The natural history of milk allergy in an observational cohort

Robert A. Wood, MD,[a]* Scott H. Sicherer, MD,[b]* Brian P. Vickery, MD,[c] Stacie M. Jones, MD,[d] Andrew H. Liu, MD,[e] David M. Fleischer, MD,[e] Alice K. Henning, MS,[f] Lloyd Mayer, MD,[b] A. Wesley Burks, MD,[c] Alexander Grishin, PhD,[b] Donald Stablein, PhD,[d] and Hugh A. Sampson, MD[b] *Baltimore and Rockville, Md, New York, NY, Chapel Hill, NC, Little Rock, Ark, and Denver, Colo*

Objective: There are few studies on the natural history of milk allergy. Most are single-site and not longitudinal, and these have not identified a means for early prediction of outcomes.
Methods: Children aged 3 to 15 months were enrolled in an observational study with either (1) a convincing history of egg allergy, milk allergy, or both with a positive skin prick test (SPT) response to the trigger food and/or (2) moderate-to-severe atopic dermatitis (AD) and a positive SPT response to milk or egg. Children enrolled with a clinical history of milk allergy were followed longitudinally, and resolution was established by means of successful ingestion.
Results: The cohort consists of 293 children, of whom 244 were given a diagnosis of milk allergy at baseline. Milk allergy has resolved in 154 (52.6%) subjects at a median age of 63 months and a median age at last follow-up of 66 months. Baseline characteristics that were most predictive of resolution included milk-specific IgE level, milk SPT wheal size, and AD severity (all $P < .001$). Baseline milk-specific IgG_4 level and milk IgE/IgG_4 ratio were not predictive of resolution and neither was expression of cytokine-inducible SH2-containing protein, forkhead box protein 3, GATA3, IL-10, IL-4, IFN-γ, or T-bet by using real-time PCR in CD25-selected, casein-stimulated mononuclear cells. A calculator to estimate resolution probabilities using baseline milk IgE level, SPT response, and AD severity was devised for use in the clinical setting.
Conclusions: In this cohort of infants with milk allergy, approximately one half had resolved over 66 months of follow-up. Baseline milk-specific IgE level, SPT wheal size, and AD severity were all important predictors of the likelihood of resolution. (J Allergy Clin Immunol 2013;131:805-12.)

Key words: *Milk allergy, natural history, food allergy, IgE*

Milk allergy is the most common food allergy in young children, with prevalence rates estimated in the range of 2% and 3%.[1,2] Although the natural history of milk allergy is generally favorable, with the majority of children showing resolution during childhood, prior studies have yielded widely varying results as to the rate of resolution.[3-16] A recent study suggested that the natural history of milk allergy might have changed over time, with slower rates of resolution and a higher proportion of children with disease persisting into adolescence and even adulthood.[15] Although these changes might be real, most differences between studies are more likely related to both study design and the specific population under investigation. For example, studies of the

From [a]the Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore; [b]the Department of Pediatrics, Mount Sinai School of Medicine, New York; [c]the Department of Pediatrics, University of North Carolina, Chapel Hill; [d]the Department of Pediatrics, University of Arkansas for Medical Sciences and Arkansas Children's Hospital, Little Rock; [e]the Department of Pediatrics, National Jewish Health, Denver; and [f]EMMES Corporation, Rockville.

*These authors contributed equally to this manuscript.

Supported by National Institutes of Health (NIH)/National Institute of Allergy and Infectious Diseases grants U19AI066738 and U01AI066560. The project was also supported by grant nos. UL1 RR025780 (National Jewish), UL1 1TR000067 (Mount Sinai), UL 1 TR000039 (Arkansas), UL 1 RR024128 (North Carolina) and UL1 RR 025005 (Johns Hopkins) from the National Center for Research Resources (NCRR), a component of the NIH. Its contents are solely the responsibility of the authors and do not necessarily represent the official view of the NCRR or the NIH.

Disclosure of potential conflict of interest: R. A. Wood has consultant arrangements with the Asthma and Allergy Foundation of America, is employed by Johns Hopkins University, has received research support from the National Institutes of Health (NIH), and receives royalties from UpToDate. S. H. Sicherer has received grants from the NIH/National Institute of Allergy and Infectious Diseases (NIAID) and has consultant arrangements with the Food Allergy Initiative. B. P. Vickery has received grants and travel support from the NIH/NIAID; has consultant arrangements with Mead Johnson; has received grants from the NIH/NIAID, the Thrasher Research Fund, the American Lung Association, and Cephalon. S. M. Jones is a member of the medical advisory board for the Food Allergy & Anaphylaxis Network (FAAN); has received grants from the NIH, the FAAN, and the National Peanut Board; has received payment for lectures, including service on speakers' bureaus, from Abbott Nutrition International, the Kentucky Society for Allergy, Asthma & Immunology, the New England Allergy Society, the American College of Allergy, Asthma & Immunology, Indiana University Medical School and Riley Children's Hospital, the Spanish Society of Allergy & Clinic Immunology, and the Oregon Allergy Asthma & Immunology Society; has served on the NIAID Safety Monitoring Committee, the Arkansas Medicaid Drug Review Committee, and the NIAID Study Section. D. M. Fleischer has received grants from the NIH/NIAID, has consultant arrangements with Sanofi-Aventis, and receives royalties from UpToDate. A. K. Henning has received grants from the NIH. A. W. Burks has received grants or has grants pending from the NIH, the Department of Defense, and the Wallace Research Foundation; is a board member for the American Academy of Allergy, Asthma & Immunology, the NIH Hypersensitivity, Autoimmune, and Immune-mediated Disease Study Section, the *Journal of Allergy and Clinical Immunology*, and the US Food and Drug Administration; is on advisory boards for ExploraMed Development, LLC, the FAAN, and ActoGeniX; has consultant arrangements with Merck & Co, Novartis Pharma AG, the Dannon Company, McNeill Nutritionals, and Schering-Plough; is employed by UNC Children's Hospital and Duke University; has received payment for lectures from Mylan Specialty; has received royalties from UpToDate; has received payment for development of educational presentations from Current Views; has stock/stock options in Allertein, Mastcell Pharmaceuticals, and Dow AgroSciences; and has received travel expenses from the European Academy of Allergy & Clinical Immunology. A. Grishin has received grants from the NIH/NIAID and has consultant arrangements with Allertein Therapeutics. D. Stablein has received grants from the NIH. H. A. Sampson has received grants from the NIH and the NIAID, has received travel support from the NIAID, is on the Danone Scientific Advisory Board, has consultant arrangements with Allertein Therapeutics and the Food Allergy Initiative, is employed by Mount Sinai Medical School, and has received royalties from Elsevier-Wiley and UpToDate. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication September 3, 2012; revised October 3, 2012; accepted for publication October 22, 2012.

Available online December 28, 2012.

Corresponding author: Robert A. Wood, MD, CMSC 1102, Johns Hopkins Hospital, 600 North Wolfe St, Baltimore, MD 21287. E-mail: rwood@jhmi.edu.

0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2012.10.060

Abbreviations used
AD: Atopic dermatitis
Ct: Cycle threshold
SPT: Skin prick test

general population,[11] especially if oral food challenges are performed at regular intervals, are more likely to demonstrate earlier resolution than studies of tertiary referral populations.[3,9,10,15]

The Consortium of Food Allergy Research enrolled infants with likely egg or milk allergy but without previously known peanut allergy in an observational study to address the immunologic, genetic, and environmental factors that affect the natural course of food allergy.[17] The primary aim of this analysis was to assess the natural history of milk allergy in the infants enrolled in this cohort with a diagnosis of milk allergy, with a particular focus on the clinical factors predicting the resolution of milk allergy over the first 5 years of life.

METHODS

Subjects, study definitions, and procedures

The subjects of this study are a subset of a larger cohort of 512 infants originally enrolled at 3 to 15 months of age at 5 sites: Mount Sinai School of Medicine, New York, New York; Duke University Medical Center, Durham, NC; Johns Hopkins University School of Medicine, Baltimore, Maryland; National Jewish Health, Denver, Colorado; and Arkansas Children's Hospital, Little Rock, Arkansas, as described previously[17]; the North Carolina subjects moved with the investigative team from Duke to the University of North Carolina–Chapel Hill in March 2012. Enrollment criteria for the whole cohort were designed to obtain atopic children with likely egg or milk allergy at risk for peanut allergy but without current peanut allergy. Briefly, enrollment required either (1) a history of a convincing immediate allergic reaction to cow's milk (and/or egg) and a positive skin prick test (SPT) response (3 mm larger than that elicited by the negative control) to cow's milk (and/or egg, if the clinical reaction was to egg) and/or (2) moderate-to-severe atopic dermatitis (AD) and a positive SPT response to milk, egg, or both.

The subgroup of children in the current study had a diagnosis of milk allergy at the time of enrollment or acquired this diagnosis after enrollment with no prior evidence of tolerance of milk (eg, enrollment diagnosis was uncertain). Study procedures were reviewed and approved by a National Institute of Allergy and Infectious Diseases Data Safety Monitoring Board and by local institutional review boards, and written signed consent forms were obtained.

Participants were considered to have milk allergy if they had either (1) a positive physician-supervised oral food challenge result or a convincing reaction (defined by symptoms within an hour of isolated ingestion that included at least urticaria and/or angioedema, difficulty breathing, wheezing, throat tightness, and/or vomiting) and sensitization to milk (milk-specific IgE level $\geq$0.35 kU_A/L and/or SPT response >3 mm) or (2) a flare of AD associated with milk ingestion along with a milk-specific IgE level of greater than 5 kU_A/L,[18] which is greater than 95% predictive of milk allergy in infants. Reactions to goat's or sheep's milk were also considered evidence of cow's milk allergy. Subjects were considered milk tolerant if they ingested whole uncooked milk products (milk, yogurt, or ice cream) in serving size quantities without symptoms either during physician-supervised oral food challenges or after introduction at home. Dietary ingestion of products with extensively heated milk (baked milk, for example as an ingredient in a muffin) was queried but was not considered evidence of resolved milk allergy.

Dietary, medical, and social histories were obtained by using questionnaires completed during enrollment interviews. A diagnosis of asthma and allergic rhinitis was based on parental report or parental report of a physician's diagnosis. A diagnosis of other food allergies included per-protocol definitions for egg and peanut,[17] whereas for other foods, this was based on a clinical diagnosis by a study physician.

Diagnosis of AD required pruritus and an eczematous rash (acute, subacute, or chronic) with typical morphology and age-specific patterns, a chronic or relapsing history, atopy (personal history, family history, or both or IgE reactivity), and xerosis. AD severity was graded based on criteria previously described and published by Rajka and Langeland.[19] Briefly, the AD severity was graded as mild, moderate, or severe by using the following parameters (see Table E1 in this article's Online Repository at www.jacionline.org)[19] to compute a score summation: (1) extent of disease (by "rule of nine" based on the proportion of body surface area with active disease), (2) course of disease (defined by history as >3 months in remission in the past year, $\leq$3 months in remission but not continuous, or continuous remission over the past year), and (3) intensity of disease (defined as mild itch rarely disturbing sleep, severe itch usually disturbing sleep, or intermediate itch/sleep disturbance), each on a 3-point scale. Summation scores of 3 to 4 indicated mild disease, 5 to 7 indicated moderate disease, and 8 to 9 indicated severe disease. Atopic disease history in parents of the enrolled infants was based on previously published definitions and was recorded by parental report.[20]

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,[21] food allergy,[22] and the American Academy of Pediatrics recommendations for allergy prevention published in 2000 to maintain uniformity and an observational approach.[23] Participants were evaluated in person at enrollment, 6 months, 12 months, and yearly thereafter, with additional telephone follow-up between each visit and instructions to contact the study site for any allergic reactions, at which time additional details were obtained.[24]

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. An SPT score was computed by subtracting the saline control measure, and a positive SPT response was defined by a score of 3 mm or greater. Tests were considered reliable if the wheal of the negative control (50% glycerin-saline) was 3 mm or smaller and wheal size elicited by the histamine control was at least 3 mm larger than the wheal size elicited by the negative control. All sites used the same lot of reagents, and training was performed to ensure consistency. The cow's milk extract was obtained from Greer (catalog no. F293).

Serum milk-specific IgE and IgG_4 levels

The concentration of specific IgE antibody to milk was measured from plasma at a central laboratory (Mount Sinai) by using the Phadia (now Thermo Fisher Scientific, Waltham, Mass) ImmunoCAP system and reported in kilounits of allergen per liter. A level of 0.35 kU_A/L or greater was considered positive. The concentration of IgG_4 antibodies to milk was also measured from plasma samples by using the Phadia ImmunoCAP system. The detection limit for IgG_4 is 0.07 mg/L.

Mononuclear cell stimulation and PCR analysis

PBMC isolation was performed with Ficoll-Paque density gradient centrifugation, and cultures were performed at each clinical site on fresh venous blood samples, as previously described.[1] Briefly, 4 million cells per condition were cultured for 48 hours in AIM-V serum-free media (Invitrogen, Carlsbad, Calif) with purified α-, β-, and κ-caseins (50 μg each/mL), and control stimulations were performed with medium alone (negative) and anti-CD3/anti-CD28 beads (positive). At the end of the culture period, cells expressing CD25 were enriched by means of selection with anti-CD25–coated paramagnetic beads, according to the manufacturer's protocol (Miltenyi Biotech, Bergisch Gladbach, Germany). Pilot experiments demonstrated approximately