

Anti-IgE treatment of eosinophil-associated gastrointestinal disorders

Shabnam Foroughi, MD,^a Barbara Foster, MS,^a NaYoung Kim, MD,^a Leigh B. Bernardino, RN,^a Linda M. Scott, CRNP,^a Robert G. Hamilton, PhD,^b Dean D. Metcalfe, MD,^a Peter J. Mannon, MD,^c and Calman Prussin, MD^a Bethesda and Baltimore, Md

Background: Eosinophil-associated gastrointestinal disorders (EGIDs) are commonly associated with atopy and are being recognized with increasing frequency. Current therapy for EGIDs is inadequate.

Objective: We sought to determine the efficacy of anti-IgE therapy in EGIDs and investigate the role of IgE in disease pathogenesis.

Methods: Nine subjects with EGIDs received omalizumab every 2 weeks for 16 weeks while other therapy was held constant. Blood absolute eosinophil counts, tissue eosinophil counts, symptom scores, and free IgE levels were serially measured. Allergen skin testing and flow cytometry for basophil activation and FcεRI were determined at baseline and at week 16.

Results: Omalizumab was associated with a decrease in absolute eosinophil count at both the week 16 (34%, $P = .004$) and combined weeks 12 to 16 (42%, $P = .012$) time points. Tissue eosinophils decreased in the duodenum (59%) and gastric antrum (69%) but did not reach statistical significance ($P = .074$ and $.098$, respectively). Esophageal eosinophil counts remained unchanged. Basophil and dendritic cell FcεRI expression and free IgE levels were all significantly decreased ($P < .005$). Omalizumab increased the concentration of allergen required to trigger half-maximal basophil activation by 170-fold. Allergen skin test wheal and erythema responses decreased by 78% and 82%, respectively. Symptom scores were decreased at both the midstudy (63%) and end of study (70%) time points ($P < .005$ for both).

Conclusion: These results demonstrate that IgE-mediated processes contribute to the generation of eosinophilic inflammation in EGIDs and suggest that anti-IgE therapy might be effective in these disorders.

Clinical implications: Anti-IgE might be a potential therapy for EGIDs. (J Allergy Clin Immunol 2007;120:594-601.)

Key words: Eosinophil, eosinophilic gastroenteritis, eosinophilic esophagitis, omalizumab, IgE, food allergy, basophil

Abbreviations used

AEC:	Absolute eosinophil count
APC:	Allophycocyanin
DC:	Dendritic cell
EC ₅₀ :	Concentration yielding 50% maximal activation
EGID:	Eosinophil-associated gastrointestinal disorders
EE:	Eosinophilic esophagitis
hpf:	High-power field
lin-1:	Lineage cocktail 1
pDC:	Plasmacytoid dendritic cell
PE:	Phycoerythrin

Eosinophil-associated gastrointestinal disorders (EGIDs), comprising eosinophilic esophagitis (EE), eosinophilic gastroenteritis, and eosinophilic colitis, are a spectrum of diseases that are being diagnosed with increasing frequency.¹ Approximately 75% of patients with EGIDs are atopic, with a high prevalence of positive food allergen skin test results.^{1,2} Some patients with EGIDs improve after institution of an amino acid-based elemental diet and will then have exacerbations after resumption of an unrestricted diet.³ In sum, these findings support the concept that food allergen-driven eosinophilic inflammation plays a major role in disease pathogenesis.

Because recognition of EGIDs as an important clinical entity is recent, there remain substantial deficits in our understanding of their pathogenesis and treatment. Mouse models of EGIDs demonstrate a T_H2-polarized inflammatory response, with important roles played by multiple cytokines, including IL-5, IL-13, eotaxin-1 (CCL11), and eotaxin-3 (CCL24).⁴ EGIDs have been hypothesized to be a mixed inflammatory disease driven by both food allergen specific IgE and T_H2 cells.⁴

Omalizumab is a humanized therapeutic anti-IgE mAb that reduces free IgE levels and is an effective treatment for allergic asthma and seasonal allergic rhinitis.⁵ A different anti-IgE therapeutic, TNX-901, was shown to increase the maximum tolerated dose of peanut by 10-fold in subjects with peanut hypersensitivity.⁶ Although no studies have specifically addressed the use of omalizumab in eosinophilic diseases, omalizumab significantly decreases peripheral blood,⁷ bronchial,⁸ and skin⁹ eosinophilia.

We thus used a clinical trial of omalizumab in subjects with EGIDs to determine the effect of omalizumab on peripheral blood eosinophilia and other measures of EGID

From ^athe Laboratory of Allergic Diseases and ^cthe Laboratory of Host Defenses, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, and ^bthe Division of Allergy and Clinical Immunology, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore.

Supported by the Intramural Research Program of the National Institute of Allergy and Infectious Diseases, National Institutes of Health.

Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

Received for publication January 26, 2007; revised June 6, 2007; accepted for publication June 7, 2007.

Reprint requests: Calman Prussin, MD, Building 10, Room 11C205, NIH, Bethesda, MD 20892-1881. E-mail: cprussin@niaid.nih.gov.

0091-6749

doi:10.1016/j.jaci.2007.06.015

disease activity. Furthermore, this allowed us to investigate the role of IgE in EGID pathogenesis and examine anti-IgE as a potential noncorticosteroid therapy for EGIDs.

METHODS

Subjects

Nine subjects with eosinophilic gastroenteritis based on typical gastrointestinal symptoms, 25 or more eosinophils per high-power field (hpf) in stomach or duodenal biopsy specimens, and negative work-up for other causes of gut eosinophilia, including helminth infection, were enrolled. Crohn's disease was ruled out by lack of pathologic findings (ulcerations, granulomata, or crypt architectural distortion) and clinical features (fistula, abdominal mass, and surgical obstructive disease) consistent with the disease. Inclusion criteria included age 12 to 76 years, a prestudy baseline absolute eosinophil count (AEC) of 500 eosinophils/mm³ or greater and evidence of atopy by skin or serologic testing, or a total serum IgE level of 100 IU/mL or greater. Exclusion criteria included immunodeficiency, the presence of the FIP1L1-PDGF-R fusion gene, and an IgE $\times$ weight product of greater than 63,000 kg $\times$ IU(IgE)/mL.

Study design

The study was a single-center open-label study conducted from December 2003 through August 2006. The National Institute of Allergy and Infectious Diseases Institutional Review Board approved the study, and all subjects signed informed consent forms. After a 3-week baseline screening period, subjects received omalizumab subcutaneously during study week 0 and then every 2 weeks for a total of 8 doses. Medications and dietary restrictions were held constant. Because doses less than that indicated in the package insert provide clinical benefit,¹⁰ subjects with IgE levels and weight beyond those allowed by the Xolair package insert were enrolled to maximize accrual. Thus 3 subjects (nos. 1-3) had a baseline serum IgE $\times$ weight product greater than that allowed by the package insert but within the study entry criteria. Subject 4 received 85 mg per dose, and all other subjects received 375 mg per dose. Each subject's omalizumab dose calculated in milligrams per kilogram per international units (IgE) per milliliter is noted in Table I. Subjects were observed for 24 hours after the first dose and for 2 hours after subsequent doses.

During the 3-week preomalizumab baseline screening, subjects underwent esophagoduodenoscopy with biopsy, lymphapheresis, and titration skin testing. Baseline laboratory measurements included complete blood count with AEC, total serum IgE levels, FcεRI expression, *in vitro* basophil activation, and a free IgE analysis. All baseline studies were repeated after 16 weeks. Total and free IgE determinations were performed by the Johns Hopkins University Dermatology, Allergy, and Clinical Immunology Reference Laboratory. Subjects underwent epicutaneous titration allergen skin testing at baseline and again at week 16. Commercial allergens (Greer Laboratories, Lenoir, NC) were used neat and at serial 3-fold dilutions to a final 1:729 dilution. Each dilution was tested in duplicate, and the wheal and erythema were measured at 15 minutes along 2 orthogonal axes. The products of the 2 orthogonal values for each dilution were averaged. Allergens studied were peanut (subjects 1, 4, and 8), *Dermatophagoides pteronyssinus* (subject 2), corn (subject 6), ragweed (subject 7), and oats (subject 9). Two subjects (subjects 3 and 5) had negative skin test responses during the baseline testing and were not included in the analysis.

Antibodies and reagents

Anti-FcεRIα (clone AER-37) was obtained from eBiosciences (San Diego, Calif). Anti-CD1c/blood dendritic cell antigen (BDCA)

(clone AD5-8E7) and BDCA-2 (clone AC144; Miltenyi Biotec, Auburn, Calif); HLA-DR, CD11c, CD63, and CD123 (BD-PharMingen, San Diego, Calif); and lineage cocktail 1 (lin-1: CD3, CD14, CD16, CD19, CD20, and CD56) and CD4 (Becton-Dickinson Biosciences, San Jose, Calif) were purchased. Biotin-labeled and unlabeled goat anti-human IgE was obtained from Biosource (Camarillo, Calif) and Kirkegaard and Perry Laboratories (Gaithersburg, Md), respectively.

Basophil activation through CD63 was measured by using published methods.¹¹ Basophils were activated with anti-IgE and clinically implicated allergens, including peanut (subjects 1, 3, 5, and 8), *Dermatophagoides farinae* (subjects 2 and 9), soy (subject 4), pecan (subject 6), and shrimp (subject 7; Greer Laboratories). Briefly, 20 μ L of stimulation buffer (20 mmol/L HEPES, 125 mmol/L NaCl, 5 mmol/L KCl, 2.4 mmol/L CaCl₂, 1 mmol/L MgCl₂, and 0.5 mmol/L glucose; Sigma-Aldrich, St Louis, Mo), IL-3 (10 ng/mL final concentration; Peprotech, Rocky Hill, NJ), and $\frac{1}{2}$ log₁₀ dilutions of allergen or anti-IgE were added to 100 μ L of heparinized whole blood, mixed, and incubated at 37°C for 15 minutes. Controls included whole blood plus stimulation buffer, with or without IL-3 (later referred to as constitutive activation) or N-formyl-methionine-leucine-phenylalanine (Sigma-Aldrich). Samples were then stained on ice with mAbs to CD63, CD123, HLA-DR, and CD4 for 20 minutes, treated with 2 mL of FACSlyse, resuspended in PBS/10% dimethyl sulfoxide, and stored at -80°C. Cryopreserved fixed cells were thawed, acquired on a FACSCalibur flow cytometer (BD Biosciences), and analyzed with FlowJo software (Tree Star, Ashland, Ore). For 6 subjects, the baseline and 16-week time points were each repeated twice on 2 consecutive days, and the results were averaged; for 3 subjects, each time point was only examined once. Basophils were identified as CD123⁺HLA-DR⁻CD4⁻ cells. The percentage of CD63⁺ basophils was determined for each concentration of allergen or anti-IgE, and the concentration yielding 50% of the maximal response (EC₅₀) was determined by using a sigmoidal dose-response curve fit with Prism software (GraphPad, San Diego, Calif). Some dose-response curves were flat because either all concentrations (including the negative control) exhibited maximal activation or because omalizumab abrogated basophil activation (no response at all concentrations). These were arbitrarily assigned a minimum or maximum EC₅₀ value, respectively.

Flow cytometric analysis of FcεRI expression and surface IgE by basophils and dendritic cells (DCs) was performed with a 6-color adaptation of published methods.^{12,13} PBMCs were prepared from EDTA-anticoagulated blood by using 1.077 g/mL Ficoll-diatrizoate (Sigma) density gradient separation, fixed in 4% room temperature paraformaldehyde for 5 minutes, resuspended in PBS/10% dimethyl sulfoxide (Sigma), and stored at -80°C. Cryopreserved fixed cells were thawed, blocked in PBS/1% BSA/5% nonfat milk powder (PBS/BSA/milk) on ice for 30 minutes, and then stained on ice with the following mAbs. For FcεRI expression, cells were stained with mAbs to lin-1 fluorescein isothiocyanate, FcεRI phycoerythrin (PE), CD123 PE/cyanin 5, BDCA-1 allophycocyanin (APC), BDCA-2 biotin, and CD11c PE/cyanin 7; washed; and stained with streptavidin APC/cyanin 7 (Becton-Dickinson Biosciences). For surface IgE binding, cells were stained with mAbs to lin-1 fluorescein isothiocyanate, anti-IgE biotin (Biosource), CD123 PE/cyanin 5, and HLA-DR APC; washed; and stained with streptavidin PE (Becton-Dickinson Biosciences). Streptavidin staining was performed in PBS/1% BSA without milk. For FcεRI staining, basophils were identified as CD123^{bright}lin-1⁻BDCA-2⁻, myeloid DCs were identified as BDCA-1⁺CD11c⁺lin⁻, and plasmacytoid DCs (pDCs) were identified as CD123⁺BDCA-2⁺lin-1⁻ subpopulations, respectively. For surface IgE binding, basophils were identified as CD123^{bright}lin-1⁻HLA-DR⁻ and pDCs were identified as

TABLE I. Study subjects

Subject no./ symbol*	Disease location (E/G/D)	Predominant symptom	Age (y)	Sex	Weight (kg)	Baseline IgE (U/mL)	Omalizumab dose (mg/kg/ IU [IgE]/mL)	Duration of disease (y)	Concurrent therapy	Allergen sensitivity	Baseline AEC × 10 ⁹ /L
1 —	E/G/D	Abdominal pain	37	M	80	583	0.0080	2	None	Peanut, egg, wheat, carrot, apple, mites	2732
2 ▲	G	Abdominal pain	48	M	66	780	0.0073	9	Budesonide 6 mg qD, omeprazole, fexofenadine, doxepin, ketotifen	Peanut, soy, wheat, pork, corn, shrimp, cod, walnut, mites	1779
3 ▼	E/G/D	Abdominal pain, bloating, dysphagia	45	M	76	555	0.0089	4	Budesonide 9 mg qD, cetirizine, montelukast	Peanut, egg, wheat, carrot, apple, mites	1889
4 ☀	E/G/D	Abdominal pain	40	M	78	42	0.02	5	Prednisone 25 mg QOD, omeprazole	Peanut, egg, wheat, corn, soy, milk	2485
5 ●	E/D	Diarrhea	60	M	64	370	0.016	1	None	None	564
6 ■	E/G/D	Abdominal pain	30	M	71	228	0.017	14	Prednisone 10 mg qD, esomeprazole, loratadine, fluticasone/salmeterol inhaler	Peanut, egg, soy, pork, corn, pecan, mites	4221
7 +	G/D	Abdominal pain, bloating, vomiting	49	F	68	223	0.025	10	Budesonide 6 mg qD, esomeprazole, fexofenadine, nasal fluticasone, fluticasone/salmeterol inhaler	Peanut, soy, wheat, shrimp, chicken, walnut, mites, ragweed	768
8 ×	E/G/D	Abdominal pain, bloating, nausea	42	F	76	268	0.018	2	None	Peanut, soy, wheat, egg, corn, shrimp, cod	1134
9 ◆	E/G/D	Bloating, nausea, early satiety	33	F	61	266	0.023	8	Lansoprazole	Peanut, pork, oats, mites	1040

Allergen sensitivity is defined as a skin test wheal of 3 mm or greater than that elicited by the control.

E, Esophagus; G, stomach; D, duodenum; M, male; F, female; qD, every day; QOD, every other day.

*Symbols are representative of symbols in figures.

CD123⁺HLA-DR⁺lin-1⁻ subpopulations, respectively. Data were acquired on LSRII (FcεRI) or FACSCalibur (IgE) flow cytometers (both BD Biosciences) and analyzed with Flowjo software (Tree Star). Typically, 300,000 to 600,000 total events were acquired to obtain 1000 or more basophils for analysis. FcεRIα and IgE fluorescence were quantitated as molecules of equivalent phycoerythrin by using 8 peak Rainbow Calibration Particles (Spherotech, Lake Forest, Ill), as per the manufacturer's instructions. Free IgE levels were measured by using a solid-phase immunoenzymetric assay in which IgE was captured with anti-human IgE (clone

HP6061) and detected with biotinylated FcεRI, as previously described.¹⁴

Esophagoduodenoscopy and tissue eosinophil counting

Endoscopic biopsy specimens were taken from the distal third of the esophagus, gastric antrum and body, and duodenum. A minimum of 5 biopsy specimens were taken from each site, formalin fixed, and hematoxylin and eosin stained. Specimens were not collected for a

given tissue site in 2 subjects (subject 3, esophagus; subject 4, gastric body) and were not included in the analysis. A blinded investigator determined the number of eosinophils per 40× hpf in 120 consecutive fields for each tissue site.

Symptom score

An EGID symptom score was modified from the Crohn's Disease Activity Index.¹⁵ The score separately rated symptoms from 0 to 3 in the following fields: stomach pain, nausea, vomiting, bloating, early satiety, dysphagia, and general well-being. Symptom scores were recorded daily during the 3 weeks before starting omalizumab and during study weeks 7 to 8 and 15 to 16, and for each period, the mean value per day was calculated. An example of the symptom scorecard and scoring scale for each field is noted in Fig E1 in this article's Online Repository at www.jacionline.org.

Statistical analysis

The predesignated primary end point was the percentage decrease in AEC at week 16 (average of ≥2 determinations) compared with the baseline preomalizumab value (average of ≥2 determinations). Unless otherwise noted, median values were used as indicators of central tendency. The Wilcoxon signed-rank test was used to compare paired data. The Spearman rank correlation test was used to test correlative data.

RESULTS

Subjects

Of the 23 patients with EGIDs screened, 9 subjects fulfilled the study inclusion criteria and were enrolled (Table 1).

Adverse events

A total of 71 doses of omalizumab were administered with no severe adverse events. Laboratory studies done for the assessment of safety, including a Chem-20 panel, complete blood count, and prothrombin and partial thromboplastin times, were not adversely affected (data not shown).

Omalizumab's effect on peripheral blood and gut eosinophils

Omalizumab caused a significant decrease in the AEC by week 16 ($P = .004$; median and mean decrease of 34% and 47%, respectively; Fig 1). Similar reductions were apparent when analyzing the mean of the combined weeks 12, 14, and 16 AECs ($P = .012$; median and mean decrease of 42% and 34%, respectively).

Although only 3 subjects were known to have EE before study entry, 7 of 9 had esophageal biopsy specimens that were diagnostic for concurrent EE with greater than 25 eosinophils/hpf. As shown in Fig 2, A through C, omalizumab therapy was associated with a downward trend in tissue eosinophilia in the duodenum (median decrease of 59%, $P = .074$), gastric antrum (decrease of 69%, $P = .098$), and gastric body (decrease of 54%, $P = .25$), although these results did not reach statistical significance. In contrast, in Fig 2, D, esophageal eosinophil counts trended upward during the study (median increase of 25%, $P = 0.47$). Changes in tissue eosinophil count did not correlate with changes in blood AEC (data not shown).

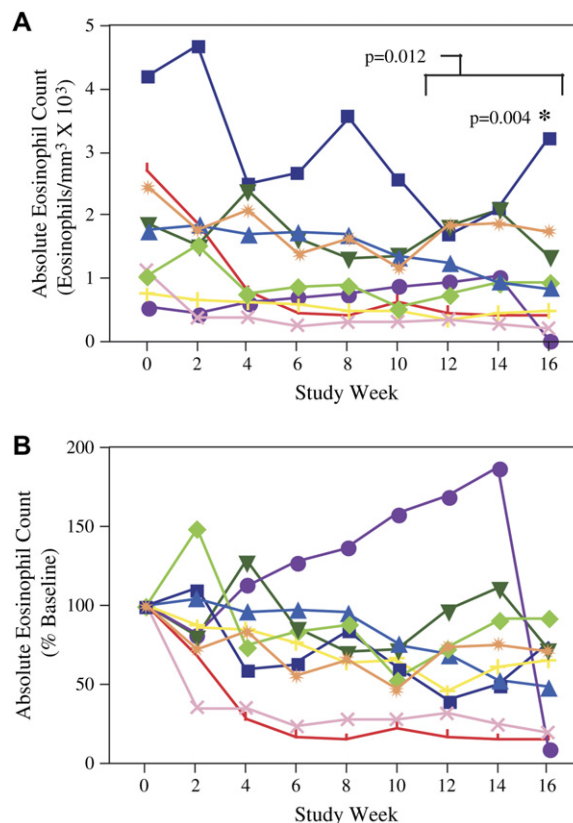


FIG 1. Effect of omalizumab on peripheral blood eosinophil counts. Peripheral blood AECs were determined at baseline (week 0) and every 2 weeks thereafter for the duration of the study. The actual values for each time point (A), as well as the value as a percentage of the preomalizumab baseline (B), are shown.

Efficacy of omalizumab in blocking IgE

We next examined whether omalizumab effectively blocked IgE in the study population. Omalizumab treatment significantly decreased serum free IgE levels at the 8-, 12-, and 16-week time points (median decrease of 76%, 80%, and 79%, respectively; $P < .005$ for all; Fig 3, A). Similarly, cell surface-bound IgE was decreased 98.4% in the basophil (Fig 3, B) and 96% in the pDC (data not shown) populations. Omalizumab treatment resulted in a significant decrease in FcεRI surface expression in basophils and DCs (75%, 81%, and 61% decrease for the basophil, myeloid DC, and pDC populations, respectively; $P < .005$ for all; Fig 3, C-E).

We further verified IgE blocking using a CD63 translocation-based functional assay of basophil activation. At baseline, CD63 expression was 9.5% in the control and 85.9% and 87.4% in the maximally activated allergen and anti-IgE activated conditions, respectively. Omalizumab therapy blocked *in vitro* basophil activation and shifted the dose response to both anti-IgE (Fig 4, A) and allergen (data not shown). As seen in Fig 4, B and C, omalizumab caused a large and significant increase in the EC₅₀ to both allergen and anti-IgE (median of 171-fold and 136-fold shifts, respectively; $P < .005$ for both). Additionally, omalizumab significantly decreased constitutive basophil activation by

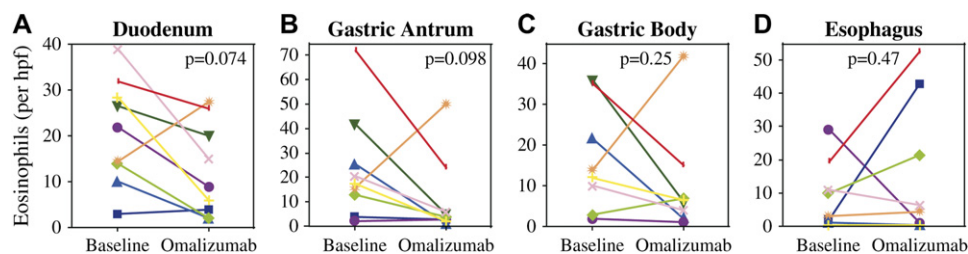


FIG 2. Effect of omalizumab on tissue eosinophil counts. Esophagogastroduodenoscopy was performed at baseline and again after 16 weeks of omalizumab therapy, and the median number of eosinophils per hpf in the duodenum (A), gastric antrum (B) and body (C), and esophagus (D) were determined. Data are shown as the median value for each subject.

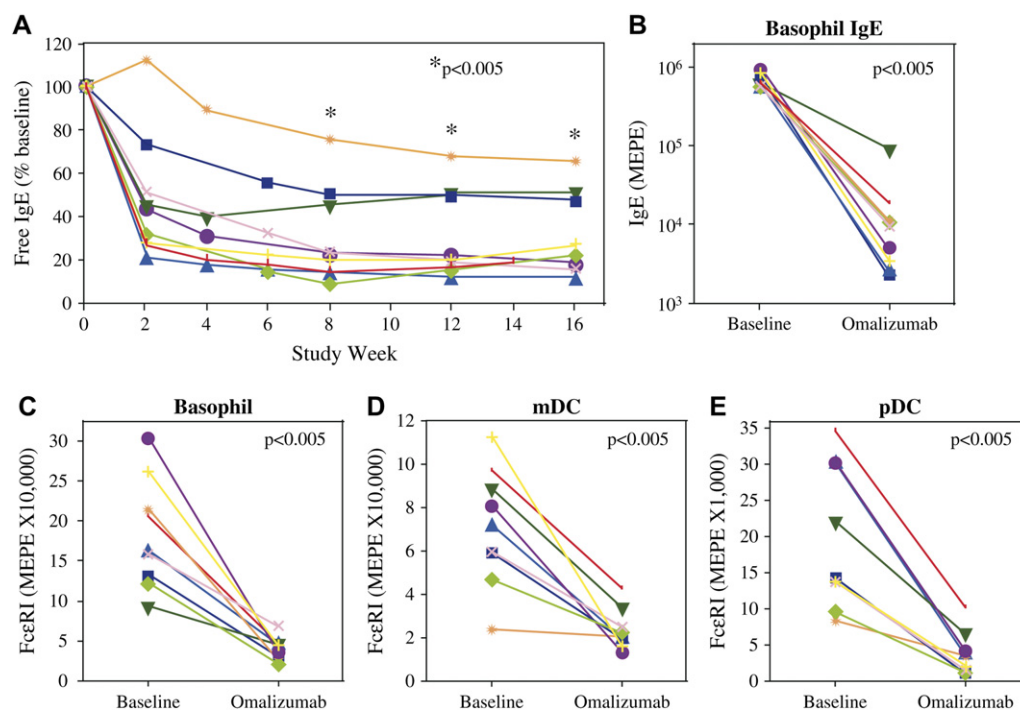


FIG 3. Efficacy of omalizumab in blocking IgE. Changes in the levels of serum free IgE (A), basophil-associated IgE (B), FcεRI expression by peripheral blood basophils (C), myeloid DCs (mDC; D), and pDCs (E) are shown. The above were determined at baseline and again after 16 weeks of omalizumab. Free IgE was determined at baseline and serially thereafter. * $P < .005$ for each time point relative to baseline. Fluorescence intensity was quantitated as molecules of equivalent phycoerythrin (MEPE).

84% and 89% in whole blood without and with added IL-3, respectively (Fig 4, D and E). Basophil responses to N-formyl-methionine-leucine-phenylalanine were unchanged (Fig 4, F). As shown in Fig 5, omalizumab caused a significant reduction in allergen skin test wheal (median reduction of 78%, $P = .016$) and erythema (median reduction of 82%, $P = .016$) responses. In sum, these data demonstrate that omalizumab effectively blocked IgE in the patients with EGIDs and inhibited downstream events, such as FcεRI expression, IgE-mediated basophil activation, and immediate hypersensitivity.

Symptom scores

EGID symptom scores improved significantly at both the midstudy (weeks 7-8) and end of study (weeks 15-16)

time points, with median reductions of 63% and 70%, respectively ($P < .005$ for both, Fig 6). The 4 subjects with the highest baseline symptom scores had less improvement compared with the 4 subjects with the lowest baseline symptom scores (32% vs 87% reduction, respectively). Improvements in EGID symptoms did not correlate with changes in blood or tissue eosinophils or indices of IgE blocking (data not shown).

DISCUSSION

In this report we examine the safety and efficacy of anti-IgE therapy in patients with EGIDs. We show that omalizumab therapy is associated with significant

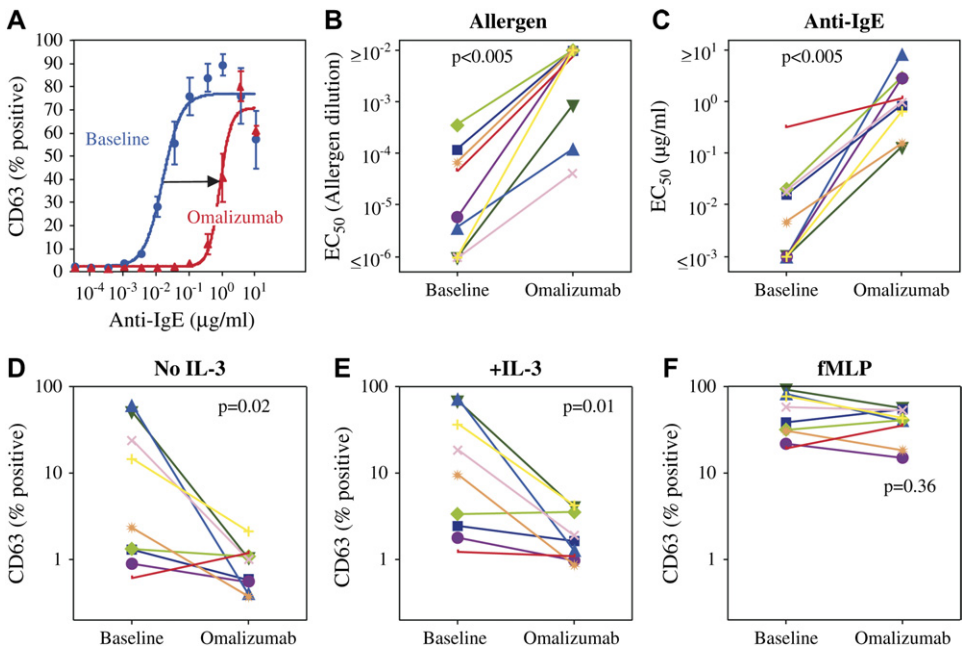


FIG 4. Efficacy of omalizumab in blocking basophil activation. Basophil CD63 expression after *in vitro* activation by anti-IgE (**A**) and allergen (data not shown) was measured, and the EC₅₀ was calculated for each, respectively (**B** and **C**). **D** and **E**, Constitutive basophil CD63 expression without or with added IL-3. **F**, Basophil CD63 expression after N-formyl-methionine-leucine-phenylalanine activation. The results were determined at baseline and again after 16 weeks of omalizumab.

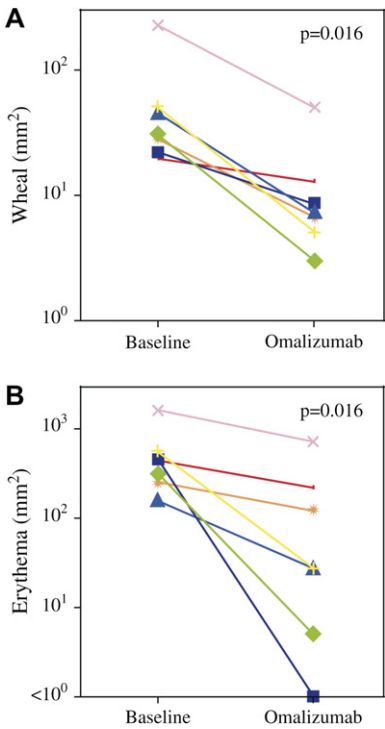


FIG 5. Efficacy of omalizumab in blocking immediate hypersensitivity. Wheal (**A**) and erythema (**B**) after food and aeroallergen titration skin testing at baseline and after 16 weeks of omalizumab. Each result is the mean of 2 skin tests.

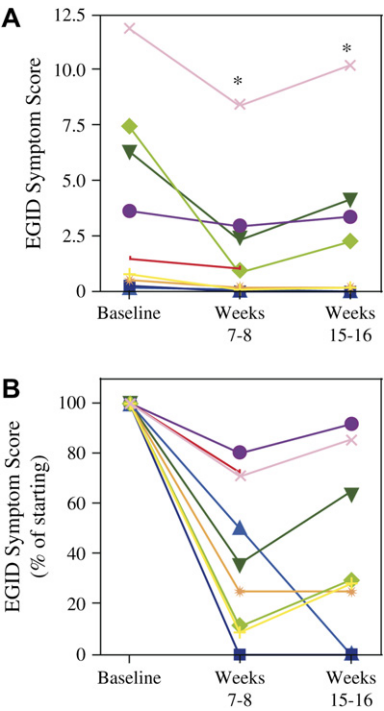


FIG 6. Effect of omalizumab on EGID symptom scores. **A**, EGID symptom scores at baseline, midstudy (weeks 7-8), and end of study (weeks 15-16). **B**, EGID symptom scores were calculated as a percentage of the baseline value for each subject. **P* < .005 for each time point relative to baseline.

decreases in peripheral eosinophilia and gastrointestinal symptoms and a trend toward lower eosinophil numbers in the gastric antrum and duodenum. Omalizumab was well tolerated. These results demonstrate that IgE-mediated processes contribute to the generation of the eosinophilic inflammation in EGID and suggest that anti-IgE therapy might be effective in these disorders.

In a recent study of children with EE, AEC correlated with tissue eosinophilia and disease activity, supporting its use as a biomarker of EGID disease activity.¹⁶ We thus used AEC as the primary end point to measure the effect of anti-IgE therapy on EGID disease activity. Almost all study end points improved in association with omalizumab therapy, and this supports the need for a multicenter placebo-controlled study to definitively study anti-IgE therapy in EGIDs.

The partial AEC response found in most subjects suggests that there are both IgE-dependent and IgE-independent inflammatory pathways operating in EGIDs.¹ The magnitude of AEC decrease was variable between subjects, suggesting heterogeneity in disease mechanism (Fig 1). This is most clearly seen in the 2 subjects who had the largest and most rapid decrease in AEC. This finding is not simply due to more effective IgE blocking in these subjects because the data from Figs 3 and 4 indicate less effective IgE blocking in these subjects. Conversely, subject 4, who had the lowest IgE level, had a dramatic increase in tissue eosinophilia. This suggests that patients with EGIDs with IgE-predominant disease might preferentially respond to omalizumab.

Omalizumab decreased both stomach and duodenum tissue eosinophilia, although these results did not achieve statistical significance (Fig 2). We performed a retrospective power analysis that indicated a minimum of 17 subjects was needed to give a 90% likelihood of detecting the magnitude of tissue eosinophil decrease we noted. This suggests that the lack of significance in our study is likely due to the small sample size (type 2 statistical error). In contrast to the decreases noted in the stomach and duodenum, the number of esophageal eosinophils trended upward. Despite this increase in eosinophil number, there was not a concomitant increase in esophageal symptoms. Although the mechanistic basis for this finding is unclear, it further underscores the dichotomy between the esophagus and stomach/duodenum as distinct inflammatory sites in EGIDs, with differing epidemiology, pathophysiology, and therapeutic response.

We used multiple techniques to verify that IgE and immediate hypersensitivity were effectively blocked (Figs 3-5). However, the level of IgE blockade in our study was substantially less than the 99% decrease obtained in early-phase studies,¹⁷ suggesting that more potent anti-IgE drugs might have greater efficacy in these disorders. We unexpectedly found increased CD63 expression in the baseline samples,¹⁸ which suggests that basophils in EGIDs are constitutively active or have been activated *in vivo*. This finding is reminiscent of previous reports in subjects with food allergy^{19,20} and chronic urticaria.²¹ Additionally, omalizumab decreased this constitutive

basophil activation. Taken together, these results suggest that constitutive CD63 expression is a consequence of *in vivo* activation by allergen, and its decrease with omalizumab therapy reflects a reduction in IgE-mediated basophil activation *in vivo*.

This study was open label; that is, subjects' diets and medications were held constant, and the only variable introduced was omalizumab. This uncontrolled design is subject to sources of error, including placebo effect, changes in disease activity, and changes in therapy. Additionally, although no correlation between AEC decrease and aeroallergen season was found, it is possible that some subjects' symptoms improved because of decreases in pollen allergen levels during the study. Thus it is not possible to attribute the symptom improvement to the study drug alone. To measure EGID disease symptoms, we modified the well-accepted Crohn's Disease Activity Index. To our knowledge, this represents the first report of a symptom score to measure EGID disease activity. This scoring system is not validated, and thus additional efforts are needed to validate this or similar symptom scores.

EGIDs represent a spectrum of diseases with increasing incidence, which lack safe and effective treatments. Progress in understanding EGID pathogenesis is needed to improve therapy. Our results demonstrate that omalizumab is effective in decreasing peripheral blood eosinophilia in EGIDs and suggest that IgE-mediated processes play a major role in the generation of eosinophilic inflammation in EGIDs. These results suggest that anti-IgE therapy, either alone or in combination with other antagonists, might be an effective treatment for EGIDs.

We thank Dr Martha Quezada for diagnostic review of pathology materials and Drs Amy Klion and Erica Brittain for helpful discussion. We also thank Pragya Gangele, Gettie Butts, and Dorrette Sutherland for their valuable assistance in endoscopy.

REFERENCES

1. Rothenberg ME. Eosinophilic gastrointestinal disorders (EGID). *J Allergy Clin Immunol* 2004;113:11-28.
2. Spergel JM, Beausoleil JL, Mascarenhas M, Liacouras CA. The use of skin prick tests and patch tests to identify causative foods in eosinophilic esophagitis. *J Allergy Clin Immunol* 2002;109:363-8.
3. Markowitz JE, Spergel JM, Ruchelli E, Liacouras CA. Elemental diet is an effective treatment for eosinophilic esophagitis in children and adolescents. *Am J Gastroenterol* 2003;98:777-82.
4. Blanchard C, Wang N, Rothenberg ME. Eosinophilic esophagitis: pathogenesis, genetics, and therapy. *J Allergy Clin Immunol* 2006;118:1054-9.
5. Holgate S, Casale T, Wenzel S, Bousquet J, Deniz Y, Reisner C. The anti-inflammatory effects of omalizumab confirm the central role of IgE in allergic inflammation. *J Allergy Clin Immunol* 2005;115:459-65.
6. Leung DY, Sampson HA, Yunginger JW, Burks AW Jr, Schneider LC, Wortel CH, et al. Effect of anti-IgE therapy in patients with peanut allergy. *N Engl J Med* 2003;348:986-93.
7. Noga O, Hanf G, Kunkel G. Immunological and clinical changes in allergic asthmatics following treatment with omalizumab. *Int Arch Allergy Immunol* 2003;131:46-52.
8. Djukanovic R, Wilson SJ, Kraft M, Jarjour NN, Steel M, Chung KF, et al. Effects of treatment with anti-immunoglobulin E antibody omalizumab on airway inflammation in allergic asthma. *Am J Respir Crit Care Med* 2004;170:583-93.

9. Beck LA, Marcotte GV, MacGlashan D, Togias A, Saini S. Omalizumab-induced reductions in mast cell FcεRI expression and function. *J Allergy Clin Immunol* 2004;114:527-30.
10. Hochhaus G, Brookman L, Fox H, Johnson C, Matthews J, Ren S, et al. Pharmacodynamics of omalizumab: implications for optimised dosing strategies and clinical efficacy in the treatment of allergic asthma. *Curr Med Res Opin* 2003;19:491-8.
11. Sainte-Laudy J, Sabbah A, Vallon C, Guerin JC. Analysis of anti-IgE and allergen induced human basophil activation by flow cytometry. Comparison with histamine release. *Inflamm Res* 1998;47:401-8.
12. Foster B, Metcalfe DD, Prussin C. Human dendritic cell 1 and dendritic cell 2 subsets express FcεRI: correlation with serum IgE and allergic asthma. *J Allergy Clin Immunol* 2003;112:1132-8.
13. Prussin C, Griffith DT, Boesel KM, Lin H, Foster B, Casale TB. Omalizumab treatment downregulates dendritic cell FcεRI expression. *J Allergy Clin Immunol* 2003;112:1147-54.
14. Hamilton RG, Marcotte GV, Saini SS. Immunological methods for quantifying free and total serum IgE levels in allergy patients receiving omalizumab (Xolair) therapy. *J Immunol Methods* 2005;303:81-91.
15. Yoshida EM. The Crohn's Disease Activity Index, its derivatives and the Inflammatory Bowel Disease Questionnaire: a review of instruments to assess Crohn's disease. *Can J Gastroenterol* 1999;13:65-73.
16. Konikoff MR, Blanchard C, Kirby C, Buckmeier BK, Cohen MB, Heubi JE, et al. Potential of blood eosinophils, eosinophil-derived neurotoxin, and eotaxin-3 as biomarkers of eosinophilic esophagitis. *Clin Gastroenterol Hepatol* 2006;4:1328-36.
17. MacGlashan DW Jr, Bochner BS, Adelman DC, Jardieu PM, Togias A, McKenzie-White J, et al. Down-regulation of Fc(epsilon)RI expression on human basophils during in vivo treatment of atopic patients with anti-IgE antibody. *J Immunol* 1997;158:1438-45.
18. Sanz ML, Gamboa PM, Antepara I, Uasuf C, Vila L, Garcia-Aviles C, et al. Flow cytometric basophil activation test by detection of CD63 expression in patients with immediate-type reactions to betalactam antibiotics. *Clin Exp Allergy* 2002;32:277-86.
19. Sampson HA, Broadbent KR, Bernhisel-Broadbent J. Spontaneous release of histamine from basophils and histamine-releasing factor in patients with atopic dermatitis and food hypersensitivity. *N Engl J Med* 1989;321:228-32.
20. Shreffler WG. Evaluation of basophil activation in food allergy: present and future applications. *Curr Opin Allergy Clin Immunol* 2006;6:226-33.
21. Vasagar K, Vonakis BM, Gober LM, Viksman A, Gibbons SP Jr, Saini SS. Evidence of in vivo basophil activation in chronic idiopathic urticaria. *Clin Exp Allergy* 2006;36:770-6.

What's new online?

Enjoy these new benefits and more:

- Track the impact of your article with Citation Alerts that let you know when your article (or any article you'd like to track) has been cited by another Elsevier-published journal.
- Save your online searches and get the results by e-mail.

Visit www.jacionline.org today to see what else is new online!

Eosinophilic Gastroenteritis Symptom Score Card

- Complete this symptom score card each day before going to bed
- Your answers should describe the proceeding 24 hours
- Start completing this form on a daily basis starting today.

	Su	M	Tu	W	Th	F	Sa
	Date:	Date:	Date:	Date:	Date:	Date:	Date:
Diarrhea: Number of liquid or very soft stools							
Stomach Pain: (0=none, 1=mild, 2=moderate, 3=severe)							
Nausea: (0=none, 1=once, 2=twice, 3=three times or more)							
Bloating Sensation After Meals: (0=none, 1=once, 2=twice, 3=three times or more)							
Feeling Full Unexpectedly (e.g. After a Small Meal): (0=none, 1=once, 2=twice, 3=three times or more)							
Vomiting: (0=none, 1=once, 2=twice, 3=three times or more)							
Difficulty swallowing: (0=none, 1=mild, 2=moderate, 3=severe)							
General well being: (0=generally well, 1=slightly under par, 2=poor, 3=very poor/terrible)							

FIG E1. Symptom score. An EGID symptom score was modified from the Crohn's Disease Activity Index. The score separately rated symptoms from 0 to 3 in the following fields: stomach pain, nausea, vomiting, bloating, early satiety, dysphagia, and general well-being. Symptom scores were recorded daily during the 3 weeks before starting omalizumab and during study weeks 7-8 and 15-16, and for each period, the mean value per day was calculated. An example of the symptom scorecard and scoring scale for each field is shown with the following notations:

- Complete this symptom score card each day before going to bed.
- Your answers should describe the proceeding 24 hours.
- Start completing this form on a daily basis starting today.