Immunologic features of infants with milk or egg allergy enrolled in an observational study (Consortium of Food Allergy Research) of food allergy

Scott H. Sicherer, MD,[a] Robert A. Wood, MD,[b] Donald Stablein, PhD,[c] A. Wesley Burks, MD,[d] Andrew H. Liu, MD,[e] Stacie M. Jones, MD,[f] David M. Fleischer, MD,[e] Donald Y. M. Leung, MD, PhD,[e] Alexander Grishin, PhD,[a] Lloyd Mayer, MD,[a] Wayne Shreffler, MD, PhD,[a] Robert Lindblad, MD,[c] and Hugh A. Sampson, MD[a] *New York, NY, Baltimore and Rockville, Md, Durham, NC, Denver, Colo, and Little Rock, Ark*

Background: Immune features of infants with food allergy have not been delineated.

Objectives: We sought to explore the basic mechanisms responsible for food allergy and identify biomarkers, such as skin prick test (SPT) responses, food-specific IgE levels, and mononuclear cell responses, in a cohort of infants with likely milk/egg allergy at increased risk of peanut allergy.

Methods: Infants aged 3 to 15 months were enrolled with a positive SPT response to milk or egg and either a corresponding convincing clinical history of allergy to milk or egg or moderate-to-severe atopic dermatitis. Infants with known peanut allergy were excluded.

Results: Overall, 512 infants (67% male) were studied, with 308 (60%) having a history of a clinical reaction. Skin test responses, detectable food-specific IgE, or both revealed sensitization as follows: milk, 78%; egg, 89%; and peanut, 69%. SPT responses and food-specific IgE levels were discrepant for peanut (15% for IgE ≥0.35 kU_A/L and negative SPT response vs 8% for positive SPT response and IgE <0.35 kU_A/L, $P = .001$). Mononuclear cell allergen stimulation screening for *CD25*, cytokine-inducible SH2-containing protein *(CISH)*, forkhead box protein 3 *(FOXP3)*, *GATA3*, *IL10*, *IL4*, *IFNG*, and T-box transcription factor *(TBET)* expression by using casein, egg white, and peanut revealed that only allergen-induced *IL4* expression was significantly increased in those with clinical allergy to milk (compared with nonallergic subjects) and in those sensitized to peanut, despite the absence of an increase in *GATA3* mRNA expression.

Conclusions: Infants with likely milk/egg allergy are at considerably high risk of having increased peanut-specific IgE levels (potential allergy). Peanut-specific serum IgE levels were a more sensitive indicator of sensitization than SPT responses. Allergen-specific IL4 expression might be a marker of allergic risk. Absence of an increase in *GATA3* mRNA expression suggests that allergen-specific IL-4 might not be of T-cell origin. (J Allergy Clin Immunol 2010;125:1077-83.)

Key words: *Food allergy, sensitization, atopy*

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Food allergy is estimated to affect approximately 4% to 6% of young children, with egg, milk, and peanut allergy being the most common.[1,2] Food allergy appears to be increasing in prevalence in westernized countries, with specific evidence for an increase in peanut allergy in children within the past decade.[3-5] Although allergy to egg and milk typically resolves over time, peanut allergy typically persists,[6] and studies indicate that milk and egg allergies

From [a]the Elliot and Roslyn Jaffe Food Allergy Institute, Division of Allergy and Immunology, Department of Pediatrics, Mount Sinai School of Medicine, New York; [b]the Department of Pediatrics, Division of Allergy and Immunology, the Johns Hopkins University School of Medicine, Baltimore; [c]the EMMES Corporation, Rockville; [d]the Department of Pediatrics, Duke University Medical Center, Durham; [e]the Division of Pediatric Allergy and Clinical Immunology, National Jewish Health, Denver; and [f]the Department of Pediatrics, the University of Arkansas for Medical Sciences, Little Rock.

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Additional Site Investigators: D. Atkins, T. T. Perry, A. M. Scurlock, M. Masilamani, and B. Vickery. Coordinators and support: D. Brown, M. Mishoe, S. Walsh, L. Talarico, S. Noone, M. Beksinska, J. Grabowska, K. Mudd, S. Driggers, P. Steele, J. Kamilaris, S. Carlisle, T. Hubbart, A. Hiegel, L. Christie, M. Groetch, J. Slinkard, J. Stone, S. Leung, K. Morgan, and K. Brown-Engelhardt.

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Reprint requests: Scott H. Sicherer, MD, Division of Allergy/Immunology, Mount Sinai Hospital, Box 1198, One Gustave L. Levy Place, New York, NY 10029-6574. E-mail: scott.sicherer@mssm.edu.

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Abbreviations used
AD: Atopic dermatitis
Ct: Threshold cycle
SPT: Skin prick test
TBET: T-box transcription factor gene

are also becoming more persistent.[7,8] Although genetic factors clearly predispose a subject to food allergy,[9] the observed recent increases and persistence of childhood food allergies must be attributable to environmental factors. Food allergy can be life-threatening or fatal[10] and seriously affects quality of life.[11] Considering the increasing prevalence and seriousness of this disease, studies to determine risk factors, prevention strategies, better diagnostic tests, and definitive treatments are needed.

The Consortium of Food Allergy Research, funded by the National Institutes of Allergy and Infectious Diseases, is comprised of 5 clinical sites in the United States with established expertise in food-induced allergic disorders that are undertaking observational and treatment studies of food allergy. To address the immunologic, genetic, and environmental factors that affect the course of food allergy, we established a cohort of infants with likely egg allergy, milk allergy, or both who are predicted to be at increased risk to have or develop peanut allergy. These children will be followed longitudinally to determine the course of their egg and milk allergies, as well as the development or resolution of peanut allergy.

Insights into the basic mechanisms responsible for the development of food allergies are lacking. In the current study we sought to determine whether mononuclear cell expression of key cytokine and regulatory genes were markers of current milk/egg allergy or associated with sensitization to peanut in these infants. In what we believe to be the largest and most comprehensive study to date, we present data confirming the expected importance of *IL4* but do not detect an associated increase in *GATA3* transcripts or a shift in the ratio of *GATA3*/T-box transcription factor *(TBET)*, findings that raise questions as to the role of T cells in the early development of food allergy. The infants enrolled in the study have demographic features representing common food allergy presentations; we found clinically important observations, including an unexpectedly high rate of peanut sensitization, and we characterize discrepancies in common diagnostic tests in this large cohort, observing that serum testing for peanut sensitization is more sensitive than skin prick tests (SPTs).

METHODS

Study rationale, design, and enrollment criteria

We aimed to study markers of development of peanut allergy and the natural course of egg/milk allergy, and therefore we did not intend to enroll children with likely peanut allergy, only those "at risk." We developed enrollment criteria for this cohort based on the results of various studies of childhood food allergies and atopic dermatitis (AD),[12-16] estimating that children with a convincing clinical reaction to milk, egg, or both with a positive SPT response to the responsible food and/or children with moderate-to-severe AD and a positive SPT response to milk, egg, or both would be likely (approximately 20% to 30%) to have a peanut allergy and have a 25% to 50% chance of resolving their egg or milk allergy by 5 to 7 years of age. We enrolled children aged 3 to 15 months to allow adequate recall of their feeding history and to reduce the chance that they already would have a peanut allergy. Infants known to have a peanut allergy or a peanut-specific IgE level of greater than 5 kU_A/L (representing a more likely current peanut allergy, as described in the Methods section of this article's Online Repository at www.jacionline.org) before screening were therefore excluded from enrollment because they already had evidence of peanut allergy/sensitization and therefore could not be evaluated for the basic question to be addressed prospectively. To maintain uniformity and an observational approach, the study design includes evaluations, care for food allergy, and instructions on dietary management that were uniform among the 5 clinical centers and reflect practice parameters for AD,[17] food allergy,[18] and the American Academy of Pediatrics recommendations for allergy prevention published in 2000, which were current at the initiation of the study.[19]

Enrollment required either (1) a history of a convincing immediate allergic reaction to cow's milk (and/or egg), as described further in the Methods section of this article's Online Repository, and a positive SPT (≥3 mm larger than that elicited by the negative control) to cow's milk (and/or egg if the clinical reaction was to egg); (2) moderate-to-severe AD at the time of enrollment (as described further in the Methods section of this article's Online Repository) and a positive SPT response to milk, egg, or both; or (3) both.

Children were excluded if they had chronic disease (other than asthma, AD, or rhinitis), required therapy (eg, heart disease and diabetes), were participating in any interventional study, were unable to discontinue antihistamines for routine tests, had a sibling enrolled in the observational study, or already had confirmed or convincing evidence of peanut allergy (see the Methods section of this article's Online Repository). Study procedures were reviewed and approved by a National Institute of Allergy and Infectious Diseases Data Safety Monitoring Board and by local site institutional review boards, and written signed consent was obtained.

Definitions of atopic diseases and categorization of food allergy

Atopic diseases (asthma, allergic rhinitis, and AD) were diagnosed and graded by severity as described in the Methods section of this article's Online Repository. Food allergy was diagnosed based on clinical criteria (as described in the Methods section of this article's Online Repository) because as an observational natural history study, repeated scheduled oral food challenges could not be imposed. At enrollment, diagnoses were categorized as confirmed or convincing when there was a clear clinical history and sensitization to the causal food, and in this report these children are categorized as allergic. Those ingesting the food or lacking sensitization are categorized as not allergic. These 2 clinical categories are the primary clinical end points evaluated in the current study. Additional enrollees with potential allergy are described in the Methods section of this article's Online Repository. The total study group was also evaluated with regard to sensitization status, as described below.

SPTs

SPTs were performed with the GreerPick (Greer Laboratories, Lenoir, NC), with participants avoiding antihistamines for at least 5 half-lives of the specific agent. Tests were performed on the infant's back, and at 15 minutes, the wheal was outlined in pen and transferred by tape to paper. The size of the longest diameter and its longest perpendicular were averaged. Additional details are described in the Methods section of this article's Online Repository.

Plasma food-specific IgE assay

The concentrations of specific IgE antibody to egg, milk, and peanut were measured from plasma at a single central laboratory by using the ImmunoCAP system (Phadia, Uppsala, Sweden) and reported in kU_A/L. Those at or greater than 0.35 kU_A/L are described as IgE sensitized.

Mononuclear cell stimulation and PCR analysis

PBMC isolation was performed by means of Ficoll-Paque density gradient centrifugation, and cultures were performed at each clinical site on fresh venous blood samples. The laboratory processing protocol was standardized, and reproducibility across sites was confirmed in pilot studies. Four million