

Effects of omalizumab, a humanized monoclonal anti-IgE antibody, on nasal reactivity to allergen and local IgE synthesis

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Background: Treatment with omalizumab has been shown to reduce serum free IgE concentrations and to have beneficial effects on allergic airway disease. However, its effect on IgE synthesis is unknown.

Objective: To determine whether omalizumab therapy affects nasal reactivity to allergen and local IgE production.

Methods: Nineteen patients with perennial allergic rhinitis were treated with intravenous omalizumab every 2 weeks for 26 weeks in an open-label study. Serum free and total IgE concentrations were measured at baseline and every 2 weeks throughout the study. Nasal challenge to dust mite allergen was performed at baseline and after 12 and 24 weeks of treatment. Nasal lavage fluid obtained before and after each nasal challenge was evaluated for mite-specific antibodies, plaque-forming cells, and productive ϵ messenger RNA (mRNA).

Results: During treatment, serum free IgE concentrations were decreased by 97% to 99%, and the nasal response to allergen challenge was significantly reduced on days 80 and 164. The postchallenge increase in nasal lavage mite specific IgE was significantly reduced by treatment with omalizumab on day 168. IgE plaque-forming cells and productive ϵ mRNA were not significantly affected by omalizumab treatment.

Conclusions: Omalizumab treatment markedly reduced serum free IgE and the clinical response to nasal allergen challenge. However, the absence of an effect on IgE-secreting B cells and ϵ mRNA in nasal lavage fluid suggests that omalizumab treatment for 6 months does not significantly modulate synthesis of nasal IgE.

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INTRODUCTION

IgE is thought to play an essential role in mediating allergic responses in humans.^{1,2} Treatment with a recombinant humanized monoclonal anti-IgE antibody (omalizumab) has been shown to dramatically reduce serum concentrations of free IgE,³ to down-regulate expression of the high-affinity receptor for IgE on blood basophils,⁴ and to markedly attenuate early and late asthmatic reactions to inhaled allergens.⁵ Clinical trials in adults and children with asthma^{6,7} or allergic rhinitis⁸ have demonstrated significant improvements in clinical symptoms, medication requirements, and quality of life.

Although the administration of anti-IgE has been shown to effectively reduce serum free IgE levels, data from animal experiments⁹ have demonstrated that omalizumab use also inhibits antigen-induced increases in IgE levels and reduces circulating numbers of CD23⁺ B lymphocytes. These data suggest that treatment with anti-IgE might potentially alter the natural history of allergic disease via long-term modulation of IgE synthesis. The purpose of the present study was to

evaluate the effects of long-term administration of high doses of omalizumab on IgE synthesis after nasal allergen challenge in atopic patients.

PATIENTS AND METHODS

Patients

Men and women aged 18 to 65 years were eligible for this study if they had been diagnosed as having perennial allergic rhinitis for at least 2 years. At the screening visit, all patients were required to have a serum IgE concentration between 85 and 550 IU/mL, a positive skin prick test reaction to *Der-matophagoides farinae* or *Dermatophagoides pteronyssinus* (>5-mm wheal), and a body weight between 70% and 130% of predicted based on sex and height. Patients were excluded from the study if they were pregnant or lactating; had clinically significant renal, hepatic, or hematologic abnormalities; were receiving allergy immunotherapy; were receiving an intranasal, inhaled, or systemic corticosteroid; or had received a monoclonal antibody preparation during the past year. The protocol was approved by the Western Institutional Review Board, Olympia, WA, and all participants provided written informed consent.

Study Design

In this randomized, open-label, single-center study, patients were divided into 2 groups according to their baseline IgE concentration: low IgE levels were defined as 85 to 300

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IU/mL, and high IgE levels were defined as more than 300 to 550 IU/mL. Within each IgE stratification group, patients were randomized to receive either low-dose (0.015 mg/kg/IU/mL) or high-dose (0.030 mg/kg/IU/mL) treatment with omalizumab, administered intravenously every 2 weeks for 26 weeks. At the time the study was conducted, only the intravenous formulation was available. Serum total and free IgE concentrations were measured at baseline and every 2 weeks throughout the study. Nasal allergen challenge was performed at baseline (day -4) and after 80 and 164 days of treatment, and nasal lavage was performed immediately before and 96 hours after each nasal allergen challenge.

Serum IgE and Anti-Omalizumab Antibodies

Serum concentrations of free and total IgE were measured by enzyme-linked immunosorbent assay (ELISA) with the use of an Fc-receptor-IgG chimeric antibody and monoclonal anti-IgE.¹⁰ The lower limits of sensitivity for this assay were 0.78 ng/mL for free IgE and 1.2 ng/mL for total IgE. Serum samples were also screened for the presence of anti-omalizumab antibodies (anti-Fc and anti-Fab) using an ELISA assay, described previously.¹⁰

Nasal Allergen Challenge and Lavage

Nasal allergen challenge was performed at baseline (day -4) and after 80 and 164 days of treatment. An atomized solution of dust mite allergen was administered in 2 challenge doses per nostril. The dosage was increased until the onset of symptoms and a positive clinical symptom score was observed. Symptom scores (sneezing, rhinorrhea, congestion, and itching domains) after nasal allergen challenge with dust mite allergen on days 80 and 164 of the study are summarized by treatment group. A symptom score of 0, 1, or 2 was assigned for each symptom. The responses corresponding to scores 0, 1, and 2, respectively, were as follows: for sneezing, 0, 1 to 3, and more than 3 sneezes; for rhinorrhea, none, mild, and abundant; for congestion, none, mild, and bilateral nasal blockage; and for itching, none, itchy eyes or throat, and watery and itchy eyes and itchy palate and throat.¹¹ These 4 domains were combined to determine a total symptom score (0–8 points). Patients avoided the use of antihistamines for 5 days, intranasal corticosteroids for 14 days, intranasal cromolyn sodium for 14 days, intranasal ipratropium bromide for 3 days, and oral or topical decongestants for 2 days before each nasal challenge.

Nasal lavage was performed 96 hours after each allergen challenge at baseline (day 0) and on days 84 and 168. Lavage samples were collected by flushing each nostril with 5 mL of a normal saline solution prewarmed to 37° C.¹² The procedure was repeated at approximately 5-minute intervals to collect a total of 6 samples.

Mite-Specific Antibodies

Concentrations of total dust mite specific IgE, IgG, and IgA in nasal lavage fluid were measured by ELISA, as previously reported.¹³ Microtiter plates (Nunc, Roskilde, Denmark) were coated with 10 mg/mL of dust mite extract (Der f or Der p;

Bayer, Elkhart, IN), and alkaline phosphatase-labeled anti-IgE, anti-IgG, or anti-IgA (1:3,000 concentration; Tago, Burlingame, CA) was used for detection. All samples were performed in duplicate, and they were repeated if there was more than a 5% variation between the 2 results.

Antibody-Secreting Cells

IgA-, IgE-, and IgG-secreting B cells were measured from nasal lavage fluid using a filter spot ELISA, as previously reported.¹⁴ Standard curves were constructed for IgE- and IgA-producing cells using the 2C4/F3 IgE-producing and GM1056 IgA-producing mature B-cell lines, respectively. Positive reactions were defined as circular granulated foci with diameters greater than 0.05 mm. Samples were collected in quintuplicate and serially diluted. Each assay was demonstrated to be specific because no plaques were detected when using myeloma cells that produce other immunoglobulin isotypes.

Productive and Germline ϵ Complementary DNA

Productive and germline ϵ complementary DNA (cDNA) after reverse transcription was measured in cells recovered from nasal lavage samples by polymerase chain reaction (PCR), as previously described.¹⁵ Briefly, transcripts that contain membrane exon region sequences were used for the specific detection of ϵ germline. The 5' primer was located in the "I" exon (AGGCTCCACTGCCCGGCACAGAAAT), and the 3' primer was in the 3' untranslated region of the M2' exon (GACGGGGATGCCAGGCAGTTGGATC). Specific detection of productive ϵ transcripts (those containing VDJ-CHe sequence) were measured using a 5' primer in the conserved sequence derived from all of the human J regions (ACCCTGGTCACCGTCTCCTCA), and the 3' primer was the same as that for ϵ germline transcripts. The PCR amplifications were performed, and PCR products were used for Southern blotting to confirm the presence of the cDNAs. Twenty milliliters of each PCR product was run on an 8% nondenaturing polyacrylamide gel at 100 V for 5 hours, dried on a Bio-Rad 543 gel drier (Bio-Rad Laboratories, Hercules, CA) for 45 minutes at 65° C, and then exposed to Kodak XAR-5 film (Eastman Kodak, Rochester, NY) for 8 to 12 hours. Densitometry was performed by scanning the autoradiograph on an Apple One 24-bit, and the resultant pattern was analyzed using the Scan Analysis program (Biosoft, Cambridge, England), which delineates peaks and integrates the area under each peak. The housekeeping gene β -actin cDNA was measured in each sample using the primers CGC-GAGAAGATGACCCAGATC and ATCACGATGCCAGT-GGTACGG and was used to normalize the amount of productive and germline cDNA.

Statistical Analysis

Demographics, baseline characteristics, and allergy history are summarized by dose group. Symptom scores after nasal allergen challenge (sneezing, rhinorrhea, congestion, itching, and total score) are summarized by dose group and study visit; change from baseline was evaluated using the Wilcoxon signed rank test. Change from baseline in analyte levels

measured in nasal lavage fluid (both prechallenge levels and the difference between prechallenge and postchallenge levels) were evaluated using the Wilcoxon sign rank test for both dose groups pooled. To mitigate the effect of missing data on comparisons of nasal lavage results over time, these analyses included only patients with both prechallenge and postchallenge results at baseline, day 84, and day 168.

RESULTS

Patient Characteristics

Demographic and baseline characteristics were similar across dose levels (Table 1). Mean patient age was 35 years (range, 22–49 years), and 63% of patients were men. Sixty-eight percent of patients were white, 21% were Asian, and 11% were Hispanic. Baseline IgE levels ranged from 89 to 1,010 IU/mL. All patients had a history of perennial allergic rhinitis, as required by the study protocol. Other allergic diseases were present at the following rates: allergic asthma, 35%; atopic dermatitis, 30%; urticaria, 20%; and documented food allergy, 10%.

Total and Free Serum IgE Concentrations

Serum free IgE concentrations declined rapidly in a dose-dependent manner after initial dosing by 97% to 99%. Concentrations averaged 7.06 and 6.32 ng/mL in the low-dose group and 2.97 and 2.00 ng/mL in the high-dose group on days 84 and 168 (Table 2). Serum total IgE levels increased approximately 3- to 4-fold in both dose groups between baseline and day 84. This increase in total IgE concentration is a result of the reduction in the rate of elimination of IgE owing to its incorporation into complexes with omalizumab. Serum free IgE concentrations demonstrated an incremental decline (2–3 ng/mL, approximately 30%) between day 84 and day 168. Serum total IgE concentration also showed a very slight overall decline (3%–7%) between day 84 and day 168 (Table 2).

Nasal Allergen Challenge

Symptom scores. Nasal allergen challenge symptom scores decreased significantly during treatment with omalizumab in both dose groups (Table 3). In the low-dose group, the mean total symptom score declined from 5.40 at baseline to 1.80 on day 84 ($P = .002$) and 2.10 on day 164 ($P = .008$). In the high-dose group, the mean total symptom score declined from 5.00 at baseline to 1.44 on day 84 and 1.67 on day 168 ($P = .004$ for both). Symptom scores decreased most in the sneezing and rhinorrhea domains, but significant decreases were observed in all symptom domains (Fig 1).

Mite-specific antibodies. At baseline, nasal allergen challenge elicited a 330% postchallenge increase in total mite specific IgE levels (Table 4) but no change in mite specific IgA or IgG levels (data not shown). Treatment with omalizumab did not significantly alter prechallenge concentrations of total nasal mite specific IgE but reduced the postchallenge increase in total mite specific IgE by 35% on day 168 ($P = .02$). Treatment with omalizumab did not substantially alter prechallenge or postchallenge concentrations of mite specific IgA or IgG (data not shown).

Plaque-forming cells. Nasal allergen challenge performed at baseline produced a 420% postchallenge increase in the number of IgE plaque-forming cells (Table 4) but caused little or no change in the number of IgA or IgG plaque-forming cells (data not shown). Treatment with omalizumab did not attenuate the prechallenge numbers or postchallenge increases in IgE plaque-forming cells on days 80 and 164 (Table 4). Similarly, omalizumab administration did not alter prechallenge or postchallenge numbers of IgA or IgG plaque-forming cells (data not shown).

Productive ϵ messenger RNA. At baseline, nasal allergen challenge resulted in a 500% postchallenge increase in productive ϵ messenger RNA (mRNA) transcripts (Table 4). Neither prechallenge levels nor postchallenge increases in

Table 1. Demographic and Baseline Characteristics by Omalizumab Treatment Group

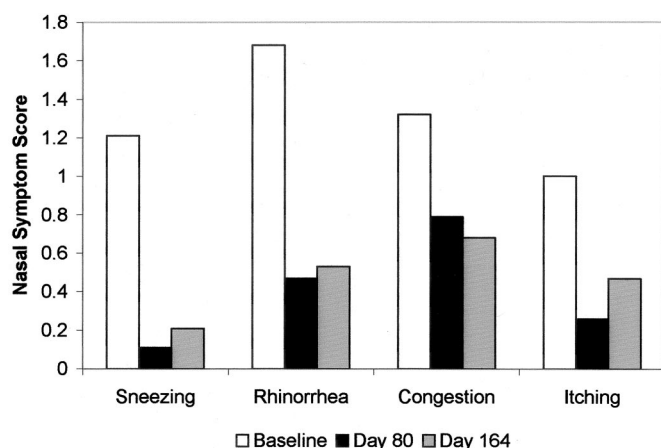
	Low-dose group (n = 10)	High-dose group (n = 9)	Total (N = 19)
Age, y			
Mean (SD)	32 (7)	38 (9)	35 (8)
Median	32	37	33
Range	23–49	22–49	22–49
Male, %	70	56	63
Race, %			
White	80	56	68
Asian	0	44	21
Hispanic	20	0	11
Weight, kg			
Mean (SD)	82 (12)	72 (10)	77 (12)
Median	84	71	77
Range	58–98	59–87	58–98
Baseline IgE, IU/mL			
Mean (SD)	342 (327)	197 (86)	274 (250)
Median	191	174	186
Range	89–1,010	116–385	89–1,010

Table 2. Serum Free and Total IgE Concentrations by Omalizumab Treatment Group and Study Visit

	Low-dose group (n = 10)	High-dose group (n = 9)	Total (N = 19)
Free IgE, mean (SD), ng/mL			
Baseline	224 (102)	251 (144)	237 (120)
Day 84	7.06 (4.66)	2.97 (1.26)	5.12 (3.99)
Day 168	6.32 (3.63)	2.00 (0.764)	4.27 (3.43)
Total IgE, mean (SD), ng/mL			
Baseline	342 (328)	197 (86.2)	274 (250)
Day 84	901 (281)	791 (272)	849 (275)
Day 168	878 (336)	737 (288)	811 (314)

Table 3. Nasal Reactivity to Dust Mite Allergen by Omalizumab Treatment Group and Study Visit

	Total nasal symptom score, mean (SD)		
	Low-dose group (n = 10)	High-dose group (n = 9)	Total (N = 19)
Baseline	5.40 (1.07)	5.00 (1.22)	5.21 (1.13)
Day 84	1.80 (1.32)*	1.44 (1.24)*	1.63 (1.26)†
Day 168	2.10 (2.47)*	1.67 (1.00)*	1.89 (1.88)†

* $P < .01$.† $P < .001$.Figure 1. Nasal allergen challenge symptom scores. Data are pooled for the low- and high-dose omalizumab treatment groups. $P < .05$ for all symptoms comparing changes from baseline on days 80 and 164.

productive ϵ mRNA were significantly affected by treatment with omalizumab (Table 4).

Safety and Tolerability

The most commonly reported adverse events overall were headache (49%), pain (32%), pharyngitis (32%), and back pain (28%). Adverse events were generally mild or moderate in severity. Most adverse events were judged to be unrelated to treatment. Adverse events possibly or probably related to the study drug and occurring in 5% or more of patients were headache (15%), dizziness (9%), and acute urticaria (9%). Acute urticaria occurred in 2 patients after administration of the initial dose, 1 in the low-dose group and 1 in the high-dose group. One of these patients had mild-to-moderate ur-

ticarial reactions after redosing. In patients with acute urticaria, there were no increases observed in plasma histamine or tryptase concentrations or in complement levels. In addition, there were no antibodies detected to either omalizumab or the human high-affinity IgE receptor, and results of epicutaneous skin testing to omalizumab were negative. There were no serious adverse events, no clinically significant changes in laboratory values, and no clinically meaningful changes in vital signs or physical examination results.

DISCUSSION

In the present study, we sought to investigate the effect of long-term omalizumab administration on local IgE synthesis in patients with perennial allergic rhinitis. In an experimental mouse model, rat-derived anti-IgE was used and resulted in a rapid and substantial reduction in IgE synthesis.¹⁶ However, in the present study of omalizumab therapy in patients with allergic rhinitis, local production of ϵ mRNA was not altered, and there was only a slight reduction in serum total IgE concentration after 168 days of administration.

The effect of omalizumab treatment on the early nasal response to allergen challenge was large (63% reduction in total nasal symptom scores) and similar to that seen in studies of omalizumab in a bronchial allergen provocation model.⁵ Although the present study did not use a control group, the effect observed in our study was far greater than that seen with historical placebo controls, which is usually on the order of a 10% to 15% decrease in total nasal symptoms.^{17,18} These results provide further support for the strong effects of omalizumab treatment on immediate airway reactivity to inhaled allergen exposure.

Our results demonstrate that a single, acute nasal allergen challenge caused substantial increases in IgE plaque-forming cells, productive ϵ mRNA, and mite specific IgE antibodies

Table 4. IgE Production Markers in Nasal Lavage Fluid*

	Prechallenge level	Postchallenge change
Mite specific IgE, IU/mL	n = 15	n = 15
Baseline	3.10 (1.04)	10.24 (9.19)
Day 84	3.21 (1.01)	8.70 (8.98)
Day 168	2.55 (1.34)	6.65 (7.90)†
IgE plaque-forming cells, $\times 10^6$	n = 10	n = 10
Baseline	1.48 (0.75)	6.20 (4.33)
Day 84	1.22 (0.71)	4.69 (3.61)
Day 168	1.01 (0.66)	3.02 (2.79)
Productive ϵ messenger RNA, % β -actin	n = 17	n = 17
Baseline	0.73 (1.04)	3.70 (2.13)
Day 84	0.46 (0.71)	3.14 (2.28)
Day 168	0.30 (0.30)	2.87 (1.64)

* Data are given as mean (SD) and are pooled for the low- and high-dose omalizumab groups.

† $P < .05$ compared with baseline change.

96 hours after challenge. These effects are specific for IgE-producing cells because neither mite specific IgA and IgG levels nor numbers of IgA and IgG plaque-forming cells were increased after nasal allergen challenge.

Treatment with omalizumab resulted in a mild reduction in the mite specific IgE response to allergen challenge on day 168. Although statistically significant, this minor decrease in allergen specific IgE is of questionable biologic relevance. To determine whether the effect on postchallenge mite specific IgE can be attributed to a reduction in IgE synthesis, our analysis also evaluated the effect of omalizumab treatment on productive ϵ mRNA transcripts. Neither prechallenge nor postchallenge ϵ mRNA levels were significantly affected by treatment with omalizumab. Further evidence that suggests that there was no significant effect on IgE synthesis is based on the analysis of total IgE concentrations during the 24 weeks of drug administration. Observations of serum total IgE concentrations demonstrated only minimal (3%–7%) reductions between day 84 and day 168. These changes in serum total IgE concentration observed in our study occurred much more slowly and were substantially smaller than the decline observed in the experimental mouse model.¹⁶ However, it is difficult to make direct comparisons between the human and animal data because the mouse studies used a rat anti-mouse antibody with different binding characteristics than omalizumab.

With respect to adverse effects, in this cohort of 19 patients, 2 developed acute urticaria. This high incidence of urticaria probably relates to the high doses of drug given rapidly by the intravenous route. A review of phase 2 and 3 placebo-controlled clinical studies of omalizumab given subcutaneously reveals that 1.3% of omalizumab-treated patients and 1.7% of placebo-treated patients reported acute urticaria. Of the urticaria reported, only 0.4% in the omalizumab and 0.1% in the placebo groups was considered by investigators to be drug related. Regarding the mechanism of the urticaria, the fact that it usually occurred after administration of the first dose of drug and that the skin test reaction to omali-

zumab and the blood assay for anti-omalizumab antibodies were negative would suggest that the urticaria was not due to immunologic hypersensitivity.

In summary, omalizumab therapy markedly reduced serum free IgE levels and significantly suppressed the clinical response to nasal allergen challenge. However, there was no evidence that IgE synthesis was significantly modulated after 6 months of high-dose omalizumab administration.

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