

LETTER TO THE EDITOR

Involvement of poly (γ -glutamic acid) as an allergen in late-onset anaphylaxis due to fermented soybeans (*natto*)

Dear Editor,

Fermented soybeans, commonly called *natto*, which is a Japanese traditional food, have been reported to induce immunoglobulin (Ig)E-mediated anaphylaxis approximately half a day after its ingestion.¹ The hypothesized mechanism of late onset in *natto* anaphylaxis is the delayed absorption or release of *natto* allergens from *natto* mucilage consisting of mainly poly (γ -glutamic acid) (PGA) into the bowel rather than an immunological phenomenon.¹ Recently, we reported a case of *natto* allergy, in which an anaphylactic reaction was also induced by PGA added as a seasoning to a commercial food product.² In this study, we investigate whether PGA has allergenicity in three other patients with late-onset anaphylaxis to *natto*.

Three consecutive patients diagnosed with late-onset anaphylaxis to *natto* at our department over a 7-year period (June 2003 to May 2010), whose cases were described previously, were enrolled in the present study.³ These three patients, all male, with ages rang-

ing 26–37 years, were diagnosed on the basis of a convincing history of anaphylaxis 9–14 h after ingestion of *natto* and a positive skin prick test (SPT) and/or a positive challenge test with *natto* (Table 1). This study was approved by our institutional review board. Consent related to the SPT and histamine release test (HRT) with PGA was given by three and two patients, respectively.

Skin prick tests were performed according to standard procedures.⁴ The elicited response was considered positive when the average wheal diameter induced by the allergen was 50% or more of the positive control response induced by histamine chloride at 10 mg/mL. In all three patients, the results of SPT with *natto* (Okamenatto; Takanofoods, Ibaragi, Japan) were positive by the prick-prick method, whereas those with soybean (Torii Pharmaceutical, Tokyo, Japan) and a saline solution containing *Bacillus natto* powder (50 mg/mL; Yuzo Takahashi Laboratory, Yamagata, Japan) were negative.⁵ Moreover, all patients were subjected to SPT using three different-sized PGA (Wako Pure

Table 1. Clinical characteristics and result of skin prick tests in three cases of late-onset anaphylaxis to fermented soybeans (*natto*)

Case no.	1	2	3
Age/sex	37 M	26 M	29 M
Interval between <i>natto</i> ingestion and onset (h)	9–13	10	9–14
Symptoms	U, Col, Con, Dy, Di, V	U, Col, Con, Dy, Di, V	U, Con, Dy
Challenge test	Positive (13 h after ingestion)	Positive (6 h after ingestion)	NT
Skin prick test (mm, score)			
Histamine chloride (10 mg/mL)	5.9	7.0	6.9
Saline as the negative control	0	0	0
Fermented soybeans (<i>natto</i>)	15.1	11.2	4.2
PGA (mean MW 200 000–500 000)	10.2	13.6	8.0
PGA (mean MW 1 500 000–2 500 000)	10.5	9.0	7.0
PGA (mean MW 4 000 000–6 000 000)	14.2	12.5	10.9
Glutamic acid	2.3	0	3.2
L-Glutamic acid	4.5	0	0
D-Glutamic acid	0	0	2.9
Seasoning containing monosodium glutamate	NT	0	NT
Soybeans extract	0	0	0
Bacillus bacteria	1.9	0	0

Col, collapse; Con, loss of consciousness; Di, diarrhea; Dy, dyspnea; MW, molecular weight; NT, not tested; PGA, poly (γ -glutamic acid); U, urticaria; V, vomiting.

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Chemical Industries, Osaka, Japan), which had mean molecular weights of 200 000–500 000, 1 500 000–2 500 000 and 4 000 000–6 000 000 at 10 mg/mL in phosphate-buffered saline (PBS). In all three patients, results of SPT with the three different-sized PGA solutions were strongly positive, whereas the glutamic acid (Johnson Matthey, Bedford, MA, USA) and D- and L-glutamic acid (Wako Pure Chemical Industries) at 10 mg/mL in PBS induced no wheals or wheals that were much smaller than those induced by PGA. Similarly, in case 2, the result of SPT was negative to a seasoning containing monosodium glutamate as a major

ingredient (Ajinomoto, Tokyo, Japan) at 10 mg/mL in saline. The results of SPT with *natto*, PGA solution and glutamic acid were negative in five control subjects.

Furthermore, to investigate the histamine release activity of PGA having molecular weights of 200 000–500 000, we measured the level of histamine released by heparinized whole blood cells after exposure to PGA at 1, 0.1 or 0.01 mg/mL for 30 min at 37°C using an enzyme immunoassay kit (Immunotech, Marseilles, France) and indicated the obtained value as a percentage of the total cellular histamine release. The results are displayed as the means of

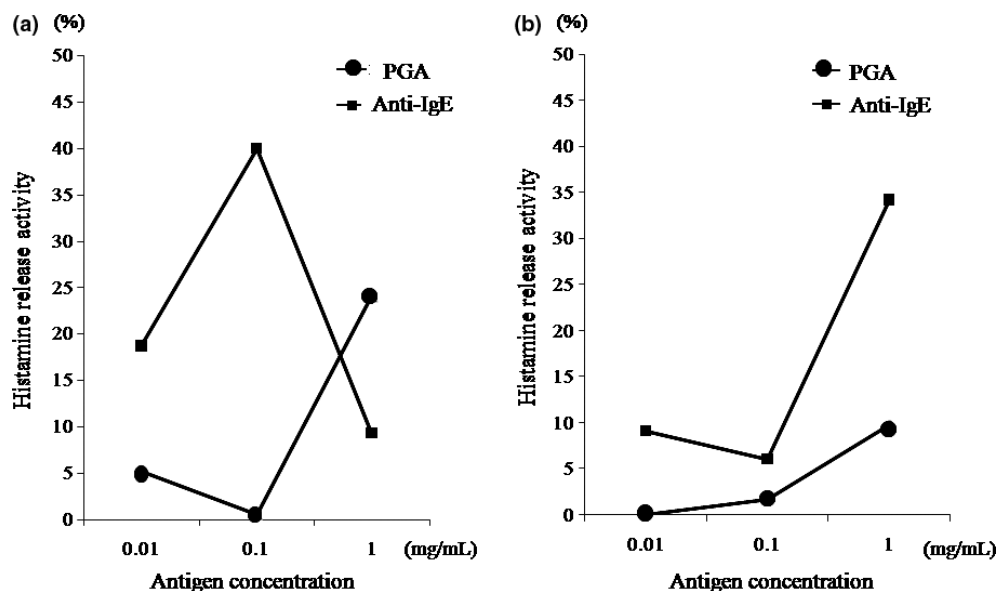


Figure 1. Histamine release test with poly (γ -glutamic acid) (PGA). The obtained values in histamine release tests were indicated as a percentage of the total cellular histamine release. PGA at a high concentration induced 24.1% and 9.8% of the total cellular histamine release in case 1 (a) and 2 (b), respectively. IgE, immunoglobulin E.

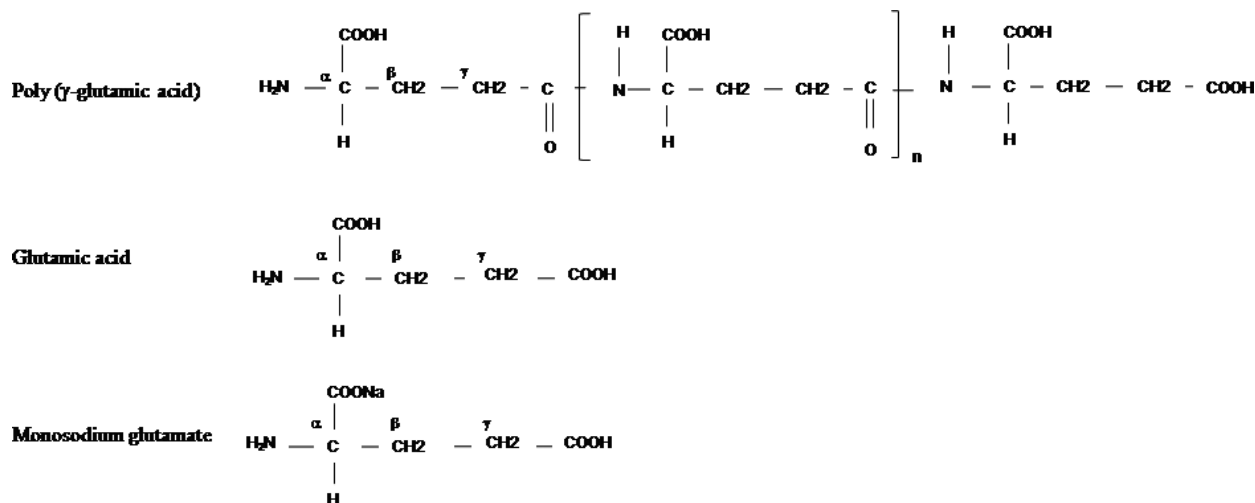


Figure 2. The structural formula of poly (γ -glutamic acid), glutamic acid and monosodium glutamate.

duplicate determinations. PGA induced a specific and dose-dependent histamine release from the whole blood of the three patients but not from that of the five control subjects. Inductions of 24.1% and 9.8% were observed at a high concentration of PGA in case 1 and 2, respectively (Fig. 1).

In IgE immunoblotting, which was performed by a previously described method, IgE-binding bands at approximately 5 and 38 kDa were found in the sera of patients 1 and 2, respectively, whereas none was detected in the sera of patient 3.³

The results of this study strongly indicated that PGA could be a relevant allergen in late-onset anaphylaxis to *natto*. PGA is newly produced by *Bacillus subtilis* in fermentation after mixing soybeans and *natto* bacteria. Certainly, in the present cases, the results of SPT were negative to soybeans and *natto* bacteria, but were positive to PGA. Additionally, PGA is known as a water-soluble, biodegradable biopolymer consisting of D- and L-glutamic acid with a high molecular weight ranging from 100 000 to over 1 000 000.⁶ In PGA, the peptide bonds are between the amino group of glutamic acid and the carboxyl group at the end of the glutamic acid side-chain (Fig. 2). As glutamic acid showed little allergenicity in this study, IgE epitopes might be provided by the structure of the peptide bonds between glutamic acids. In *natto*-induced anaphylaxis after sensitization by PGA, the late onset might be due to the slow absorption of the small peptides of PGA as PGA biodegrades in the gastrointestinal tract.

However, in the present cases, IgE-binding bands at approximately 5 and 38 kDa were also detected in IgE immunoblotting. Therefore, it is necessary to investigate whether the 5- and 38-kDa proteins might be associated with PGA.

Recently, PGA has been used in a wide range of fields such as foods, dietary supplements, cosmetics and medicine.⁷ Therefore, patients with *natto* allergy should be instructed to avoid not only *natto* but also the various materials containing PGA.

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