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;■■■:■■■-■■■.)

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).

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

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Supported in part by the National Medical Research Council (Singapore NMRC/EDG/0039/2008).

Disclosure of potential conflict of interest: A. J. Nauta, J. Bindels, and K. Knipping are employed by Danone Research. D. J. Delsing and L. H. Ulfman are employed by FrieslandCampina. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication May 9, 2012; revised August 13, 2012; accepted for publication August 29, 2012.

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0091-6749/$36.00

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http://dx.doi.org/10.1016/j.jaci.2012.08.048

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

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

TABLE I. Clinical features of subjects presenting with anaphylaxis to CMF with scGOS

Patient no.	Sex	Race	Atopy phenotype	Age at reaction	No. of episodes	Milk eliciting reaction	Volume eliciting reaction (mL)	Time (min)	Reaction	Type of treatment	Place of treatment
1	F	Chinese	AR AS AD AC	5 y, 6 mo	3	Gain IQ Step3 (CMF2)	(1) 100 (2) 120* (3) 40	10	RS Resp	AH, β_2-agonist, oxygen	Hospital
2	F	Chinese	AR AD	6 y, 6 mo	1	Mamil Gold Step4 (CMF1)	250	30	AE RS Resp	AH, steroids	Hospital
3	M	Chinese	AR	38 y	1	Mamil Gold Step3 (CMF1)	180	10	AE RS Resp	Adrenaline, AH, steroids, β_2-agonist, oxygen	Hospital
4	F	Chinese	AR	6 y	1	Mamil Gold Step4 (CMF1)	250	15	AE GU GI RS Resp	Steroids	Outpatient clinic
5	M	Malay	AD AR AS	8 y	1	Gain IQ Step3 (CMF2)	180	5	AE GU RS Resp	Adrenaline, AH, steroids, β_2-agonist, oxygen	Hospital

CMF1 and CMF2 denote the respective CMF brands, as listed above. These abbreviations are also referred to as such in the main text.
AC, Allergic conjunctivitis; *AD*, atopic dermatitis; *AE*, angioedema; *AH*, antihistamines; *AR*, allergic rhinitis; *AS*, asthma; *F*, female; *GI*, vomiting; *GU*, generalized urticaria; *LU*, localized urticaria; *M*, male; *Resp*, wheeze, dry persistent cough, or dyspnea; *RS*, rhinorrhea and bouts of sneezing.
*The patient underwent an oral provocation challenge with CMF2 in the hospital and had similar respiratory-related symptoms.

a type 1 hypersensitivity reaction. The threshold dose resulting in the reaction was relatively high. Although it was reported to be as low as 40 mL, 3 of our patients consumed 250 mL of CMF containing scGOS on first exposure. They sought medical treatment after their first known exposure of CMF containing scGOS (3 subjects with CMF1 containing both scGOS and lcFOS and 2 subjects with CMF2 containing only scGOS). They had angioedema, profuse rhinorrhea, and wheezing within 1 hour of exposure. Four of the 5 patients were admitted for inpatient hospitalization because of oxygen desaturation and wheezing. Patient 1 underwent an oral challenge with the CMF2, which elicited the initial reaction. This challenge resulted in a similar event of desaturation to 88%, cyanosis, and wheezing within 20 minutes of ingestion of the CMF2. Interestingly, patient 4 had previously taken the earlier stages (steps 1, 2, and 3, which are appropriate for progressive age groups) of the same brand of CMF, Mamil (without supplemented scGOS), uneventfully. She then had acute symptoms on her first exposure to Mamil Step 4 (CMF1) in 2007 when scGOS-supplemented formula was introduced in Singapore.

SPTs

SPT responses are summarized in Table II. SPT responses were positive to both CMF1 and CMF2 containing scGOS, except for patient 3, who had a negative response to CMF1 with prebiotics and scGOS at 7.2 mg/mL (the concentration found in the milk formula). However, a positive skin test response to scGOS was only obtained after doubling the dose of scGOS to 14.4 mg/mL. Responses to lcFOS and CMF1 and CMF2 without scGOS supplementation were negative in all patients. SPT responses to scGOS fractions were negative to DP2 in all patients but positive for all other fractions (ie, DP3, DP4, DP5, and DP6). Two patients (patients 3 and 4) had negative SPT responses to scGOS at the lower dilution but positive responses at the higher concentration (Table II). SPT responses to this panel of extracts were negative in a healthy atopic adult volunteer. In a control population SPT responses to scGOS (7.2 and 14.4 mg/mL) were also found to be negative in 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.

BATs

After incubation with milk formula CMF1 supplemented with prebiotics (scGOS and lcFOS), all patients' basophils showed clear dose-dependent upregulation of CD203c. An average 6-fold increment in the mean fluorescence intensity of CD203c was induced by using CMF1 and CMF2 containing 10 μg/mL scGOS. Similar results were obtained by using the standard scGOS and low-protein scGOS. BAT results were negative to lcFOS and CMF1 without prebiotic supplementation (Fig 1, *A*). The scGOS fractions were used to determine the minimum length of scGOS in the induction of basophil activation. Results showed that DP3, DP4, DP5, and DP6, but not DP2, induced the upregulation of CD203c (Fig 1, *B*). The BAT was conducted in the plasma-free condition to determine whether any plasma protein was required to induce basophil activation by scGOS. Results showed that patients' basophils reacted to DP3, DP4, DP5, and DP6 in a dose-dependent manner (Fig 1, *C*). Furthermore, scGOS did not induce the upregulation of CD203c on the basophils of 1 healthy atopic adult and 8 scGOS-tolerant child control subjects (data not shown). Similar

TABLE II. SPT responses

	Patient no.				
	1	2	3	4	5
CMF					
Positive histamine	7 × 5	10 × 7	5 × 5	6 × 6	9 × 7
Negative control	0	0	0	0	0
Cow's milk	0	0	0	0	0
CMF1 with prebiotics scGOS + lcFOS	7 × 6	3 × 3	1 × 1	4 × 4	6 × 5
CMF1 without prebiotics	0	0	0	0	0
CMF2 with prebiotic scGOS	7 × 6	5 × 3	5 × 5	6 × 6	14 × 10
Standard Vivinal GOS, low-protein GOS and fractionated scGOS, DP2, DP3, DP4, DP5, and DP6 (numerals refer to wheal diameters)					
Positive histamine	6 × 5	5 × 5	5 × 5	6 × 6	9 × 7
Negative control	0	0	0	0	0
Standard Vivinal GOS (7.2 mg/mL)	3 × 3	7 × 5	0	5 × 5	3 × 3
Low-protein GOS (7.2 mg/mL)	6 × 5	7 × 6	0	3 × 3	0
Low-protein GOS (14.4 mg/mL)	ND	ND	4 × 4	ND	0
DP2 dilution (3.6 mg/mL)	0	0	0	0	0
DP2 dilution (7.2 mg/mL)	0	0	0	0	0
DP3 dilution (3.6 mg/mL)	3 × 3	5 × 5	4 × 4	3 × 3	3 × 3
DP4 dilution (3.6 mg/mL)	4 × 5	13 × 5	4 × 4	4 × 4	5 × 5
DP5 dilution (3.6 mg/mL)	3 × 3	4 × 4	4 × 4	5 × 5	6 × 5
DP6 dilution (3.6 mg/mL)	3 × 4	4 × 4	5 × 5	0	5 × 4
DP6 Dilution (7.2 mg/mL)	ND	ND	ND	5 × 5	ND

GOS, Galacto-oligosaccharide; *ND*, not done.

results were also obtained by analyzing the CD63 marker (data not shown).

Basophils of control subjects were passively sensitized with sera from 3 of our 5 patients to further examine IgE dependency of the oligosaccharide-induced basophil degranulation. As shown in Fig 2, *A*, there was a clear basophil degranulation of basophils sensitized with sera from patients 1 and 2, but this was not seen in the patient 3 or in the control subject before passive sensitization. Passively sensitized basophils were preincubated with the phosphatidylinositol 3-kinase inhibitor wortmannin to further determine the role of IgE-mediated activation through the oligosaccharides.[12] As can be seen in Fig 2, *B*, low-protein scGOS and oligosaccharide (DP6)–induced basophil degranulation by the sera of patients 1 and 2 was diminished in the presence of wortmannin, indicating that the effect was IgE mediated. As a control, anti-IgE–induced degranulation of the basophils was blocked in the presence of wortmannin, whereas N-formyl-methionyl-leucyl-phenylalanine–induced basophil degranulation was not affected (Fig 2, *B*).

DISCUSSION

This study describes 5 cases of acute anaphylactic reactions triggered by the unusual allergen scGOS. Our data indicate that the acute anaphylactic reactions seen in our patients represented an IgE-mediated immediate allergy and that the allergen trigger was scGOS or, more specifically, galacto-oligosaccharides of 3 sugar units or greater.

The clinical picture was similar in all our patients in that they were cow's milk tolerant and had acute allergic symptoms with wheezing within half an hour after consumption of children's CMFs containing prebiotics (Table I). The volumes of milk that were needed to elicit these symptoms in our subjects were between 40 and 250 mL, which is much higher than those reported for patients with IgE-mediated cow's milk protein allergy, in whom the threshold median dose at challenge to elicit symptoms was 40 mg, which is equivalent to approximately 2 mL of milk.[13] In our first patient oral challenge testing to the formula confirmed the allergy. This patient was the catalyst to initiate further investigations to determine the cause of these anaphylactic events in which the predominant respiratory symptoms were uniform in all cases presented.

Our investigations point to an IgE-mediated mechanism with positive SPT responses to scGOS, but not lcFOS, which is the other prebiotic present in the CMF1 formula (Table II). Additionally, the SPT responses were negative to the same formula without scGOS supplementation. *In vitro* BAT results[14] support the SPT responses (Fig 1). Activation of both of the basophil activation markers CD63 and CD203c showed a dose-dependent response to the milk formula supplemented with scGOS and scGOS alone but not lcFOS. Additionally, no basophil activation was seen when using basophils from a healthy adult volunteer and 8 children who were consuming scGOS-supplemented CMF on a regular basis. As further substantiation of the role of scGOS as the allergen, basophils of a healthy control subject passively sensitized with IgE from our subjects also demonstrated basophil activation in 2 of the 3 patient's sera tested. This reaction involved the phosphatidylinositol 3-kinase activation pathway. The failure of the sera of patient 3 to activate passively sensitized basophils could be due to a relatively lower titer of IgE specific to scGOS. This is supported by the weaker SPT responses, and a positive test response was only elicited by using a double concentration of scGOS (Table II). Taken together, our data strongly support the notion that scGOS was indeed the allergen.

scGOS (FrieslandCampina Domo) supplemented to the milk formulas CMF1 and CMF2, which triggered these allergic reactions in our patients, is prepared with a β-galactosidase derived from *Bacillus circulans* and is a well-characterized mixture of more than 50 different oligosaccharides.[15] These oligosaccharides are composed of 1 to 7 galactose units linked to a glucose molecule in proportion of DPs as follows: DP5 or greater, 6.6%; DP4, 10.8%; DP3, 22.1%; DP2, 37.4%; and DP1, 22.5%.[9] The

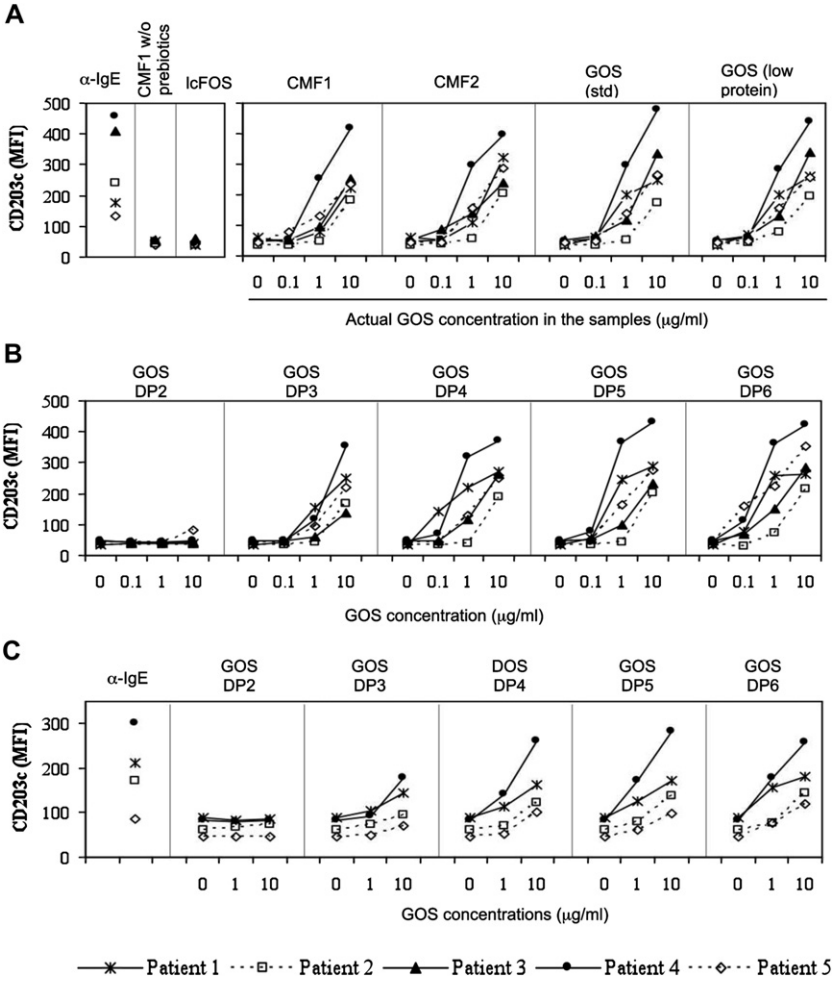


FIG 1. Dose-dependent effect of scGOS on basophil activation. Expression of CD203c on IgE^{high} cells was analyzed by using flow cytometry. **A,** Anti-IgE (1 μg/mL), lcFOS (10 μg/mL), CMF1 without prebiotics *(CMF1 w/o prebiotics)*, indicated doses of CMFs, and scGOSs. **B,** scGOS fractions DP2, DP3, DP4, DP5, and DP6. **C,** Patients' blood was washed with Dulbecco PBS to remove the plasma. The cells were then incubated with scGOS fractions DP2, DP3, DP4, DP5, and DP6 and anti-IgE for 15 minutes at 37°C. *MFI*, Mean fluorescence intensity.

β-glycosidic linkage between the monomer units is mainly the linear linkage 1→4 Gal, although other β-glycosidic linkages, among which are found branched linkages, can also include the following: 1→6 Gal, 1 →2 Glc, 1→3 Glc, 1→4 Glc, 1→6 Glc, 1→2 Gal, and 1→3 Gal.[9] We also investigated the component or components in the scGOS mixture binding capacity to IgE. To this end, the scGOS mixture was fractionated by DP. SPTs showed positive responses from DP3 onward, which was supported by *in vitro* BATs in which DP3 and higher showed the presence of basophil activation markers. The basophil activation appeared to plateau from DP4 and upward.

The possibility that traces of contaminating protein derived from *B circulans*, which was used in the scGOS manufacturing process, could be the allergen was considered and excluded because the protein concentration of the scGOS mixture and scGOS fractions were either very low (about 50 ng of protein per 1 mg of scGOS) or not detectable. Furthermore, in subjects 3, 4, and 5, purified enzyme β-galactosidase obtained from the manufacturer showed absence of sensitization by SPTs and BATs, both with basophils obtained from the patient (subject 5), as well as passively sensitized control basophils (subjects 1 and 2, data not shown). Furthermore, IgE immunoblots and ELISA with the plasma of 5 patients did not show the presence of β-galactosidase IgE antibodies (data not shown). Taken together, our investigations strongly support scGOS as the eliciting allergen and substantiate our notion that a pure low-molecular-weight carbohydrate could cross-link IgE on mast cells and trigger clinical anaphylaxis.

scGOS is considered a functional food and has been widely used in Japan since the 1980s and has been a supplement in CMF in Europe since 2000.[16] The product Vivinal scGOS, which is the scGOS used in formula in this study, received "generally regarded as safe"[17] status from the US Food and Drug Administration in September 2008. Despite the availability of scGOS in Japan and Europe for more than 2 decades, acute reactions to scGOS-containing products have not been reported. In Singapore scGOS has been available in infant milk and follow-on formula for children up to the age of 10 years since 2007. It is a cultural practice that children in Singapore and much of southeast Asia consume cow's milk as CMF rather than pasteurized milk, even beyond 6 years of age.

Even though we have established scGOS as the allergen triggering anaphylaxis in our patients, it is possible that a primary

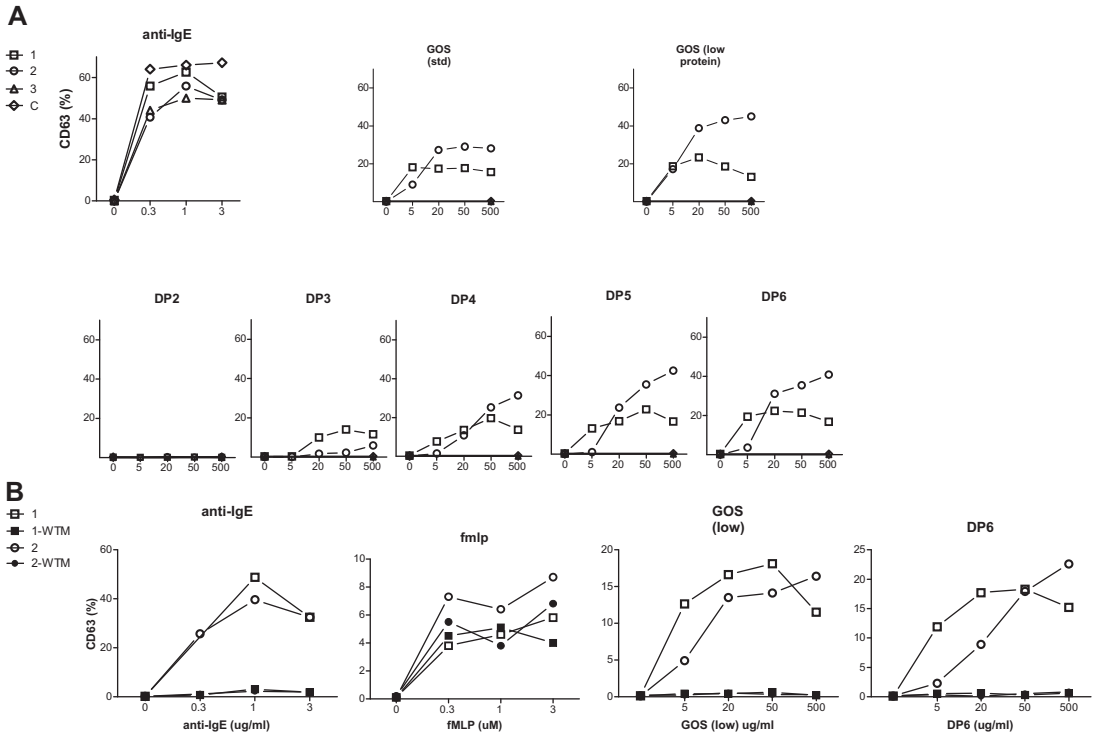


FIG 2. Dose-dependent effect of scGOS fractions on activation of *in vitro*–sensitized control basophils. Basophil activation was analyzed by using flow cytometry and is expressed as the percentage of $CD63^+$ cells within the $CD203c/CD123^+$ cells. **A,** Anti-IgE, standard scGOS, low-protein scGOS, and the scGOS fractions DP2, DP3, DP4, DP5, and DP6 at indicated doses were tested with sera from patients 1, 2, and 3 and a healthy control subject *(C)*. **B,** Inhibition of scGOS-induced basophil degranulation by the phosphatidylinositol 3-kinase inhibitor wortmannin. Control PBMCs were incubated with sera from patients 1 and 2. After 5 minutes of preincubation with 300 nmol/L wortmannin at 37°C, basophils were stimulated for 30 minutes at 37°C with anti-IgE, N-Formyl-L-methionyl-L-leucyl-L-phenylalanine *(fMLP)*, low-protein scGOS, and the scGOS fraction DP6 at indicated doses. *GOS*, Galacto-oligosaccharide.

sensitizer might be an agent specific only to the southeast Asian region. First, there is a very recent report of acute allergic reactions after the ingestion of new milk products containing scGOS in 16 Vietnamese children aged 2 to 15 years.[18] The authors suspected that the trigger was the supplemented scGOS because these children had no prior history of cow's milk allergy,[18] but no further investigations were made to confirm their suspicion. It is plausible that the Vietnamese subjects were allergic to scGOS because they presented in a very similar fashion as our patients from Singapore.

Second, there have been reports in the Japanese literature of an uncommon allergy in Japanese oyster shuckers. These workers had allergic asthma triggered by means of inhalation of Hoya (sea squirts that grow on oyster shells) antigen and then subsequently also acquired acute allergy to an scGOS-containing lactic acid beverage.[19] The authors concluded that cross-reactive carbohydrate epitopes in the Hoya (sea squirt) antigen were the primary sensitizer, even though their sugar moieties are quite different from those of scGOS.[20]

These observations are also reminiscent of the studies relating to the carbohydrate moiety α-gal as the IgE-binding epitope in patients with anaphylaxis to the mAb cetuximab and delayed acute allergic reactions to red meat.[21] These reactions were restricted to a geographic region of the southeastern United States. Although the primary sensitizing agent has not yet been identified, a possible link to tick bites as the primary sensitizing agent has been suggested. In this region bites from dog ticks, seed ticks, and "chiggers" (the larval form of harvest mites) are very common.[22] Of note is the relative older age of our patients (children ≥5 years of age and 1 adult), even though the exposure to these scGOS-containing CMFs occurs mainly in infants and young children. It is possible that the primary sensitizer in our cases might be an aeroallergen, and this explains the relatively later age of onset. Our literature search and personal communication with Professor Hans Kamerling, an expert on oligosaccharide structures (Chemical Glycobiology, University of Groningen, The Netherlands) did not reveal known naturally occurring aeroallergens containing the β-gal linkages similar to scGOS. Furthermore, an oligosaccharide mixture, such as scGOS, is also not known to occur naturally in other foods. Only 3 components present in scGOS occur in human breast milk: lactose, 4′-galactosyl-lactose, and 6′galactosyl-lactose.[23]

Rare cases of anaphylaxis have also been reported against inulin (fructosyl oligo-saccharides and polysaccharides), although the reactivity was most likely against inulin bound to proteins.[24,25] Additionally, IgE-mediated reactions to erythritol, a 4-carbon sugar prepared from glucose by means of fermentation and naturally occurring in certain foods (mushroom, watermelon, pear, grape, wine, beer, soy sauce, and cheese), have been described in Japanese patients, and the authors suggested that this sugar molecule might function as a hapten, resulting in acute allergy.[26] The possibility that low-molecular-weight scGOS could

act as a hapten[27] was also considered. However, our BAT experiments performed in a protein-free system showed scGOS is still able to induce basophil activation, although the magnitude of CD203c upregulation was only slightly reduced compared with the reaction performed using whole blood. It was also shown that the anti-IgE positive control did not require the presence of serum protein for IgE cross-linking. Therefore our results suggested that prior protein binding to scGOS was not necessary.

In conclusion, a series of rare and unusual cases of IgE-mediated allergy occurring in southeast Asia and resulting from consumption of milk formula specifically supplemented with the prebiotic scGOS has occurred, posing a concern for these subjects because scGOS is a widely used functional food. Our investigations show that the allergen was the low-molecular-weight prebiotic scGOS, although primary sensitization is possibly induced by another allergen. scGOS is widely available in Japan and Europe and has been shown to have very good safety records. Further research is needed to identify the causative sensitizing agent and the allergenic structure or structures in scGOS.

Clinical implications: This study shows that the prebiotic scGOS, a mixture of low-molecular-weight oligosaccharides, could trigger IgE-mediated anaphylaxis in atopic subjects. Further research is needed to identify the causative sensitizing agent and the allergenic structure or structures in scGOS.

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