

Development of a Monoclonal Anti-Immunoglobulin E Antibody (Omalizumab) for the Treatment of Allergic Respiratory Disorders

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Immunoglobulin E (IgE) mediates many of the inflammatory processes that underlie the symptoms of asthma and other allergic respiratory disorders. Recently, a recombinant, humanized, monoclonal antibody (mAb) that binds to and neutralizes IgE has been developed for the treatment of these disorders. Preclinical and clinical studies have shown that this mAb, directed against IgE and known as omalizumab, inhibits the binding of IgE to its receptors on effector cells, reduces IgE synthesis by B cells in response to allergen exposure, decreases the expression of IgE receptors, and attenuates both immediate and delayed inflammatory airway responses following exposure to inhaled allergen. Omalizumab is nonanaphylactogenic, and clinical experience to date suggests that omalizumab is safe and well tolerated by patients. These results suggest that specific inhibition of IgE may be an important new therapeutic option for the treatment of asthma and related disorders.

Keywords: asthma; IgE; anti-IgE; monoclonal antibody; allergic rhinitis

Immunoglobulin E (IgE) is essential for development of the inflammatory processes that produce the symptoms of asthma, seasonal allergic rhinitis, and other allergic respiratory disorders (1, 2). In response to an initial exposure to allergen, IgE is released from B lymphocytes and binds to IgE receptors located on mast cells and other effector cells. On subsequent re-exposure to allergen, the allergen molecules bind to and form crosslinks between these IgE–receptor complexes (3). This crosslinking of IgE receptors triggers the immediate release of histamine and other inflammatory mediators from storage vesicles within the effector cells (cellular “degranulation”) (Figure 1). Also within minutes, potent mediators derived from arachidonic acid are synthesized and released (4, 5). By 1 h after IgE-mediated stimulation, effector cells initiate the synthesis of numerous cytokines and other factors that produce a second, more gradual, inflammatory response (6–9). Because of the central importance of IgE in asthma and other allergic respiratory diseases, researchers have long hypothesized that preventing these IgE-mediated processes should reduce the severity of these disorders.

Recently, a highly specific monoclonal antibody (mAb), which binds to circulating IgE and prevents it from making contact with its receptors on effector cells, has been developed for the treatment of asthma, allergic rhinitis, and other allergic disorders. There are several reasons why an mAb that inhibits IgE would be an important clinical advance for the treatment of allergic airway diseases (10). In contrast to conventional im-

muno-therapy, in which the patient is gradually desensitized to a particular allergen, targeting IgE with mAb therapy should attenuate the allergic response regardless of the allergen involved. Unlike antihistamines, which provide only symptomatic relief, an mAb directed against IgE targets the basic pathophysiologic processes underlying the allergic response. Inhaled corticosteroids, although effective for many patients, can produce a variety of side effects (e.g., reduction in bone mineral density, cataract formation, possible retardation of growth in children) (11) and may vary in effectiveness because of the propellant used or the characteristics of the inhaler (12). However, for a monoclonal anti-IgE antibody to be clinically useful, it must be nonanaphylactogenic; that is, the mAb must not itself permit the crosslinking of IgE–receptor complexes, because this would trigger cellular degranulation and produce an allergic response to the mAb.

This article briefly reviews the development of a nonanaphylactogenic mAb directed against IgE, and the early preclinical and clinical studies that first demonstrated the effectiveness of this approach as a potential therapy for asthma and hypersensitivity disorders.

STRATEGY FOR INHIBITING IgE

Two types of IgE receptors are expressed on the surfaces of effector cells: a high-affinity receptor (FcεRI), which is primarily found on mast cells and basophils, and a low-affinity receptor (FcεRII, also known as CD23), which is located on numerous cell types including B cells, macrophages, and platelets. Most researchers believe that IgE produces an allergic response primarily by binding to its high-affinity receptors, although some experimental evidence suggests that the binding of IgE to low-affinity receptors may also be of some importance (13). The structure of the IgE molecule is shown in Figure 2. The Fc segments of the two heavy chains of IgE each consist of CH2, CH3, and CH4 domains. The binding site for both the high-affinity IgE receptor and the low-affinity IgE receptor is located in the CH3 domain (14).

An mAb directed against this receptor-binding portion of IgE was developed through hybridoma techniques. In this procedure, mice are immunized with human IgE, and their B lymphocytes are harvested and fused with myeloma cells, producing immortalized “hybridoma” cells that secrete the antibody of interest (15). A single hybridoma cell line is then used to generate large quantities of the antibody, which is the reason for its designation as “monoclonal.” The resulting monoclonal anti-IgE antibody (which was known as MAE-11) was then “humanized” by removing murine elements that could trigger an immune response upon administration of the mAb to humans. The residues on the light chains of the mAb that are specific for IgE binding were grafted onto a human IgG₁ framework (16). A few murine residues are essential to maintain the stability of the IgE-binding portion of the mAb but are not sufficient to produce an allergic response on exposure

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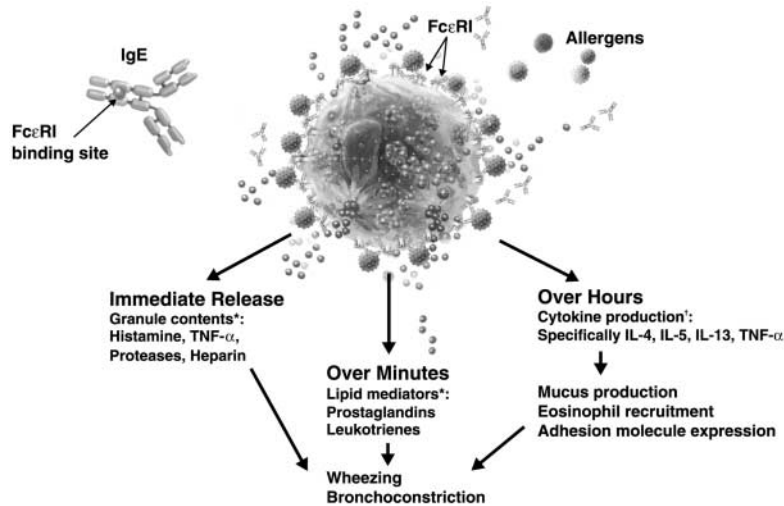


Figure 1. Immunoglobulin E (IgE)-dependent release of inflammatory mediators. Proteins rapidly elute from allergenic particles, such as pollen grains (shown), and result in crosslinking of two or more cell-bound IgE molecules. Crosslinking of a critical mass of cell-bound IgE molecules by allergenic proteins results in the degranulation of effector cells and the release of an impressive array of mediators that are causally linked to the pathophysiology of asthma and allergic rhinitis. Mast cell degranulation is the key event that launches the symptoms of the EAR. *Preformed granule contents and newly generated lipid mediators also result in nasal symptoms, including sneezing, nasal congestion, runny nose, and watery eyes. †Secretion of cytokines from mast cells located in the upper airway results in the characteristic nasal symptoms associated with delayed hypersensitivity inflammatory reactions. TNF- α = tumor necrosis factor- α ; IL = interleukin.

to the humanized mAb. The completed mAb, which is known as anti-IgE (omalizumab) (Figure 3), binds to the receptor-binding portion of IgE. Because the epitope that is recognized by omalizumab encompasses binding regions for both high-affinity and low-affinity IgE receptors, omalizumab eliminates the ability of IgE to bind to both types of receptors (14). However, omalizumab is not able to crosslink IgE molecules that are already bound to the cell surface. The binding of the Fc ϵ RI to one CH3 domain of one heavy chain inhibits or prevents the binding of omalizumab to the CH3 region of the other heavy chain. Thus, omalizumab can bind only to IgE that is in circulation (10, 17). The precise reason for this is not understood, but it is likely that the binding of IgE to its cell-surface receptor produces a conformational change in the IgE molecule that makes the second heavy chain unavailable to the monoclonal anti-IgE antibody. In contrast, the binding of omalizumab to free IgE in circulation does not produce a similar loss of binding affinity for omalizumab in the contralateral

heavy chain. Thus, each molecule of IgE may be simultaneously bound by two molecules of omalizumab. This would appear to raise the possibility of the formation of large complexes of IgE and omalizumab, which could precipitate an immune reaction. However, detailed analyses of IgE–omalizumab complex formation have shown that the largest complexes formed are stable cyclic hexamers (18). Table 1 summarizes the characteristics of omalizumab.

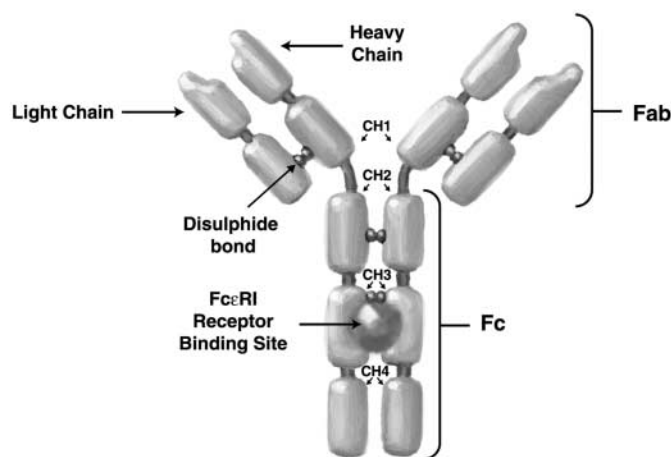
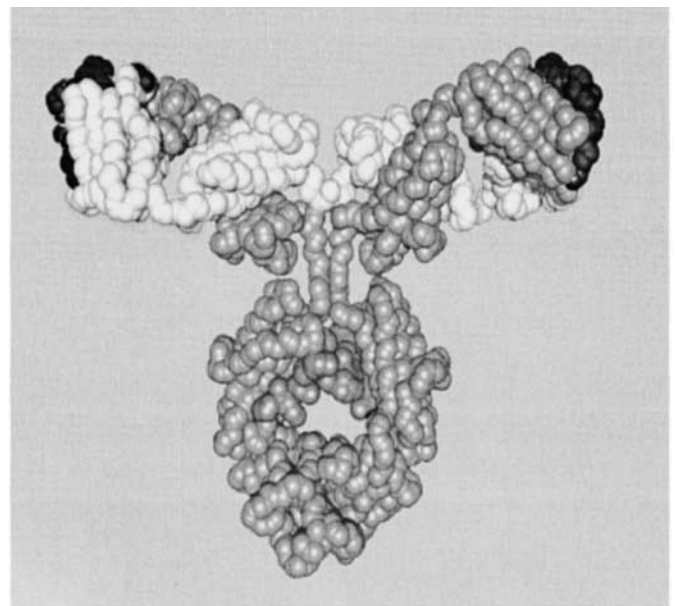


Figure 2. The structure of the IgE molecule. The receptor-binding sites for both high-affinity and low-affinity IgE receptors are located in the CH3 portion of the heavy chain. When IgE is free in circulation, the Fc ϵ RI-binding sites on the two heavy chains of the IgE molecule do not obstruct one other and are therefore available to bind two molecules of anti-IgE antibody. When IgE binds to its receptor, however, a conformational change results in the second heavy chain of the immunoglobulin, thereby preventing omalizumab from binding to cell-bound IgE and stimulating degranulation.



■ Heavy Chain □ Light Chain ■ Mouse CDR

Figure 3. Structure of the omalizumab molecule. Omalizumab is a recombinant humanized mAb containing 5% murine sequence and 95% human sequence. To generate omalizumab, the IgE-binding portion of a murine monoclonal antibody was grafted onto a human IgG framework. A small number of murine residues are essential to maintain stability of the IgE-binding region but are not sufficient to provoke an immune response when omalizumab is administered to humans. CDRs = complementary-determining regions (i.e., mouse antigen-binding regions).

TABLE 1. CHARACTERISTICS OF OMALIZUMAB

IgG1 human framework containing murine antibody complementary-determining regions (m.w. = 150 kD)
Humanized monoclonal antibody against IgE (95% human)
Binds circulating IgE regardless of IgE specificity
Does not activate complement
Does not bind cell-bound IgE to result in activation (nonanaphylactogenic mechanism)
Forms small, biologically inert omalizumab-IgE complexes

Definition of abbreviation: Ig = immunoglobulin.

EFFECTS OF OMALIZUMAB ON IgE EXPRESSION, IgE RECEPTOR BINDING, AND RECEPTOR DENSITY

In Vitro Studies

Shields and colleagues reported the results of a detailed analysis of the effects of omalizumab on IgE-mediated inflammatory processes *in vitro* (17). Omalizumab prevented the ability of IgE to bind to human lung mast cells and blocked the muscle contraction and release of histamine on exposure of passively sensitized lung tissue to ragweed antigen. Moreover, omalizumab did not trigger the release of histamine from IgE-sensitized human basophils, suggesting that it does not crosslink IgE-receptor complexes on these cells. Omalizumab also inhibited IgE synthesis by B lymphocytes in response to cat-pelt antigen.

One intriguing possibility is that the inhibition of IgE production may also cause a decrease in the number of cell-surface IgE receptors. It has long been known that the density of IgE receptors on effector cells is correlated with free serum IgE levels (19), and it has also been shown that IgE receptor density is increased by exposure to allergen (20, 21). Therefore, MacGlashan and colleagues evaluated whether omalizumab administration would affect the number of IgE receptors on effector cells (22). These investigators noted that the number of high-affinity receptors on the cell surface is ordinarily quite high, ranging from about 10^4 to 10^6 per cell, whereas the quantity of IgE required to produce degranulation is small, being on the order of perhaps as few as a few hundred molecules. This implies that a large reduction in circulating IgE (at least 99%) would be required to completely abolish IgE receptor-mediated biologic responses. MacGlashan and coworkers found that the number of high-affinity IgE receptors on the surfaces of basophils obtained from humans who had been treated with omalizumab decreased from a baseline of about 200,000 per cell to a posttreatment level of about 8,000 per cell: a reduction of about 93% (Figure 4). This is potentially of considerable clinical significance, because reducing the number of IgE receptors might amplify the inhibitory effect of omalizumab on inflammatory processes.

Preclinical Studies

Schoenhoff and colleagues examined the pharmacokinetic and pharmacodynamic profile of omalizumab in cynomolgus monkeys (23). Omalizumab was cleared slowly from circulation, with an elimination half-life following intravenous or subcutaneous administration of 1 to 4 wk. Administration of omalizumab produced a dose-related decrease in the free serum IgE concentration and an increase in total IgE (free IgE plus IgE complexed with omalizumab), which was the result of reduced clearance of IgE that was bound to omalizumab. Omalizumab was also found to prevent an allergic skin reaction produced by ragweed antigen in cynomolgus monkeys; this inhibition persisted for several weeks following the last dose of omalizumab (17).

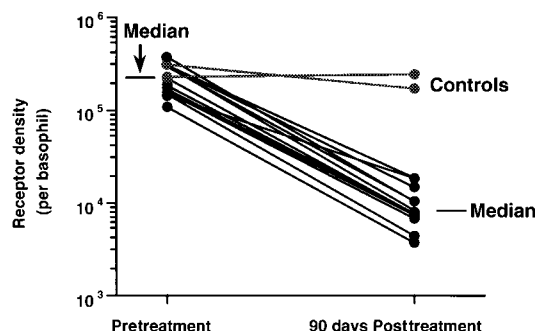


Figure 4. Expression of endogenous FcεRI on basophils of subjects undergoing omalizumab treatment. The figure shows the results for 12 treated patients and 2 controls. The data were generated with the acetate buffer "stripping" technique. The shaded symbols indicate the control subjects, and the closed circles indicate the treated patients. The graph plots the results of IgE measurements for cells fully sensitized, and therefore essentially plots total receptor densities. Adapted by permission from MacGlashan DW Jr, Bochner BS, Adelman DC, et al. *J Immunol* 1997;158:1438-1445.

INITIAL CLINICAL EXPERIENCE WITH OMALIZUMAB

Pharmacokinetic/Pharmacodynamic Studies

The first study of omalizumab in humans investigated the tolerability and pharmacokinetic/pharmacodynamic profile of increasing single doses of omalizumab in 59 healthy individuals or patients with allergies (24). The concentration of free IgE in serum decreased rapidly after administration of omalizumab and gradually returned to baseline over a period of several weeks after the last dose. Omalizumab was well tolerated by the patients, and no anaphylactic reactions were observed during the study. Similar results were obtained in two subsequent multiple-dose studies; the first enrolled 24 adult patients with either allergic rhinitis or mild asthma (25), and the second enrolled 34 children and adolescents with moderate to severe allergic asthma (26). In these multiple-dose studies, free IgE in serum decreased with an increasing dose of omalizumab, gradually returned to baseline after the last omalizumab treatment, and was not associated with any allergic reactions or medication-related adverse events.

On the basis of these encouraging initial findings, a single-blind, Phase I clinical trial was initiated to examine the safety and tolerability of omalizumab, in comparison with placebo, among 12 adult patients with moderate to severe asthma (27). Patients were treated with either placebo or omalizumab (0.15 mg/kg subcutaneously or 0.50 mg/kg intravenously) on a weekly basis for 3 wk; clinical evaluations were performed for a total of 6 wk. No adverse responses to treatment or changes in physiologic or laboratory values were observed during the study. At the end of 3 wk of treatment, the concentration of free IgE in serum had decreased to an average of 60% of its baseline value among patients who received the lower dose of omalizumab and to 20% of its baseline value among patients who received the higher dose.

Together, the results of these early studies confirmed that omalizumab can be safely administered to both adults and children with allergic respiratory disorders and that treatment with omalizumab rapidly and consistently reduces the quantity of free IgE in circulation.

EFFECT OF OMALIZUMAB ON ALLERGIC SKIN REACTIVITY

An open-label study was conducted to test the hypothesis that treatment with omalizumab would prevent allergic skin reactivity to a skin-prick test with dust mite antigen (28). A total of

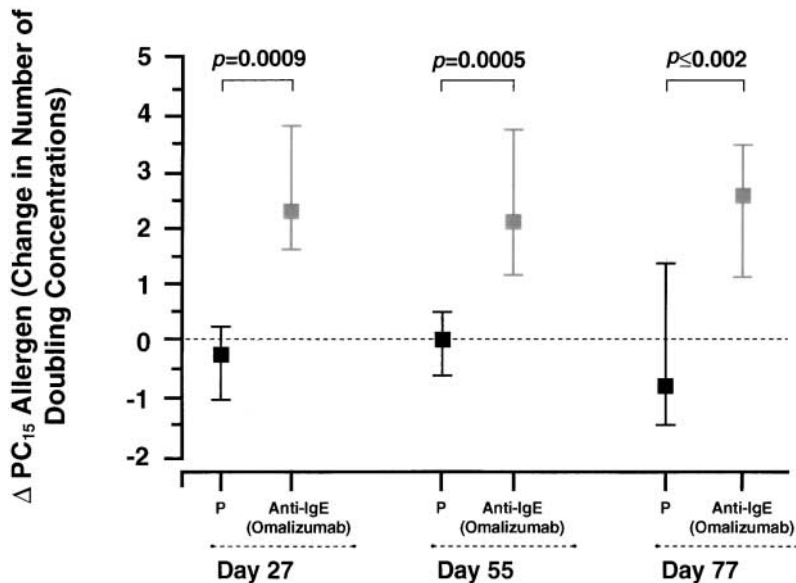


Figure 5. Administration of omalizumab, but not placebo (p), significantly increases the quantity of antigen required to produce a 15% reduction (PC₁₅) in FEV₁. Adapted by permission from Boulet LP, Chapman KR, Cote J, et al. *Am J Respir Crit Care Med* 1997;155:1835–1840.

47 patients, all of whom had perennial allergic rhinitis and who exhibited positive skin-prick reactivity to dust mite antigen, were randomly assigned to receive one of two dosages of omalizumab, with the amount adjusted on the basis of the patients' baseline serum concentration of IgE (0.015 or 0.30 mg/kg per IU/ml IgE every other week). Skin reactivity was tested after 6 mo of treatment, at which time the skin wheal area in response to injection of several different dilutions of dust mite extract was significantly decreased from baseline by omalizumab treatment ($p \leq 0.001$). At baseline, the mean summed wheal area of all dilutions of dust mite extract tested was 155 mm² (with an SEM of ± 31 mm²) for the low-dose omalizumab group and 181 ± 43 mm² for the high-dose group. After 6 mo of treatment, the summed wheal area in response to dust mite extract was 61 ± 15 mm² for the low-dose group and 34 ± 11 mm² for the high-dose group. Urticarial reactions were noted after the first dose of omalizumab in four patients; three of these patients elected to remain in the study, and no further reactions were noted with subsequent omalizumab injections in any patient.

EFFECT OF OMALIZUMAB ON AIRWAY HYPERRESPONSIVENESS

When exposed to aeroallergen, patients with asthma exhibit an early asthma response (EAR) characterized by a rapid decrease in FEV₁ over a period of minutes, and associated with breathlessness. Within 3 to 6 h of apparent recovery, there is a reduction in FEV₁ and dyspnea termed the late asthmatic re-

sponse (LAR) (29). The EAR is thought to be a classic Type 1 hypersensitivity reaction, whereas the LAR is caused by the synthesis and release of several mediators that attract eosinophils, neutrophils, and other cells to the site of inflammation, where they contribute to vascular smooth-muscle contraction and inflammation (30). IgE is widely regarded as an important mediator of the EAR; the role of IgE in the LAR is generally considered to be less well established, although evidence obtained from animal studies suggests that IgE may participate in both responses. The late-phase results from an influx of inflammatory cells produced by mediators released in the early phase, and from the subsequent release of additional mediators in the airways (13).

Recently, two clinical trials examined the effects of omalizumab on the airway response to inhaled allergen among patients with mild allergic asthma (29, 31). These studies were primarily designed to assess whether omalizumab could modify the response to allergen; subsequent clinical trials, with more direct clinical applicability, are described in detail elsewhere in this supplement (please see Casale TB: "Anti-IgE Therapy in Allergic Rhinitis"; and Busse WW: "Anti-IgE Therapy in Asthma").

Boulet and colleagues studied the effects of omalizumab treatment on the EAR to inhaled allergen (29). The EAR was assessed by determining the quantity of allergen required to produce a 15% reduction in FEV₁ (the provocative concentration of the allergen causing a 15% reduction, [PC₁₅] in FEV₁). A total of 20 adult patients with mild allergic asthma were enrolled in this randomized, double-blind, placebo-controlled

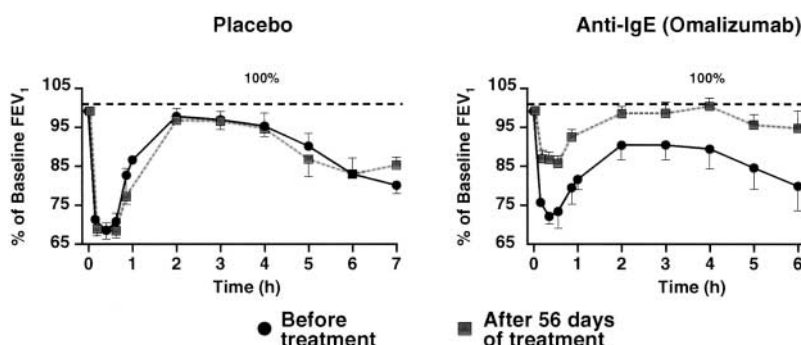


Figure 6. Treatment with omalizumab attenuates both the EAR and the LAR after allergen exposure in patients with mild allergic asthma. FEV₁ after allergen exposure is expressed as a percentage of the baseline value for the placebo (left panel) and omalizumab (right panel) groups, both before treatment (circles) and after treatment (squares). Adapted by permission from Fahy JV, Fleming HE, Wong HH, et al. *Am J Respir Crit Care Med* 1997;155:1828–1834.

clinical trial. Patients were assigned to receive omalizumab in an initial dose of 2.0 mg/kg given intravenously, with subsequent doses of 1.0 mg/kg at Weeks 1, 2, 4, 6, 8, and 10, or placebo. Omalizumab treatment was well tolerated by most patients, and 19 of the 20 patients finished the trial. Treatment was discontinued for one patient after the appearance of an urticarial rash following the first dose of omalizumab. No anaphylactic responses were noted. Administration of omalizumab reduced the serum IgE concentration in all 10 patients who received omalizumab, and reduced it below the lower limit of detection in seven patients. Treatment with omalizumab, but not placebo, increased the PC₁₅ of the antigen used in the study (Figure 5). By Week 11, the PC₁₅ had increased by an average of 2.7 doubling doses, which, the investigators noted, made the antibody treatment comparable or superior to the effectiveness of inhaled corticosteroids. Omalizumab also reduced airway hyperresponsiveness after challenge with methacholine. No significant difference in asthma symptoms in the two treatment groups was observed during the study; however, the authors noted that the primary purpose of the study was to examine the effect of omalizumab on the response of the airway to allergen exposure and that the patients enrolled in this study had relatively mild asthma symptoms. An evaluation of clinical efficacy would require a larger study and the enrollment of patients with more severe asthma.

In a second, similar study, Fahy and colleagues examined the effect of omalizumab treatment on both the EAR and LAR among 19 patients with mild asthma (31). Patients were treated with omalizumab at 0.5 mg/kg given intravenously at weekly intervals for 8 wk. Again, omalizumab was safe and well tolerated; the incidence of adverse events was similar for the treatment and placebo groups, and no patients developed antibodies to omalizumab. Free IgE in circulation decreased significantly during the treatment period in the omalizumab group but not in the placebo group ($p < 0.001$). Treatment with omalizumab significantly attenuated both the EAR and the LAR ($p < 0.02$) (Figure 6). These results suggest that omalizumab prevents the immediate response to allergen exposure, as well as the more delayed response, in patients with mild allergic asthma.

A Phase II multicenter, double-blind, placebo-controlled study was performed to evaluate the efficacy of omalizumab in adolescents and adults (ages 11 to 50 yr) with moderate-to-severe asthma (32). After a 4-wk run-in period during which β -agonist and corticosteroid (oral and/or inhaled) dosages were adjusted for optimal control of asthma, 317 study patients were randomized to receive low-dose omalizumab (2.5 μ g/kg body weight/ng IgE/ml); high-dose omalizumab (5.8 μ g/kg body weight/ng IgE/ml); or placebo intravenously on days 0 (half-dose), 4 (half-dose), and 7 (full dose), and then once every 2 wk for 20 wk. The regimen of corticosteroids was continued during the first 12 wk of treatment and was tapered during the next 8 wk.

During the 20-wk treatment period, significantly more patients receiving placebo experienced an asthma exacerbation than did those receiving either high- or low-dose omalizumab ($p = 0.03$ and $p = 0.01$, respectively) (32). Omalizumab also diminished the need for adjunctive therapies, including inhaled corticosteroids and β -agonists, without impairing disease control or raising the risk of exacerbations (32). A 50% or greater reduction in dose of oral corticosteroids was reported in 78% of the subjects in the high-dose group ($p = 0.04$) and in 57% of the subjects in the low-dose group ($p = 0.23$), versus 33% of the subjects in the placebo group. In terms of complete discontinuation of oral corticosteroids, a

greater percentage of patients in both the high- and low-dose omalizumab groups than in the placebo group were able to discontinue oral corticosteroids completely (33% in the high-dose group, 43% in the low-dose group, and 17% in the placebo group). This trend, however, did not reach statistical significance.

The study investigators also noted a significant improvement in asthma symptom scores in both the high- and low-dose omalizumab groups as compared with patients given placebo ($p = 0.008$ and $p = 0.005$, respectively). The improvement in asthma symptom scores continued during the steroid-withdrawal phase of the study (32). In addition, omalizumab treatment significantly improved the morning peak expiratory flow rate (PEFR) in the high-dose omalizumab group as compared with the placebo group during the steroid-stable phase ($p = 0.007$) as well as during the steroid-withdrawal phase of the study ($p = 0.02$). There was also a significant improvement in quality of life with omalizumab as compared with placebo in both the high- and low-dose omalizumab groups ($p < 0.001$ and $p = 0.007$, respectively). Omalizumab therapy was safe and well tolerated, and there was no evidence of anti-omalizumab antibodies.

CONCLUSIONS

IgE is a crucial mediator of the physiologic processes that result in allergic airway diseases, including allergic asthma and seasonal allergic rhinitis. It has been hypothesized that, by blocking the ability of IgE to bind to IgE receptors on mast cells and other effector cells, it should be possible to attenuate the allergic response to inhaled allergens, thereby reducing the symptoms of asthma and allergic rhinitis.

Omalizumab, an mAb, has recently been developed for the treatment of asthma and allergic rhinitis. This antibody binds to IgE and prevents it from making contact with its receptors. Preclinical and clinical studies have shown that omalizumab reduces the level of free IgE in circulation and reduces the number of high-affinity IgE receptors on basophils. Initial clinical studies suggest that omalizumab can be safely administered to both adult and pediatric patients with allergies and that omalizumab reduces the early and late airway responses to inhaled allergen. These findings suggest that the use of mAb directed against IgE is an important potential new therapeutic strategy for the treatment of asthma, allergic rhinitis, and related allergic conditions.

References

1. Busse WW, Coffman RL, Gelfand EW, Kay AB, Rosenwasser LJ. Mechanisms of persistent airway inflammation in asthma: a role for T cells and T-cell products. *Am J Respir Crit Care Med* 1995;152:388-393.
2. Busse WW. Inflammation in asthma: the cornerstone of the disease and target of therapy. *J Allergy Clin Immunol* 1998;102:S17S-S22.
3. Schulman ES, Yassin R, Peters S. Mechanisms of mast cell activation. In: Busse WW, and Holgate S, editors. *Asthma and rhinitis*. Oxford, UK: Blackwell Science; 2000.
4. Schulman ES, MacGlashan DW, Peters SP, Schleimer RP, Newball HH, Lichtenstein LM. Human lung mast cells: purification and characterization. *J Immunol* 1982;129:2662-2667.
5. MacGlashan DW, Schleimer RP, Peters SP, Schulman ES, Adams GK III, Newball HH, Lichtenstein LM. Generation of leukotrienes by purified human lung mast cells. *J Clin Invest* 1982;70:747-751.
6. MacGlashan DJ, White JM, Huang SK, Ono SJ, Schroeder JT, Lichtenstein LM. Secretion of IL-4 mRNA and protein in resting and stimulated basophils. *J Immunol* 1994;152:3006-3016.
7. Jaffe JS, Glaum MC, Raible DG, Post TJ, Dimitry E, Govindarao D, Wang Y, Schulman ES. Human lung mast cell IL-5 gene and protein expression: temporal analysis of upregulation following IgE-mediated activation. *Am J Respir Cell Mol Biol* 1995;13:665-675.

8. Jaffe JS, Raible DG, Post TJ, Wang Y, Glaum MC, Butterfield JH, Schulman ES. Human lung mast cell activation leads to IL-13 mRNA expression and protein release. *Am J Respir Cell Mol Biol* 1996;15:473-481.
9. Schulman ES, and Raible DG. Mast cells and eosinophils. In: Fishman AP, editor. *Pulmonary diseases and disorders*, 3rd ed. New York: McGraw-Hill; 1998. p. 289-301.
10. Chang TW. The pharmacological basis of anti-IgE therapy. *Nat Biotechnol* 2000;18:157-162.
11. Jackson LD, Polygenis D, McIvor RA, Worthington I. Comparative efficacy and safety of inhaled corticosteroids in asthma. *Can J Clin Pharmacol* 1999;6:26-37.
12. Shaw RJ. Inhaled corticosteroids for adult asthma: impact of formulation and delivery device on relative pharmacokinetics, efficacy, and safety. *Respir Med* 1999;93:149-160.
13. Coyle AJ, Wagner K, Bertrand C, Tsuyuki S, Bews J, Heusser C. Central role of immunoglobulin (Ig) E in the induction of lung eosinophil infiltration and T helper 2 cell cytokine production: inhibition by a non-anaphylactogenic anti-IgE antibody. *J Exp Med* 1996;183: 1303-1310.
14. Presta L, Shields R, O'Connell L, Lahr S, Porter J, Gorman C, Jardieu P. The binding site on human immunoglobulin E for its high affinity receptor. *J Biol Chem* 1994;269:26368-26373.
15. Breedveld FC. Therapeutic monoclonal antibodies. *Lancet* 2000;355:735-740.
16. Presta LG, Lahr SJ, Shields RL, Porter JP, Gorman CM, Fendly BM, Jardieu P. Humanization of an antibody directed against IgE. *J Immunol* 1993;151:2623-2632.
17. Shields RL, Whether WR, Zioncheck K, O'Connell L, Fendly B, Presta LG, Thomas D, Saban R, Jardieu P. Inhibition of allergic reactions with antibodies to IgE. *Int Arch Allergy Immunol* 1995;107:308-312.
18. Fox JA, Hotaling TE, Struble C, Ruppel J, Bates DJ, Schoenhoff MB. Tissue distribution and complex formation with IgE of an anti-IgE antibody after intravenous administration in cynomolgus monkeys. *J Pharmacol Exper Ther* 1996;279:1000-1008.
19. Malveaux FJ, Conroy MC, Adkinson NF, Lichtenstein LM. IgE receptors on human basophils: relationship to serum IgE concentration. *J Clin Invest* 1978;62:176-181.
20. Rajakulasingam K, Till S, Ying S, Humbert M, Barkans J, Sullivan M, Meng Q, Corrigan CJ, Bunge J, Grant JA, Kay AB, Durham SR. Increased expression of high affinity IgE (FcεRI) receptor-α chain mRNA and protein-bearing eosinophils in human allergen-induced atopic asthma. *Am J Respir Crit Care Med* 1998;158:233-240.
21. Saini SS, MacGlashan D, Adelman D, Jardieu P, Togias A, Lichtenstein LM, et al. Culture with IgE increases IgE density and FcεRI expression on human basophils. *J Allergy Clin Immunol* 2000;99:S102.
22. MacGlashan DWJ, Bochner BS, Adelman DC, Jardieu PM, Togias A, McKenzie-White J, Sterbinsky SA, Hamilton RG, Lichtenstein LM. Down-regulation of FcεRI expression on human basophils during in vivo treatment of atopic patients with anti-IgE antibody. *J Immunol* 1997;158:1438-1445.
23. Schoenhoff M, Bates D, Rupel J, Fei D, Fox JA, Thomas D, Jardieu P. Pharmacokinetics/dynamics following administration of a recombinant humanized monoclonal anti IgE antibody in the cynomolgus monkey. *J Allergy Clin Immunol* 1995:356.
24. Froehlich J, Schoenhoff M, Tremblay T, Ruppel J, Jardieu P. Initial human study with a humanized recombinant anti-IgE monoclonal antibody: safety, tolerance and pharmacokinetic (PK)/dynamic profile. *Clin Pharmacol Ther* 1995;57:162.
25. Froehlich J, Schoenhoff M, Jardieu P, Ruppel J, Fei D, Buckley P, Bush R. Multiple doses of a recombinant humanized monoclonal anti IgE antibody are safely tolerated and decrease free serum IgE to undetectable levels. *J Allergy Clin Immunol* 1995:356.
26. Bisberg D, Froehlich J, Schoenhoff M, Mendelson J. Multiple administrations of the anti-IgE recombinant monoclonal antibody E25 (rhuMAb-E25) reduces free IgE levels in a dose dependent manner in adolescents and children with moderate to severe allergic asthma. Paper presented at: American College of Chest Physicians 25th Annual Meeting, 1999, Chicago, IL.
27. Corren J, Froehlich J, Schoenhoff M, Spector S, Rachelefsky G, Schanker H, Patnaik M, Siegel S. Phase I study of anti-IgE recombinant humanized monoclonal antibody rhuMAb-E25 (E25) in adults with moderate to severe asthma. *J Allergy Clin Immunol* 1996;97:245.
28. Togias A, Corren J, Shapiro G, Reimann JD, von Schlegell A, Wighton TG, Adelman DC. Anti-IgE treatment reduces skin test (ST) reactivity. *J Allergy Clin Immunol* 1998;101:S171-S171.
29. Boulet L-P, Chapman KR, Côte J, Kalra S, Bhagat R, Swystun VA, Laviolette M, Cleland LD, Deschesnes F, Su JQ, et al. Inhibitory effects of an anti-IgE antibody E25 on allergen-induced early asthmatic response. *Am J Respir Crit Care Med* 1997;155: 1835-1840.
30. Zwieman B. The late-phase reaction: role of IgE, its receptor and cytokines. *Curr Opin Immunol* 1993;5:950-955.
31. Fahy JV, Fleming HE, Wong HH, Liu JT, Su JQ, Reimann J, Reimann J, Fick RO, Boushey HA. The effect of an anti-IgE monoclonal antibody on the early- and late-phase responses to allergen inhalation in asthmatic subjects. *Am J Respir Crit Care Med* 1997;155:1828-1834.
32. Milgrom H, Fick RB, Su JQ, Reimann JD, Bush RK, Watrous ML, Metzger WJ. Treatment of allergic asthma with monoclonal anti-IgE antibody. *N Engl J Med* 1999;341:1966-1973.