

suggested that inability to detoxify ROS because of null genotypes of *GSTM1* and *GSTT1* and decreased capacity of the inflammatory effect of *CC16* because of the presence of the 38A allele through the low level of *CC16* concentration should perpetuate the inflammatory process, activate bronchoconstrictor mechanisms, and precipitate asthma symptoms. Other studies are needed to elucidate the cooperative or possible antagonistic effect of these polymorphisms with other candidate genes, as well as the effect of environmental factors in asthma.

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Late-onset anaphylaxis to *Bacillus natto*-fermented soybeans (natto)

To the Editor:

Onset of an anaphylactic reaction usually occurs within 30 minutes after exposure to the relevant allergen. Isolated

late anaphylactic reactions without early-phase reactions are rarely reported in patients with food-induced anaphylaxis.¹ We here report the first case, to our knowledge, of IgE-mediated cutaneous, respiratory, and abdominal symptoms occurring 10 to 12 hours after consuming natto (ie, soybeans fermented by the bacteria *Bacillus natto* [subtilis]).

A 36-year-old man presented with a 2-month history of recurrent generalized urticaria and anaphylaxis. He had taken antihistamines, but had urticaria >2 to 3 days a week and more recently had at least 3 severe anaphylactic reactions accompanied by dyspnea, chest tightness, abdominal cramps, palpitation, vomiting, dizziness, and headache. These 3 episodes required emergency care. Symptoms resolved with intravenous antihistamines. His medical history revealed asthma in childhood. There was no family history of food allergy or of anaphylaxis.

He was treated with antihistamines such as loratadine and hydroxyzine daily and was instructed to keep a diet diary. Thereafter, he had 3 anaphylactic reactions over a period of 47 days. Symptoms resolved 1 to 3 hours after receiving dexchlorpheniramine. In 2 of 3 episodes, he ate dinner containing natto at 6 PM or 8 PM, respectively, and then anaphylactic reactions developed at 6 AM while sleeping. He had never realized that these reactions were elicited by something he had eaten, because he had 2 of these 3 episodes while sleeping. His diet diaries, however, revealed that he had ingested natto 10 to 12 hours before the onset of symptoms in all 3 instances, indicating an association between natto and these 3 anaphylactic events.

Total IgE concentration was 710 IU/mL (range for age, 1-300). Specific IgE antibodies were detected against egg white (a score of 2+), but not against soybean or the other foods, by CAP RAST (Pharmacia, Uppsala, Sweden). Responses to all skin prick tests (SPTs) were read at 15 minutes and interpreted according to European Academy of Allergy and Clinical Immunology guidelines.² All results of SPTs with multiple commercial foods allergens (eg, soybean, rice, and egg white; Torii Pharmaceutical Co, Tokyo, Japan) were negative. The vehicle control was negative. According to Dreborg and Foucard,³ the results of the prick-by-prick test with the raw ingredients included in the dinner the patient had eaten 12 hours before an episode that occurred while he was sleeping were negative for all ingredients except natto (Okamenatto; Takanofoods Co Ltd, Ibaragi, Japan). Results for natto revealed a mean wheal diameter of 5 mm and a mean flare diameter of 20 mm, whereas the 10 control subjects remained negative for wheals and flares. Subsequent SPTs with a saline solution containing *B natto* powder (50 mg/mL; Yuzo Takahashi Laboratory, Yamagata, Japan) and *B natto* cultivated on agar were negative. In addition, to test for allergy to natto, we prepared our own extract. A total of 25 mL 0.125 mol/L ammonium bicarbonate was poured over 1 g commercial natto (Okamenatto) followed by homogenization in blender for 30 minutes at 4°C until a smooth paste was achieved. After centrifugation at 3000 rpm for 30 minutes, the supernatant was dialyzed against saline and filtered through a 0.45-μm membrane. Then the natto

extract was lyophilized to make a powder. The SPTs results with the natto extract (4 mg/mL in PBS) revealed strong positivity (mean wheal diameter, 13 mm; mean flare diameter, 52 mm), whereas the 5 control subjects remained negative for wheals and flares. Further, the prick-by-prick tests were negative for other soy products such as tofu and miso.

The patient tolerated oral challenge by the other ingredients of the suspect meal (except for natto) in open challenge manner. Oral challenge with natto was not undertaken because both the treating physician and the patient believed the risk was unwarranted on the basis of a convincing history of severe reactions with no alternative explanation and a strong positive test response.

Specific IgE antibody for the self-prepared natto extract was detected by using fluorometric ELISA, as described previously.⁴ The patient showed IgE antibodies to the natto extract. None of the 6 control subjects showed IgE antibodies to the natto extract. The mean OD of IgE antibody levels to natto in ELISA was 2.34 in the patient, whereas the mean OD ranged from 0.55 to 0.65 (mean $\pm$ 3 SD, 0.57 ± 0.10) in control subjects. Furthermore, the natto extract and molecular weight markers (Daiichi, Tokyo, Japan) were separated by SDS-PAGE as described and analyzed by Coomassie blue staining. SDS-PAGE analysis of the natto extract showed several bands ranging from <14 kD to ~38 kD in molecular weight. In particular, the natto extract had 1 marked band at <14 kD and 2 thin bands, 1 at 28 kD and 1 at 38 kD. Further immunoblotting experiments with the patient's serum revealed IgE binding to ~28-kD and 38-kD proteins in the natto extract (Fig 1), whereas sera from 2 control subjects never bound to the bands of the natto extract.

At the time of this report, the patient had been followed up for 1 year, and no reactions had occurred since he started to avoid natto.

Natto is a traditional Japanese food that has recently made an appearance in Western cultures and diets, much like sushi, as a healthy food. The strongest allergen of soybean, Gly m Bd 30K, is not found in fermented soy foods such as natto, soy sauce, and miso, indicating that these fermented soy foods may be hypoallergenic.⁵

Soybean (ie, unfermented soy products) is well known as a stable class 1 food allergen. However, allergic reactions to natto have never been reported. The major allergens of soybean have been designated as Gly m Bd 60K, Gly m Bd 30K, and Gly m Bd 28K, respectively. Gly m Bd 30K and Gly m Bd 28K have molecular weights of 34,000 and 26,000, respectively. On the basis of his specific IgE and SPT results, our patient is sensitive to natto but not to unfermented soybean or *B natto*. Furthermore, IgE immunoblotting showed that his serum bound different molecular weight proteins from the 3 major allergens of soybean. Consequently, our results indicated that the patient was sensitive to allergens peculiar to natto, and not to the residues of soybean allergens. Therefore, it was strongly suspected that the natto allergens might be produced during fermentation

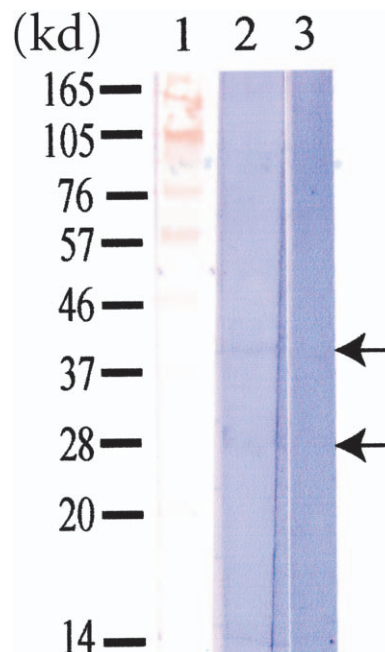


FIG 1. IgE immunoblots with sera of the patient and a control subject. Molecular weight is given in kilodaltons. A 28-kD protein and a 38-kD protein were found to be positive in the patient. Lanes 1, 2, and 3 indicate molecular weight markers, patient serum, and control subject serum, respectively.

with *B natto* and might already exist in natto before ingestion and digestion. It was found that most of the crude proteins of natto existed as peptide polymers having molecular weights larger than 60,000. It is known that the viscous substance of natto contains poly γ -glutamic acid (PGA), which is produced by *B natto* in fermentation. PGA is a water-soluble, biodegradable biopolymer and has molecular weights ranging from 100,000 to >1,000,000. Recent research has shown that drugs bound to PGA can be released from porous macromolecules such as PGA in long-term controlled release systems, and that PGA can be used in drug delivery applications for the controlled release of some drugs.⁶ It could take a long time for an allergic subject to absorb allergens sufficient to induce symptoms, because the natto allergens bound to PGA may be released slowly as PGA biodegrades in the gastrointestinal tract. Therefore, we speculated that in natto-induced anaphylaxis, late onset might be caused by slow release of natto allergens from porous biodegradable macromolecules of PGA into the gastrointestinal tract. Further studies are necessary to clarify the mechanism of late-onset anaphylaxis to natto.

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Peanut allergy: Sensitization by peanut oil-containing local therapeutics seems unlikely

To the Editor:

Recent findings have renewed the debate on the allergenicity of peanut oils by describing an association between the use of skin preparations containing peanut oil and peanut allergy in children.¹ Peanut oil use is widespread in pharmaceutical products, such as vitamins and skin ointments, but also in more specialized products, such as bone cement for orthopedic surgery. Obligated to local pharmacopeias, only peanut oil that meets the specified requirements (eg, refining) is used in pharmaceuticals. Refined food oils could also contain a certain amount of unrefined peanut oil to improve taste and flavor.

We investigated the allergenicity of crude peanut oils, peanut oils at different steps of the refining process,

refined peanut oils from supermarkets, pharmaceutical peanut oils, and a number of current products containing peanut oil, like vitamins, by means of ELISA and immunoblotting (Table I and Fig 1). These methods are supplementary to each other.²

Crude oils contained significant amounts of protein, whereas in refined oils and products thereof, no protein was detectable (<0.3 ng/mL). The ELISA results were confirmed by measuring nitrogen as a marker for protein (data not shown). Because hydrophobic peanut proteins, such as oleosins,³ might be extracted inefficiently during sample preparation for ELISA, we investigated representative peanut oil samples directly by using immunoblot technology with sera of patients with peanut allergy. It is clear from Fig 1 that crude peanut oil contains immunoreactive protein bands at molecular weights typical for peanut, whereas refined oils do not show any reactivity. The intensity of the protein bands of extracted peanut oil (Fig 1, lane 2) is an order of magnitude higher compared with that of the direct measurement (Fig 1, lane 1). This is in full agreement with the oil/buffer ratio of 10:1 applied during extraction. Therefore it is unlikely that significant amounts of protein remain in the oil phase after the extraction.

Lack et al¹ posited that the cutaneous route of exposure to the residual proteins of peanut oil might be a plausible determinant of allergic sensitization. Our data show that in refined peanut oils the protein levels are less than 0.3 ng/mL. Hsieh et al⁴ reported that allergen exposure through the skin in BALB/c mice could serve as a sensitization pathway for food allergy. An amount of 100 µg of ovalbumin applied to a 1 × 1 cm patch for 1 week was needed to require a positive challenge result. The concentration used (10 mg/mL) is more than a million-fold higher than the detection limit of our ELISA in this experiment.

TABLE I. Peanut protein concentration in peanut oil-containing products

Product class	Description	Sample no.	Protein (ng/mL) detected with ELISA
Crude oils	Crude oil*	1	6470
	Crude oil*	2	90
	Cold pressed oil*†	3	2550
Samples from the refining process	Neutralized groundnut oil*	4	<0.3
	Bleached groundnut oil*	5	<0.3
	Deodorized groundnut oil*	6	<0.3
Refined food oils	Arachid oil	7	<0.3
	Groundnut oil	8	<0.3
Pharmaceutical ingredients	Refined arachid oil	9	<0.3
	Arachid oil	10	<0.3
	Arachid oil	11	<0.3
Pharmaceutical products	Vitamin K (1)	12	<0.3
	Vitamin K (2)	13	<0.3
	Vitamin K (3)	14	<0.3
	Vitamin K (4)	15	<0.3
	Bone cement	16	<0.3

*Not commercially available. Only supplied for this study.

†Prepared at our own pilot facilities for oil processing.