Anaphylaxis to cow's milk formula containing short-chain galacto-oligosaccharide

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Background: On the basis of the proven prebiotic effects of oligosaccharides in cow's milk formula (CMF) in infants, CMFs are supplemented with oligosaccharides.
Objective: We present a series of 5 cases of cow's milk–tolerant but atopic patients with a history of respiratory allergies. All had anaphylaxis after the ingestion of CMF supplemented with short-chain galacto-oligosaccharide (scGOS). The allergen trigger was investigated.
Methods: Clinical histories were collated. Skin prick tests (SPTs) and basophil activation tests (BATs) were carried out with the eliciting CMF that triggered anaphylaxis, with or without supplemented prebiotics (scGOS) and with scGOS fractions containing oligosaccharides of different chain lengths.
Results: The median age of presentation was 6 years (range, 5-38 years). Anaphylaxis occurred within 30 minutes of the first known exposure to CMF supplemented with prebiotics in all patients. Only 1 patient was subjected to oral challenge, which resulted in an anaphylactic reaction. All patients demonstrated IgE sensitization through SPTs and BATs to scGOS and fractions of scGOS containing 3 sugar units or greater but not to cow's milk or long-chain fructo-oligosaccharide. Eight child control subjects tolerant to regular ingestion of scGOS-supplemented CMF and 1 adult volunteer were found to have negative results to scGOS through SPTs and BATs. In addition, *in vitro* BATs with donor basophils sensitized with sera from 2 of the 3 reported cases showed reactions to scGOS. The scGOS-induced basophil activation was inhibited in the presence of wortmannin, a phosphatidylinositol 3-kinase inhibitor.
Conclusions: This study describes an unusual form of IgE-mediated anaphylaxis triggered by low-molecular-weight oligosaccharides in scGOS. The primary sensitizer for this phenomenon requires further investigation. (J Allergy Clin Immunol 2012;130:1361-7.)

Key words: *Galacto-oligosaccharide allergy, Asia, children, basophil activation test, skin prick test*

Prebiotics are defined as a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth, activity, or both of 1 or a limited number of bacteria in the colon and thus improve the host's health.[1] Naturally occurring prebiotics are found in fruits, vegetables, and human milk. Commercial oligosaccharides have been used to supplement cow's milk formula (CMF) for infants and children to approach the beneficial effects found in prebiotic human milk oligosaccharides. These oligosaccharides include short-chain galacto-oligosaccharides (scGOSs), polydextrose, lactulose, inulin, short-chain fructo-oligosaccharides, and long-chain fructo-oligosaccharides (lcFOSs), as well as combinations of these products. Some of these oligosaccharides have proved prebiotic and clinical effects in human subjects.[2-4]

The conventional dogma in the treatment of allergy is that allergens are proteins/glycoproteins and that IgE binding usually involves linear, conformational, or both protein epitopes corresponding to their amino acid sequence, tertiary structure, or both, respectively. Although IgE has been shown to bind avidly to carbohydrate epitopes, the clinical significance of cross-reactive carbohydrate determinants in grass pollen–sensitized subjects has been questioned.[5,6] However, recent studies have demonstrated that some carbohydrate epitopes might have allergenic potential. This was demonstrated in reports of anaphylaxis to the mAb cetuximab, a chimeric (mouse/human) mAb administered by means of intravenous infusion for the treatment of metastatic colorectal and head and neck cancer.[7] In these studies the allergic response was shown to be solely due to the carbohydrate moiety of the mAb galactose α-1, 3-galactose (α-gal).[7] This same α-gal allergy has been shown to be responsible for some anaphylaxis cases to red meat in the United States.[8]

In the current study we report 5 cases of anaphylaxis associated with CMF supplemented with prebiotics. All patients had a history of atopy. Unlike those with cow's milk allergy, all our patients were 5 years and older at the time of presentation. Our investigations focus on the scGOS present in the milk formula as the specific eliciting allergen, as shown by positive skin prick test (SPT) and IgE-mediated basophil activation test (BAT) results to this prebiotic (scGOS).

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Abbreviations used
α-gal: Galactose α-1, 3-galactose
BAT: Basophil activation test
CMF: Cow's milk formula
DP: Degree of polymerization
lcFOS: Long-chain fructo-oligosaccharide
scGOS: Short-chain galacto-oligosaccharide
SPT: Skin prick test

METHODS

Cases

All 5 patients were identified from the allergy clinics of Kandang Kerbau Women's and Children's Hospital and National University Hospital between the period from December 2007 to January 2012. Patients presented with symptoms of anaphylaxis and were treated with adrenaline injections in 2 patients and β_2-agonist with oxygen in 3 patients with wheeze associated with desaturation (Table I). They were seen for the evaluation of anaphylaxis, and the common trigger was assessed to be CMF supplemented with prebiotics. Their case histories were reviewed, and a structured questionnaire was applied to all subjects to obtain information on their past exposure to CMF and other commercial products supplemented with prebiotics. In addition, we included 8 children 5 years and older (age range, 5-8 years), who were consuming scGOS-containing CMF regularly. Four of these 8 child control subjects were atopic and sensitized to house dust mites.

This study was approved by the hospital's institutional ethical review board, and written consent was obtained from the patients' parents and the only adult patient.

Reagents

Standard and low-protein Vivinal scGOS syrups consisting of 45% scGOS were obtained from FrieslandCampina Domo (Amersfoort, The Netherlands). lcFOSs were obtained from Orafti (Raftiline HP; Orafti, Wijchen, The Netherlands). β-Galactosidase (Biolacta; Amano, Nagoya, Japan), the enzyme used for the synthesis of scGOS, was provided by FrieslandCampina Domo (Borculo, The Netherlands). scGOS contains a mixture of oligomers with a basic structure of a lactose core with various degrees of polymerization (DPs) of galactose units, which vary between 1 and 7.[9] lcFOS contains a mixture of polymers with a basic glucose-fructose core and various DPs of fructose units, which vary between 7 and 60 (average, 23).[10] The scGOS oligomer mixture was also fractionated by means of mass distribution by using HPLC, such that specific fractions each contained separately 2, 3, 4, 5, and 6 or more units of sugar, which were referred to in as DP2, DP3, DP4, DP5, and DP6, respectively. The prebiotics supplementing commercial CMFs used in the study were Mamil Step 4 (CMF1; Danone Dumex, Malaysia; contains scGOS and lcFOS mixtures) and *Gain EyeQ (CMF2; Abbott; contains scGOS mixtures only). The commercial milk formula without prebiotic supplementation (CFM1 without prebiotics) was prepared by Danone.

SPTs

All SPTs were conducted on the patients' backs or forearms with commercial cow's milk (Greer Laboratories, Lenoir, NC), reconstituted commercial CMF that was consumed by the patients, and the commercial formula (without supplemental prebiotics), standard Vivinal scGOS, low-protein scGOS (product used to supplement extensively hydrolyzed), scGOS fractions (2, 3, 4, 5, and 6 or more sugar units), and lcFOS. Histamine and saline were used as positive and negative controls, respectively. According to the manufacturer's instructions for CMF1, the reconstituted milk contained 7.2 mg/mL scGOS and 0.8 mg/mL lcFOS. Hence the 7.2 mg/mL scGOS and 3.6 mg/mL scGOS fractions were used. When the patient had a negative reaction to the above concentration, a 2-fold concentration was used. The wheal size for each allergen was recorded and used as the degree of skin test reactivity.

BATs

The CMF, scGOS, and lcFOS products were reconstituted and diluted serially to their final working concentrations. To prepare CMF with a final concentration of 10 μg/mL scGOS, 230 μg of CMF1 and 308 μg of CMF2 was reconstituted in 1 mL of PBS. To rule out the activation of basophils by other compounds in the CMF, 230 μg of milk powder without supplemental prebiotics was reconstituted in 1 mL of PBS. Heparinized peripheral blood aliquots (100 μL) were preincubated at 37°C for 5 minutes and then incubated with 100 μL of PBS, serially diluted milk, scGOS, scGOS fractions and lcFOS, β-galactosidase, or anti-IgE antibody (G7-18; BD Biosciences, San Jose, Calif) for 15 minutes (37°C). After incubation, cells were washed in PBS-EDTA (20 mmol/L) and then incubated with phycoerythrin-labeled anti-human IgE (Ige21; eBioscience, San Jose, Calif), biotin-labeled anti-human CD203c (NP4D6; BioLegend, San Jose, Calif), and fluorescein isothiocyanate–labeled anti-human CD63 (MEM-259, BioLegend) mAbs for 20 minutes at 4°C. After washing the cells with 1% BSA/PBS, allophycocyanin-conjugated streptavidin (BD Biosciences) was added and incubated for 15 minutes at 4°C. Thereafter, samples were subjected to erythrocyte lysis with 2 mL of FACS Lysing Solution (BD Biosciences). Cells were then washed, resuspended in 1% BSA/PBS, and analyzed by means of FACSCalibur (BD Biosciences). Basophils were detected on the basis of side-scatter characteristics and expression of IgE (IgE^{high}). The blood was subjected to centrifugation and the plasma was removed to perform BATs under plasma-free conditions. The remaining cells were washed 3 times with Dulbecco PBS. After the last wash, the Dulbecco PBS was added to the cells to reconstitute the sample back to its original volume.

Indirect BAT

The indirect BAT was performed as described previously.[11] Briefly, PBMCs were isolated from citrate-anticoagulated blood of a donor with house dust mite allergy by using Ficoll density centrifugation. Autologous IgE was dissociated from the basophils by means of lactic acid stripping. Basophils were sensitized with patient's IgE by means of incubation with the patient's plasma for 90 minutes at 37°C. Cells were resuspended in RPMI (1×10^6 PBMCs per 75 μL). Subsequently, basophil activation was performed by adding 75 μL of allergen diluted in RPMI/IL-3 (2 ng/mL; R&D Systems, Minneapolis, Minn) to equal volumes of cells and incubating for 30 minutes at 37°C. The reaction was stopped by adding 25 μL of cold PBS/EDTA (20 mmol/L). Cells were stained with phycoerythrin-conjugated mouse anti-human CD63 and fluorescein isothiocyanate–conjugated mouse anti-human CD123 (BioLegend) and allophycocyanin-conjugated mouse anti-human CD203c (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany). Basophil activation was analyzed by means of flow cytometry with a FACSCanto II (BD Biosciences) and is expressed as the percentage of $CD63^+$ cells within the CD203c/$CD123^+$ cells. In the case of inhibition with wortmannin (Enzo Life Sciences, Plymouth Meeting, Pa), basophils were incubated for 5 minutes at 37°C before adding the stimuli, including anti-IgE (Kirkegaard & Perry Laboratories, Gaithersburg, Md) and N-Formyl-L-methionyl-L-leucyl-L-phenylalanine (Sigma Chemicals, St Louis, Mo).

RESULTS

Case studies

The clinical features of the 5 patients are summarized in Table I. The median age of presentation was 6 years (range, 5-38 years). There was only 1 adult patient included, and he reacted after ingestion of his child's unfinished milk formula. The sex ratio was 3 female/2 male patients. There were 4 Chinese patients and 1 Malay patient. All patients were atopic, with allergic rhinitis in all, allergic asthma in 3, and atopic dermatitis in 2. None of the subjects had a prior history of food allergy. All 5 patients were clinically tolerant to cow's milk; however, they reacted to their first known exposure to scGOS-supplemented CMF.

All subjects had symptoms typical of an IgE-mediated allergic reaction in terms of their onset of symptoms from the time of ingestion (within 30 minutes), as well as the typical symptoms of