levels and FcεRI expressed. These results have important implications for the use of omalizumab in the treatment of allergic disease.

METHODS

Subjects

Subjects were between 19 and 50 years of age and had a history of ragweed SAR requiring pharmacotherapy for at least 2 years. All subjects had a positive skin prick test reaction to mixed giant/short/Western ragweed at the screening visit defined by a ragweed-induced wheal at least 5 mm larger in diameter than diluent control and a positive intranasal challenge to ragweed as defined below. Women of childbearing potential had a negative urine pregnancy test result and used effective means of contraception during the study.

Subjects were excluded from study participation if they had any of the following: a history of asthma; past or present immunotherapy; exposure to omalizumab; severe anaphylactic or anaphylactoid reactions; rhinitis medicamentosa; clinically significant perennial rhinitis, as manifested by current symptoms of sneezing, rhinorrhea, or nasal congestion requiring pharmacologic intervention; the presence of a severely deviated nasal septum, septal perforation, structural nasal defect, or large nasal polyps causing obstruction; or evidence of acute or significant chronic sinusitis. Subjects were also excluded if they had taken β -adrenergic antagonists in any form, had had an upper respiratory or sinus infection requiring treatment with an antibiotic agent within 2 weeks before the screening visit, used medications that would affect assessment of the effectiveness of the study medication (ie, antihistamines of any form, intranasal or oral decongestants, corticosteroids of any form, leukotriene modifiers, intranasal anticholinergics, and tricyclic antidepressants), used prohibited concomitant medications without appropriate washout, or had an IgE level of >700 IU/mL.

Study design

The study was a 6-week, single-center, double-blind, placebo-controlled trial of 24 subjects enrolled at Creighton University conducted from December 2001 to March 2002. The study was approved by the Creighton University Institutional Review Board. At the completion of the trial, the data were analyzed in a blinded fashion.

Eligible subjects were randomly assigned to receive either omalizumab (0.016 mg/kg per IgE [IU/mL]) or placebo in a 2:1 ratio on days 0 and 28. The treatment schedule was based on the data show-

ing the pharmacokinetic and pharmacodynamic relation between baseline IgE levels, omalizumab, and the expected suppression of IgE necessary for clinical benefit.^{5-7,10,11}

Serum total IgE levels were measured during the screening visit, and serum free IgE levels were measured on days 3, 28, and 42. Blood was drawn for evaluation of expression of FcεRI receptors on basophils on days 0, 7, 14, 28, and 42. Complete blood cell count, comprehensive chemistry profile, and urinary analysis were done periodically during the trial, and weekly clinical assessments were done to evaluate for adverse events.

Nasal challenge

Subjects were randomly assigned into 2 groups of 12 (groups A and B) so that no individual would be challenged more frequently than once every 2 weeks (Table I), thus lessening the likelihood of a priming effect from repeated nasal challenges done at shorter time intervals. There were 8 active and 4 placebo-treated subjects in each group. One person performed all of the acoustic rhinometry measurements in the same manner to ensure that the measurements were done in identical fashion.

During the screening visit, baseline acoustic rhinometry (Hood Laboratories, Pembroke, Mass) and spirometry (Koko, Pulmonary Data Services, Louisville, Colo) were performed. Two sprays of nasal saline (vehicle control) were then administered to each nostril (2 sprays = 0.2 mL) through a nasal spray pump. Ten minutes after saline administration, acoustic rhinometry and spirometry were repeated. Saline challenge was repeated a total of 3 times, and the last saline challenge was used as the baseline.

Five serial doses of ragweed were prepared at 0.00054 AU, 0.0054 AU, 0.054 AU, 0.54 AU, and 5.4 AU from the mixed giant, short, and Western ragweed extract (1:20 wt/vol, 66.67 AU/mL antigen E) obtained from Hollister-Stier (Spokane, Wash). Each serial dose of ragweed was administered to the nostrils through the nasal spray pump. Ten minutes after each administration of ragweed solution, acoustic rhinometry and spirometry were performed. The challenges continued at the next higher ragweed dose until there was a 30% decrease in nasal volume as determined by acoustic rhinometry, the final ragweed dose of 5.4 AU was reached, or a 20% decrease in FEV₁ was reached. Subjects were excluded if they did not achieve a 30% decrease in nasal volume or the FEV₁ declined by $\geq 20\%$ during the challenge. No subject in the study had decreases in FEV₁ of $\geq 20\%$. The additive provocative dose required to elicit a 30% decrease in nasal volume (PD₃₀) was calculated.

On subsequent visits, depending on the group (A: days 7, 21, and 35; B: days 14, 28, and 42), subjects returned for repeat nasal challenges. After baseline saline challenges were done, subjects were given their individualized calculated cumulative PD₃₀ dose of ragweed, based on their serial ragweed challenge results from the screening visit. Acoustic rhinometry and spirometry were recorded 10 minutes after PD₃₀ ragweed administration.

Free and total IgE measurements

Serum concentrations of total IgE were measured at baseline, and serum free IgE was measured on days 3, 28, and 42. Total IgE was measured by Associated Regional and University Pathologists

(ARUP, Salt Lake City, Utah) using the Immulite 2000 immunoassay. Serum free IgE that was not coupled to omalizumab was measured by Genentech with a solid-phase ELISA, as previously described.⁵

Basophil cell preparation

PBMCs for basophil analysis were isolated as previously described.¹²⁻¹⁴ PBMCs were isolated from EDTA anticoagulated blood with the use of a 1.083 g/mL ficoll-diatrizoate (Sigma, St Louis, Mo) density gradient separation. The cells were washed twice in PBS, and 20×10^6 cells were fixed in 3 mL of 4% paraformaldehyde for 5 minutes at room temperature. Samples were then washed once in cold PBS/0.1% BSA, and the pellets were resuspended in PBS/10% dimethyl sulfoxide (Sigma) and stored at -80°C .

Antibodies

Anti-CD2 (clone LT2) FITC and anti-CD19 FITC (clone FMC63) were obtained from Serotech (Raleigh, NC); anti-HLA-DR/DP/DQ FITC (clone TU39), CD4 APC (clone RPA-T4), and anti-IL 3R/CD 123 PE (clone 7G3) were acquired from PharMingen (San Diego, Calif); and anti-CD14 PE/Cy5 (clone Tuk4) and anti-CD19 PE (clone SJ25-C1) were obtained from Caltag (Burlingame, Calif). Anti-CD14 FITC (Immunotech, Marseille, France), anti-CD16 FITC (clone B-E16, BioSource, Camarillo, Calif), and goat anti-mouse IgG1 Alexa 647 (Molecular Probes, Inc, Eugene, Ore) were obtained from the manufacturers. Anti-FcεRI (clone 22E7) was a gift from F. Hoffmann-La Roche Ltd (Nutley, NJ).

Antibody staining and flow cytometry

To minimize intrasubject variation, all samples from a given donor were processed on the same day. FcεRIα staining was performed by using a nonpermeabilizing adaptation of previously described procedures.¹²⁻¹⁴ All buffers were used at 4°C . Cryopreserved fixed cells were thawed, washed in PBS/BSA, and then blocked in PBS/1% BSA/5% nonfat milk powder (PBS/BSA/milk) for 1 hour on ice before mAb staining. Cells were incubated with an exclusion panel of FITC-labeled mAbs to CD2, CD16, CD19; HLA-DR/DP/DQ and CD123 PE, CD14 PE/Cy5 (all non-IgG1 isotype); and anti-FcεRI (mouse IgG1 isotype) for 30 minutes in PBS/BSA/milk, washed twice, and incubated with goat anti-mouse IgG1 Alexa 647 in PBS/BSA/milk for 30 minutes and again washed. Basophils were identified as a distinct cluster of CD123 bright cells that stained negative with the exclusion panel and negative for CD14. Basophil expression of FcεRIα in mean fluorescence intensity (MFI) units was determined after gating on the basophil population.

Data were acquired with a 2-laser, 4-parameter FACS Calibur flow cytometer (BD Biosciences, San Jose, Calif) and analyzed with Cellquest (BD Biosciences) and FlowJo (Tree Star, San Carlos, Calif) software. Typically 300,000 to 600,000 total events were acquired to obtain ≥ 1000 basophils for analysis.¹⁴

Statistics

ANOVA and Tukey-Kramer multiple comparison tests were used to analyze data from the nasal volumes, the IgE levels, and the basophil FcεRI expression. Groups A and B were combined in the statistical analysis to achieve sufficient power. The data were analyzed by comparing the differences between baseline and treatment periods for the active and placebo groups. *P* values of $<.05$ were considered statistically significant. It should be noted that this study was powered to show differences in the omalizumab group between baseline and subsequent visits; it was not powered to determine differences between the active and placebo groups. Correlative data in were analyzed by means of the Spearman rank correlation test. For

correlations, for each subject, all IgE and for FcεRI determinations on study drug were averaged with like results and a single representative percentage fraction while on study drug relative to the day 0 baseline was determined. Linear regression analysis and calculation of correlation coefficients were performed with the use of Prism software (GraphPad Software, San Diego, Calif).

RESULTS

Demographics

A total of 24 subjects, 16 active and 8 placebo-treated, were enrolled in this single-center study at Creighton University. The mean age for the treated active group was 30.5 years versus 29.4 years for the placebo group. There were 5 men and 11 women in the omalizumab group and 3 men and 5 women in the placebo group. The mean PD₃₀ ragweed dose for the active group was 1.85 AU versus 0.98 AU for the placebo group. The starting IgE level and basophil FcεRI receptor levels were 281 ng/mL and 806.2 MFI units, respectively, for the active group, compared with 108 ng/mL and 741.4 MFI units for the placebo group.

Effect on IgE levels

Three days after omalizumab administration, the mean IgE level decreased 96.1%, from 281 ± 55 ng/mL to 10.9 ± 1 ng/mL for the active group ($P < .001$). On days 28 and 42 for the active group, the free IgE levels were 28.1 ± 3.7 ng/mL ($P < .001$) and 17.5 ± 2.5 ng/mL ($P < .001$), respectively. The placebo group did not have any statistical differences in IgE values between any of the visits and baseline or between any visit and any other. IgE values for the active group were significantly less than those for the placebo group at all visits after screening ($P < .001$).

Effect on nasal challenge

The mean baseline PD₃₀ doses for the active and placebo groups were 1.85 AU and 0.98 AU, respectively. The 30% nasal volume decrease at baseline was decreased to 20.4% at 7 to 14 days ($P < .001$), 20.1% at 21 to 28 days ($P < .001$), and 12.2% at 35 to 42 days ($P < .001$) for the omalizumab group. The placebo group did not have any statistical differences in nasal responses at any time compared with baseline (Fig 1). Fig 2 shows representative nasal challenge data from one individual given placebo and one individual treated with omalizumab. As can be seen, the subject given placebo did not have a significant change in the reduction in nasal volume during subsequent ragweed challenges throughout the 42-day trial period. However, the subject given omalizumab had a blunted response to ragweed challenge at all time points.

Identification of peripheral blood basophils

Using 4-color flow cytometry, basophils were identified as a cluster of CD123 bright cells that stained negative for both CD14 and the exclusion panel (a lineage cocktail of antibodies against CD2, CD16, CD19, and MHCII), separating them from T cells, B cells, monocytes, macrophages, neutrophils, natural killer cells, and dendritic cells (Fig 3, a). The number of basophils as a

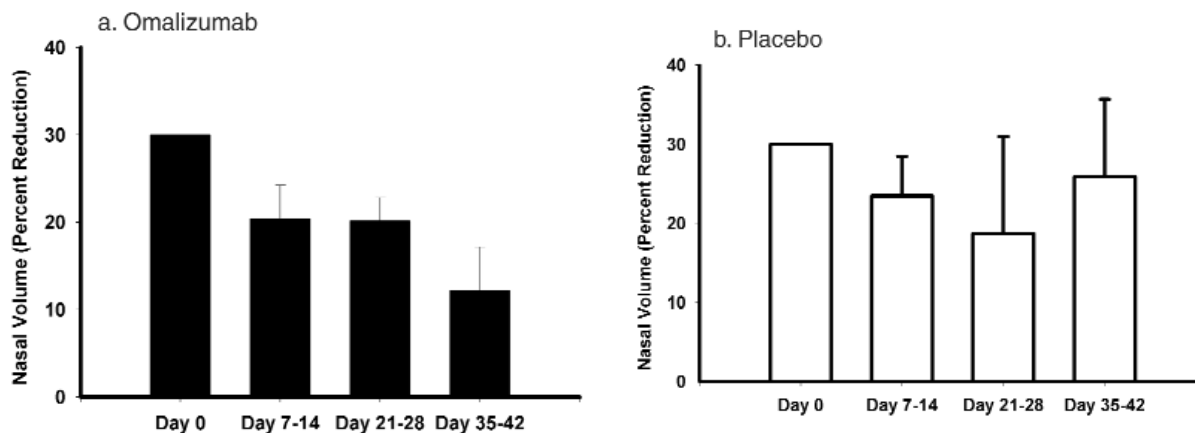


FIG 1. Effect of omalizumab on nasal volume reduction after ragweed challenge. **a.** Omalizumab group showed significant blunting of reduction in nasal volume after PD₃₀ dose of ragweed ($P < .001$) at each visit compared with baseline. **b.** In the placebo group, there was no statistical significance between visits to baseline and to each other. Data are medians $\pm$ 95% CIs (** $P < .001$).

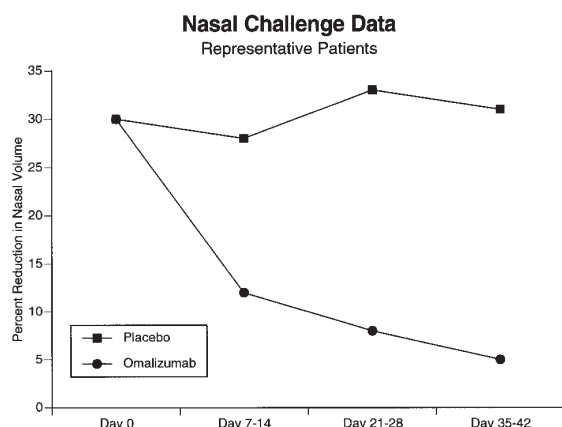


FIG 2. Nasal challenge data on representative placebo-treated and omalizumab-treated subjects. The subject given placebo maintained a 30% reduction in nasal volume after ragweed nasal challenge compared with the subject given omalizumab, who had gradual blunted response to ragweed challenge, with onset at 7 to 14 days.

percentage of total PBMCs did not change significantly throughout the study in either the treatment group or the placebo group (data not shown).

Effect of omalizumab on basophil Fc ϵ RI receptor expression

Basophil Fc ϵ RI α expression was determined by flow cytometry with the use of an anti-Fc ϵ RI α subunit Ab, 22E7, which binds to both occupied and unoccupied receptors. Fig 3, *b* shows the change in Fc ϵ RI expression from a representative omalizumab-treated subject after 42 days. Fig 4 shows group median data indicating that omalizumab caused a significant decrease in basophil Fc ϵ RI expression at all time points versus baseline ($P < .001$) and versus placebo treatment ($P < .001$). Maximum inhibition of Fc ϵ RI expression occurred within 14 days of omalizumab treatment, and this reduction was main-

tained for the duration of the study (median change, -73% ; mean change, -70%). No significant changes in Fc ϵ RI expression were found in the placebo group at any time point (Fig 4, *b*).

Previous data indicated that there is a correlation between serum IgE levels and the expression of high-affinity IgE receptors on inflammatory cells.¹⁵ To better define this relation, we compared the baseline serum IgE and basophil Fc ϵ RI expression with that observed while the study drug was being received. As shown in Fig 5, there was a high correlation between the reduction in baseline serum IgE (ie, lowest percentage of baseline serum IgE levels) and the reduction in basophil Fc ϵ RI expression (ie, greatest reduction from baseline). This relation between the reduction in IgE levels and basophil Fc ϵ RI expression was significant.

DISCUSSION

The primary purpose of this study was to determine the onset of action of omalizumab by using a challenge model to better describe how this drug might be used clinically. The second objective of the study was to determine if this clinical end point correlated with immunologic parameters that could explain this onset of action.

We found that omalizumab rapidly inhibited ragweed-induced nasal responses and that this inhibition was seen as early as 7 to 14 days and was even more pronounced by the 35th to 42nd days. These data imply that patients with SAR would have measurable protection with a single dose of omalizumab given within 2 weeks of an anticipated pollen season. Presumably, a second dose would be needed to offer full protection for the duration of a typical pollen season.

In this study, we found 2 potential mechanisms that could account for the rapid in vivo onset of action. The first mechanism was the decrease in IgE by 96.1% within 3 days. The second mechanism was the rapid down-regulation of the Fc ϵ RI receptors on basophils seen by 7

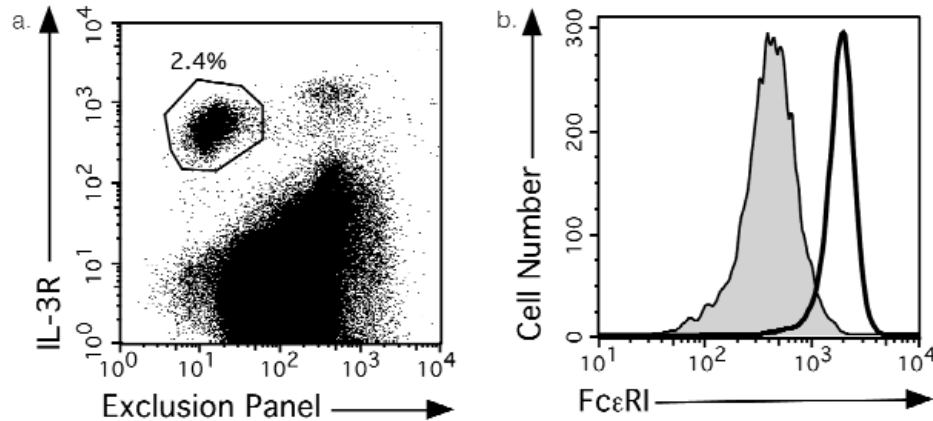


FIG 3. Basophil FACS plots. **a**, Flow cytometric dot plot shows gating used to identify the basophil population (polygon). **b**, Representative basophil FcεRI staining before study (open histogram) and on day 42 of omalizumab (shaded histogram).

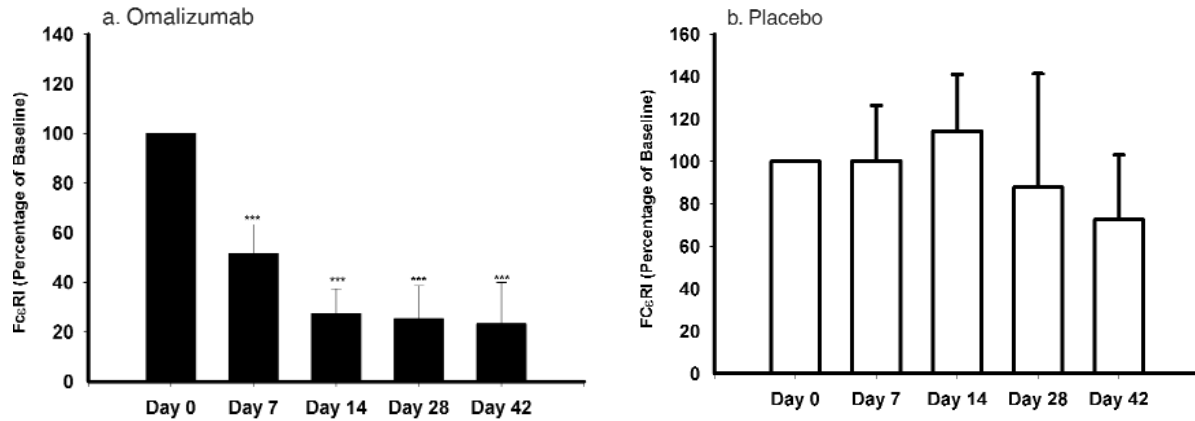


FIG 4. Effects of omalizumab on basophil FcεRI. Percentage of baseline MFI, median $\pm$ 95% CI. **a**, Significant reduction in FcεRI on basophils at all time points vs baseline ($P < .001$, Tukey-Kramer) in the omalizumab-treated group. **b**, No significant change in FcεRI on basophils in the placebo-treated group.

days and maximally occurring within 14 days (70%). In a preclinical study, MacGlashan et al¹ showed that omalizumab markedly reduced the density of high-affinity IgE receptors on human basophils after 90 days of multiple intravenous infusions. Basophils from these subjects showed a 90% reduction in their release of histamine after challenge with antigen, providing evidence that IgE receptor density is correlated with in vitro allergen-induced mediator release. MacGlashan et al described 3 subjects whose FcεRI expression on basophils declined within a similar time interval as compared with our study. Our data also confirm the relation between blood IgE levels and FcεRI expression (Fig 5).

This study also provides insight into the duration of IgE binding to high-affinity IgE receptors on circulating inflammatory cells. Serum free IgE was rapidly and maximally decreased within 3 days of omalizumab treatment. In contrast, maximal FcεRI downregulation did not occur until day 14. The kinetics of inhibition of basophil FcεRI expression and nasal PD₃₀ response appeared to parallel one another and were delayed relative to the drop in free IgE. This suggests that the downregulation of FcεRI on

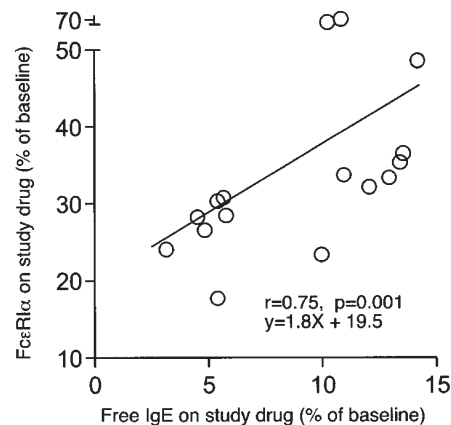


FIG 5. Correlation of basophil FcεRIα expression with serum IgE. For each subject, the fraction of IgE and FcεRI while receiving omalizumab relative to day 0 baseline was determined and plotted as described in the Methods section.

critical effector cells is required for the clinical inhibition of immediate hypersensitivity responses by anti-IgE. However, the study was not designed to measure nasal

PD₃₀ responses at sufficiently early time points (3 days) to more fully address this question. These data also suggest that the resident time of IgE bound to its high-affinity receptor was <14 days; however, this may only be applicable when IgE levels are rapidly lowered. Nonetheless, since the anti-FcεRI antibody used in this study recognized both occupied and unoccupied receptors, this implied that IgE binding to FcεRI was a dynamic process that may be altered with omalizumab in a way that would help explain its mechanism and onset of action. Although basophils are associated more with late-phase responses, we used them as surrogates for what might be expected to occur on resident mast cells as well.

Our study is not without its limitations, and we had to make certain assumptions in doing the study. We assumed that the method used to measure serum IgE levels before the administration of omalizumab was equivalent to that used to measure free serum IgE after omalizumab administration. The method used to measure the free IgE levels was not able to differentiate values >150 ng/mL; thus we were required to measure prestudy IgE levels by using a conventional clinical laboratory assay rather than the same free IgE assay used for all determinations while the patient received the study drug. Total IgE levels after administration of omalizumab were not used, because this would have included the coupled omalizumab-IgE complexes and the free IgE (unbound).

Another possible criticism of this study is that the baseline nasal challenge response measured as a cumulative PD₃₀ value with a complete series of escalating ragweed doses might be different from that observed with a single PD₃₀ dose. In the placebo group, there was a slight decrease in ragweed-induced nasal volume responses between the baseline visit and the other visits, but the differences were not statistically significant. However, the omalizumab treatment resulted in significant differences at all time points versus baseline. Thus, it is unlikely that the use of this technique substantially influenced the overall findings of this study.

Finally, although flow cytometric analysis on live cells is more commonly used, the logistics of the study and the off-site collaboration did not allow this. We thus used fixed cells, as we have extensively in past studies.¹²⁻¹⁴ A strength of this approach is that it allowed us to analyze all data from a given subject at the same time, thus minimizing intrasubject variability caused by day-to-day variation in staining procedures and instrument performance. An additional benefit of this approach is the ability to archive multiple samples for later, possibly unanticipated analysis. As such, the use of fixed archived samples is ideally suited to mechanistic studies associated with clinical trials of immunotherapeutic drugs.

In conclusion, our study demonstrates that the onset of action of omalizumab in blunting ragweed-induced nasal responses is within 2 weeks, and this effect correlated with immunologic changes that may define mechanisms of action. We have associated clinical response with 2

mechanisms of action: decreased serum-free IgE and decreased FcεRI receptor expression on an effector cell that is important in allergic rhinitis. Basophil expression of the IgE receptor might be regarded as a surrogate marker of similar changes in mast cells. This rapid clinical and biological onset of action in vivo has important implications for understanding the clinical utility and mechanism of action of anti-IgE therapy.

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