METHODS

Subjects

All blood specimens were obtained by venipuncture with informed consent by using protocols approved by the institutional review board. Basophils isolated from a total of 38 FA and 18 nonallergic children were investigated for histamine release, with additional markers (eg, IL-4, syk, and CD203c/CD63) also investigated when sufficient numbers of cells were isolated. In particular, basophil responses from 13 of 14 subjects, who were part of a previous study exploring dendritic cell response in children allergic to cow's milk,[E1] were investigated for SBHR and IL-4. The remaining subjects consisted of 24 children who were followed at baseline and during a protocol involving SLIT/OIT.[E1] Baseline values for several of the basophil parameters investigated in this SLIT/OIT subgroup are restated herein to demonstrate correlative associations not previously reported. Basophils from this group were used to investigate SBHR, CD203c/CD63, and intracellular syk levels. Supporting information regarding all subjects has been previously reported,[E1] with pertinent information summarized in Table E1. As described later, plasma specimens were also collected from many of these subjects and were used in the passive sensitization experiments described herein.

For all subjects, the diagnosis of food allergy was based on a convincing history of reaction following exposure to cow's milk and a milk-specific IgE level of more than 0.10 KU_A/L (UniCAP; Phadia, Uppsala, Sweden). Milk was strictly being avoided by each subject at the time blood was drawn. Sensitization to foods (defined by food-specific IgE level of >0.35 KU_A/L) currently being ingested on a regular basis, current medications, and presence of other allergic diseases (eczema, allergic rhinitis, asthma) were determined by patient and parental interviews and review of medical records. Control subjects had no history of acute reaction to any food, had never avoided any foods, and were currently tolerating milk in their diet.

Cell preparation and culture

Blood specimens anticoagulated with ethylenediaminetetraacetic acid were subjected to double Percoll density centrifugation, as described in detail elsewhere.[E2] Briefly, plasma was saved and stored at −20°C. Cells accumulating on the 61% Percoll density consisted of 3% to 15% basophils. These basophil-enriched cells (BECs) were cultured in media alone or stimulated in medium containing crude milk extract (1-10 μg/mL), goat anti-human IgE antibody (1-100 ng/mL), calcium ionophore A23187 (500 ng/mL), or f-Met peptide (10^{-6} mol/L). Culture medium consisted of Iscove's modified Dulbecco's medium supplemented with 5% FCS, nonessential amino acids, and 10 μg/mL gentamicin (pH, 7.2-7.4). This conditioned medium was chosen over several others (that also supported SBHR) because of its capacity to support cytokine secretion by basophils.[E2] Histamine release was measured after 30 minutes and IL-4 release after 4 hours of culture. For the passive transfer experiments using plasma, BEC suspensions from nonallergic donors were used as is (ie, for the CD203c assays), or additionally underwent negative selection by using the Stemsep basophil enrichment cocktail (StemCell Technologies, Vancouver, British Columbia, Canada) to purify basophils (>97%) for the SBHR and IL-4 assays. In both instances, cells were treated with lactic acid solution (pH, 3.9)[E3] for 30 seconds on ice to partially remove cell surface–bound IgE, washed, and then passively sensitized for 30 minutes with plasma containing up to 500 ng/mL of total IgE (determined by the UniCap measurements). Control plasma from nonallergic subjects was used in some experiments, and was supplemented with in-house JK myeloma IgE to bring the final concentration to 500 ng/mL. For some conditions, omalizumab (10 μg/mL) and/or IL-3 receptor (α and β subunits)-blocking antibodies (2 μg/mL), or isotype control antibodies were added during the passive sensitization step.

Histamine and cytokine measurements

A portion of each culture supernatant was assessed for histamine by using automated fluorimetry, with values reported as a percentage of total histamine content determined by lysing an equivalent number of basophils, as originally described.[E4] The remaining supernatant was assayed for IL-4 by using ELISA (eBioscience, San Diego, Calif).

Flow cytometry

The measurement of intracellular syk protein in basophils was performed as previously described.[E5] Surface staining of CD203c and CD63 was performed as previously reported.[E6] The expression of all 3 markers is reported as normalized net mean fluorescence intensity defined as the difference between staining with specific antibody and isotype-labeled cells corrected for instrument variability by using CaliBRITE allophycocyanin calibration beads (BD Biosciences, Franklin Lakes, NJ). Basophils within BEC suspensions were identified as IL-3 receptor $(CD123)^+$, blood dendritic cell antigen-2^- cells.[E5,E7]

Serologic measurements

Measurements of total IgE, milk-specific IgE, Fx5 multitest, and the Phadiatop multiallergen screen were performed on plasma by the Johns Hopkins University Dermatology Allergy and Clinical Immunology Reference Laboratory by using a fluorescent-based enzyme immunoassay performed on the ImmunoCAP 250 (Phadia, Kalamazoo, Mich). Phadiatop is a single measurement that detects IgE antibody specific for the 10 most common aeroallergens, and the Fx5 is a single measurement that detects IgE to the 6 most common food allergens (egg, milk, soy, peanut, wheat, and fish).

Statistics

Analyses were performed by using Prism Software (GraphPad Software, San Diego, Calif). Comparisons were by done by using *t* tests when compared data were normally distributed. Otherwise, comparisons were by done by using the Mann-Whitney *U* test. Pearson correlations were performed (all data were normally distributed) as indicated. Significant *P* values defined as less than .05 are shown.

REFERENCES

E1. Frischmeyer-Guerrerio PA, Guerrerio AL, Chichester KL, Bieneman AP, Hamilton RA, Wood RA, et al. Dendritic cell and T cell responses in children with food allergy. Clin Exp Allergy 2011;41:61-71.

E2. Schroeder JT, Kagey-Sobotka A. Assay methods for measurement of mediators and markers of allergic inflammation. In: Rose NR, Hamilton RG, Detrick B, editors. Manual of clinical laboratory immunology. American Society for Microbiology Press, Washington DC, 2002. p. 899-909.

E3. Pruzansky JJ, Grammer LC, Patterson R, Roberts M. Dissociation of IgE from receptors on human basophils, I: enhanced passive sensitization for histamine release. J Immunol 1983;131:1949-53.

E4. Schroeder JT, MacGlashan DW Jr, Kagey-Sobotka A, White JM, Lichtenstein LM. IgE-dependent IL-4 secretion by human basophils: the relationship between cytokine production and histamine release in mixed leukocyte cultures. J Immunol 1994;153:1808-17.

E5. Ishmael SS, MacGlashan DW Jr. Syk expression in peripheral blood leukocytes, $CD34^+$ progenitors, and CD34-derived basophils. J Leukoc Biol 2010;87:291-300.

E6. Celik GE, Schroeder JT, Hamilton RG, Saini SS, Adkinson NF. Effect of in vitro aspirin stimulation on basophils in patients with aspirin-exacerbated respiratory disease. Clin Exp Allergy 2009;39:1522-31.

E7. Frischmeyer-Guerrerio PA, Schroeder JT. Cellular immune response parameters that influence IgE sensitization. J Immunol Methods 2012;383:21-9.

TABLE E1. Baseline characteristics for subjects

Group	FA	Controls
N	38	18
Male, n (%)	23 of 38 (60.5)	5 of 18 (27.8)
Age (y), median (range)	7.9 (4.2-16.9)	12.3 (2.2-17.9)
Total IgE (KU_A/L), median (range)	1121.5 (79-5960)	24.9 (<2-1056)
Cow's milk-specific IgE (KU_A/L), median (range)	52.5 (1.9-1108.0)	<0.10 (<0.10-1.52)
History of anaphylaxis to cow's milk, n (%)	33 of 38 (86.8)	0 of 18 (0)
Allergic rhinitis, n (%)	24 of 38 (63.2)	4 of 18 (36)
Other food allergies, n (%)	35 of 38 (92.1)	0 of 18 (0)
Asthma, n (%)	35 of 38 (92.1)	0 of 18 (0)
Atopic dermatitis, n (%)	29 of 38 (76.3)	0 of 18 (0)