

Late-onset Anaphylaxis after Ingestion of *Bacillus Subtilis*-fermented Soybeans (Natto): Clinical Review of 7 Patients

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ABSTRACT

Background: Allergic reactions after ingestion of fermented soybeans have rarely been reported. Fermented soybeans were recently reported to be a causative food of IgE-mediated, late-onset anaphylaxis without early phase responses. The objectives of our study are to clarify the clinical and laboratory features and to characterize the allergens in allergy due to fermented soybeans.

Methods: Seven patients with suspected hypersensitivity to fermented soybeans, from whom informed consent had been obtained, underwent skin prick-prick tests with fermented soybeans and challenge test with fermented soybeans. Additionally, specific IgE against fermented soybeans and the allergens of fermented soybeans were detected by ELISA and IgE-immunoblotting, respectively.

Results: Seven male patients, aged 26 to 42 years (mean age, 33.1 years), participated. All patients reported generalized urticaria and dyspnea; 5, loss of consciousness; 2, collapse; 2, vomiting; and 2, diarrhea after fermented soybean ingestion. The interval between fermented soybean ingestion and onset of symptoms was 5 to 14 hours (mean, 9.6 hours). All patients were positive on skin prick-prick tests with fermented soybeans. In 2 patients, oral challenge with fermented soybeans was positive 5.5 and 13 hours after ingestion. In ELISA, all 5 patients tested showed elevated IgE levels to the fermented soybean extract. Furthermore, IgE-immunoblotting using 5 patients' sera showed six bands, of which three bands at 38, 28, and 26-kd were bound to sera from 4 patients.

Conclusions: Cases with hypersensitivity after ingestion of fermented soybeans most frequently correspond to IgE-mediated, late-onset anaphylactic reactions due to fermented soybeans.

KEY WORDS

anaphylaxis, *Bacillus subtilis* (natto), fermented soybeans, food allergy, late onset

INTRODUCTION

Natto (*i.e.*, soybeans fermented by the bacteria *Bacillus subtilis* (natto)) is a traditional and popular food in Japan. Nevertheless, allergic reactions after ingestion of natto have rarely been reported. We recently reported the first case of IgE-mediated, late-onset anaphylaxis induced approximately half a day after ingestion of natto.¹ The hypothesized mechanism of late-onset anaphylaxis to fermented soybeans is due to

delayed absorption or release into the bowel rather than an immunologic phenomenon. In addition, we confirmed late-onset anaphylaxis induced half a day after ingestion of natto by provocation test.² An anaphylactic reaction was provoked by natto in a dose-dependent manner and levels of chemical mediators, such as plasma histamine and plasma tryptase, were transiently elevated during provocation tests. These two reports indicated that natto might be the typical causative food that induced a peculiar clinical course

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Received 11 August 2006. Accepted for publication 28 December 2006.

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of IgE-mediated, late-onset anaphylaxis without early phase reactions after its ingestion.

In this report, we clarified the clinical and laboratory features in 7 patients with natto allergy and characterized the allergens involved.

METHODS

PATIENTS

Consecutive patients with convincing histories of allergic reactions following ingestion of natto, seen at the Dermatology Department of Yokohama City University Hospital in Yokohama over a 5-year period (October 2000–November 2005), were selected; 4 of them were described previously.¹⁻³

These patients were diagnosed on the basis of documented allergic reactions following natto ingestion and results of several examinations. The patients with a convincing history of anaphylaxis following ingestion of natto and with a positive skin prick test (SPT) with natto, positive natto-specific IgE, and/or a positive challenge test were considered allergic to natto.

PREPARATION OF NATTO EXTRACT

To test for allergy to natto we prepared our own extract. Twenty-five ml of 0.125M ammonium hydrogen carbonate was poured over 1 g of commercial natto (Okamenatto; Takanofoods Co. Ltd., Ibaragi, Japan) followed by homogenization in a 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 pore diameter membrane (Millipore, Bedford, MA, U.S.A). Then the natto extract was lyophilized to make a powder that was stored at -40°C until use.

SKIN PRICK TESTS AND PRICK-PRICK TESTS

SPT was performed according to the standard procedure⁴ with a commercial allergen (Torii Pharmaceutical Co, Tokyo, Japan) prepared at 1 : 20 wt/vol for soybean, the natto extract (4 mg/mL in phosphate-buffered saline (PBS) solution) we had prepared, and a saline solution containing *Bacillus natto* powder (Yuzo Takahashi laboratory, Yamagata, Japan) (50 mg/ml). We also performed SPT with the prick-prick technique⁵ with all ingredients in the meals containing natto which the patients had eaten approximately half a day prior to episodes that could be precisely recalled. Responses were read at 15 minutes and were graded according to the standard methods recommended by the European Academy of Allergy and Clinical Immunology. SPT using the natto extract (4 mg/mL in PBS) was also performed on 5 control subjects. Histamine chloride (10 mg/mL) and the vehicle served as positive and negative controls, respectively. The prick-prick tests with natto were also carried out on 5 control subjects.

PROVOCATION TESTS

Open challenge tests were carried out with natto in 2 patients, whose informed consent had been obtained (the other 5 patients did not consent to the oral challenge test). In this study, open challenge test was selected rather than double-blind, placebo-controlled food challenge, which is the gold standard for the diagnosis of food allergies,⁶ because these patients were apprehensive about blind provocation. Initially, 10 g of natto (Nattoichi; Asahimatsushokuhin, Saitama, Japan) without any seasoning were eaten. More than 24 hours after the first challenge, 25 g or 50 g of natto without any seasoning was consumed.

LABORATORY TESTS

Serum total IgE and specific IgE levels (CAP RAST; Pharmacia, Uppsala, Sweden) for soybean were measured.

SPECIFIC IGE AGAINST NATTO

Serum samples were obtained for further immunologic evaluation with the informed approval of the institutional review board of Yokohama City University Hospital.

Serum specific IgE antibody for the natto extract was detected using fluorometric ELISA as described previously.⁷ In all experiments, duplicate samples were tested. The mean optical density (O.D.) value plus 3 SD of the mean of the control sera were used as the cut-off limit for positivity. None of these 5 subjects had any known food allergies. Serum levels of total IgE in the control subjects ranged from 17 to 570 IU/mL, with a geometric mean of 244 IU/mL.

SDS-PAGE AND IMMUNOBLOTTING

The natto extract (40 mg/mL in PBS) and the molecular weight markers (Daiichi, Tokyo, Japan) were separated by sodiumdodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as described elsewhere,⁸ and analyzed by Coomassie-blue staining. Thereafter, the proteins were transferred to polyvinylidene difluoride (PVDF) membrane (Daiichi) in a transfer cell (Daiichi).

The transferred PVDF membrane was incubated with 2% of the patients' and the control subjects' sera, respectively, at 4°C overnight after blocking with skim milk, and IgE antibodies bound to the antigen were visualized using peroxidase-labeled anti-human IgE antibodies (KPL, Gaithersburg, M.D., U.S.A.).

RESULTS

PATIENTS

Seven patients, all male, whose ages ranged from 26 to 42 years (mean age, 33.1 years), were given a diagnosis of hypersensitivity to natto. All 7 patients reported generalized urticaria and dyspnea; 5, loss of consciousness; 2, collapse; 2, vomiting; and 2, diarrhea after natto ingestion (Table 1). All patients ex-

Table 1 Clinical characteristics and test results of 7 patients with allergic reactions after natto ingestion.

Patient no.	Sex	Age (y) at onset	Symptoms	Interval between ingestion and onset (h)	Natto extract		Soybean		Serum total IgE (IU/ml)	Provocation test
					Specific † IgE (O.D.)	SPT ‡ (mm)	Specific IgE (UA/ml)	SPT ‡ (mm)		
1	M	36	U, Dy	9	0.162	13	<0.35	0	710	NT
2	M	27	U, Con, Dy	9	0.158	11.4	<0.35	0	699	NT
3	M	42	U, Con, Dy	12	0.134	10	<0.35	0	169	NT
4	M	35	U, Dy	5	0.317	7.5	3.94	3.3	8871	NT
5	M	37	U, Col, Con, Dy, Di, V	9–13	0.805	7.2	0.51	0	4219	+
6	M	29	U, Con, Dy	9–14	NT	10.8	<0.35	0	329	NT
7	M	26	U, Col, Con, Dy, Di, V	10	NT	16.6	0.37	0	522	+

SPT, Skin prick test; U, urticaria; Con, consciousness loss; Col, collapse; Di, diarrhea; Dy, dyspnea; V, vomiting; NT, not tested.

† Mean of duplicate determinations. O.D. for 5 control subjects' sera ranged from 0.110 to 0.135 (mean \pm 3SD: 0.124 \pm 0.027).

‡ Mean diameter of a wheal. The five control subjects remained negative for wheals and flares in response to the natto extract.

perienced one to five anaphylactic reactions with at least two of the main indicators of anaphylaxis (generalized urticaria, hypotension, and dyspnea),^{9,10} resulting in emergency treatment with epinephrine, antihistamines, or systemic corticosteroids. The interval between natto ingestion and onset of symptoms was 5 to 14 hours (mean, 9.6 hours). The prodromal symptoms were pruritus and flushing, followed by whealing and generalized urticaria in all patients. All patients began to avoid natto and experienced no further symptoms.

SKIN PRICK TESTS AND PRICK-PRICK TESTS

All patients were positive to natto in prick-prick test with natto and SPT with the natto extract, whereas the results of the prick-prick test, with the raw ingredients in the respective meals each patient had eaten half a day prior to an episode that could be precisely recalled, were negative for all ingredients except natto in all patients. In addition, results of SPTs with soybean allergen and a saline solution containing *Bacillus natto* powder (Yuzo Takahashi Laboratory, Yamagata, Japan) (50 mg/ml) were negative in all patients. The 5 control subjects remained negative for wheals and flares for natto and for the natto extract.

PROVOCATION TESTS

In patient 5, the oral challenge with 50 g of natto was positive 13 hours after its ingestion, whereas the challenge with 10 g of natto was negative. Thirteen hours after the ingestion of 50 g of natto, the patient developed anaphylactic reactions accompanied by generalized urticaria, dyspnea, chest tightness, and decreased oxygen levels in the blood detected by pulse oximetry. Plasma histamine levels and tryptase levels were significantly elevated in association with symptoms. In patient 7, the oral challenge with 25 g of natto was positive 5.5 hours after its ingestion, whereas the challenge with 10 g of natto was negative. The remaining 5 patients were not subjected to

the oral challenge test because they had experienced prior anaphylactic reactions. Thus, it was felt that the risk of the oral challenge was unwarranted.

LABORATORY TESTS

Total IgE concentration was 169–8871 IU/ml (mean, 2934, range for age: 1–300). Specific IgE antibodies were detected against soybean in 3 of 7 patients.

SPECIFIC IGE AGAINST NATTO

All 5 patients tested showed IgE antibodies to the natto extract. The mean O.D. of IgE antibody levels to natto in ELISA were 0.134–0.805 (mean, 0.315) in the patients, whereas the mean O.D. ranged from 0.110 to 0.135 (mean \pm 3SD: 0.124 \pm 0.027) in the control subjects (Table 1).

SDS-PAGE AND IMMUNOBLOTTING

SDS-PAGE of the natto extract showed multiple protein bands with molecular weight mainly ranging from less than 5-kd to about 38-kd. The most intense bands were observed at about 28 and 38-kd, under reduced and non-reduced conditions.

As shown in Figure 1, when serum samples from each of 5 patients allergic to natto were tested for IgE binding to the crude natto extract by immunoblotting, all samples showed positive results, whereas no samples from control subjects did. The apparent molecular weights of IgE-binding bands ranged from approximately 5-kd to 38-kd. Bands with molecular weight of approximately 26, 28, and 38-kd were detected in four of five sera (80%) (not in the serum of patient 5), and IgE-binding bands at approximately 5, 24, and 27-kd were shown in the sera of patients 5, 2, and 1, respectively.

DISCUSSION

We report the first clinical review of allergic reactions to natto in 7 patients. All 7 patients experienced anaphylactic reactions within 5–14 hours, without early

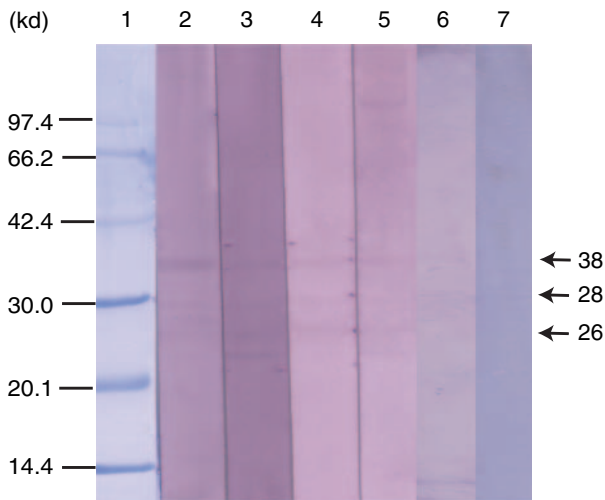


Fig. 1 IgE immunoblots with sera of 5 patients and a control subject. Molecular weight is given in kilo Daltons (kDa). Bands with molecular weight of approximately 26, 28, and 38-kd were detected in four of five sera with the exception of the serum of patient 5, and IgE-binding bands at approximately 5, 24, and 27-kd were shown in the sera of patients 5, 2, and 1, respectively. Lanes 1, 2, 3, 4, 5, 6, and 7 indicate molecular weight markers, patient 1 serum, patient 2 serum, patient 3 serum, patient 4 serum, patient 5 serum, and control subject serum, respectively.

phase reactions, after ingestion of natto, suggesting that cases with hypersensitivity after ingestion of natto might most frequently correspond to late-onset anaphylaxis due to natto, although very few cases with hypersensitivity to natto have been reported so far. Natto might be the typical causative food of a novel clinical pattern of IgE-mediated, late-onset anaphylaxis without early phase reactions.

Natto is a traditional Japanese food that has made recent inroads, like Sushi, into Western cultures and diets as a healthy food. In addition, it was reported that by sandwich enzyme-linked immunosorbent assay, the strongest allergen of soybean, Gly m Bd 30K, was not found in fermented soy foods such as natto and soy sauce, indicating that these fermented soy foods might be hypoallergenic.¹¹ Recently, several health supplements containing natto extract have been for sale all over the world on the internet, reflecting the fact that effects of natto on a number of physiological functions, such as antioxidant activity,¹² anti-hypertensive activity,¹³ thrombolytic activity,¹⁴ and anti-bone loss activity¹⁵ have been reported and remarked. Therefore, people in countries other than Japan might have a risk of natto sensitization.

We identified 7 patients who suffered from allergic reactions after natto ingestion seen in our hospital over a period of about 5 years. Surprisingly, all subjects experienced anaphylactic reactions to natto, several of which were accompanied by hypotension and

loss of consciousness. Notably, all subjects were men and all were cases of adult onset, and 2 of the 7 patients only began to eat natto after reaching adulthood.

Moreover, in the current study, we succeeded in confirming six IgE-binding proteins by immunoblot analysis using sera from the 5 patients, probably because a more highly concentrated extract of natto than that reported previously for immunoblotting was used. Consequently, three proteins, at 26, 28, and 38-kd in the natto extract, were considered as the major allergens because these three proteins were bound by IgE in 4 of 5 patients' sera. IgE-immunoblotting revealed that 4 subjects with the exception of patient 5 were sensitive to several proteins in natto. The patients were found to be sensitive to natto but not to unfermented soybeans or to the natto bacteria of *B natto*, based on specific IgE and SPT results. Consequently, our results indicate that the patients were 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 newly produced during fermentation and might already exist in natto before ingestion and digestion.

The mechanism of late-onset allergy due to natto remains unclear. The viscous substance of natto was reported to contain poly (γ -glutamic acid; (PGA)), which is produced by *Bacillus natto* in fermentation. PGA is a water-soluble, biodegradable biopolymer and has molecular weights ranging from 100,000 to over 1000,000.¹⁶ It was reported that drugs bound to PGA can be released from porous macromolecules such as PGA in long-term controlled release systems, and PGA can be used in drug delivery applications for the controlled release of some drugs.¹⁷ It could take a long time for allergens to achieve a concentration in the blood sufficient to induce symptoms because the natto allergens bound to PGA may be slowly released as the PGA biodegrades in the gastrointestinal tract. Therefore, we hypothesized that in natto-induced anaphylaxis, late onset might be due to slow release of natto allergens from porous biodegradable macromolecules of PGA to the gastrointestinal tract. However, it has been reported that tyramine, a kind of vaso-active amine, was detected in 7 of 42 natto products (16.7%), but histamine was not.¹⁸ Therefore, tyramine in natto might trigger symptoms via a non-immunologic mechanism after natto ingestion. Further studies are needed to elucidate the mechanism of late-onset allergy due to natto.

This clinical review revealed that allergic reactions after ingestion of natto could mostly show a novel clinical course of IgE-mediated, late-onset anaphylaxis without early phase reactions. This peculiar clinical feature might make it difficult to diagnose natto allergy, and natto allergy could have been previously undiagnosed or underestimated. Therefore, it is

important to realize that natto could cause late-onset type allergic reactions without an immediate/early response after its ingestion. In addition, natto allergy should be considered as potentially responsible for severe anaphylactic reactions.

ACKNOWLEDGEMENTS

This work was supported in part by grants from the Yokohama City University Hospital, the Grants-in-Aid for Scientific Research (B) program of the Ministry of Education, Culture, Sports, Science and Technology of the Japanese Government (project No. 18790796, to N.I.).

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