

epinephrine. In addition, peanut-tolerant children often experience failure of peanut introduction into the diet 3 years after a negative DBPCFC result. Our results indicate that HRQL improves in peanut-tolerant children after successful peanut introduction and not after failed peanut introduction or in children with peanut allergy.

The high rate of accidental allergic reactions despite the explicit advice to avoid peanut and inappropriate management despite instructions on how and when to use epinephrine, as seen in this study, is of clinical concern. A recent analysis of the European Anaphylaxis Register demonstrated that more than 85% of patients with severe anaphylaxis did not use their epinephrine autoinjectors, despite clear recommendations.⁷ Children with peanut allergy and their families might benefit from multidisciplinary allergy care, including regular clinical consultations with an experienced physician and dietitian to evaluate any accidental ingestion of peanut and continuing education on peanut avoidance and use of emergency medications.⁸

Almost one third of peanut-tolerant children did not introduce peanut, despite guidance after the DBPCFC. The importance of peanut introduction in peanut-tolerant children is supported by our results that indicate that HRQL improved only in peanut-tolerant children after successful peanut introduction and remained unchanged after failed peanut introduction. Further research and discussion are needed to investigate whether food aversion and anxiety, which were the main reason not to introduce, can be relieved when psychological guidance is provided.

Our study is limited by the relatively small sample size and the possibility of recall bias over a 3-year time period. The study is strengthened by a prospective study design, including a well-defined patient cohort of children who all underwent a DBPCFC for peanut and a high response rate after 3 years of follow-up.

In conclusion, despite adequate diagnostic procedures and guidance, living with peanut allergy remains a challenge during childhood. In peanut-tolerant children peanut introduction often fails. However, peanut introduction is meaningful to improve HRQL.

We thank Coco Dekkers, Department of Dermatology/Allergology, University Medical Center Utrecht, Utrecht, The Netherlands, for performing the data analysis of HRQL.

Hannah M. Kansen, MD^{a,b}

Thuy-My Le, MD, PhD^b

André C. Knulst, MD, PhD^b

Diana M. W. Gorissen, MD^c

Cornelis K. van der Ent, MD, PhD^d

Yolanda Meijer, MD^d

Francine C. van Erp, MD, PhD^b

From ^athe Department of Pediatric Pulmonology and Allergology, Wilhelmina Children's Hospital, Utrecht University, and ^bthe Department of Dermatology/Allergology, University Medical Center Utrecht, Utrecht University, Utrecht, The Netherlands, and ^cthe Department of Pediatrics, Deventer Hospital, Deventer, The Netherlands. E-mail: h.m.kansen-2@umcutrecht.nl.

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

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Available online September 27, 2019.
<https://doi.org/10.1016/j.jaci.2019.09.011>

Allergy to prebiotic galacto-oligosaccharides: House dust mites—the putative primary sensitizer



To the Editor:

Galacto-oligosaccharides (GOS) have been increasingly used globally to supplement commercially available milk formulations and dairy products for their health benefits.¹ Intriguingly, when GOS were introduced into Southeast Asian countries as an ingredient in milk formula, a few acute allergic reactions and anaphylactic responses to GOS were promptly observed.²⁻⁴ Anaphylaxis to GOS occurred on first consumption in all our patients with allergic reactions, which was an indication of the presence of pre-existing GOS sensitization. GOS allergy is unique because the allergen is a pure carbohydrate. Vivinal GOS (vGOS) (FrieslandCampina, Amersfoort, The Netherlands) is produced with a β -galactosidase enzyme derived from *Bacillus circulans*. The composition of GOS has been shown to contain mostly β -1,4 linkages between glucose and galactose or between galactoses.⁵ However, β -1,6 and β -1,3 linkages are also found in vGOS, although to a lesser extent. GOS does not contain any fucose or sialic acid moieties. In view of the geographic restriction of GOS allergy to Southeast Asian countries when GOS is used to supplement milk formulas globally, we postulated that GOS allergy might be a result of sensitization to an agent that is likely geographically confined to Southeast Asia. We sought to identify the primary sensitizer for GOS allergy.

The clinical symptoms/reactions, skin prick test responses, and basophil activation test results to vGOS in 33 patients with GOS allergy, including 23 subjects who reacted to vGOS after consumption of vGOS-containing milk or water and 10 GOS-sensitized subjects who did not consume GOS before testing, are shown in Table I. Sera from these subjects were tested to determine the sensitization profile to 54 allergens (see Table E1 in this article's Online Repository at www.jacionline.org for the list of allergens) by using the EUROLINE multiplex immunoblot assay (EUROIMMUNE, Lübeck, Germany) and to *Curvularia lunata* and *Aedes communis* by using the

TABLE I. Clinical and immunologic features of GOS-sensitized subjects

Patient no.*	Age of onset (years)	Dose eliciting reaction (g)	Symptoms	SPT to vGOS (mm)	BAT threshold dose† (μg/mL)	Specific IgE‡				
						GOS (ng/mL)	<i>B tropicalis</i> (kU/L)	<i>D pteronyssinus</i> (kU/L)	Cow's milk (kU/L)	Shellfish mix (kU/L)
1	6	0.2	RS, Resp	3 × 3	0.1	103.6	94	100	1.7	<0.35
2	7	1.9	AE, RS, Resp	7 × 6	1	41.5	66	90	1.7	0.35
3 (1)	38	1.5	AE, RS, Resp	5 × 4	10	2.5	29	1.4	<0.35	<0.35
4	6	1.9	AE, GU, GI, RS, Resp	5 × 5	1	<1.14	100	87	0.52	18
5	8	0.4	AE, GU, RS, Resp	3 × 3	1	<1.14	86	45	0.35	17.4
6	2	1.1	AE, GU, RS, Resp	5 × 4	1	<1.14	0.43	0.7	5.5	0.35
7	30	2	AE, LU	ND	ND	<1.14	2.7	<0.35	0.43	62
8	6	1.9	AE, GU, GI, Resp	4 × 4	1	9.4	18	<0.35	1	<0.35
9	3	1.6	AE, LU, RS, Resp	3 × 7	1	<1.14	5.5	9.5	11.4	68
10	40	Unknown	AE, GU, RS, Resp	5 × 6	1	<1.14	92	5.5	<0.35	<0.35
11	32	1.5	AE, RS, Resp	5 × 5	1	1.8	0.6	0.43	<0.35	1.4
12	29	1.9	AE, GU, RS	5 × 5	0.1	<1.14	1.7	0.35	<0.35	<0.35
13	33	1.5-1.9	AE, RS, Resp	5 × 5	10	1.2	1	0.43	<0.35	20
14	26	0.7	AE, RS, Resp	5 × 5	10	2.1	40	37	0.43	<0.35
15	10	0.9	AE, RS, Resp	5 × 5	ND	1.5	8.5	7.5	0.43	27
16	13	1.5	AE, LU, GI, Resp	10 × 8	0.1	<1.14	2.4	72	0.5	1
17	2	0.5	AE, LU, RS, Resp	8 × 6	10	1.7	16.4	59	1	3.4
19	37	2	RS, Resp	6 × 6	10	<1.14	7.5	12.4	<0.35	<0.35
18	30	2	AE, GU, RS	4 × 5	1	<1.14	81	2.4	0.35	<0.35
20 (2)	40	2	AE, RS	5 × 6	10	1.8	61	5.5	<0.35	0.43
21 (3)	27	0.6	AE, RS, Resp	8 × 5	0.1	1.6	40	2.4	<0.35	0.35
22 (4)	43	2	Resp	5 × 8	>1000§	2.7	42	2.4	<0.35	<0.35
23	22	4	LU, GI, RS	3 × 3	1	1.3	84	48	<0.35	<0.35
24	NA	NA	NC	3 × 3	500	1.3	72	57	0.35	<0.35
25	NA	NA	NC	3 × 3	10	<1.14	81	36	0.43	<0.35
26	NA	NA	NC	6 × 5	1	1.4	53	45	2.7	6.5
27	NA	NA	NC	4 × 4	1	1.5	100	92	<0.35	<0.35
28	NA	NA	NC	8 × 5#	>1000§	<1.14	59	100	0.43	<0.35
29	NA	NA	NC	5 × 4	10	<1.14	0.6	5.5	0.43	<0.35
30	NA	NA	NC	3 × 3	10	<1.14	4.5	74	<0.35	0.43
31 (5)	NA	NA	NC	4 × 3	1	12.9	70	35	<0.35	0.52
32	NA	NA	NC	4 × 6	10	84.1	85	95	0.35	15.4
33	NA	NA	NC	5 × 5	10	1.14	13.4	29	<0.35	<0.35

AE, Angioedema; BAT, basophil activation test; GI, vomiting; GU, generalized urticaria; LU, localized urticaria; NA, not applicable; NC, not challenged; ND, not done; Resp, wheeze, dry persistent cough, or dyspnea; RS, rhinorrhea and bouts of sneezing; SPT, skin prick test.

*Subjects 1 to 17 had GOS allergy, subjects 19 to 23 had allergic symptoms on vGOS oral challenge, and subjects 24 to 33 were sensitized to GOS but did not undergo oral GOS challenge. The 5 subjects described in Fig 1 are indicated inside parentheses.

†The lowest dose that induced both upregulation of CD203c and expression of CD63 on basophils was used.

‡GOS-specific IgE levels were determined by means of ELISA. An sIgE level of 1.14 ng/mL or greater was considered positive. Levels of *B tropicalis*- and *D pteronyssinus*-specific IgE were determined by using EUROLINE. An sIgE level of 0.35 kU/L or greater was considered positive.

§The subject had a negative BAT response to the highest dose (1000 μg/mL) of GOS used in the BAT assay.

#Results of SPT to 14.4 mg/mL of vGOS.

ImmunoCAP assay (Phadia, Uppsala, Sweden). This study was approved by the ethics review boards of both National University Hospital and Kandang Kerbau Women's and Children's Hospital, and written informed consent was obtained from all participants.

Of the 56 allergens tested, the common group of allergens to which all patients with GOS allergy were sensitized was dust mites. All patients with GOS allergy had specific IgE to *Blomia tropicalis*, *Dermatophagoides farinae*, and *Dermatophagoides microceras*. The sensitization rates to *Tyrophagus putrescentiae* and *Dermatophagoides pteronyssinus* were 97% and 94%, respectively, in patients with GOS allergy. The sensitization rates to *D pteronyssinus*, *D farinae*, *D microceras*, and *T putrescentiae* ranged from 79% to 83% in atopic control subjects. Atopic control subjects were atopic subjects (n = 24) with a positive

skin prick test response to *B tropicalis* but not to GOS. Sensitization rates to the rest of allergens were all less than 50%, except for cow's milk and shellfish mix 1, with approximately 55% of both patients with GOS allergy and control subjects reacting to these allergens.

B tropicalis and *D pteronyssinus* are 2 common mite species found in Singapore. Five serum samples with high levels of GOS-specific IgE (Table I and see Fig E1 in this article's Online Repository at www.jacionline.org) and available serum were selected for determining the IgE cross-reactivity between GOS and *B tropicalis*/*D pteronyssinus* by using a competitive ELISA. A control extract, *Curvularia* species, which showed low IgE sensitization rates (3%) in patients with GOS allergy, was included. Both dust mite extracts inhibited GOS-specific IgE in a dose-dependent manner for all 5 subjects except for one subject

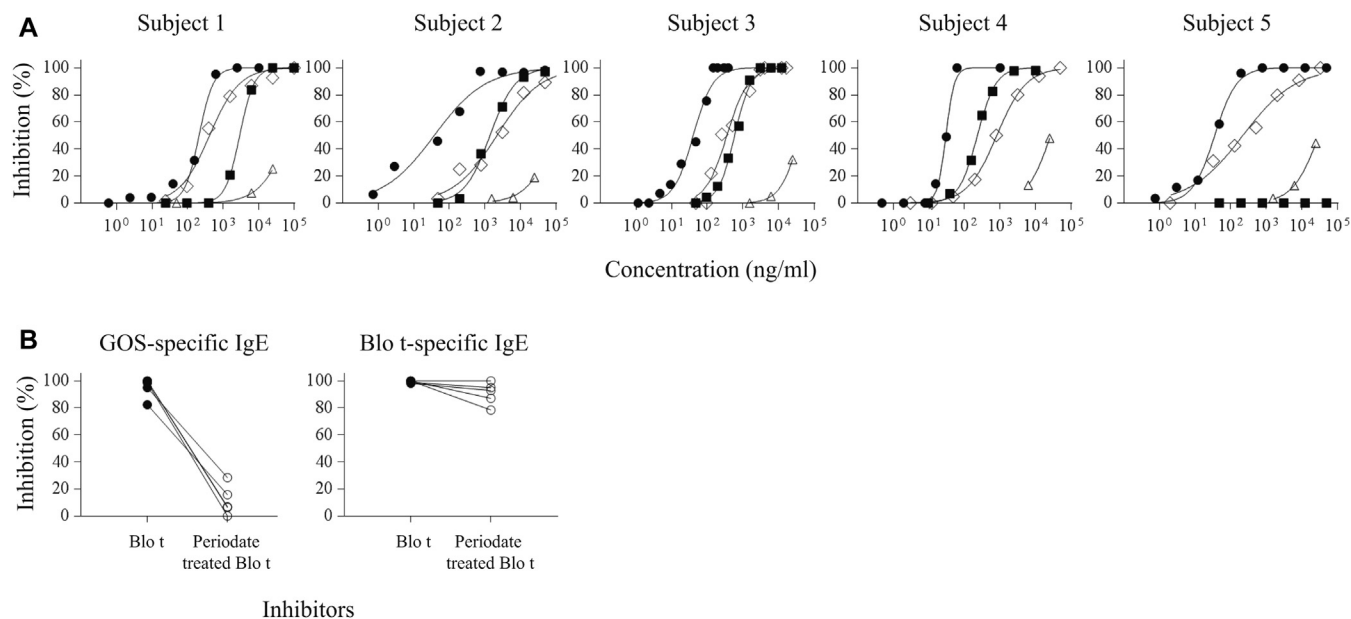


FIG 1. Competitive ELISA of GOS-specific IgE. **A**, Sera of 5 patients with GOS allergy were preabsorbed with various concentrations of *B tropicalis* (●) or *D pteronyssinus* (■) extract, GOS (◇), or *Curvularia* extract (Δ), as indicated. The level of GOS-specific IgE was detected. **B**, Sera ($n = 5$) were preabsorbed with *B tropicalis* extract or sodium periodate-treated *B tropicalis* extract. Levels of GOS-specific and *B tropicalis*-specific IgE were detected. Results are presented as the percentage of inhibition of GOS-specific IgE (left) or *B tropicalis*-specific IgE (right) compared with that seen in sera without preabsorption.

in whom there was no inhibition observed with *D pteronyssinus* extract (Fig 1, A). *B tropicalis* extract was a very potent inhibitor because the concentration of *B tropicalis* extract needed to inhibit 50% of GOS-specific IgE (IC₅₀) was 11 times less than that of GOS itself (86 and 954 ng/mL, average IC₅₀ values of 5 subjects for *B tropicalis* and GOS, respectively). The IC₅₀ for *D pteronyssinus* extract (1032 ng/mL, average IC₅₀ values of 4 subjects) was comparable with that for GOS. On the other hand, *Curvularia* species extract showed minor ability (approximately 40%) to inhibit GOS-specific IgE only at high concentrations (30 μ g/mL; Fig 1). IgE cross-reactivity between mite culture medium and GOS was measured. The results of preliminary experiments make it unlikely that the mite culture medium contributes to GOS cross-reactivity of *Blomia* species extract (data not shown). *B tropicalis* instead of *D pteronyssinus* was selected as the most relevant allergen for further evaluation of IgE cross-reactivity with GOS. This mite is highly prevalent in tropical regions and could explain the geographic restriction of GOS allergy to Southeast Asia.

Another reason that *Dermatophagoides* species might not be the primary sensitizer for GOS allergy is the global importance of *D pteronyssinus* and *D farinae* allergens. *Dermatophagoides* species sensitization is common in Europe, where GOS-supplemented formula and dairy products have been widely available in the market for years, and yet there have not been any reports of GOS-related allergy in that region.

Several dust mite allergens were shown to be glycosylated.^{6,7} GOS and *B tropicalis* extracts were subjected to deglycosylation by means of periodate oxidation. The epitope for GOS-specific IgE was sensitive to periodate oxidation because periodate-treated GOS lost its ability to inhibit GOS-specific IgE (see Fig E2, A, in this article's Online Repository at

www.jacionline.org). There was a decrease in GOS-specific IgE inhibition from 80% to 100% in untreated extracts to 0% to 30% in periodate-treated, deglycosylated *B tropicalis* extracts (Fig 1, B). Periodate treatment did not significantly affect the protein-specific IgE epitopes. Periodate-treated *B tropicalis* extract was still able to inhibit more than 75% of *B tropicalis*-specific IgE (Fig 1, B). We went on to validate our findings by treating *B tropicalis* extract with Proteinase K (New England Biolabs, Ipswich, Mass) and trypsin. The proteolysed *B tropicalis* extract showed 50% to 95% reduction in its ability to inhibit *B tropicalis*-specific IgE. In contrast, proteolysed *B tropicalis* extract still retained its ability to inhibit GOS-specific IgE, which was comparable with the untreated *B tropicalis* extract (>97%; see Fig E2, B). Taken together, these results indicate that protein-specific IgE epitopes in the *B tropicalis* extract do not play a role in inhibition of GOS-specific IgE. The carbohydrate moieties of the *B tropicalis* allergens were responsible for the IgE cross-reactivity between GOS and *B tropicalis*.

The presence of a primary sensitizer for carbohydrate-induced anaphylaxis has been demonstrated in 2 other studies: (1) α -gal allergy, in which the deer tick was a primary sensitizer in the United States,⁸ and (2) allergy to GOS-supplemented beverages in a group of oyster shuckers having sea squirt allergy in Japan.⁹ Antibody cross-reactivity between the crude extract of sea squirt body fluid and the GOS was observed. In this study we have identified *B tropicalis* as a potential primary sensitizer for allergy to GOS in Singaporean subjects and determined that the cross-reactive IgE epitope or epitopes are carbohydrates and not proteins. Based on our small study, this is the first time that *B tropicalis* contains glycoallergens that have IgE cross-reactive epitopes specific to GOS.

We thank Ms Corinne Kwek for her assistance with the subjects, the subjects for their participation, and Linqiu Cao and Mireille Gadella (FrieslandCampina, Amersfoort, The Netherlands) for providing the enriched DP4-GOS sample.

Li Yuan Gabriella Nadine Lee, BSc (Hons)^{a,b}

Youjia Zhong, MCI^b

Si Yuan Leow^a

Shan Chun Lim^a

Hongmei Wen^{a,b}

Jian Yi Soh, MMed^{a,b}

Wen Chin Chiang, MRCPCH^c

Dianne J. Delsing, PhD^d

Bee Wah Lee, MD^{a,b}

Chiung-Hui Huang, PhD^{a,b}

From ^athe Department of Paediatrics, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; ^bKhoo Teck Puat-National University Children's Medical Institute, National University Health System, Singapore; ^cthe Department of Paediatrics, Kandang Kerbau Women's and Children's Hospital, Singapore; and ^dFrieslandCampina, Amersfoort, The Netherlands. E-mail: paechh@nus.edu.sg.

This work was supported by the National Medical Research Council (grants NMRC/CIRG/1368/2013 and NMRC/CIRG/1487/2018).

Disclosure of potential conflict of interest: D. J. Delsing is employed by FrieslandCampina, Amersfoort, The Netherlands. The rest of the authors declare that they have no relevant conflicts of interest.

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Available online October 15, 2019.

<https://doi.org/10.1016/j.jaci.2019.10.003>

IgG Fc glycosylation as an axis of humoral immunity in childhood



To the Editor:

In children, antibody levels and repertoire diversity mature with age. A further determinant of humoral immunocompetence, still poorly characterized in the pediatric population, is IgG Fc glycosylation. Paired biantennary glycans at asparagine 297 in each heavy chain modulate the ability of IgG to bind complement, Fc γ receptors, and other ligands. The availability of more than 30 glycoforms provides the opportunity to “fine-tune” IgG effector capacity.¹ Variation in IgG Fc glycan use with

age is recognized, but small sample sizes and contrasting reports leave the pediatric end of this spectrum poorly defined.²⁻⁶ We sought to test the hypothesis that IgG Fc glycans vary predictably with age in childhood and to explore the possibility that altered IgG Fc glycosylation might contribute to otherwise unexplained respiratory tract infections in children.

We obtained blood samples from 267 children aged 9 months to 18 years: 145 healthy children from Boston Children's Hospital, 82 healthy children from Erasmus Medical Center (Rotterdam, The Netherlands), and 40 children evaluated at the Immunology Clinic at Boston Children's Hospital for unexplained recurrent respiratory tract infections (RRI; see the Methods section in this article's Online Repository at www.jacionline.org for recruitment procedures). Unexplained RRIs were defined as recurrent infections of the middle ear, sinuses, and/or lung sufficient to trigger immunologic consultation during which the attending immunologist elected to perform quantitative assessment of humoral immunity but for which no cause was ultimately determined. Patients were excluded if RRIs were attributed to an environmental cause; a predisposing condition, such as cystic fibrosis; or a diagnosable immunodeficiency, such as hypogammaglobulinemia, IgG subclass deficiency, specific antibody deficiency, or a defect in complement or cellular immunity (see Table E1 in this article's Online Repository at www.jacionline.org). Patients with IgA levels of less than the lower limit of normal in the absence of other immune abnormalities were described as “low IgA” and evaluated as a distinct subgroup (see Table E2 in this article's Online Repository at www.jacionline.org). Subjects receiving immunoglobulin supplementation at any point before sampling were excluded.

IgG Fc glycans were quantitated using liquid chromatography and electrospray ionization mass spectrometry (LC-ESI-MS). Glycopeptide fragments from IgG₂ and IgG₃ are indistinguishable by this method and are reported together. Methods and statistical strategies are provided in the Methods section in this article's Online Repository.

Individual glycoforms, as well as summary measurements of galactosylated, sialylated, bisected, and fucosylated glycans, were plotted as continuous variables and by age group: 9 months to 2 years (n = 59), 2 to 5 years (n = 76), and 5 to 18 years (n = 92). Galactosylation exhibited age-dependent variability consistent across IgG subclasses (Fig 1, A, and see Figs E1-E4 in this article's Online Repository at www.jacionline.org). Digalactosylated (G2) forms were overrepresented in younger children, largely at the expense of monogalactosylated (G1) glycans. The agalactosylated (G0) fraction was highest in children aged 2 to 5 years. Younger children exhibited more sialylated and core-fucosylated glycans (Fig 1, A), which are typically considered anti-inflammatory, as well as alteration in glycans of unclear functional effect, including those featuring a bisecting N-acetylglucosamine or variant hybrid structures (IgG₁ is shown in Fig 1, A; Figs E1 and E4 show other isotypes). Thus, compared with older children, younger children exhibited a distinct pattern of IgG Fc glycosylation.

To test whether IgG Fc glycans varied in a regular manner, we assessed the ability of combinations of glycoforms to differentiate children by age. An elastic net multinomial classifier was trained in the Boston healthy cohort, yielding a classification accuracy of approximately 80% across age categories (Fig 1, B). Validation in the Dutch cohort confirmed

METHODS

Skin prick tests and basophil activation tests to GOS

Skin prick tests and basophil activation tests to vGOS were conducted, as described previously.^{E1} The wheal size for each allergen was recorded and used as the degree of skin test reactivity. One hundred microliters of blood was stimulated with up to 1000 µg/mL vGOS. The threshold dose was recorded as the lowest dose that triggered both the expression of CD63 and CD203c above the cutoff value of a positive basophil activation test result, as determined in our previous study.^{E2}

Screening for allergen sensitization profile

Levels of specific IgE to a panel of 54 allergens and cross-reactive carbohydrate determinants were measured by using the Multiplex Immunoblot (EUROLINE "Atopy Indonesia 1"; DP 3707-1601-1 E; EUROIMMUNE). Multiplex immunoblots was performed according to the manufacturer's instructions. Briefly, test strips were incubated with subjects' sera using the volume-optimized version, as described in the manufacturer's instructions. Results were analyzed with EUROLIneScan (EUROIMMUN) software. In addition, levels of specific IgE to *A. communis* and *C. lunata* were measured by using the ImmunoCAP assay, which was run with the Phadia 100 system (Phadia AB, Uppsala, Sweden). In both assays results were considered positive when the level of specific IgE to a particular allergen measured was greater than 0.35 kU/L.

Preparation of mites and crude fungal extracts

Cultured *D. pteronyssinus* was purchased from Mahidol University (Bangkok, Thailand). *B. tropicalis* mites were obtained in house, as described previously.^{E3} In brief, the *B. tropicalis* mite was cultured with TetraMin fish feed (Tetra, Blacksburg, Va). The mite culture was separated through a series of 500- and 125-µm sieves by using a mechanical sieve shaker in which vibration was applied for about 20 minutes to remove most of the culture media. Mites with sizes of greater than 120 µm were transferred to a modified Tullgren funnel, in which mites were harvested by using the heat-escape method, which collects living mites escaping from cultures exposed to low heat. A mixture of spores from *Curvularia* species was a kind gift from Associate Professor Chew Fook Tim (Department of Biological Science, National University of Singapore).

Mites and spores were kept at −80°C until use. Frozen mites were homogenized with a mortar and pestle in the presence of liquid nitrogen. PBS containing 0.1% aprotinin (Sigma-Aldrich, St Louis, Mo), 1 mmol/L phenylmethylsulfonyl fluoride (Sigma-Aldrich), 15 µmol/L *trans*-epoxysuccinyl-L-leucylamido(4-guanidino)butane (Sigma-Aldrich), and 10 µmol/L Pepstatin A (Sigma-Aldrich) was used for protein extraction at 4°C overnight. The supernatant was obtained after centrifugation at 14,000g for 10 minutes and kept at −80°C until use. The protein extract of *Curvularia* species was obtained by using a similar method, except the extraction buffer was changed to 12.5 mmol/L ammonium bicarbonate. After centrifugation, the supernatant was lyophilized and reconstituted in PBS. Total protein concentrations were determined by using a Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Hercules, Calif).

Generation and purification of the allergenic GOS-enriched fraction for ELISA

To synthesize a GOS-fraction enriched in allergenicity, an enzymatic reaction was started with 19% lactose wt/wt (Lactochem; FrieslandCampina, Amersfoort, The Netherlands) and a lactase (Lactoles L3; Amano, Yokohama, Japan) dosage of 50 LU/g lactose at 50°C for 24 hours while stirring with a magnet bar. The obtained GOS solution was loaded to an active carbon column (PN2 type; Norit NV, Amersfoort, The Netherlands) and subsequently eluted with 5 bed volumes of water to remove nonadsorbed components, 5 bed volumes of 5% ethanol aqueous solution (Sigma), and finally 5 bed volumes of 30% ethanol aqueous solution. After evaporation of water and ethanol from the 30% ethanol fraction, water was added to reach a concentration of greater

than 45% (wt/wt). Subsequently, this concentrated fraction was further refractioned by using a Bio-Gel Column (Bio-Rad Laboratories). The degree of polymerization 4 (DP4) fraction was collected, pooled, and further concentrated to approximately 20% solution (wt/wt) by using a Rotavap (Rotary vacuum evaporation; Salm en Kipp, Breukelen, The Netherlands). The sample was stored at −20°C until use. The dose required to induce basophil activation for this enriched DP4 fraction is 3 times lower than that of the DP4 of vGOS (data not shown).

Competitive IgE ELISA

The DP4-GOS fraction was conjugated to human serum albumin (HSA; Sigma-Aldrich) by means of the Maillard reaction to bind the enriched DP4-GOS fraction to the solid phase. In brief, the DP4-GOS fraction and HSA were dissolved in distilled water and then mixed together at a 1:1 (wt/wt) ratio. The mixture was lyophilized. The Maillard reaction was conducted by placing the lyophilized sample in an airtight container containing saturated sodium chloride solution to maintain relative humidity at about 75%. The container was put in an incubator at 50°C for 9 days. The sample (HSA-GOS) was then reconstituted in PBS.

Specific IgE to GOS was measured by means of ELISA. All the ELISA procedures with 25 µL/well were carried out at room temperature for 1 hour unless specified. Half-area ELISA plates (Corning, Corning, NY) were coated with 5 µg/mL HSA-GOS extract in 0.1 mol/L NaHCO₃, pH 8.3. After washing with Tris-buffered saline containing 0.05% Tween-20 (TBS-T; Sigma-Aldrich), plates were blocked with 100 µL of 1% BSA (Sigma-Aldrich) in TBS-T.

Sera were incubated overnight with serially diluted *B. tropicalis*, *D. pteronyssinus*, and *Curvularia* species extracts or GOS together with 50 µg/mL HSA at 4°C. Preabsorbed sera were incubated overnight with HSA-GOS-coated wells in duplicates at 4°C. Plates were washed and incubated with horseradish peroxidase-conjugated anti-human IgE (B3102E8; Southern Biotechnology, Birmingham, Ala) at room temperature for 1 hour. Signals were developed with 3, 3', 5, 5'-tetramethylbenzidine (Invitrogen, Vienna, Austria), and reactions were stopped with the addition of 12.5 µL of 1 mol/L sulfuric acid. Absorbance of 450/570 nm was determined by using the Synergy 2 Multi-Detection Microplate Reader (BioTek, Winooski, Vt). For semiquantification purposes, a standard curve was generated by adding serially diluted semipurified human IgE (Chemicon International, Temecula, Calif) to wells coated with 2 µg/mL anti-human IgE. GOS-specific IgE titers were extrapolated from standard curves. The percentage of inhibition was calculated as follows:

$$(1 - \text{IgE level with inhibitor} / \text{IgE level without inhibitor}) \times 100$$

In some experiments sera were preincubated with deglycosylated or proteolysed *B. tropicalis* extract. GOS-specific IgE or *B. tropicalis*-specific IgE levels were measured thereafter.

Deglycosylation of *B. tropicalis* extract by means of periodate oxidation

The lyophilized *B. tropicalis* extract (100 µg) was reconstituted in 250 µL of 0.1 mol/L sodium acetate, pH 5.5. Deglycosylation was performed by adding 250 µL of 20 mmol/L Sodium (meta)periodate (Sigma-Aldrich) and incubated at room temperature for 30 minutes in the dark. The deglycosylated *B. tropicalis* extract was then dialyzed against PBS. The total protein concentration was subsequently determined by using a Bio-Rad Protein Assay Kit (Bio-Rad Laboratories).

Proteolysis of *B. tropicalis* extract with trypsin and Proteinase K

The lyophilized *B. tropicalis* extract (100 µg) was reconstituted in 25 µL of 50 mmol/L Tris buffer (pH 8.0) with 0.1% SDS and 20 mmol/L dithiothreitol and heated at 95°C for 10 minutes. The denatured extract was first treated with 5 µg of trypsin (Pierce Biotechnology, Rockford, Ill) at 37°C for 24 hours and subsequently treated with 20 µg of Proteinase K at 50°C for 1 hour. The

sample was subjected to heat inactivation at 95°C for 10 minutes after each digestion.

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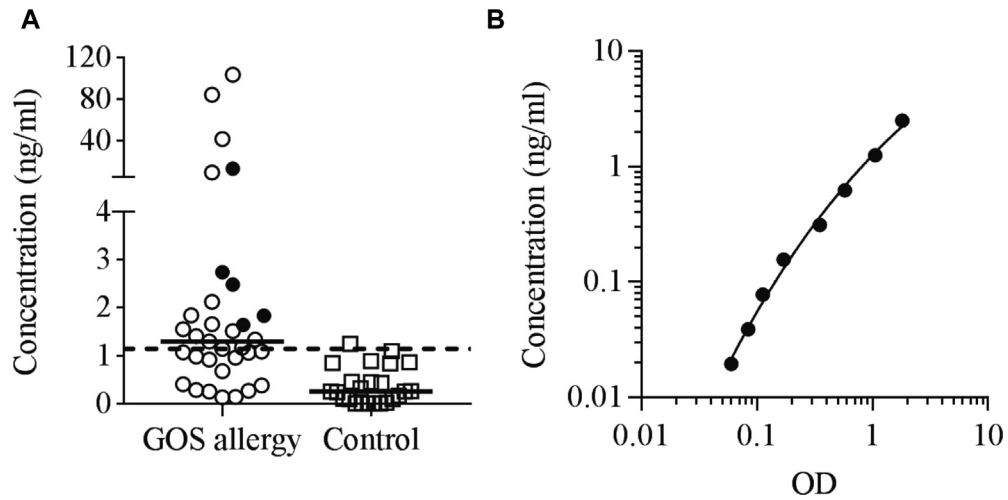


FIG E1. Detection of GOS-specific IgE by using ELISA. **A**, An ELISA plate was coated with HSA-GOS conjugate and HSA. Sera of patients with GOS allergy ($n = 33$) and atopic control subjects ($n = 24$) were preabsorbed with HSA and then added to both HSA-GOS- and HSA-coated wells. **B**, The titer of GOS-specific IgE was extrapolated from the standard curves. The level of GOS-specific IgE was determined after subtracting the level of HSA-specific IgE. Five sera selected for the ELISA inhibition study are presented as shaded circles in Fig E1, A. Solid line, Median IgE level of the respective group. Dashed line, Mean + 2 SD of the IgE level of the control group.

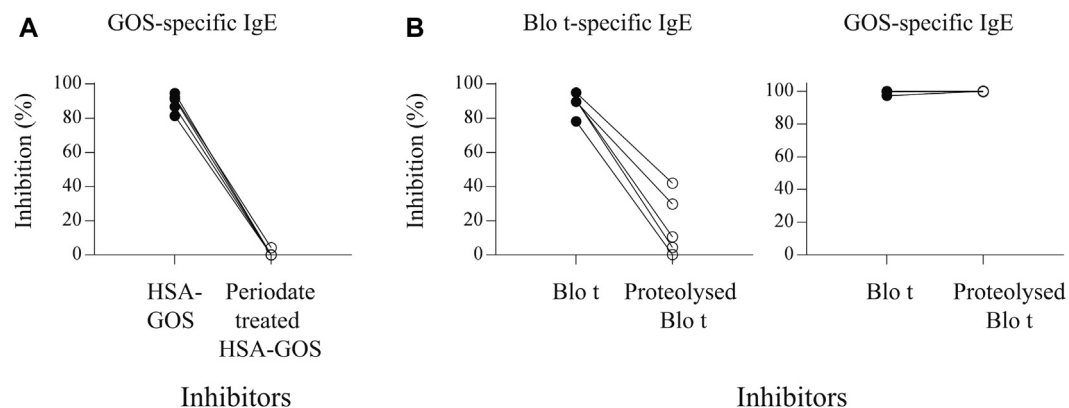


FIG E2. GOS-specific IgE is sensitive to periodate oxidation. Sera of patients with GOS allergy ($n = 5$) were preabsorbed with HSA-GOS conjugates or periodate-treated HSA-GOS (**A**) or *B. tropicalis* extract or proteolysed *B. tropicalis* extract (**B**). Levels of *B. tropicalis*-specific IgE and GOS-specific IgE were detected. The ability of HSA-GOS, periodate-treated HSA-GOS, *B. tropicalis* extract, or proteolysed *B. tropicalis* extract in inhibition of GOS sIgE or *B. tropicalis*-specific IgE (as indicated) compared with sera without preabsorption was presented as a percentage of inhibition.

TABLE E1. List of allergens in EUROLINE Atopy Indonesia 1 panel

Aeroallergens/venom	Mite	● <i>Dermatophagoides pteronyssinus</i> ● <i>Dermatophagoides farinae</i> ● <i>Dermatophagoides microceras</i>	● <i>Tyrophagus putrescentiae</i> ● <i>Glycyphagus domesticus</i>
	Pollen	● Bermuda grass ● Timothy grass ● Grass mix 5 (Sweet vernal grass, Bermuda grass, Timothy grass, cultivated rye)	● <i>Blomia tropicalis</i> ● Acacia ● White pine (Australian) ● Oil palm ● Kapok
	Mold	● Mold mix 1 (<i>Penicillium notatum</i>, <i>Cladosporium herbarum</i>, <i>Aspergillus fumigatus</i>, <i>Alternaria alternata</i>)	● <i>Candida albicans</i>
	Animal dander	● Cat ● Dog ● Horse	● Feather mix 1 (chicken feathers, duck feathers, goose feathers)
	Insect	● Honeybee venom	● Cockroach (German)
	Egg/milk/nut	● Cow's milk ● nBos d 4 ● nBos d 5 ● nBos d 6	● nBos d 8 ● Egg white ● Cheddar cheese
	Fish/shellfish	● Codfish ● Tuna ● Salmon	● Crab ● Shrimp (Pacific) ● Lobster
Food allergens	Meat	● Duck meat ● Beef (cooked)	● Pork (cooked) ● Chicken
	Others	● Tomato ● Garlic ● Strawberry ● Kiwi	● Coffee ● Wheat flour ● Gluten ● Chocolate
			● Peanut ● Hazelnut ● Almond ● Shellfish mix 1 (spiny lobster, oyster, clam) ● Lamb ● Soybean ● Baker's yeast ● Glutamate