
Case report

Late-onset anaphylaxis to fermented soybeans: the first confirmation of food-induced, late-onset anaphylaxis by provocation test

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Background: Late-onset anaphylactic reactions without early-phase reactions are rarely reported. The hypothesized mechanism of late-onset anaphylaxis to fermented soybeans is delayed absorption or release into the bowel rather than an immunologic phenomenon.

Objectives: To investigate the mechanisms of late-onset anaphylaxis to fermented soybeans in 2 patients and to characterize the allergens involved in anaphylaxis caused by fermented soybeans.

Methods: Two patients underwent skin prick-by-prick tests with fermented soybeans as is. We used an open challenge for the provocation test of anaphylaxis and measured changes in plasma histamine, plasma tryptase, serum eosinophil cationic protein, and plasma leukotriene B₄ levels in 1 patient. In addition, specific IgE against fermented soybeans and the allergens of fermented soybeans were detected by enzyme-linked immunosorbent assay and immunoblotting, respectively.

Results: The results of the prick-by-prick tests with fermented soybeans as is were positive in both patients and negative in control subjects. The challenge with 50 g of fermented soybeans caused generalized urticaria and dyspnea 13 hours after ingestion of fermented soybeans in 1 patient. In addition, his plasma histamine and tryptase levels transiently elevated during the anaphylactic event. In enzyme-linked immunosorbent assay, the patients showed elevated IgE levels to the proteins of fermented soybeans. Serum IgE antibodies of patients 1 and 2 were bound to approximately 5- and 26-kDa proteins in immunoblotting of fermented soybeans, respectively.

Conclusion: To our knowledge, this is the first report of late-onset anaphylaxis provoked by the challenge test half a day after ingestion of fermented soybeans.

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INTRODUCTION

Onsets of anaphylactic reactions usually occur within 30 minutes after exposure to the relevant allergen.¹ Isolated late-onset anaphylactic reactions without early-phase reactions are rarely reported in patients with food-induced anaphylaxis.² We previously reported the first case of late-onset anaphylaxis after ingestion of natto (ie, soybeans fermented by the bacteria *Bacillus natto* [subtilis]).³ The hypothesized mechanism of late-onset anaphylaxis in response to natto is delayed absorption or release into the bowel rather than an immunologic phenomenon. The aim of this study was to investigate the mechanisms of late-onset anaphylactic reactions to natto in 2 patients. In addition, we conducted tests to characterize the allergens involved in anaphylaxis caused by natto.

CASE REPORTS

Patient 1

A 37-year-old man who had experienced 3 severe anaphylactic reactions accompanied by generalized urticaria, dyspnea, chest tightness, abdominal cramps, palpitation, vomiting, dizziness, headache, loss of consciousness, and collapse was referred to Yokohama City University Hospital, Yokohama, Japan, for evaluation. Each of these 3 reactions required emergency care, including intravenous corticosteroid administration. The last episode occurred when he awoke in bed at 8 AM. He had eaten a dinner containing natto at 7 PM the day before, and after the dinner he had had nothing to eat and did not exercise before the time the symptoms appeared. His medical history revealed seasonal allergic rhinitis and conjunctivitis. There was no family history of food allergy or of anaphylaxis. Because he experienced 1 of the latest 2 episodes on first waking, he never realized that these reactions were elicited by something he had eaten. His diet diaries, however, revealed that he had ingested the meals containing natto approximately 9 to 13 hours before the onset of symp-

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toms in the latest 2 episodes, indicating an association between natto and these anaphylactic events.

Patient 2

A 27-year-old man presented with 3 anaphylactic events accompanied by generalized urticaria, dyspnea, chest tightness, abdominal cramps, vomiting, and loss of consciousness requiring emergency care. In the latest event, he felt itching that woke him at 4 AM and found generalized urticaria; he immediately arose and went to the lavatory. However, he lost consciousness and fell as soon as he reached the lavatory. Two hours later, without having been moved or having received any treatment, he recovered consciousness. His diet diary revealed that he had ingested a dinner containing natto 9 hours before the onset of symptoms in the last instance, indicating an association between natto and these anaphylactic event.

METHODS

Laboratory Tests and Extraction of Natto Proteins

Serum total IgE and specific IgE levels (CAP RAST; Pharmacia, Uppsala, Sweden) for soybean, egg white, cow's milk, wheat, and rice were measured. To test for allergy to natto, we prepared our own extract. Twenty-five milliliters 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 3,000 rpm for 30 minutes, the supernatant was dialyzed against saline and filtered through a 0.45- μ m membrane. Then the natto extract was lyophilized to make a powder that was stored at -40°C until use.

Skin Prick Tests and Prick-by-Prick Tests

Patients and control subjects underwent skin prick tests (SPTs) with multiple commercial allergens (Torii Pharmaceutical Co, Tokyo, Japan) prepared at 1:20 wt/vol for foods such as soybeans, egg white, cow's milk, wheat, rice, and the natto extract we had prepared. Responses were read at 15 minutes and were graded according to the standard methods recommended by the European Academy of Allergy and Clinical Immunology.⁴ Skin prick tests using the natto extract (4 mg/mL in phosphate-buffered saline solution) were also performed on 5 control subjects. Histamine chloride (10 mg/mL) served as a positive control, and the vehicle served as a negative control.

According to the method of Dreborg and Foucard,⁵ prick-by-prick tests were conducted for all ingredients in the meals containing natto, which the patient had eaten approximately half a day before an episode that could be precisely recalled. Prick-by-prick tests using natto were also performed on 10 control subjects (5 men and 5 women; age range, 1–69 years; mean age, 32.2 years), including 4 healthy adults, 3 subjects with positive SPT results in response to soybean, and 3 subjects with atopic dermatitis.

Provocation Tests

A full explanation of the tests to be undertaken was given to the patient, and informed consent was obtained. Open challenge tests were performed under closely supervised conditions in the hospital. Examination of vital signs and oxygen saturation in the blood were performed before the challenge and were repeated at hourly intervals throughout the duration of the challenge. Initially, 10 g of natto (Nattoichi; Asahimatsushokuhin, Saitama, Japan) without any seasoning was eaten. More than 24 hours after the first challenge, 50 g of natto without any seasoning was consumed. For 12 hours before and during the challenge tests, eating meals was prohibited, and intravenous hydration was given. Venous blood samples were collected serially during the tests to measure plasma histamine, plasma tryptase, serum eosinophil cationic protein (ECP), and plasma leukotriene B₄ levels. In addition, the other oral challenge tests were performed by administering up to approximately 5 g of each of the ingredients in meals other than those containing natto that had also been eaten by the patients.

Immunologic Studies and 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. Specific IgE antibody for the natto extract was detected using fluorometric enzyme-linked immunosorbent assay as described previously.⁶ In all the experiments, duplicate samples were tested. The mean optical density value plus 3 SDs of the mean of the control serum sample was used as the cutoff limit for positivity. The control serum samples were from 5 atopic volunteers (mean age, 28.2 years; range, 25–35 years). None of these 5 subjects had any known food allergies. Serum levels of total IgE in control subjects ranged from 17 to 570 IU/mL, with a geometric mean of 244 IU/mL.

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis and Immunoblotting

The natto extract and the molecular weight markers (Daiichi, Tokyo, Japan) were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), as described elsewhere,⁷ and were analyzed by Coomassie blue staining. Thereafter, the proteins were transferred to polyvinylidene difluoride membrane (Daiichi) in a transfer cell (Daiichi).

The transferred polyvinylidene difluoride membrane was incubated with 2% of the patient and control subject serum samples 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, MD). The control serum samples were from 6 healthy volunteers (mean age, 39.7 years; range, 30–73 years). None of these 6 subjects had any known food allergies. Serum levels of total IgE in the control subjects ranged from 20 to 6,220 IU/mL, with a geometric mean of 1,753 IU/mL.

RESULTS

SPTs and Radioallergosorbent Assay

The total IgE concentrations in patients 1 and 2 were 4,219 and 710 IU/mL, respectively (range for age, 1–300 IU/mL). Specific IgE antibodies were detected against wheat (score, 2+), soybean (score, 1+), and rice (score, 1+) but not against egg white or cow's milk in patient 1. In patient 2, the CAP-RAST results to all foods investigated were negative.

All SPT results with multiple commercial foods allergens (eg, soybean, wheat, rice, egg white, and egg yolk) were negative in both patients. The vehicle control was negative. The results of the prick-by-prick tests using the raw ingredients in the respective meals the patients had eaten for dinner 9 to 13 hours before an episode that could be precisely recalled were negative for all ingredients except natto in both patients. Results for natto as is revealed mean wheal diameters of 17.6 and 12.2 mm and mean flare diameters of 33.6 and 31.8 mm in patients 1 and 2, respectively, whereas the 10 control subjects remained negative for wheals and flares. A subsequent prick-by-prick test with a saline solution containing *B natto* powder (50 mg/mL) was negative in both patients. In addition, the SPT results with the natto extract revealed strong positivity (mean wheal diameters of 7.2 and 11.4 mm and mean flare diameters of 35.2 and 32.6 mm in patients 1 and 2, respectively), whereas the 5 control subjects remained negative for wheals and flares. Furthermore, the prick-by-prick test results were negative for other soy products, such as tofu and miso.

Provocation Test

This test was performed using natto in patient 1 by administering increasing amounts of natto up to 50 g, after obtaining informed consent. The oral challenge with 50 g of natto had positive results 13 hours after ingestion, whereas the challenge with 10 g of natto had negative results (Fig 1). Thirteen hours after ingestion of 50 g of natto, the patient suddenly felt a strong itch on his forearm, and then he rapidly developed generalized urticaria on his trunk and extremities. He received oral dexchlorpheniramine. Because these symptoms were followed, however, by dyspnea and chest tightness, accompanied by decreased oxygen levels in blood detected by pulse oximetry, he was treated with a hypodermic injection of epinephrine and intravenous hydrocortisone. The vital signs, heart rate, respiratory rate, and blood pressure, at the occurrence of the reaction were 85/min, 18/min, and 120/78 mm Hg, respectively. Dyspnea and chest tightness gradually resolved, and generalized urticaria almost completely disappeared 1 hour later. Surprisingly, these symptoms reappeared 3 hours after the administration of intravenous corticosteroids. Therefore, he was also treated with a hypodermic injection of epinephrine and intravenous corticosteroids. The symptoms were protracted for more than 6 hours after the first treatment started.

Plasma histamine levels and tryptase levels were significantly elevated in association with symptoms (Fig 1), whereas serum ECP levels and plasma leukotriene B₄ levels did not show any significant changes (data not shown). In addition, both patients tolerated oral challenge, given in an

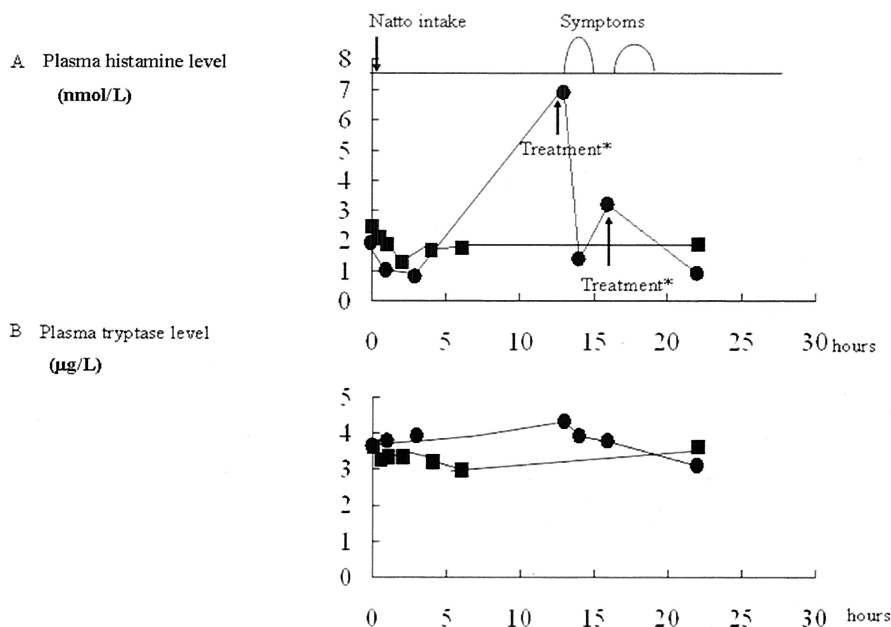


Figure 1. Changes in plasma histamine (A) and tryptase (B) levels during provocation tests in patient 1. Plasma histamine levels changed remarkably during the challenge test with 50 g of natto (black circles) but did not change during the challenge test with 10 g of natto (black squares). Asterisk indicates that treatments with intravenous corticosteroids and subcutaneous epinephrine were performed.

open manner, with the other ingredients of the suspect meal (except natto).

Specific IgE Against Natto and SDS-PAGE and Immunoblotting

The patient showed IgE antibodies to the natto extract. None of the 6 control subjects showed IgE antibodies to the natto extract. The mean optical density of IgE antibody levels to natto in enzyme-linked immunosorbent assay were 0.391 and 0.237 in patients 1 and 2, respectively, whereas the mean optical density ranged from 0.110 to 0.135 (mean $\pm$ 3 SD, 0.124 ± 0.027) in control subjects.

The SDS-PAGE analysis of the natto extract showed several bands ranging from less than 5 kDa to approximately 38 kDa in molecular weight. Furthermore, immunoblotting experiments using serum samples from patients 1 and 2 revealed IgE binding to approximately 5- and 26-kDa proteins in the natto extract, respectively (Fig 2), whereas serum samples from the 6 control subjects never bound to the bands of the natto extract.

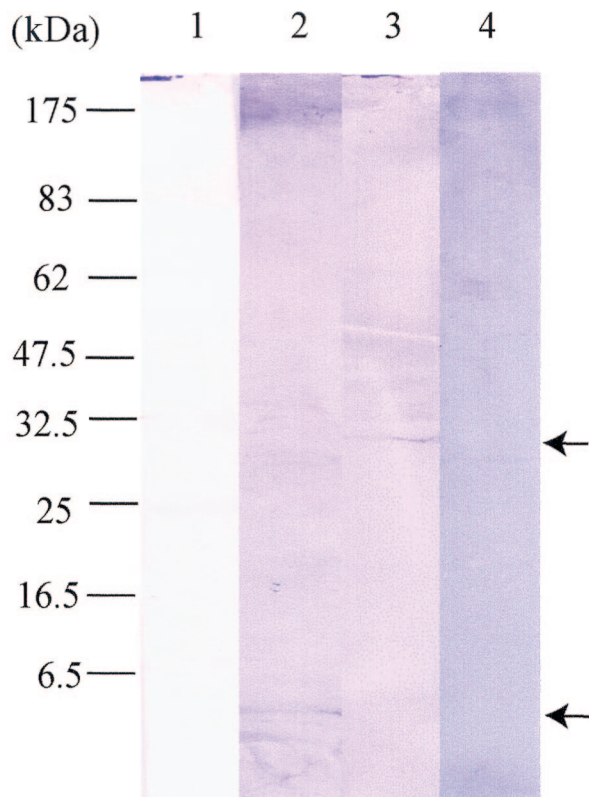


Figure 2. IgE immunoblots using serum samples from the patients and a control subject. Proteins of approximately 5 and 26 kDa were found to be positive in patients 1 and 2, respectively. Lanes 1, 2, 3, and 4 indicate molecular weight markers, patient 1 serum, patient 2 serum, and control subject serum, respectively.

Dietary Treatment

At the time of this report, patients 1 and 2 had been followed up for half a year and 2 years, respectively, and no reactions in either have occurred since they started to avoid exposure to natto.

DISCUSSION

We report the first case of natto-induced, late-onset anaphylaxis, which was confirmed by provocation tests. Furthermore, to our knowledge, this is the first case of food-induced, late-onset anaphylaxis provoked by challenge with the causative food in a dose-dependent manner. In this study, open challenge was selected rather than double-blind, placebo-controlled food challenge, which is the gold standard for the diagnosis of food allergies,⁸ because the patient was apprehensive about blind provocation.

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, by sandwich enzyme-linked immunosorbent assay, that 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.⁹

Plasma histamine and tryptase levels were significantly elevated in association with symptoms during the provocation test of natto in patient 1, whereas the levels of plasma leukotriene B₄ and serum ECP did not significantly change. These changes in the levels of chemical mediators in the blood during the provocation test indicate that in late-onset anaphylaxis due to natto, the onset of symptoms could be related to degranulation of mast cells and basophils but not to release of newly generated lipid-derived mediators and activation of eosinophils. Positive results of specific IgE, SPTs, and IgE immunoblotting with natto strongly supported IgE-mediated allergic reactions to natto, which were confirmed by changes in levels of chemical mediators during the provocation test. However, provocation testing in patient 1 also revealed an isolated late-onset anaphylactic reaction without an early phase, and the onset was approximately half a day after ingestion of natto. These results indicate that the onset of anaphylactic reaction to natto occurs much later than that of ordinary IgE-mediated, early-phase reaction, although natto allergens could induce the release of histamine and tryptase from mast cells and basophils as soon as natto allergens bind specific IgE to natto on the surface of mast cells and basophils. We succeeded in confirming by provocation test that late-onset anaphylaxis to natto is an IgE-mediated reaction accompanied by degranulation of mast cells and basophils, not a delayed reaction after an early-phase reaction.

The viscous substance of natto was reported to contain poly- γ -glutamic acid (PGA), which is produced by *B natto* in fermentation. The PGA is a water-soluble, biodegradable biopolymer with molecular weights ranging from 100 to more than 1,000 kDa.¹⁰ 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.¹¹ 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. This hypothesis was supported in part by changes in the levels of chemical mediators, such as histamine and tryptase, during the provocation test of natto. In addition, anaphylactic reactions might also be protracted owing to the slow release of natto allergens. Patients 1 and 2 were sensitive to different natto allergens having molecular weights of 5 and 26 kDa, respectively. As a matter of course, even if the natto allergens have various molecular weights, they might both be slowly released from porous biodegradable macromolecules of PGA. The patients were found to be sensitive to natto but not to unfermented soybeans or the natto bacteria of *B natto*, based on specific IgE and SPT results. Consequently, our results indicate 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 newly produced during fermentation and might already exist in natto before ingestion and digestion.

These 2 cases and the case we described previously³ indicate that allergic reactions after ingestion of natto might show a peculiar clinical course, ie, the onset of allergic reactions is approximately half a day after ingestion of natto, although allergic reactions to natto are mediated by IgE. This peculiar clinical feature might make it difficult to diagnose natto allergy, and natto allergy could, therefore, have been previously undiagnosed or underestimated. None of the known causative foods have been known to induce allergic reactions half a day after ingestion. Therefore, natto might be the

typical causative food of a novel clinical pattern of IgE-mediated, late-onset anaphylaxis.

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