

Late-Onset Anaphylaxis Due to Poly (γ -glutamic acid) in the Soup of Commercial Cold Chinese Noodles in a Patient with Allergy to Fermented Soybeans (Natto)

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ABSTRACT

Background: Fermented soybeans (natto) have been reported to induce IgE-mediated, late-onset anaphylaxis without early-phase responses. However, the relevant allergens of natto allergy have never been identified.

Case Summary: A 38-year-old man developed an anaphylactic reaction accompanied by flashing, generalized urticaria, conjunctival redness, and dyspnea 3 hours after ingestion of commercial cold Chinese noodles. He had avoided natto for the past year due to developing several anaphylactic reactions half a day after natto ingestion. The results of skin prick tests (SPTs) were strongly positive for natto and the soup of cold Chinese noodles. Furthermore, SPTs showed positive for poly (γ -glutamic acid) (PGA), which is a major constituent of natto mucilage, alone among all the ingredients of the cold Chinese noodle soup. Therefore, he was diagnosed with late-onset anaphylaxis to PGA contained in natto and the cold Chinese noodle soup.

Discussion: These results indicated that in the present case, the relevant allergen of late-onset anaphylaxis may have been PGA in all episodes and that the patient had been sensitized by PGA through natto ingestion. PGA is produced by *Bacillus subtilis* during fermentation and is a high-molecular, biodegradable polymer. The late onset is therefore, hypothesized to be due to a delayed absorption of PGA, as PGA biodegrades to peptides sufficiently small to be absorbed in the bowel. PGA has recently been applied to a wide range of fields such as foods, cosmetics, and medicine. Therefore, patients with late-onset anaphylaxis to PGA of natto should avoid not only natto but also other materials containing PGA.

KEY WORDS

anaphylaxis, fermented soybeans (natto), food allergy, late onset, poly (γ -glutamic acid)

INTRODUCTION

Poly (γ -glutamic acid) (PGA) is a major constituent of mucilage in fermented soybeans, which is commonly called natto. The hypothesized mechanism of late-onset anaphylaxis due to natto has been reported to be a slow absorption or release of natto allergens from PGA into the bowel, as PGA is a water-soluble, high-molecular polymer and slowly biodegrades in the living body.^{1,2} However, in the present case with

late-onset anaphylaxis due to natto, the anaphylactic reactions also developed 3 hours after the ingestion of cold Chinese noodles containing a small amount of PGA as a seasoning. To the best of our knowledge, late-onset anaphylaxis to PGA as an ingredient of foods other than natto has never been reported.

CLINICAL SUMMARY

A 38-year-old man developed an anaphylactic reaction accompanied by flashing, generalized urticaria, red-

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Received 10 September 2010. Accepted for publication 6 November 2010.

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Table 1 The results of skin prick tests

Allergen	Skin prick test
Fermented soybeans (natto)	3+
Soybeans	-
<i>Bacillus subtilis</i>	-
The ingredients of cold Chinese noodles	
Cold Chinese noodle soup	3+
Cold Chinese noodles	-
Boiled mung bean sprouts	-
Raw leeks	-
Mustard	-
Poly (γ -glutamic acid) in the cold Chinese noodle soup	3+
Glutamic acid	2+
Seasoning containing monosodium glutamate	1+

ness of conjunctiva, and dyspnea 3 hours after the ingestion of cold Chinese noodles, which were made at home using commercial raw noodles with soup, boiled mung bean sprouts, leeks, and mustard. He was treated with intravenous antihistamines and corticosteroids and was then referred to our hospital for examination.

Three years ago, he started to develop anaphylactic reactions accompanied by flashing, generalized urticaria, dyspnea and loss of consciousness. In typical episodes, the anaphylactic reactions occurred at 0 p.m. after the ingestion of natto, egg, and rice at 4 a.m. Also, whenever he ate natto for dinner, anaphylactic reactions developed while he was sleeping at 4-5 a.m. the next morning. He realized one year ago that every episode occurred about half a day after ingesting natto, and so for the past year he has avoided natto. The patient had a past medical history of gout. He ate Chinese foods containing monosodium glutamate as a food additive without any symptoms. Serum total IgE level was 825 IU/ml. The specific IgE measurements using the ImmunoCAP system (Pharmacia, Uppsala, Sweden) showed negative for soybeans, wheat, gluten, rice, egg white and egg yolk. Skin prick tests (SPT) with commercial extracts (Torii Pharmaceutical, Tokyo, Japan) for soybeans, rice, and wheat were negative, according to standard procedures.³ Responses to the prick test were classified as negative when the average wheal diameters were the same as the negative control, or as false positive (+), positive (2+), strongly positive (3+), and very strongly positive (4+) when the average wheal diameters were >25%, 50%, 100%, and 200%, respectively, of the positive control response induced by histamine chloride at 10 mg/ml. The elicited response was considered positive when the average wheal diameter induced by the allergen was $\geq 50\%$ of histamine as the positive control. SPTs with natto and all ingredients of the meal were performed according to the prick-

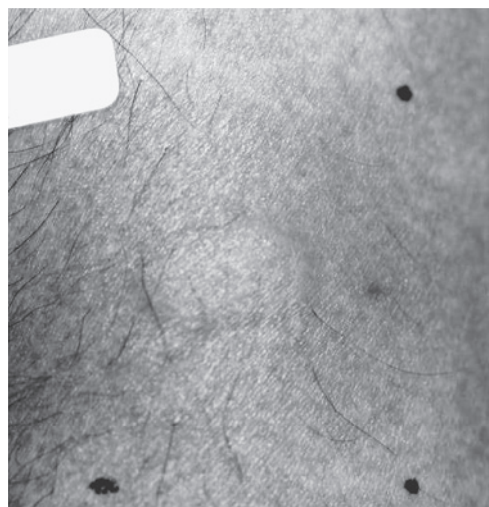


Fig. 1 Skin prick test with poly (γ -glutamic acid). Poly (gamma-glutamic acid) in the cold Chinese noodle soup at 10 mg/mL in phosphate-buffered saline induced a large wheal and flare.

prick method.⁴ SPTs showed positive for the soup of cold Chinese noodles, but not for noodles and vegetables in cold Chinese noodles (Table 1). In addition, the SPT results were positive for natto (Takanofoods, Ibaragi, Japan) but not for soybean extract and *Bacillus natto* (Yuzo Takahashi Laboratory, Yamagata, Japan) at 20 mg/ml in saline. Furthermore, the SPT results were strongly positive for PGA at 10 mg/ml in phosphate-buffered saline (PBS) among all ingredients of the soup of cold Chinese noodles, whereas they were negative in five control subjects (Fig. 1). Interestingly, the skin reactions were prolonged until approximately half a day after pricking with the PGA solution. Meanwhile, the mean diameter of a wheal induced by glutamic acid (Johnson Matthey Company, Bedford, MA, USA.), which was included in PGA, at 10 mg/ml in PBS was 4.1 mm, which was much smaller than the 15.6 mm diameter induced by PGA. The SPT results were negative for a seasoning containing monosodium glutamate as a major ingredient (Ajinomoto, Tokyo, Japan) at 10 mg/mL in saline.

These results indicated that the relevant allergen of the latest anaphylactic reaction was PGA in the soup of cold Chinese noodles. The PGA content in the soup was estimated to be less than 18 mg according to the information from the manufacturer. The provocation test was considered to be inappropriate as a further examination for diagnosis because a severe anaphylactic reaction was provoked after ingesting a small amount of PGA in the soup of cold Chinese noodles. The patient was instructed to avoid not only natto but also all materials containing PGA, such as foods and cosmetics.

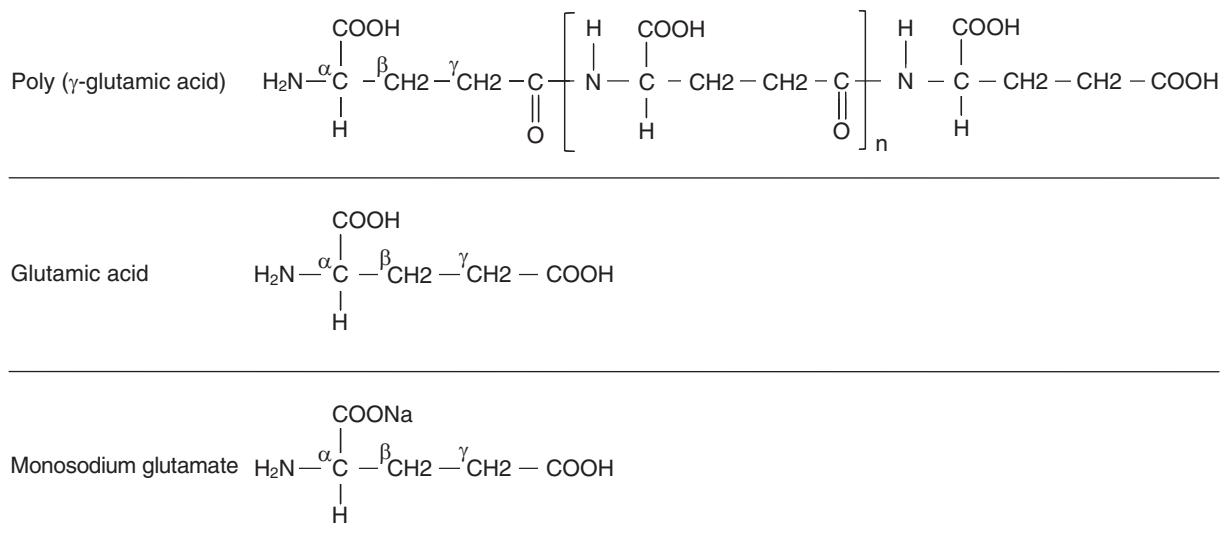


Fig. 2 The structural formula of poly (γ-glutamic acid), glutamic acid, and monosodium glutamate.

PATHOLOGICAL FINDINGS AND DISCUSSION

In the present case, the anaphylactic reactions developed 3 hours after consumption of cold Chinese noodles containing PGA as a seasoning. Before the latest anaphylaxis occurred after ingesting cold Chinese noodles containing PGA, the patient experienced several anaphylactic reactions 8-9 hours after the ingestion of natto, the mucilage of which mainly consisted of PGA. The clinical course and the results of the examinations indicated that he might have been initially sensitized by PGA through the ingestion of natto and consequently also developed anaphylactic reactions after the ingestion of the food other than natto containing PGA as an ingredient.

PGA is produced by *Bacillus natto* during fermentation after mixing the boiled soybeans and *Bacillus natto* and consists of glutamic acids. Certainly, in the present case, SPTs showed positive for natto, whereas they showed negative for soybeans and natto bacteria, suggesting that the allergen might be newly produced during fermentation. In PGA, there are peptide bonds between the amino group of glutamic acid and the carboxyl group at the end of the glutamic acid side chain (Fig. 2). PGA is a water-soluble, biodegradable biopolymer and has molecular weights ranging from 100,000 to over 1,000,000. In this case, it could take a long time for enough PGA to be absorbed to induce symptoms, as PGA may be absorbed slowly as high-molecular weight PGA biodegrades to peptides sufficiently small to be absorbed in the gastrointestinal tract. In addition, the interval between ingestion and onset of symptoms was much shorter in anaphylaxis after the ingestion of cold Chi-

nese noodles than in anaphylaxis after the ingestion of natto. This difference could be dependent on the molecular size of the PGA contained in the foods as well as other factors such as solidity that could influence the digestion and absorption. Furthermore, the prolonged skin reaction of PGA in SPT was suspected to be due to slow absorption of the PGA peptides, as PGA, with a molecular weight ranging from 100,000 to over 1,000,000, biodegrades slowly to peptides that are small enough to penetrate through the skin barrier.

Proteins having molecular weights of 26 kd, 28 kd, and 38 kd have been reported to be candidates for natto allergens in late-onset anaphylaxis.^{1,5} 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.² Therefore, in the cases sensitized by these proteins other than PGA, the late onset has been hypothesized to be due to a delayed release of natto allergens bound to PGA from PGA into the bowel.¹ In the present case, further investigations are needed because not only PGA but also allergens other than PGA may have been involved.

In the present case, the SPT results with glutamic acid, which is a minimum constituent of PGA, were much weaker than those with PGA. Some glutamic acids, which may have aggregated in the solution, could provide the IgE-epitopes, resulting in induction of a weak reaction. However, the patient has never experienced allergic symptoms after ingestion of a seasoning containing monosodium glutamate, although it was frequently added to his meals. The pa-

tient history and the SPT results suggested that the epitopes he reacted to could have come from the peptide bonds or the 3-dimensional structures in PGA but not from a monomer of glutamic acid. We speculate that there might be a portion of PGA, i.e., some linear or conformational structure of peptides in PGA, that possesses antigenicity. However, the further investigation is required to determine this more specific portion of PGA epitopes.

The minimal dose of PGA such as less than 18 mg could provoke anaphylactic reactions when the present patient ingested cold Chinese noodles. The minimal dose of allergens has been reported to have potency to induce anaphylactic reactions. A randomized, double-blind, placebo-controlled food challenge study showed that 5 mg of peanut crude protein provoked a systemic reaction in a severe peanut allergic patient.⁶ Therefore, it seems to be reasonable that the ingestion of minimal dose of PGA as an allergen, but not a crude protein of natto, could provoke a systemic reaction in the present patient, who had had several experiences of anaphylactic shock due to natto.

In the past few years, the application of PGA has been of interest in a wide range of fields such as foods, cosmetics, and medicine.⁷ In foods, PGA is added to breads, cookies, soups, and beverages, such as sport drinks. In Asia, PGA is also applied to skin-care products, such as soap and moisturizers. In medicine, application of PGA to drug delivery systems for the controlled release in anti-cancer therapy and vaccine adjuvants in the therapy for infectious diseases, such as human immunodeficiency virus, and immunotherapy for allergic diseases is expected.⁷⁻¹⁰

The present study indicated that PGA could be a relevant allergen in late-onset anaphylaxis after the ingestion of not only natto but also other foods containing PGA as an ingredient. In late-onset anaphy-

laxis to PGA of natto, both natto and all foods and materials containing PGA should be avoided.

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