

Omalizumab-induced reductions in mast cell FcεRI expression and function

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Background: By design, omalizumab binds free IgE in the circulation and prevents its attachment to the surface of mast cells and basophils, thereby preventing them from responding to allergens. Previously, omalizumab rapidly reduced free IgE levels, as well as basophil high-affinity IgE receptors, leading to significant reductions in basophil mediator response to allergen. It is assumed that tissue mast cells are similarly altered in their FcεRI density and function.

Objective: We examined the phenotypic shift of skin mast cells in parallel to that of blood basophils in 3 subjects infused with omalizumab.

Methods: Three subjects with allergic rhinitis underwent intradermal skin test titration with house dust mite antigen at days 0, 7, 70, and 196 of omalizumab treatment. As control subjects, 5 untreated subjects with allergic rhinitis were evaluated at similar time points. All subjects underwent skin biopsy 18 to 24 hours later at the site of allergen injection.

Biopsy specimens were characterized by means of immunohistochemistry for tryptase and FcεRIα immunoreactivity, as well other markers (CD3, CD45RO, CD68, cutaneous lymphocyte antigen, and major basic protein). **Results:** Omalizumab recipients, but not control subjects, demonstrated reductions in FcεRIα immunoreactivity at days 70 and 196 in parallel with reductions in the acute wheal response to allergen. However, no reductions in tryptase-positive cells were noted at these time points.

Conclusion: Reductions in free IgE levels by omalizumab leads to a rapid reduction in basophil FcεRI receptor expression. In contrast, the time course for the decrease of FcεRI expression in skin mast cells is slower and associated with decreased acute allergen wheal size. (*J Allergy Clin Immunol* 2004;114:527-30.)

Key words: Mast cells, FcεRI, omalizumab, basophils

The recent approval of a monoclonal anti-IgE (omalizumab) as an asthma therapeutic has offered an alternative treatment for allergic asthma. By design, omalizumab only

Abbreviations used

HDM: House dust mite

LPR: Late-phase reaction

binds free IgE in the circulation and prevents its attachment to the surface of mast cells and basophils, thereby preventing them from responding to allergens. In a phase I trial in subjects with allergic rhinitis, omalizumab rapidly reduced free IgE levels, as well as high-affinity IgE receptors on blood basophils.¹ This combined effect resulted in significant reductions in basophil mediator release in response to allergen stimulation. It was also noted that the shift in basophil phenotype and functional response was reversed by either discontinuation of omalizumab or reduction in the dose. Although it is assumed that tissue mast cells are similarly altered in their FcεRI receptor density and function, the supporting data are somewhat limited. In this study we examined the phenotypic shift of skin mast cells in parallel to that of blood basophils in 3 subjects infused with omalizumab.

METHODS

Subjects

The 3 subjects in this report had a history of moderately severe perennial allergic rhinitis and provided informed consent to venous blood sampling, skin testing, and skin biopsies per protocols approved by the Johns Hopkins Institutional Review Board. Subjects were participants in a phase I trial of omalizumab, as previously reported, and received omalizumab, 0.03 mg/kg/IU IgE/mL, administered intravenously on a bimonthly basis for 6 months.^{1,2} In the first week of dosing, these subjects received one third the calculated dose at days 0, 3, and 5 and the full dose at days 7 and 14 and then every 2 weeks. Five additional subjects with allergic rhinitis and with a total IgE level in the same range as the subjects receiving omalizumab (324 ± 158 IU/mL for control subjects vs 432 ± 164 IU/mL for omalizumab-treated subjects [mean $\pm$ SEM]) were enrolled as control subjects. These subjects received no treatment but underwent the same sequences of skin testing and biopsies as the omalizumab recipients.

Serum IgE measurements and basophil surface FcεRIα measurement

Omalizumab recipients had free IgE monitored during the course of the trial by Genentech.² As previously reported, venous blood was subjected to Percoll density gradient sedimentation to obtain enriched

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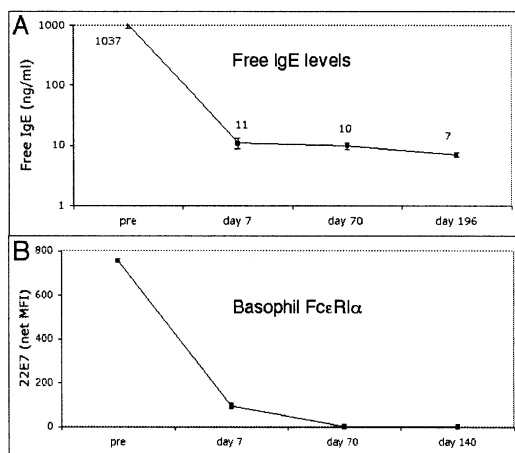


FIG 1. Free IgE levels influence basophil FcεRIα levels. **A**, Free IgE levels were reduced by greater than 99% by day 7 of omalizumab infusion and sustained through day 196. **B**, Basophil FcεRIα levels were reduced approximately 90% by day 7 and were undetectable after day 70 and day 140 of omalizumab treatment.

basophils, and surface FcεRIα was measured by means of dual-color flow cytometric analysis.¹

Skin testing

All subjects had positive skin test responses to house dust mite (HDM) and underwent intradermal skin test titrations to HDM at study enrollment. The dose of allergen required to yield a 15- to 20-mm wheal size at 15 minutes was determined and used for all subsequent skin testing. Skin biopsy specimens were obtained from the allergen-injected site 18 to 24 hours later. Acute and late-phase skin responses were calculated by using a standard protocol (the average of 2 perpendicular diameters of the reaction from a tracing of the wheal-and-flare reaction).

Skin biopsies

Subjects underwent punch biopsies (4 mm) at the site of allergen injection during the late-phase response (18-24 hours after allergen injection). All subjects had clinical evidence of a cutaneous late-phase reaction (LPR). Biopsy specimens were obtained from omalizumab recipients before their first infusion and at 7, 70, and 196 days after the initiation of infusions. The control subjects underwent biopsies at similar time intervals (baseline, day 70, and day 196), with the exception of day 7.

Immunohistochemistry

Skin biopsy specimens were fixed in formalin and paraffin embedded. Adjacent sections (5 μm) were stained to enumerate mast cells (tryptase) and FcεRIα (22E7) immunoreactivity. Murine mAbs for detection of tryptase (IgG1; Chemicon International, Inc, Temecula, Calif) were used at a final concentration of 1.2 μg/mL, and 22E7 (IgG1 unaffected by FcεRI occupancy, kindly provided by J. Kochan, Roche Pharmaceuticals, Nutley, NJ) was used at a final concentration of 0.3 to 1.5 μg/mL. Pilot studies were conducted to determine the minimal dilution of mAb 22E7 for optimal detection of FcεRIα in skin tissue by using dilutions between 10 to 0.3 μg/mL. Additional cellular markers examined included CD3, CD45RO, CD68, and cutaneous lymphocyte antigen, as well as May-Grunwald counts for eosinophils, as previously described.³ An indirect immunofluorescent assay, described previously, using a poly-

clonal antibody to eosinophil granule major basic protein, was used to quantify and localize the degree of eosinophil degranulation.⁴ A species-matched and immunoglobulin subtype-matched protein was used as a negative control for each antibody. The number of positive cells per square millimeter of dermis (subepithelial area) was enumerated by an investigator blinded to the status of the skin tissue.

RESULTS

Serum IgE levels and basophil FcεRIα

These results have been previously published and are noted here to provide background for the mast cell studies. At baseline, the 3 omalizumab recipients had a mean IgE level of 1037 ± 394 ng/mL (432 ± 164 IU/mL). One week after initiation of omalizumab, subjects had an approximately 99% reduction in free IgE levels that was maintained through day 196 (Fig 1, A). As previously reported,¹ these subjects also showed an 88% reduction in basophil surface FcεRIα expression 1 week after initiating omalizumab and a greater than 99% reduction for the remainder of the study (Fig 1, B). Furthermore, such a reduction in basophil FcεRIα densities was linked to a greater than 90% decrease in antigen-triggered (Der p 1) basophil histamine release.¹

Immunohistochemistry for tryptase and FcεRIα

Adjacent skin sections were examined for the expression of tryptase-positive cells relative to FcεRIα-positive cells in biopsy specimens from allergen-induced late-phase skin sites. The numbers of tryptase-positive (mast) cells remained stable over the course of the study (range, 87-110 cells/mm²) in omalizumab recipients, as well as in control subjects (Fig 2, A). However, the number of FcεRIα-positive cells was stable (range, 45-54 cells/mm²) during the first week in omalizumab recipients but was reduced by greater than 90% at day 70 and day 196 (Fig 2, B). The decrease in FcεRIα-positive cells was consistent in all 3 omalizumab recipients and not explained by a loss in tryptase-positive cells in adjacent sections. In contrast, no reduction in FcεRIα-positive cells was seen in the control subjects. Interestingly, a reduction in the clinical LPR size was seen in 2 of 3 omalizumab recipients. Furthermore, in all omalizumab recipients, a reduction in eosinophil infiltration (May-Grunwald) and degranulation was also observed at day 70 and day 196, with little or no effect on T-cell subsets (CD3, CD45RO, and cutaneous lymphocyte antigen) at any of the time points (data not shown). LPR size and cellularity were stable at all time points in the control subjects.

Allergen-induced wheal responses

As part of the study protocol, subjects underwent repeated skin testing with a fixed dose of allergen demonstrated to induce an acute wheal response of at least 15 mm in mean diameter. The average wheal size during the acute phase among omalizumab recipients and control subjects to a fixed dose of HDM allergen over the

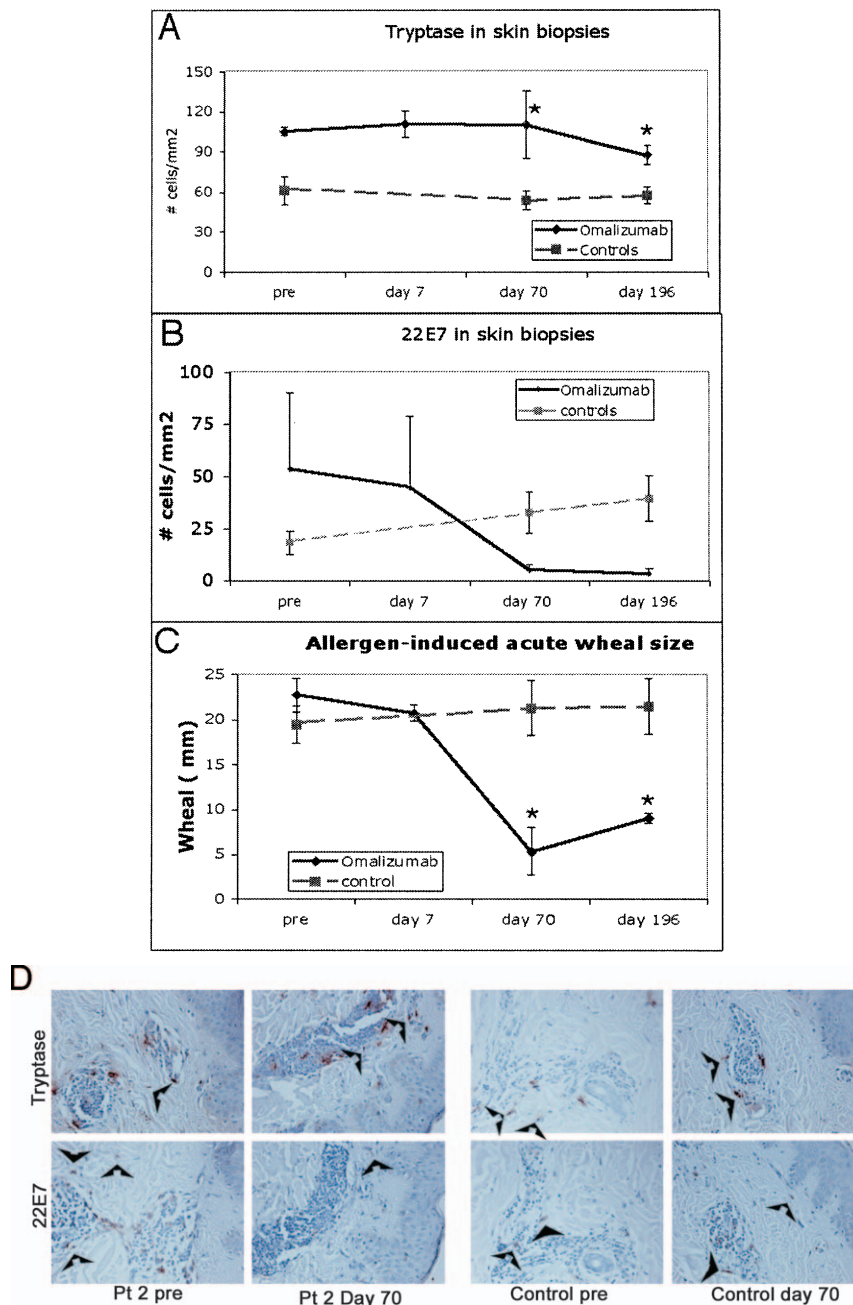


FIG 2. Free IgE levels influence mast cell FcεRIα expression and allergen-induced responses in omalizumab recipients. **A** and **B**, Biopsy specimens from omalizumab recipients, but not control subjects, show reduced 22E7 positivity (Fig 1, **B**), but in all subjects, no reduction in tryptase was seen (Fig 1, **A**). **C**, Allergen-induced acute wheal size was reduced by day 70 in omalizumab recipients. **D**, Representative sections of an omalizumab recipient (patient 2) and a control subject are shown (original magnification 400×). Arrows indicate some positive cells. * $P < .05$, omalizumab recipients versus control subjects (Mann-Whitney U test).

course of the study is shown in Fig 2, **C**. There was no significant shift by 7 days of treatment, but by day 70, a near 80% reduction in acute wheal size was noted in omalizumab recipients. The reduction in wheal size mirrored the reduction in FcεRIα positivity noted in the parallel skin biopsy specimens. No reduction in acute wheal size was seen in the control subjects.

DISCUSSION

In earlier reports we demonstrated a clear relationship between free IgE levels and FcεRI expression on blood basophils in allergic subjects undergoing omalizumab infusion. Furthermore, the decrease in basophil FcεRI expression was accompanied by a decrease in basophil

histamine response to allergen challenge.^{1,2} Separately, a reduction in mast cell FcεRI expression, function, or both was implicated by the reduction in acute skin test size after several weeks of omalizumab infusion,⁵ and this has recently been replicated in a study of omalizumab's effects on early and late-phase skin reactions.⁶

In the present study we demonstrate the behavior of 2 different FcεRI-bearing cell types, basophils and mast cells, under altered free IgE environments induced by omalizumab. With rapid reductions in free IgE levels in the circulation, we see a relatively rapid reduction in receptor expression on the basophil. However, although there was a significant decrease in expression of FcεRI associated with tryptase-positive cells, the time course for the decrease was considerably slower. At 1 week, we see little decrease in mast cell receptor expression or functional response, as measured on the basis of the allergen skin test acute wheal size. However, after 70 days of treatment, a significant decrease in mast cell FcεRI expression, but not cell number, was accompanied by a decrease in allergen-induced wheal size, as well as a reduction in the recruitment of eosinophils in the LPR. The phenotypic changes of skin mast cells observed with omalizumab gives insight into the time needed to shift tissue responses to allergen challenge. Allergen challenge studies in the lung,^{7,8} skin,^{5,6} and gastrointestinal tract⁹ support that several weeks might be necessary to observe clinical changes. A possible explanation for the phenotypic differences observed between mast cells and basophils might be the difference in the lifespan of each cell type, the half-life of allergen-specific IgE bound to FcεRI receptors, and the tissue penetration of omalizumab. As demonstrated several decades ago by Prausnitz and Kustner, local serum transfer leads to an allergen-induced acute wheal response that can persist for weeks. Thus it might be

possible in a particular allergic disease condition to dissect the contributions of the mast cell or basophil given the distinct temporal shifts in phenotype and function, and this is certainly an area for future investigation.

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