Mechanistic correlates of clinical responses to omalizumab in the setting of oral immunotherapy for milk allergy

Pamela A. Frischmeyer-Guerrerio, MD, PhD,[a] Madhan Masilamani, PhD,[b] Wenjuan Gu, MS,[c] Erica Brittain, PhD,[d] Robert Wood, MD,[e] Jennifer Kim, MD,[b] Kari Nadeau, MD, PhD,[f] Kirsi M. Jarvinen, MD, PhD,[g] Alexander Grishin, PhD,[b] Robert Lindblad, MD,[h] and Hugh A. Sampson, MD[b] *Bethesda, Frederick, Baltimore, and Rockville, Md; New York and Rochester, NY; and Stanford, Calif*

Background: In our recent clinical trial, the addition of omalizumab to oral immunotherapy (OIT) for milk allergy improved safety, but no significant clinical benefit was detected.
Objective: We sought to investigate mechanisms by which omalizumab modulates immunity in the context of OIT and to identify baseline biomarkers that predict subgroups of patients most likely to benefit from omalizumab.
Methods: Blood was obtained at baseline and multiple time points during a placebo-controlled trial of OIT for milk allergy in which subjects were randomized to receive omalizumab or placebo. Immunologic outcomes included measurement of basophil CD63 expression and histamine release and casein-specific $CD4^+$ regulatory T-cell proliferation. Biomarkers were analyzed in relationship to measurements of safety and efficacy.
Results: Milk-induced basophil CD63 expression was transiently reduced in whole blood samples from both omalizumab- and placebo-treated subjects. However, IgE-dependent histamine release increased in washed cell preparations from omalizumab- but not placebo-treated subjects. No increase in regulatory T-cell frequency was evident in either group. Subjects with lower rates of adverse reactions, regardless of arm, experienced better clinical outcomes. Pre-OIT basophil reactivity positively associated with occurrence of symptoms during OIT, whereas the baseline milk IgE/total IgE ratio correlated with the likelihood of achieving sustained unresponsiveness.
A combination of baseline basophil and serologic biomarkers defined a subset of patients in which adjunctive therapy with omalizumab was associated with attainment of sustained unresponsiveness and a reduction in adverse reactions.
Conclusions: Combining omalizumab therapy with milk OIT led to distinct alterations in basophil reactivity but not T-cell responses. Baseline biomarkers can identify subjects most likely to benefit from adjunctive therapy with omalizumab. (J Allergy Clin Immunol 2017;140:1043-53.)

Key words: *Oral immunotherapy, food allergy, milk allergy, omalizumab, desensitization, sustained unresponsiveness, basophil activation, biomarker, regulatory T cells*

Food allergy affects approximately 15 million Americans and is the most common cause of anaphylaxis outside the hospital setting.[1] No US Food and Drug Administration–approved treatment for food allergy is currently available. Recent studies suggest that oral immunotherapy (OIT), in which allergenic food is

From [a]the Laboratory of Allergic Diseases, Food Allergy Research Unit, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda; [b]the Department of Pediatrics, Division of Allergy & Immunology, Icahn School of Medicine at Mount Sinai, New York; [c]the Clinical Research Directorate/Clinical Monitoring Research Program, Leidos Biomedical Research, NCI Campus, Frederick; [d]the Biostatistics Research Branch, Division of Clinical Research, National Institutes of Health, Bethesda; [e]the Department of Pediatrics, Division of Allergy and Immunology, Johns Hopkins University School of Medicine, Baltimore; [f]the Departments of Medicine and Pediatrics, Sean N. Parker Center for Allergy and Asthma Research, Stanford School of Medicine; [g]the Department of Pediatrics, Division of Allergy and Immunology, School of Medicine and Dentistry, University of Rochester Medical Center; and [h]the EMMES Corporation, Rockville.

Omalizumab (Xolair) and omalizumab placebo for the trial were kindly provided by Genentech. The study was supported by grant AI-44236 from the National Institute of Allergy and Infectious Diseases (NIAID) and RR-026134 (Mount Sinai) and UL1 TR 001079 (Johns Hopkins) from the National Center for Advancing Translational Sciences, National Institutes of Health (NIH) and a supplemental grant from Food Allergy Research and Education. P.A.F.-G. is supported by the Division of Intramural Research, NIAID, NIH. This project was funded in in part with federal funds from the National Cancer Institute (NCI)/NIH under contract no. HHSN26120080000IE (to W.G.). The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the US Government.

Disclosure of potential conflict of interest: P. A. Frischmeyer-Guerrerio's employment is funded by the Division of Intramural Research, National Institutes of Health (NIH), and she serves on the scientific advisory board for Food Allergy Research and Education (FARE). R. Wood has received grants from FARE, the National Institute of Allergy and Infectious Diseases (NIAID), DBV, Aimmune, Astellas, and HAL-Allergy; is employed by Johns Hopkins University; and receives royalties from UpToDate. K. M. Jarvinen has received grants from the NIAID, has consultant arrangements with DBV Technologies and Merck, and has received royalties from UpToDate. A. Grishin has received a grant from the NIH/NIAID and has consultant arrangements with Allertein Therapeutics. R. Lindblad has received a grant from the NIH/NIAID/Division of Allergy, Immunology, and Transplantation. H. A. Sampson has received grants from the NIAID, NIH, and FARE; received medicines for the study from Genentech; is on the advisory board for Allertein Therapeutics; is employed by DBV Technologies and receives stock/stock options as part of his compensation; and receives royalties from UpToDate. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication November 10, 2016; revised February 10, 2017; accepted for publication March 15, 2017.

Available online April 13, 2017.

Corresponding author: Hugh A. Sampson, MD, Jaffe Food Allergy Institute, Department of Pediatrics, Box 1089, Icahn School of Medicine at Mount Sinai, New York, NY 10029. E-mail: hugh.sampson@mssm.edu.

The CrossMark symbol notifies online readers when updates have been made to the article such as errata or minor corrections

0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2017.03.028

Abbreviations used
AUC: Area under the curve
FoxP3: Forkhead box P3
HR: Histamine release
MOIT: Milk oral immunotherapy
OFC: Oral food challenge
OIT: Oral immunotherapy
PAG: PIPES/albumin/glucose
PIPES: Piperazine-N,N′-bis(2-ethanesulfonic acid)
SU: Sustained unresponsiveness
Treg: Regulatory T

mixed into a vehicle and then ingested in gradually increasing quantities, might hold promise as a treatment for food allergy.[2,3] However, enthusiasm for this therapy has been tempered by the frequent occurrence of adverse reactions and the lack of sustained protection in most subjects once treatment is discontinued.[2,3] These limitations have sparked investigations into adjunctive therapies, including omalizumab, that could improve both safety and long-term efficacy. Omalizumab is a humanized mAb that sequesters free IgE and prevents its binding to the high-affinity IgE receptor FcεRI. Recently, we completed the first double-blind, placebo-controlled trial of omalizumab in combination with OIT in patients with severe persistent cow's milk allergy.[4] Although no statistically significant benefit of omalizumab in promoting desensitization was detected compared with subjects receiving milk oral immunotherapy (MOIT) alone, omalizumab-treated subjects exhibited highly significant improvements in nearly all safety parameters.[4]

Similar to other immunotherapy trials for food allergy, we observed significant heterogeneity in the frequency and severity of adverse reactions, as well as clinical responses both within and between treatment groups.[4] Identification of biomarkers that could predict which subjects are at highest risk of reactions and which are most likely to benefit from adjunctive therapies would have tremendous value in efforts to translate these therapies into clinical practice. To accomplish this goal, a greater understanding of the immunologic mechanisms underlying successful treatment is needed, as well as how adjunctive therapies, such as omalizumab, can facilitate these effects. Suppression of mast cell and basophil reactivity, increase in food-specific IgG_4 levels, decrease in food-specific IgE levels, and reduction in T_H2 immune responses are mechanisms most often associated with successful immunotherapy to food allergens.[5,6] By preventing the interaction between circulating IgE and FcεRI, omalizumab suppresses expression of FcεRI on the surfaces of basophils, mast cells, and antigen-presenting cells, leading to reduced allergen-mediated activation of these cells.[7-9] However, some clinical studies have demonstrated that basophils from a subset of patients treated with omalizumab exhibited no change or, paradoxically, greater responsiveness to IgE receptor cross-linking after treatment.[10-12] These subjects experienced less clinical benefit from omalizumab, particularly if they had higher baseline allergen-specific/total IgE ratios.[12]

In this study we sought to dissect the effects of omalizumab on basophil and T-cell allergen-induced activation in subjects undergoing MOIT and to explore whether baseline biomarkers can predict which subjects are likely to benefit the most from adjunctive treatment with omalizumab.

METHODS

Clinical study

Participants aged 7 to 35 years were enrolled in a randomized, multicenter, double-blind, placebo-controlled trial of MOIT combined with omalizumab in the treatment of challenge-confirmed IgE-mediated cow's milk allergy. The study design and clinical outcomes have been reported previously.[4] Briefly, 57 subjects were randomized 1:1 to receive either omalizumab (n = 28) or placebo (n = 29) injections. One subject dropped out of each arm before starting injections. Omalizumab-treated subjects whose dose fell outside the package insert dosing chart received 0.016 mg/kg/IgE IU; subjects whose calculated dose was greater than 750 mg were excluded. At month 4, all subjects began MOIT dosing and were built up to a minimum maintenance dose of 520 mg of milk protein. Subjects continued blinded omalizumab or placebo injections through month 16. At month 16, all subjects were unblinded, and placebo-treated subjects discontinued placebo dosing, whereas omalizumab-treated subjects continued active injections until month 28. All randomized subjects completed a 10-g desensitization oral food challenge (OFC) at month 28 (n = 26 in the omalizumab arm and n = 24 in the placebo arm at month 28 because of 1 additional withdrawal in the omalizumab arm and 4 in the placebo arm). Subjects in whom this OFC failed discontinued MOIT (n = 2 in the omalizumab group and n = 4 in the placebo group), whereas those who had passing OIT results continued MOIT dosing until month 30, at which point they discontinued and underwent a 10-g tolerance OFC at month 32. Participants who passed the month 32 challenge were considered to have achieved sustained unresponsiveness (SU), which was defined as lack of reactivity after milk ingestion after 8 weeks of milk avoidance (n = 13 in the omalizumab group and n = 10 in the placebo group). Blood was collected at baseline and at each of the time points described above, as well as at month 22. The clinical study was approved by the institutional review boards of the Icahn School of Medicine at Mount Sinai, Johns Hopkins University, and Stanford University, and informed consent/assent was obtained from all participants.

Basophil assays

Whole heparinized blood or washed basophil-enriched suspensions were prepared, as previously described, and stimulated with milk allergen, anti-IgE, or media alone and assayed for basophil CD63 expression or histamine release (HR), respectively.[13,14] Additional details are provided in the Methods section in this article's Online Repository at www.jacionline.org.

Casein-specific regulatory T-cell assay

$CD4^+$ regulatory T (Treg) cell ($CD4^+CD25^+CD127^{lo/-}$ forkhead box P3 $[FoxP3]^+$) proliferation was assessed by means of dilution of carboxyfluorescein diacetate succinimidyl ester–labeled PBMCs, as described in the Methods section in this article's Online Repository.

Immunoglobulin measurements

Quantification of milk-specific, casein-specific, β–lactoglobulin–specific, and total IgE and IgG_4 was done by using the ImmunoCAP assay (Thermo Fisher Scientific, Waltham, Mass).

Statistical analysis

Wilcoxon rank sum tests, analysis of covariance, and paired *t* tests were used to analyze continuous variables, logistic regression and Fisher exact tests were used to analyze categorical data, and Spearman correlations were used to assess associations. We analyzed all randomized subjects, and those without OFC outcome data were considered failures.

To identify subgroups in which omalizumab might be most beneficial, we first identified promising prognostic variables from graphic displays. We then applied the approach of Shuster and van Eys,[15] fitting regression models that allowed the omalizumab effect to vary as a function of these prognostic factors and to construct regions with the strongest evidence of omalizumab advantage by using logistic regression for binary and ordinal outcomes and linear regression for continuous outcomes. Given the exploratory nature of this analysis, these resulting subgroups should be viewed as preliminary.