53rd WEEKLY NEWSLETTER

USF INTERNAL MEDICINE & PEDIATRIC DIVISIONS OF ALLERGY AND IMMUNOLOGY

Continuing with key historical articles of scientific importance in the medical literature, Thomas Casale, Professor of Medicine and Pediatrics, and Director, Joy McCann Culverhouse Clinical Research Unit, Internal Medicine Division of Allergy and Immuology, would like to highlight the early work done with omalizumab and the clinical impact of these findings.

From Thomas B. Casale, MD

240 ragweed allergic patients were dosed intravenously or subcutaneously with omalizumab. Omalizumab decreased serum-free IgE levels in a dose- and baseline-IgE dependent fashion. Only 11 subjects had IgE levels that were suppressed to undetectable levels, a sample too small to demonstrate overall clinical efficacy. We calculated that suppression of serum-free IgE to the lowest levels of detection requires an omalizumab/total IgE ratio of 10 to 15:1. We concluded that dosing should be based on baseline IgE and weight, developing a formula (0.016mg/kg/IU/mL) which continues to be the basis for omalizumab dosing tables today. We also demonstrated equivalent results with subcutaneous and IV formulations. This study changed how omalizumab was used in all future clinical trials and today in practice.

CasaleTB, Bernstein IL, Busse WW, LaForce CF, Tinkelman DG, Stoltz RR, Dockhorn RJ, Reimann J, Su JQ, Fick RB, Jr, Adelman DC: Use of an Anti-IgE Humanized Monoclonal Antibody in Ragweed-Induced Allergic Rhinitis. *J Allergy Clin Immunol* 1997; 100(1):110-121.

With warm regards,

Richard F. Lockey, MD
Distinguished University Health Professor
Joy McCann Culverhouse Chair in Allergy and Immunology
Professor of Medicine, Pediatrics & Public Health
Director, Division of Allergy and Immunology
Department of Internal Medicine

Jolan Walter, MD, PhD
Robert A. Good Endowed Chair in Immunology
Associate Professor of Pediatrics and Medicine
Chief, Division of Allergy and Immunology
Department of Pediatrics

Use of an anti-IgE humanized monoclonal antibody in ragweed-induced allergic rhinitis

Thomas B. Casale, MD,[a] I. Leonard Bernstein, MD,[b] William W. Busse, MD,[c] Craig F. LaForce, MD,[d] David G. Tinkelman, MD,[e] Randall R. Stoltz, MD,[f] Robert J. Dockhorn, MD,[g] James Reimann, PhD,[h] John Q. Su, PhD,[h] Robert B. Fick, Jr., MD,[h] and Daniel C. Adelman, MD[h] *Iowa City, Iowa, Cincinnati, Ohio, Madison, Wis., Raleigh, N.C., Denver, Colo., Evansville, Ind., Lenexa, Kan., and South San Francisco, Calif.*

Background: Increased serum levels of antigen-specific IgE are often associated with allergic respiratory disorders. RhuMAb-E25, a recombinant humanized monoclonal antibody, decreases free serum IgE by forming biologically inactive immune complexes with free IgE.
Objective: We hypothesized that rhuMAb-E25 would decrease total serum IgE and reduce symptoms.
Methods: Two hundred forty subjects were enrolled into five groups to determine the safety, tolerance, and efficacy of repeated administration of rhuMAb-E25 in adults with ragweed-induced allergic rhinitis and to explore the pharmacodynamic relationship of rhuMAb-E25 and IgE. One hundred eighty-one subjects received an initial intravenous loading dose (day 0, 1 month before ragweed season), followed by administration of rhuMAb-E25 (in mg/kg body weight) of 0.15 mg/kg subcutaneously, 0.15 mg/kg intravenously, or 0.5 mg/kg intravenously on days 7, 14, 28, 42, 56, 70, and 84. A subcutaneous placebo group and an intravenous placebo group were included. The total evaluation time included the 84-day treatment period, followed by a 42-day observation period.
Results: Adverse events were mild, and no differences were observed in the rates between the three active and two placebo treatment groups. Ragweed-specific IgE levels correlated with symptom scores. RhuMAb-E25 decreased serum free IgE levels in a dose- and baseline IgE–dependent fashion. However, only 11 subjects had IgE levels that were suppressed to undetectable levels (≤24 ng/ml), a sample too small to demonstrate significant differences and clinical efficacy. Thus the case for efficacy was not proven. Nonetheless, the study confirms that it is safe to repeatedly administer rhuMAb-E25 over a period of months.
Conclusions: Because rhuMAb-E25 decreased serum free IgE in a dose-dependent fashion and because symptom scores correlated with antigen-specific IgE levels, the results suggest that if given in adequate doses, rhuMAb-E25 should be an effective therapy for allergic diseases. (J Allergy Clin Immunol 1997;100:110-21.)

Key words: Antibodies, allergy, IgE, anti-IgE

Abbreviations used

HRP:	Horseradish peroxidase
IV:	Intravenous
PBS:	Phosphate-buffered saline
rhuMAb-E25:	Recombinant human monoclonal antibody E25
SC:	Subcutaneous

Allergic rhinitis is the most common clinical form of atopic disease, affecting approximately 25% to 30% of the U.S. population.[1, 2] Ragweed (*Ambrosia* spp.) is the most common cause of seasonal allergic rhinitis (hay fever) in North America[3] and is widespread throughout the warmer portions of the Western Hemisphere. However, it is unusual for patients with allergic rhinitis to be sensitive to ragweed alone.

Current therapies for the treatment of allergic rhinitis symptoms include allergen avoidance, pharmacologic interventions (e.g., antihistamines, sympathomimetics, topical and systemic corticosteroids, and chromones), and immunotherapy.[1, 4, 5] Although helpful, many of these pharmacologic interventions provide only moderate or partial relief of symptoms and may be associated with significant side effects. Traditional allergen immunotherapy can be used alone or in conjunction with these pharmacologic interventions to provide relief of symptoms, but it is only effective in a narrow, antigen-specific fashion. Thus a novel therapy that works in a broader, antigen-nonspecific fashion is desirable.

Because of the central role that IgE antibody plays in the cascade of biochemical events resulting in allergic reactions,[6] an emerging concept of immunotherapy is to inhibit IgE responses through the use of anti-IgE antibodies. In a previously reported study, murine monoclonal anti-IgE antibodies were produced by the immunization of mice with human IgE.[7] The screening strategy used in monoclonal selection identified an antibody that recognized IgE at the same site as the high-affinity (IgE) receptor (FcεRI). Thus this antibody has the following characteristics: binds to free IgE and not to IgG or IgA, blocks the binding of IgE to its specific high-affinity receptor, does not bind to basophil- or mast cell–bound IgE, and inhibits the synthesis of IgE in cultured IgE-

From [a]the Department of Internal Medicine, University of Iowa; [b]Cincinnati; [c]University of Wisconsin, Madison; [d]Raleigh; [e]Denver; [f]Evansville; [g]Lenexa, Kansas; [h]Genentech, Inc., South San Francisco.
Supported by Genentech, Inc., San Francisco, Calif.
Received for publication May 31, 1996; revised Oct. 31, 1996; accepted for publication Feb. 12, 1997.
Reprint requests: Thomas B. Casale, MD, Nebraska Medical Research Institute, 401 E. Gold Coast Rd., Suite 124, Papillion, NE 68046-4796.

0091-6749/97 $5.00 + 0 **1/1/81429**

TABLE I. Number of patients and dosing regimens

Dose group	No. of patients	Dosing regimen*							
		Day 0	Day 7	Day 14	Day 28	Day 42	Day 56	Day 70	Day 84
1	20	Placebo IV & SC	Placebo SC	Placebo SC	Placebo SC	Placebo SC	Placebo SC	Placebo SC	Placebo SC
2	60	0.15 IV & SC	0.15 SC	0.15 SC	0.15 SC	0.15 SC	0.15 SC	0.15 SC	0.15 SC
3	60	0.3 IV	0.15 IV	0.15 IV	0.15 IV	0.15 IV	0.15 IV	0.15 IV	0.15 IV
4	60	0.5 IV	0.5 IV	0.5 IV	0.5 IV	0.5 IV	0.5 IV	0.5 IV	0.5 IV
5	40	Placebo IV	Placebo IV	Placebo IV	Placebo IV	Placebo IV	Placebo IV	Placebo IV	Placebo IV

*Dose is expressed as milligrams per kilogram of rhuMAb-E25 versus an equivalent amount of placebo.

producing cells. To make the molecule more suitable for long-term administration to human beings, the critical amino acids responsible for binding to IgE were engrafted onto a consensus human IgG_1 framework.[8] This recombinant humanized monoclonal anti-IgE antibody (rhuMAb-E25) demonstrated biologic properties similar to those of the murine monoclonal anti-IgE antibody. RhuMAb-E25 was expected to be well tolerated in human beings, because another antibody with the identical IgG_1 framework but a different binding site (recombinant humanized anti-$p185^{HER2}$ monoclonal antibody) has been well tolerated when used for the long-term treatment of patients with breast cancer.[9] Furthermore, because rhuMAb-E25 does not bind to IgE on mast cells and basophils, there was no presumed risk of cross-linking IgE molecules on these cells and inducing acute anaphylaxis.

Given the primary role of IgE in the pathogenesis of atopic diseases, particularly seasonal allergic rhinitis, and the demonstrated ability of both murine monoclonal anti-IgE antibody and humanized anti-IgE antibody to block the biologic effects of IgE,[10] it would be predicted that decreasing total serum IgE (free and complexed with rhuMAb-E25) would in turn decrease the amounts of antigen-specific IgE available to bind to and sensitize tissue mast cells. Therefore for this study it was hypothesized that the partial reduction of IgE would result in an amelioration of IgE-mediated allergic symptoms and control of atopic disease.

Thus a multicenter, double-blind, placebo-controlled study was designed to: (1) determine the safety and tolerance of multiple intravenous (IV) or subcutaneous (SC) administrations of rhuMAb-E25 in a large number of adults with a history of seasonal ragweed-induced allergic rhinitis when dosing was initiated before and during one ragweed season; (2) determine the pharmacodynamic effects on serum free IgE of rhuMAb-E25; and (3) compare the efficacy of prophylactic administration of three dose levels of rhuMAb-E25 with that of placebo by assessment of symptoms during ragweed season in adults with a history of seasonal ragweed-induced allergic rhinitis.

METHODS

Study design

The study was a double-blind, placebo-controlled trial of 240 patients at seven centers, randomized into five arms: three active treatment and two placebo (Table I). Study drug administration occurred over a 12-week period (4 weeks before and during the 1994 ragweed season), followed by an 8-week follow-up period. Treatment was allocated according to a computerized randomization scheme prepared by the Genentech Biostatistics Department. A separate scheme for each study center allocated treatment in balanced blocks. The active treatment dosing regimens selected for this study were designed to approach different serum steady-state levels, which were expected to demonstrate a therapeutic effect. To enhance both the effect of neutralizing serum IgE (by obtaining earlier steady-state concentrations) and the expected prophylactic efficacy of rhuMAb-E25, the dosing schedule provided for more frequent dosing during the initial 3 weeks (Table I).

This study was conducted in outpatients who were screened during the 14 days before the initiation of the study. Eligible patients returned to the study center for study drug administration and physical and laboratory evaluations on days 0, 7, 14, 28, 42, 56, 70, and 84. Patients remained in the study center for at least 2 hours after each study drug administration for observation of potential acute allergic reactions (e.g., flushing, urticaria, pruritus, bronchospasm, lightheadedness, or other signs and symptoms of anaphylaxis). Patients returned to the study center for follow-up physical and laboratory evaluations on study days 98, 112, 126, and 140.

Patients withdrawing from the study because of adverse events (clinical or laboratory) were treated and followed up according to established, acceptable medical practice. All pertinent information concerning the outcome of such treatment was recorded in the medical record. If the investigator removed a patient from the study or if the patient declined further study participation, evaluations scheduled for day 140 were completed at the time of patient withdrawal.

Patients and investigators remained blinded to the study drug assignments throughout the study. A clinical pharmacist, who was not blinded, provided the study staff with syringes of study drug for patient administration labeled with the patient identification number, the protocol number, volume of study drug, and the route of administration.

Although patients were to receive no therapy for allergic rhinitis other than the study drug, rescue medication was made available to all participants. Tavist-D tablets were provided in a known quantity and could be taken by patients for relief of discomfort caused by allergic rhinitis symptoms scored as 2 or

more on the daily diary score cards. The number of Tavist-D tablets taken was recorded in the diary, confirmed by pill counts at each visit, and recorded at each center.

Human subjects

All patients were at least 18 years old and had a documented history of seasonal allergic rhinitis or rhinoconjunctivitis requiring treatment for at least the two previous seasons and demonstrated well-characterized positive reactivity to ragweed allergen. Women of childbearing potential had a negative serum pregnancy test result and, in the opinion of the investigator, had been using an effective means of contraception.

Patients were excluded from study participation if they had rhinitis medicamentosa or moderate to severe perennial vasomotor, infectious, or atrophic rhinitis; structural nasal abnormalities (e.g., septum deviation) severe enough to cause obstruction; history of asthma requiring daily maintenance therapy; adverse reactions to the rescue medication (Tavist-D); clinically significant upper respiratory tract infection in the 7 days before enrollment; desensitization therapy (immunotherapy) with a change in maintenance dose within the past 12 months; treatment with any systemic corticosteroid within 3 days during the 14 days before enrollment; treatment with any prescription medication for rhinitis, urticaria, eczema, hay fever, or asthma within 14 days before enrollment; long-term treatment with over-the-counter or prescription drugs for rhinitis, urticaria, eczema, hay fever, or asthma; history of other cardiovascular, pulmonary, hepatic, hematopoietic, renal, neurologic, or metabolic dysfunction that in the opinion of the investigator would contraindicate taking an investigational drug or might affect the interpretation of the results of the study; previous therapy with any monoclonal antibody preparation, including rhuMAb-E25; or treatment with an investigational drug within 30 days before day 0. Pregnant or lactating women were also excluded. Patients who demonstrated reactivity to rhuMAb-E25 in either the skin prick or intradermal challenge test were excluded from the study but were followed up for safety evaluation as deemed appropriate by the investigator.

This study was carried out in compliance with United States Food and Drug Administration Good Clinical Practices and local ethical and legal requirements. To safeguard the rights of the individual, each patient provided written informed consent before enrollment in the study. The informed consent was approved by the institutional review board of each participating center and was constructed in accordance with local ethical and legal requirements.

Symptoms diary

Patients were instructed to maintain a daily diary of their allergic rhinitis symptoms. The patients scored their eye and nose symptoms in a diary twice daily (morning and evening) throughout the study. Symptom scores used were: 0, no symptoms; 1, mild symptoms (present but not troublesome); 2, moderate symptoms (frequently troublesome but not sufficient to interfere with normal daily activity or nighttime sleep); and 3, severe symptoms (sufficiently troublesome to interfere with normal daily activity or nighttime sleep). Other concurrent symptoms or complaints, Tavist-D use, and local pollen counts were also recorded in the diary. Diary entries were reviewed by the study coordinator and transferred to a case report form.

For both morning and evening symptom scores, one or more nasal symptoms of at least moderate severity (score = 2) and a combined symptom score of at least 5 (for the symptoms of blocked nose, runny nose, sneezing, and itchy eyes) were required to be classified as a "symptom day." The mean numbers of symptom days in the active and placebo regimens were compared by using the Wilcoxon rank-sum test, stratified by study center for "before season," "during season," and "after season," respectively.

Efficacy assessments

The primary efficacy variables were the average of all daily symptom scores during the ragweed season and the total number of days that patients experienced symptoms.

Quality-of-life questionnaires

Patients were instructed to complete two quality-of-life questionnaires before study drug administration on day 0 and during the course of the study. The Juniper Rhinoconjunctivitis Quality-of-Life questionnaire[11] was administered before study drug administration on days 0, 7, 14, 28, 56, and 84 and on day 140. Patients were asked to complete the MOS 36-item short-form health survey (SF-36), a self-administered quality-of-life questionnaire, before study drug administration on day 0 and on days 84 and 140.

Safety assessments

All patients who received study drug were closely monitored and evaluated for safety after the initial study drug administration and throughout the study period. Adverse event information was collected immediately before the first study drug administration and throughout the study. Adverse events included new or increased severity of symptoms relative to baseline, adverse changes noted on physical examination, and clinically significant laboratory findings.

Severity of adverse events was assessed by the investigator at the individual site. "Severe" was defined as an adverse event that caused severe discomfort, severely limited or prevented normal function, was a definite hazard to the patient's health, or may have required a prolonged hospitalization. "Moderate" was defined as a symptom that was uncomfortable to the patient, produced some degree of impairment to normal function, resulted in a substantive change from baseline, and may have required treatment but was not hazardous to health. "Mild" was defined as a symptom that was an annoyance to the patient but did not affect baseline status, did not hinder the patient's normal functioning level, may have been intermittent or continuous, and may or may not have required treatment with medication.

End-point titration with ragweed allergen

End-point titration for ragweed allergen skin testing was performed before study drug administration on days 0, 28, 56, and 84 and on days 112 and 140. All end-point titration began with a 1:1,000,000 dilution.

Antibodies to rhuMAb-E25

Serum samples were obtained before skin prick challenge testing with rhuMAb-E25 and on days 28, 84, and 140. These samples were frozen as soon as possible after collection and were evaluated for the presence of antibodies to rhuMAb-E25. Human anti-rhuMAb-E25 antibodies were detected by using two solid-phase ELISA methods: one assay to detect anti-rhuMAb-E25 Fab responses and a separate assay to detect anti-rhuMAb-E25 Fc responses.

To measure anti-rhuMAb-E25 Fab antibody, rhuMAb-E25 Fab fragments were adsorbed to high-binding flat-bottom polystyrene plates (Costar, Cambridge, Mass.) at 3 μg/ml in phos-

TABLE II. Selected baseline characteristics of enrolled patients

	Placebo* (*n* = 59)	0.15 SC (*n* = 60)	0.15 IV (*n* = 61)	0.5 IV (*n* = 60)	Overall (*n* = 240)
Age range (yr)	20-59	18-65	18-55	18-66	18-66
(mean ± SD)	35 ± 9.2	32 ± 9.8	35 ± 9.3	33 ± 10.7	34 ± 9.8
Sex					
Male	33 (56%)	27 (45%)	28 (46%)	28 (47%)	116 (48%)
Female	26 (44%)	33 (55%)	33 (54%)	32 (53%)	124 (52%)
Race					
Caucasian	49 (83%)	55 (92%)	59 (97%)	46 (77%)	209 (87%)
Black	7 (12%)	4 (7%)	1 (2%)	10 (17%)	22 (9%)
Other	3 (5%)	1 (1%)	1 (1%)	4 (6%)	9 (4%)
Weight range (kg)	48-153	46-120	44-127	49-105	44-153
(mean ± SD)	(79 ± 19)	(77 ± 17)	(77 ± 18)	(74 ± 14)	(77 ± 17)
Height range (cm)	152-191	145-193	150-193	152-191	145-193
(mean ± SD)	(173 ± 11)	(172 ± 10)	170 ± 10)	(171 ± 10)	(171 ± 10)
Asthma history	5 (8.5%)	6 (10.0%)	15 (24.6%)	7 (11.7%)	33 (13.8%)
Baseline IgE (IU/ml) (mean ± SD)	221 ± 300	235 ± 477	267 ± 710	238 ± 269	240 ± 472

*Pooled data for both placebo groups.

phate-buffered saline (PBS) overnight at 2° to 8° C. The plates were washed with 0.05% Tween-20 in PBS, then incubated with blocking/sample buffer (0.2% bovine gamma globulin [Sigma Chemical Co., St. Louis, Mo.]/0.25% CHAPS [Sigma]/0.5% bovine serum albumin [Intergen Co., Purchase, N.Y.]/0.05% Tween-20/0.01% thimerosal in PBS) for 1 hour. The standards and samples were diluted in blocking/sample buffer. The minimum sample dilution is 1:100. After the plates were washed, the diluted samples and standards were incubated in the wells for 1 hour. The plates were washed and incubated with a 1:60,000 dilution of protein G–horseradish peroxidase (HRP) (Bio-Rad, Hercules, Calif.) in 0.5% bovine serum albumin/0.05% Tween-20/0.01% thimerosal in PBS for 1 hour. The plates were washed and then developed with 0.4 mg/ml o-phenylenediamine and 4 mmol/L H_2O_2 in PBS. The color reaction was stopped with 4.5N H_2S0_4. The absorbance at 492 nm was measured with an SLT EAR 340AT ELISA Reader (Research Triangle Park, N.C.).

A standard curve to determine the anti-rhuMAb-E25 Fab response was constructed by using dilutions of cynomolgus monkey anti-rhuMAb-E25 serum. The titer values of the anti-rhuMAb-E25 serum dilutions were initially determined relative to a negative control serum. In subsequent assays the previously determined titer values of the anti-rhuMAb-E25 serum calibrators were fitted with a four-parameter logistic log program, and the fit was used to calculate the titers of the samples. Any samples that were reactive in the assay (titer ≥ 2.0) were analyzed in an additional ELISA, in which the anti-rhuMAb-E25 Fab assay described above was performed with the addition of 25 μg/ml rhuMAb-E25 to the diluted sample. Samples were considered reactive for anti-rhuMAb-E25 Fab antibodies if the addition of rhuMAb-E25 to the sample resulted in a titer decrease of more than 0.4 titer units.

The measurement of anti-rhuMAb-E25 Fc antibody was performed by using the anti-rhuMAb-E25 Fab protocol described above, except that rhuMAb-E25 Fc at 1 μg/ml in sodium carbonate buffer (pH 9.6) was coated on the plate in place of the rhuMAb-E25 Fab and anti-human IgG (Fab-specific)–HRP at a dilution of 1:75,000 was used in place of the protein G–HRP. The other assay steps and data reduction were performed as described above.

Skin testing for reactivity to rhuMAb-E25

Skin testing for reactivity to rhuMAb-E25 was performed during the screening period and on day 140.

Free and total IgE concentrations

Serum concentrations of total serum IgE and free serum IgE were measured at baseline and at various time points during the study. Total IgE was measured by using a commercial kit (IMx Total IgE; Abbott Laboratories, Abbott Park, Ill.). The lower limit of detection is 2.4 ng/ml. Concentrations of rhuMAb-E25 in this assay up to 1 mg/ml do not interfere in the detection of total IgE.

Human free IgE was measured by using a solid-phase ELISA. High-binding flat-bottom polystyrene plates (Costar) were coated overnight at 2° to 8° C with 100 ng of human IgE receptor α-chain IgG chimera (FcεRI-IgG)[12] in 100 μl of carbonate buffer (pH 9.6). The plates were washed with 0.05% Tween-20 in PBS, then incubated with 200 μl of assay diluent (0.5% bovine serum albumin [Intergen Co.]/0.05% Tween-20/0.01% thimerosal in PBS) for 1 to 2 hours. The plates were sealed and frozen until use. All subsequent assay steps were performed at room temperature. For the ELISA, plates were thawed and washed, and 50 μl of sample or standard was added in duplicate to 50 μl of assay diluent in the wells and incubated for 1 hour. FcεRI-IgG captures only free IgE, that is, IgE that is not in a complex with rhuMAb-E25. The plates were washed, and 100 ng of biotinylated monoclonal anti-human IgE in 100 μl of assay diluent was added to the wells and incubated for 1 hour. The plates were washed, and 100 μl of avidin-HRP (Vector Laboratories, Inc., Burlingame, Calif.), diluted 1:2000 in assay diluent, was added to the wells and incubated for 30 minutes. The plates were washed and then developed with 0.4 mg/ml o-phenylenediamine and 4 mmol/L H_2O_2 in PBS. The color reaction was stopped with 4.5N H_2SO_4. The absorbance at 492 nm was measured with an SLT EAR 340AT ELISA Reader. The concentration of free IgE was calculated for the

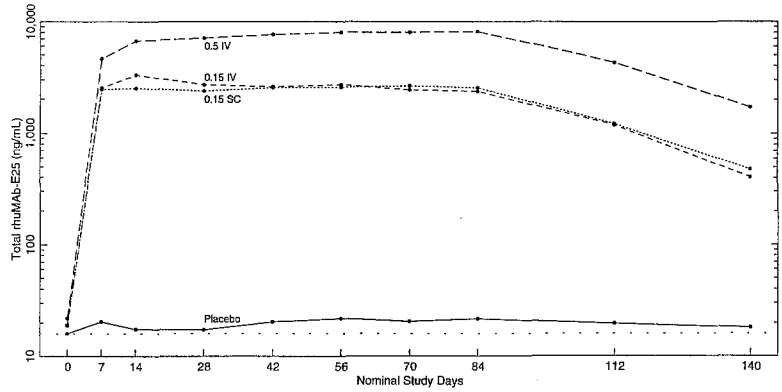

FIG. 1. Geometric means of total rhuMAb-E25 by treatment group. Times are 5 minutes before dose; total rhuMAb-E25 has lower limit of quantification of 16 ng/ml, indicated by the faint dotted line.

TABLE III. Adverse events observed in more than 10% of patients

	Placebo (n = 59)	0.15 SC (n = 60)	0.15 IV (n = 61)	0.5 IV (n = 60)	Overall (n = 240)
Headache	31 (53%)	25 (42%)	41 (67%)	31 (52%)	128 (53%)
Infection	16 (27%)	13 (22%)	13 (21%)	7 (12%)	49 (20%)
Pain	10 (17%)	12 (20%)	6 (10%)	7 (12%)	35 (15%)
Pharyngitis	9 (15%)	12 (20%)	10 (16%)	10 (17%)	41 (17%)

standard curve by using a four-parameter logistic log fit for the standards.

Ragweed-specific IgE

Ragweed-specific IgE concentration was measured by using the Pharmacia CAP system RAST FEIA (Pharmacia, Uppsala, Sweden). In brief, the ragweed-specific IgE in the sample binds to ragweed allergen that has been covalently coupled to the solid support. The captured IgE is detected with an enzyme-labeled anti-IgE antibody, followed by a fluorescent substrate.

The presence of rhuMAb-E25 in the sample will interfere somewhat with the detection of ragweed IgE. High concentrations of rhuMAb-E25 spiked into ragweed IgE–positive sera reduced the amount of detected ragweed IgE by up to 25%.

Ragweed pollen counts

The ragweed season was determined for each study center by using local daily pollen counts. Because of the variability in the start of the season and in the magnitude of pollen measurements at each study center, the start of ragweed season was defined as the sustained elevation of daily pollen counts over baseline (before season), and the end of ragweed season was defined as the return of daily pollen counts to baseline.

Statistical analysis

For all analyses, morning and evening symptom scores were averaged after a preliminary assessment of their possible differences.

Three pairwise comparisons with placebo were performed by using Wilcoxon rank-sum tests, stratified by study center, for each nose and eye symptom score and for averaged nose symptom scores, averaged eye symptom scores, and averaged all symptom scores. The validity of the pooling of data across centers was assessed by a preliminary test for center-by-treatment interaction based on a two-way analysis of variance model with ranked data.

RESULTS

Demographics

A total of 240 patients were enrolled at seven centers located in the Midwest and on the East Coast of the United States. Patients were treated with rhuMAb-E25 or placebo weekly for the first 2 weeks, then biweekly through day 84 (Table I). All treatment groups were well balanced except for the 0.15 IV group, which had a slightly higher proportion of Caucasian patients and a higher rate of asthma at study entry (Table II). Twenty-nine (12%) patients discontinued use of study medication during the course of study: 22 patients voluntarily withdrew, five patients were discontinued because of adverse events, one patient in the 0.5 IV dose group was discontinued because of noncompliance, and one patient in the 0.15 SC dose group was removed on the basis of the physician's decision.

Adverse reactions

Overall, most reported adverse events were mild to moderate in severity and, in the opinion of the investigators, were unlikely to be related to study drug. One serious adverse event leading to withdrawal was reported during the 140-day study period. This one serious adverse event was due to colitis and was believed to be unrelated to study drug administration. Five other patients withdrew from the study because of adverse events. One of the five adverse events was thought to be

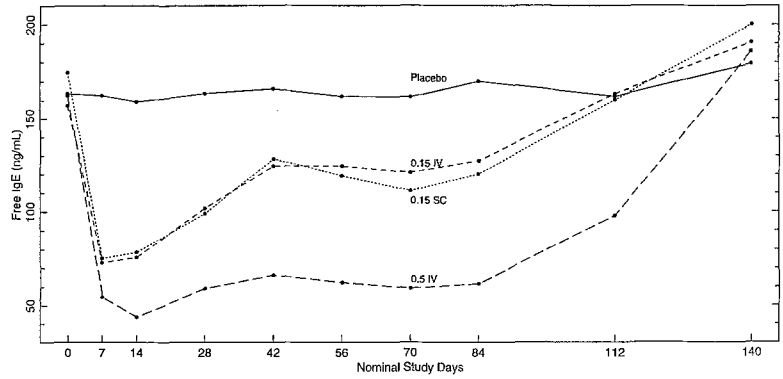

FIG. 2. Geometric means of free serum IgE by treatment group. Times are 5 minutes before dose; free IgE has limits of quantification of 10 ng/ml and 165 ng/ml.

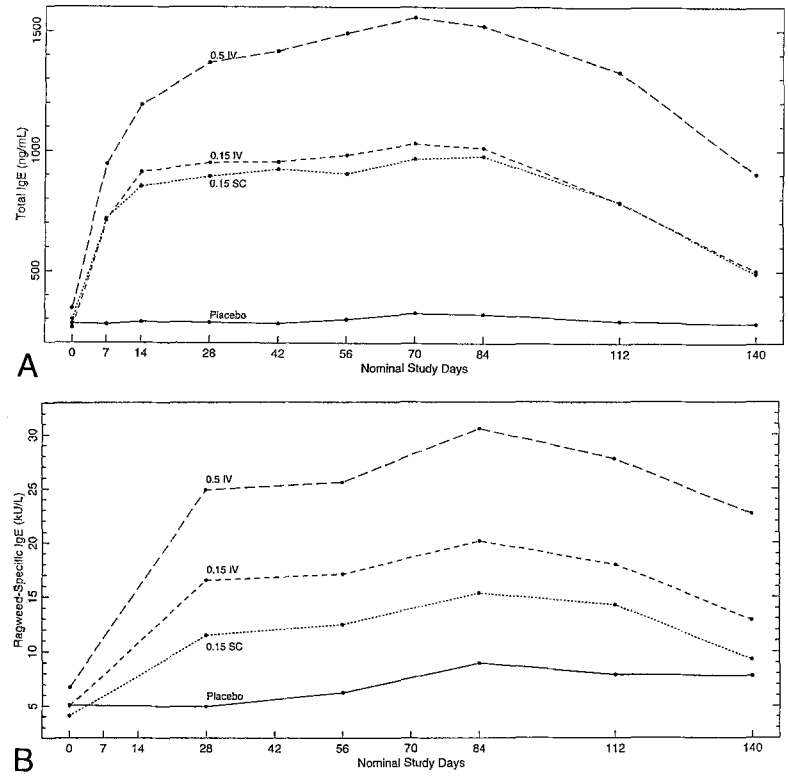

FIG. 3. Geometric means of serum total IgE **(A)** and ragweed-specific total IgE **(B)** by treatment group. Times are 5 minutes before dose.

possibly caused by study drug. This event, which resulted in an early withdrawal, was reported as a "mild asthma attack." The patient, in dose group 3, complained of increased cough and "a lot of mucus" but had no wheezing or spirometric evidence of bronchospasm and remained hemodynamically stable. This event began 45 minutes after dosing, was treated medically, and lasted for 1 hour. However, similar reactions were not observed in other patients with allergic asthma. There were no significant differences overall in adverse events among treatment groups. There were also no significant differences in adverse events among treatment groups with respect to sex or age.

Table III summarizes the most frequently observed adverse events during the study. No differences were found between the placebo and treatment groups.

Skin testing with rhuMAb-E25

Epicutaneous and intradermal skin tests with rhuMAb-E25 were performed twice on all patients, once during the screening period and again on day 140. A small number of subjects at the screening visit had a

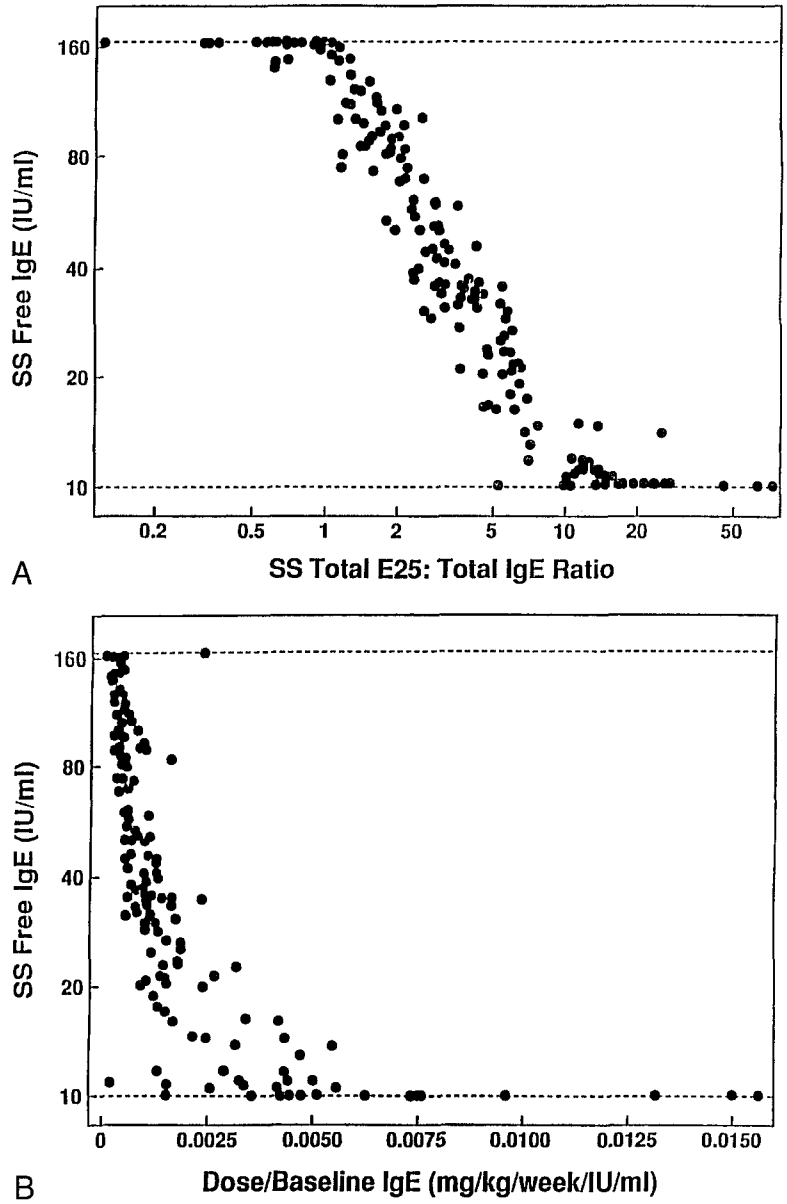

FIG. 4. A, Steady-state *(SS)* free IgE against steady-state total E25/total IgE ratio in treated patients. Steady-state concentrations are geometric means of predose values over days 42, 56, 70, and 84. **B,** Steady-state free IgE against dose/baseline IgE in dosed patients. Steady-state free IgE is geometric mean of predose values over days 42, 56, 70, and 84.

positive skin test response to rhuMAb-E25 and were excluded from further participation. All skin test results were required to be negative during the screening period for enrollment. On day 140, there were no differences in the rate of positive results between the placebo and active treatment groups (data not shown). Furthermore, none of the patients who had positive skin test responses exhibited adverse reactions to administration of rhuMAb-E25. No patients were found to have human anti-human antibodies to rhuMAb-E25 at the completion of the study.

Pharmacokinetics and pharmacodynamics

Total rhuMAb-E25 cleared slowly with a terminal half-life of 2.9 ± 0.7 weeks (mean ± SD) after multiple-dose SC and IV administration of rhuMAb-E25. Kinetic profiles of trough concentrations of total rhuMAb-E25 in the 0.15 SC and 0.15 IV dose groups were similar over the course of the study, consistent with results after SC and IV dosing in previous phase I studies.[13] Steady-state concentrations of total rhuMAb-E25 were generally achieved by days 14 to 28 in all dose groups (Fig. 1). The geometric means of total rhuMAb-E25 slowly declined

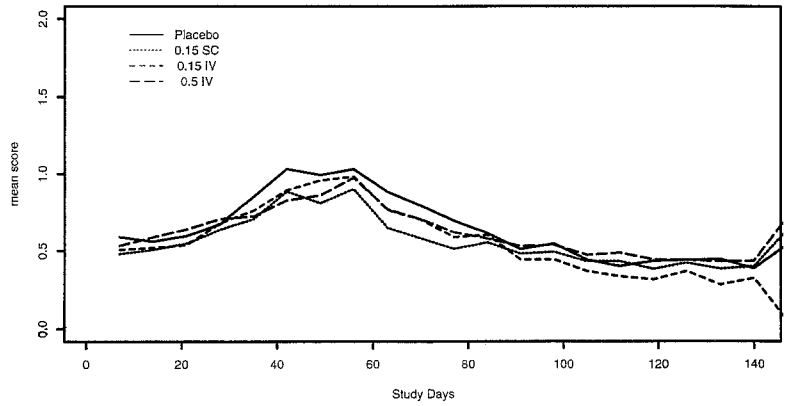

FIG. 5. Mean daily eye and nose symptom scores by study days.

in all three treatment groups after discontinuation of therapy.

Free, total, and ragweed-specific total IgE

Administration of rhuMAb-E25 decreased serum concentration of free IgE in a fashion that was dependent on dose of rhuMAb-E25 administered and the patient's baseline IgE, which was consistent with previous phase I studies. Higher doses of rhuMAb-E25 resulted in more complete suppression of free IgE concentrations (Fig. 2). Serum free IgE concentrations were suppressed rapidly after IV dosing with rhuMAb-E25 and slowly returned to their baseline values after termination of dosing. In the 0.15 SC and 0.15 IV dose groups, serum concentrations of free IgE at steady state (trough concentrations on days 42, 56, 70, and 84) were approximately 60% of baseline free IgE. There were no significant differences in the pharmacodynamics between the SC and IV routes of administration, demonstrating that functionally the two groups were comparable. In the 0.5 IV dose group, serum concentrations of free IgE at steady state were approximately 30% of baseline free IgE and reached the lowest level of detection (≤24 ng/ml) in patients with baseline IgE less than 40 IU/ml. Free IgE was suppressed to a greater extent during the first few weeks of the trial when doses of rhuMAb-E25 were administered weekly.

Because of formation of rhuMAb-E25–IgE complexes, which have a longer serum half-life than free IgE (unpublished observations), total serum IgE (free and complexed IgE) and ragweed-specific total IgE increased in treated patients during the course of the study by twofold to threefold, on average, in the 0.15 SC and 0.15 IV dose groups and by fourfold to fivefold, on average, in the 0.5 IV group (Fig. 3). Increases in total IgE concentrations were dependent on the dose of rhuMAb-E25 administered and the patient's baseline IgE. Increases of fivefold to sixfold, on average, were observed in patients who had free serum IgE suppressed to the lowest levels of detection at steady state. Total serum IgE and ragweed-specific total IgE levels declined after termination of therapy but generally did not approximate those levels measured in the placebo group on day 140.

TABLE IV. Daily symptom scores

Dose of rhuMAb-E25	Mean ± SD (*n*)	
	Before season	Peak season
Placebo	0.64 ± 0.54 (58)	0.91 ± 0.65 (56)
0.15 SC	0.53 ± 0.42 (58)	0.73 ± 0.48 (54)
0.15 IV	0.57 ± 0.54 (61)	0.82 ± 0.66 (59)
0.50 IV	0.63 ± 0.50 (60)	0.77 ± 0.52 (56)

The magnitude of symptoms, the change of symptoms from preseason to peak season, and the difference among the treatment groups were all smaller than predicted and not significantly different ($p > 0.5$).

To investigate the pharmacodynamic relationship between trough serum concentrations of total rhuMAb-E25, free IgE, and total IgE at steady state, steady-state free IgE was plotted against the steady-state ratio of total rhuMAb-E25 to total IgE (Fig. 4, *A*). To assess the likely effect of administering a fixed dose per kilogram of body weight and international units per milliliter of baseline IgE, free IgE at steady state was plotted against the dose per baseline IgE of rhuMAb-E25 expressed in milligrams per kilogram per week for every international unit per milliliter of baseline IgE (Fig. 4, *B*). This figure shows that there is a clear relationship between starting IgE levels and the dose of rhuMAb-E25 required to effectively suppress this level (i.e., for any starting serum IgE concentration, higher doses of rhuMAb-E25 result in lower concentrations of free IgE).

Symptom scores

All centers reported ragweed pollen counts typical for their respective regions. As expected, pollen counts in the Midwest were greatest (up to 2000 grains/m^3), whereas those for the West were lowest. All centers had well-defined ragweed seasons on the basis of daily pollen counts.

The average daily symptom score of patients receiving placebo was slightly higher than that of patients treated with rhuMAb-E25 during the season, but this difference

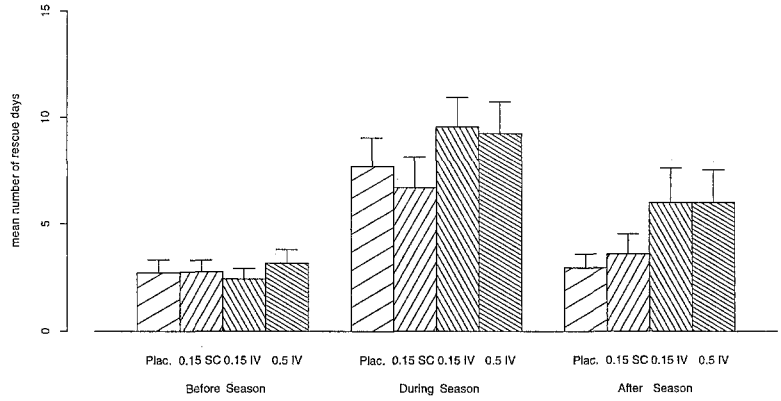

FIG. 6. Mean (± SEM) number of days rescue medication was used by treatment group. *Plac.,* Placebo.

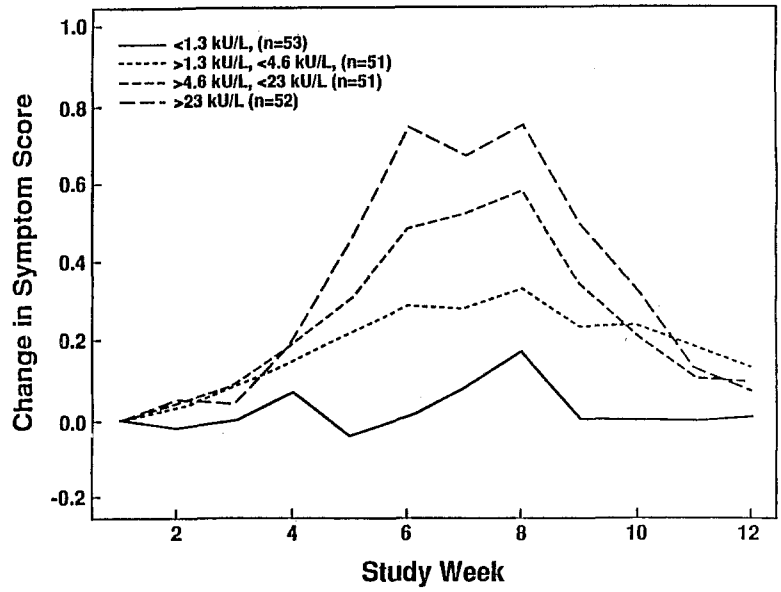

FIG. 7. Weekly total symptom score by baseline ragweed-specific total IgE (all treatment groups combined).

was not statistically significant. Overall, the change in symptoms from before the season to the peak season was small. The averaged symptom score before the season was approximately 0.6 for patients receiving placebo and 0.5 to 0.6 for treated patients. During the season, the averaged symptom scores were 0.91 for patients receiving placebo and 0.7 to 0.8 for treated patients (Table IV). Even during the peak of the season (the 2-week period with highest daily pollen counts), the averaged symptom scores were only 1.0 for patients receiving placebo and 0.8 to 0.9 for treated patients (Fig. 5). The magnitude of symptoms, the change of symptoms from before the season to the peak season, and the difference among the treatment groups were all smaller than predicted; whereas the variability of symptom scores was higher than estimated. The standard deviation was up to 0.9 within each treatment group. In addition, the variability among the study centers was large, and the treatment effects were not consistent among study sites. The differences in rescue medication use between the treated groups and the placebo group were not significant (Fig. 6). There was also no significant treatment effect on quality-of-life scores. Overall, the study failed to demonstrate a significant improvement in treated subjects.

Symptom scores correlated significantly with baseline ragweed-specific IgE levels ($\rho = 0.33$; $p = 0.0001$) (Fig. 7). Subjects with the lowest levels of baseline ragweed-specific total IgE (<1.3 kU/L) had little or no increase in symptoms during the ragweed season, whereas a progressively greater increase in symptoms during the ragweed season was seen in patients with progressively higher baseline levels of ragweed-specific total IgE. The group with the highest baseline levels had symptom score increases of approximately 0.7 units, on average during the season, compared with an average increase of approximately 0.4 units for all patients combined. There was little indication of a correlation between symptoms

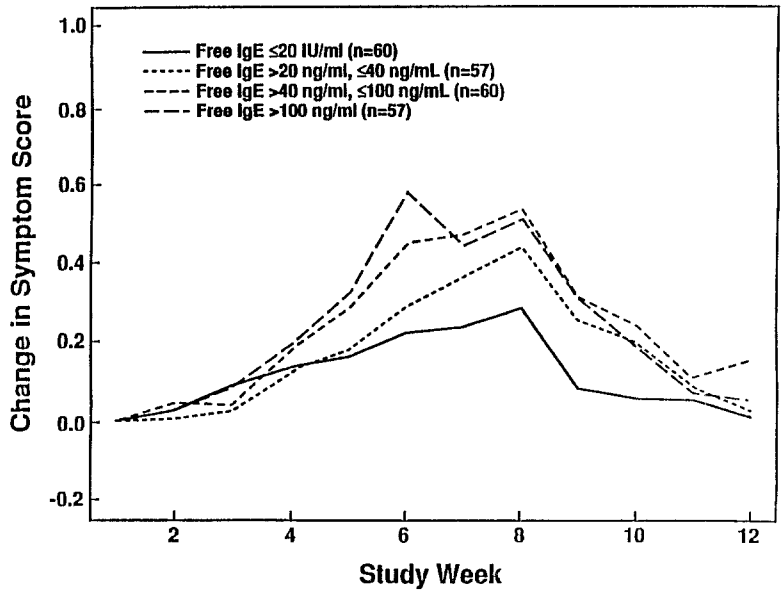

FIG. 8. Weekly total symptom score by free IgE level (all treatment groups combined). Free IgE is geometric mean of predose values on days 14, 28, 42, 56, and 70.

TABLE V. End-point titration to ragweed allergen

Study day	Treatment	*n*	Mean change (SEM)	*p* Value	
				Within	Overall
Day 28	Placebo	56	−0.071 (0.095)	0.451	0.093
	0.15 SC	54	−0.111 (0.096)	0.250	
	0.15 IV	60	−0.150 (0.091)	0.102	
	0.50 IV	58	0.155 (0.093)	0.097	
Day 56	Placebo	55	0.145 (0.097)	0.133	0.028
	0.15 SC	51	0.039 (0.100)	0.696	
	0.15 IV	58	−0.121 (0.094)	0.201	
	0.50 IV	54	0.278 (0.097)	0.005	
Day 84	Placebo	55	0.036 (0.104)	0.726	0.107
	0.15 SC	51	0.000 (0.108)	1.000	
	0.15 IV	57	−0.158 (0.102)	0.123	
	0.50 IV	54	0.204 (0.105)	0.053	

Data are mean change from baseline across treatment groups (base 10 logarithmic scale). Probability (*p*) values are determined from analysis of variance.

and baseline total IgE levels, possibly because ragweed-specific total IgE and total IgE at baseline are only weakly correlated at baseline ($\rho = 0.4$ in log units). However, symptom scores during the ragweed season appeared to be lower in patients with free serum IgE levels (average of trough samples on days 14, 28, 42, 56, and 70) less than 40 ng/ml (Fig. 8).

End-point titration

Skin test reactivity to ragweed allergen, as measured by average end-point titration, remained unchanged in the placebo, 0.15 SC dose, and 0.15 IV dose groups over the course of the study. In the 0.5 IV dose group, there was a marginally significant increase in average end-point titration relative to baseline (day 0) on days 28 through 84, corresponding to an increase of approximately two thirds in the concentration of ragweed allergen needed to obtain the standard allergic response (Table V).

DISCUSSION

The results of this phase II, multicenter, double-blind, placebo-controlled study in adults with a history of seasonal ragweed-induced allergic rhinitis indicate that prolonged administration of a humanized recombinant monoclonal antibody directed against human IgE is safe in a large number of patients and demonstrates an important biologic dose-response effect and functional comparability of SC and IV administration. Although the data do not demonstrate statistically significant

differences in efficacy between active- and placebo-treated patients, they do provide a clearer understanding of the required dosing strategies for this new class of therapeutic agents to produce clinical activity in an IgE-mediated disease.

A total of 181 patients received treatment with rhuMAb-E25 for up to 84 days. To assess the safety of rhuMAb-E25, all patients were carefully monitored for adverse events with blood chemistries, complete blood counts, urinalyses, and physical examinations. In addition, patients were assessed for de novo immunologic responses (in vitro and in vivo) to the administration of the humanized protein, rhuMAb-E25. Overall, reported adverse events were mild to moderate in severity and, in the opinion of the investigators, were unlikely to be related to study drug. Four of the five patients who withdrew from the study because of adverse events did so after completing the treatment phase of the study. Moreover, there were no significant differences in the rates of adverse events among any of the treatment groups (Table III). The one serious adverse event (an episode of colitis) during the study was not attributed to the study drug.

Skin testing with rhuMAb-E25 demonstrated a positive rate of approximately 10% and was consistent across all treatment groups. Specifically, the rate of positive test results was similar between patients receiving placebo and those receiving active treatment. Thus it is unlikely that these test results indicate sensitization of patients by prolonged administration of rhuMAb-E25. Furthermore, it can be inferred that as a result of the lack of immunogenicity, rhuMAb-E25 can be safely given to patients without screening by skin testing before administration.

The most important biologic concept to emerge from this study is the understanding of the pharmacodynamic relationship between rhuMAb-E25 and both unbound and complexed IgE. This relationship suggests that rhuMAb-E25 may alter the equilibrium that exists in vivo between cell-bound FcεRI and free IgE. The pharmacodynamic data from this study demonstrate that consistent suppression of serum free IgE to the lowest levels of detection requires an initial rhuMAb-E25/total IgE (free IgE and IgE complexed with rhuMAb-E25) ratio of approximately 10 to 15:1 (Fig. 4, *A* and *B*). This analysis implies that dosing should be calculated on the basis of individual baseline IgE values. In this study dosing of rhuMAb-E25 at approximately 0.005 mg/kg/week for each international unit per milliliter of baseline IgE suppressed serum free IgE to the lowest level of assay detection at steady state.

The assessment of efficacy in this study must therefore be interpreted in the context of the pharmacodynamic data. According to these analyses, only 11 patients in the highest dose group (0.5 mg/kg) were adequately treated to achieve full suppression of serum free IgE concentrations, a sample too small to demonstrate statistically significant differences between the active and placebo treatment groups. However, symptom scores were shown to correlate with antigen-specific IgE; thus, lower symptom scores would be expected in patients with lower levels of free IgE during the season, because they should have little antigen-specific IgE. There is some evidence of this, because patients with the lowest level of free IgE during the ragweed season (≤45 ng/ml) had symptom scores that were approximately 0.2 units lower, on average, than the other patients. Because the number of patients with serum free IgE suppressed to the lowest levels of detection was low and because the variability in the symptom scores was high, no further investigation of a possible treatment effect in patients with the greatest suppression of serum free IgE levels was done.

The data from this study confirm the results of earlier studies[13] that rhuMAb-E25 is well tolerated and safe at the dose levels administered. Pharmacodynamically, it is apparent that if baseline serum IgE concentration is taken into consideration in determining dose level, then dose-dependent suppression of free IgE to or below the lowest levels of detection can be achieved. The original hypothesis, that partial reduction of serum free IgE would lead to amelioration of seasonal ragweed allergic rhinitis symptoms, needs to be modified. Our data suggest that if sufficient quantities of rhuMAb-E25 are given to fully suppress serum free IgE levels, then allergic rhinitis symptoms will likely decrease or be ameliorated. Because equivalent results were found with IV and SC dosing regimens, this form of therapy is practical and likely could be administered in regimens similar to traditional immunotherapy. Therefore further investigation of rhuMAb-E25 in the treatment of the symptoms of allergic diseases is warranted. Indeed, a safe and effective therapy for allergic diseases that is not antigen-specific would provide a novel and useful tool for the armamentarium of the clinical allergist/immunologist.

We thank the study coordinators, nurses, and pharmacists for their assistance in carrying out these studies and the following Genentech individuals for their invaluable contributions: John Curd, MD, Juergen Froehlich, MD, Paula Jardieu, PhD, V. Miles Rios, Jr., CCRA, Jane Ruppel, BA, Monika Schoenhoff, PhD, and Thomas M. Tremblay, RN. We also thank Darla Bartels and Vicki Sears for secretarial and editorial support.

REFERENCES

1. Kaliner M, Lemanske R. Rhinitis and asthma. JAMA 1992;68: 2807-29
2. Naclerio R. Allergic rhinitis [review]. N Engl J Med 1991;325:860-9.
3. Evans R III. Epidemiology and natural history of asthma, allergic rhinitis, and atopic dermatitis. In: Middleton E Jr, Reed CE, Ellis EF, Atkinson NF Jr, Yunginger JW, Busse WW, editors. Allergy: principles and practice. 4th ed. St. Louis: Mosby; 1993. p. 1109-36.
4. Ito K, Nakagawa T, Morita Y, Okudaira H, Takaishi T, Ishii A, et al. Immunologic analysis of steroid-dependent asthma. Ann Allergy 1989;62:15-20.
5. Kemp J. New drugs in the treatment of asthma. Immunol Allergy Clin North Am 1991;11:233-49.
6. Bahna S. A 21-year salute to IgE. Ann Allergy 1989;62:471-5.
7. Presta LG, Lahr SJ, Shields RL, Porter JP, Gorman CM, Fendly BM, et al. Humanization of an antibody directed against IgE. J Immunol 1993;151:2623-32.
8. Shields R, Whether WR, Zioncheck K, O'Connell L, Fendly B,

Presta LG, et al. Inhibition of allergic reactions with antibodies to IgE. Int Arch Allergy Immunol 1995;107:308-12.
9. Baselga J, Tripathy D, Mendelsohn J, Benz C, Dantis L, Moore J, et al. Phase II study of recombinant human anti-HER2 monoclonal antibody (rhumAb HER2) in stage IV breast cancer (BC): HER2-shedding dependent pharmacokinetics and antitumor activity [abstract]. Proc Am Soc Clin Oncol 1995;14:103.
10. Saban R, Haak-Frendscho M, Zine M, Ridgway J, Gorman C, Presta LG, et al. Human Fc ERI-IgG and humanized anti-IgE monoclonal antibody MaE11 block passive sensitization of human and rhesus monkey lung. J Allergy Clin Immunol 1994;94:836-43.
11. Juniper E, Guyatt G. Development and testing of a new measure of health status for clinical trials in rhinoconjunctivitis. Clin Exp Allergy 1991;21:77-83.
12. Haak-Frendscho M, Ridgway J, Shields R, Robbins K, Gorman C, Jardieu P. Human IgE receptor α-chain IgG chimera blocks passive cutaneous anaphylaxis reaction in vivo. J Immunol 1993;151:351-8.
13. Froehlich J, Schoenhoff M, Jardieu P, Ruppel J, Fei D, Buckley P, et al. Multiple doses of a recombinant humanized monoclonal anti-IgE antibody are safely tolerated and decrease free serum IgE to undetectable levels [abstract]. J Allergy Clin Immunol 1995;95:356.