Anti-IgE treatment of eosinophil-associated gastrointestinal disorders

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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 reults.[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 skin[9] 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

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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/mm^3 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 μL of stimulation buffer (20 mmol/L HEPES, 125 mmol/L NaCl, 5 mmol/L KCl, 2.4 mmol/L $CaCl_2$, 1 mmol/L $MgCl_2$, and 0.5 mmol/L glucose; Sigma-Aldrich, St Louis, Mo), IL-3 (10 ng/mL final concentration; Peprotech, Rocky Hill, NJ), and ½$\log_{10}$ dilutions of allergen or anti-IgE were added to 100 μL 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 $CD123^+HLA\text{-}DR^-CD4^-$ 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 (EC_{50}) 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 EC_{50} 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 $CD123^{bright}lin\text{-}1^-BDCA\text{-}2^-$, myeloid DCs were identified as $BDCA\text{-}1^+CD11c^+lin^-$, and plasmacytoid DCs (pDCs) were identified as $CD123^+BDCA\text{-}2^+lin\text{-}1^-$ subpopulations, respectively. For surface IgE binding, basophils were identified as $CD123^{bright}lin\text{-}1^-HLA\text{-}DR^-$ and pDCs were identified as