

CASE REPORT

Two cases of pollen-food allergy syndrome to soy milk diagnosed by skin prick test, specific serum immunoglobulin E and microarray analysis

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ABSTRACT

Oral allergy syndrome to soy milk is classified as a phenotype of pollen-food allergy syndrome (PFAS). As causative antigens, Gly m 4 (Bet v 1 homolog, 17 kD) and oleosin (23 kD), have been reported. In this study, we report two cases of PFAS to soy milk. Both cases showed positive reactions to soy milk in skin prick tests (SPT) and to Gly m 4 in specific serum immunoglobulin (Ig)E antibody. When we measured specific serum IgE antibody of soy-related proteins using a new laboratory testing method, microarray analysis, both cases showed a positive reaction for Bet v 1. One case was weakly positive for a soybean protein, β -conglycinin. Other results for reactivity to soy, peanut, cross-reactive carbohydrate determinants and profilin were negative. Based on these results, we diagnosed the two cases as PFAS to Gly m 4. We also performed protein microarray analysis and found it useful as a screening test for immediate allergy, such as PFAS.

Key words: class 2 food allergy, Gly m 4, microarray analysis, pollen-food allergy syndrome, soy milk.

INTRODUCTION

Although soy antigens were considered to cause class 1 food allergy only via digestion,^{1,2} recent reports indicate soy products also induce respiratory allergy via inhalation³ and can evoke contact urticaria or protein dermatitis on the skin.^{4,5} More recently, not only class 1 but also class 2 food allergy to soy milk accompanied by pollinosis have been reported by many investigators.^{6–8} Regarding class 2 allergy, Gly m 4 (SAM22),⁹ a Bet v 1 homolog, has been identified as an allergen causing such pollen-food allergy syndrome (PFAS) to soy milk.

In this study, we report two cases in which an immediate allergy were induced by soy milk. We performed microarray assay, which can screen for a large number of allergens using a small amount of

serum, and compared the results of this microarray assay, skin prick test (SPT) and specific serum immunoglobulin (Ig)E antibody (ImmunoCAP, Phadia, Uppsala, Sweden).

CASE REPORT

Case 1

A 37-year-old Japanese woman developed oral and pharyngeal symptoms and became unconscious after drinking soy milk. When she had moved her place of residence at the age of 27 years, she developed perennial rhinitis that tended to be exacerbated in the spring. Around the same time, atopic dermatitis began to appear that was exacerbated during pollen season. She had also experienced a tingling sensation in her oral cavity and an obstructive

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Received 20 December 2007; accepted 8 August 2008.

feeling in her throat when she ate apples, kiwis, pears, watermelons, peaches, melons, pineapples, tomatoes and strawberries.

Case 2

A 47-year-old Japanese woman developed oral and pharyngeal symptoms and runny nose, itchy eyes and wheezing after drinking soy milk. She experienced swelling of the lips after eating soy-related foods or bean sprouts. Approximately 10 years before, she began having rhinitis symptoms primarily in the spring. She had never experienced allergic symptoms, such as atopic dermatitis or urticaria other than rhinitis.

Measurement of specific IgE antibodies

Commercially available assays for specific serum IgE antibody (ImmunoCAP, Phadia) were performed for soy bean, common silver birch, alder, rGly m 4, rBet v 1, rBet v 2 and rBet v 6s. Levels of specific IgE antibody more than 0.7 kU/L were considered positive.

SPT

We used both refrigerated and heated commercially available soy milk. Prick-to-prick tests were performed for soybean milk, soy bean curd, bean sprouts, miso, green soy beans, strawberry, kiwi, apple, watermelon, pineapple, tomato, melon and latex extracts.¹⁰ Recombinant (r) antigens, including rBet v 1 and 2 and Art via-GAM,¹¹ were purchased from Biomay (Vienna, Austria), and Japanese cedar were purchased from Torii Pharmaceutical (Tokyo, Japan).

Protein microarray assay for detection of IgE responses in sera

Immunoglobulin E reactivity with 14 different allergens and allergen extracts were tested in a multiplexed immunoassay.¹² Soybean and peanut extracts, were ground, freeze-dried and extracted in phosphate buffer at neutral pH. Allergenic components were either purchased or purified essentially according to previously published methods and included Gly m T1 (Sigma-Aldrich, St Louis, MO, USA), SBA (Vector Laboratories, Peterborough, UK). β -conglycinin¹³, glycinin¹³, Ara h 2¹⁴, Ara h 1¹⁴, Ara h 3¹⁵ and cross-reactive carbohydrate determinants¹⁶ (CCD). Recombinant allergenic components were produced with a histidine tag and purified using immobilized

metal affinity chromatography and included rGly m 4,^{9,17,18} rAra h 8,¹⁹ rBet v 1²⁰ and rPhl p 12.²¹ Allergens and extracts were covalently immobilized to polystyrene particles (0.11- μ m diameter; Bangs Laboratories, Carmel, IN, USA). Particles were spotted in duplicate in an array format on a nitrocellulose-based capillary flow membrane using a nano-plotter (Type 2.0 system; GeSim, Grosserkmannsdorf, Germany). The assay procedure included sequential additions of 30 μ L washing buffer (phosphate buffer at neutral pH with blocking agents and detergent), serum (30 μ L), washing buffer (30 μ L), fluorophore-conjugated monoclonal anti-human IgE antibody (20 μ L) and washing buffer (2 $\times$ 30 μ L), before reading the fluorescence intensity of each spot using a GenePix 4000B laser scanner (Molecular Devices, Sunnyvale, CA, USA). IgE reactivity was reported as response units corrected for background binding adjacent to each spot and the average response of four IgE negative sera plus 3 standard deviations.

RESULTS

Results of the test for specific IgE antibodies (Table 1)

In case 1, laboratory examinations revealed a serum total IgE level of 119 IU/mL, and negative specific serum IgE antibody against soy. The patient's serum had positive specific serum IgE antibody to rGly m 4 (9.3 UA/mL) and rBet v 1 (47.1 UA/mL).

In case 2, laboratory examinations revealed a serum total IgE level of 131 IU/mL, and the level of specific serum IgE antibody against soy was very low (0.43 UA/mL). The patient's serum had positive specific serum IgE antibody to rGly m 4+ (6.4 UA/mL) and rBet v 1 (18.5 UA/mL).

Results of SPT (Table 1)

Case 1 showed positive reactions for commercially available soy milk, cedar pollen, rBet v 1, rBet v 2, strawberry, apple, kiwi and bean sprout.

Case 2 showed positive reactions for commercially available soy milk, *Artemisia* pollen antigen, rBet v 1, soybean curd and bean sprout (Fig. 1).

Results of protein microarray assay (Table 2)

The microarray results indicated a medium response to rBet v 1 for both patients (as already

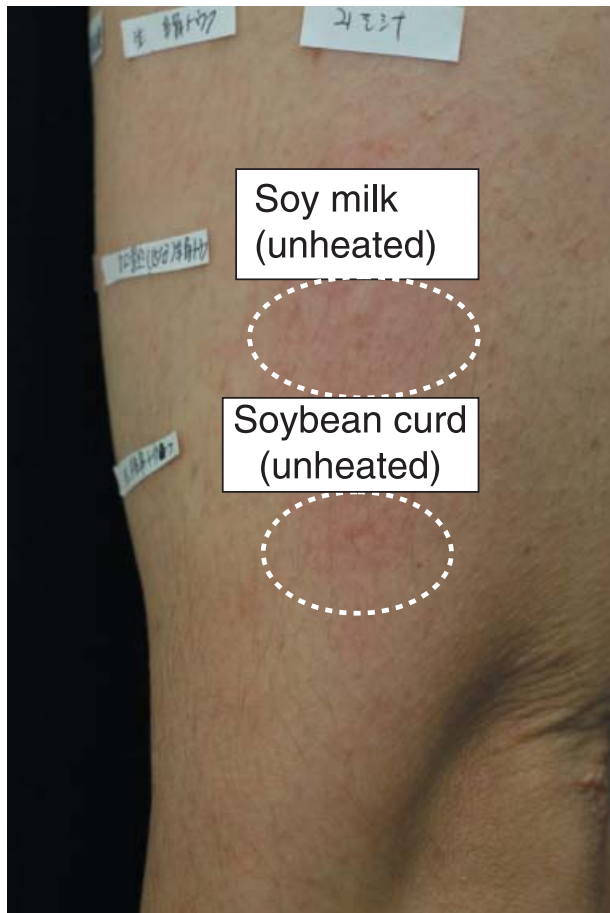


Figure 1. Results of skin prick tests with soy products in case 2.

seen with rBet v 1 ImmunoCAP analysis). A low response was also detected for case 2 to the soybean protein β -conglycinin. No other soy/peanut related or well-known cross-reacting (like cross-reactive carbohydrate determinants and profilin) IgE-specific responses were detected.

DISCUSSION

Based on the clinical symptoms, the results of SPT, serological tests and microarray assays, we diagnosed these two cases as having PFAS to soy milk, which is a class 2 food allergy. We speculated that the causative antigen was Gly m 4 (Bet v 1 homolog) and that sensitization to pollen antigens induced these symptoms. Mittag *et al.* examined 22 patients who developed concurrent allergies to birch pollen and soy milk.¹⁸ Of these patients, 96% (21/22) were positive for rGly m 4-specific IgE antibody and 100% were positive for rBet v 1-specific IgE antibody. In addition, IgE binding to soy milk and IgE binding to birch pollen were inhibited by 80% and 60%, respectively, by rGly m 4. Furthermore, Kleine-Tebbe *et al.* suggested that Gly m 4 (also referred to as SAM22) is highly cross-reactive to Bet v 1, which belongs to the pathogenesis-related proteins-10 protein family and are defense-related proteins.²² In contrast, Rihs *et al.* demonstrated that specific IgE antibody to rGly m 3, recombinant soy profilin, was

Table 1. Microarray results for the patients

				Case 1	Case 2
Array components	Soy bean	Soy Glycinin	11S globulin/legumins		
	Soy components	β -Conglycinin	7S/Vivilin		Low response
		Gly m TI	Trypsin inhibitor/ α -amylase inhibitor		
		SBA	Agglutinin		
	PR-10	rBet v 1/rGly m 4/rAra h 8		Medium response	Medium response
	Profilin	rPhl P 12			
	CCD	MUXF3			
	Peanut	Peanut			
		Ara h 1	7S/Vivilin		
	Peanut component	Ara h 2	2S albumin, trypsin inhibitor/ α -amylase inhibitor		
		Ara h 3	11S globulin/legumins		

The results for the different array components was converted from fluorescence units into response units and corrected for background response (background around all individual array components presented as average response units for four immunoglobulin E-negative sera plus 3 standard deviations). CCD, cross-reactive carbohydrate determinant; SBA, Gly m Lectin.

Table 2. Background information for two patients

			Case 1	Case 2
Age			37 years	45 years
Sex			Female	Female
Personal history			Atopic dermatitis, polinosis	Polinosis
Clinical symptoms			Soybean milk, apple, kiwi,	Soybean milk
inducible foods			pear, watermelon, peach, melon, pineapple, tomato, strawberry	
Non-specific IgE antibody (IU/mL)			119	131
Specific IgE	CAP FEIA	Gly m?	9.3	6.4
antibody (UA/mL)		rBet v 1	47.1	18.5
		rBet v 2	<0.35	<0.35
		rBet v 6 (isoflavone reductase of birch)	<0.35	<0.35
		Soy bean	<0.35	0.43
		Common silver birch	31.9	15.9
		Alder	29.6	11.7
		Japanese cedar	14.6	3.5
Skin prick test (score)		Soybean milk (heated)	3	3
		Soybean milk (unheated)	4	4
		Soybean curd	Negative	3
		Bean sprouts	3	4
		Miso	Negative	Negative
		Green soybeans	Negative	Negative
		rBet v1 (Biomay)	4	3
		rBet v2 (Biomay)	2	Negative
		Art v1a-GAM (Biomay)	Negative	4
		rHev b 6.02	Negative	Negative
		rHev b 8	Negative	Negative
		Wormwood (Torii)	Negative	4
		Japanese cedar (Torii)	3	3
		Ragweed (Torii)	0	1
		Orchard grass (Torii) 0 3		
		Strawberry	3	NT
		Kiwi	3	NT
		Apple	3	NT
		Watermelon, pineapple, tomato, melon, latex extracts	Negative	NT

NT: not tested.

confirmed in nine of 13 patients (70%) allergic to soy milk and that IgE binding to rBet v 2 was inhibited by rGly m 3.²³ These results suggested that Gly m 3, which is cross-reactive to another important antigen causing cedar pollen allergy, Bet v 2 (profilin), was also possibly an allergen causing PFAS to soy milk. In summary, Gly m 4 (soy Bet v 1 homolog), Gly m 3 (soy profilin) are possible allergens that cause PFAS to soy milk.

In the present study, we performed the SPT, specific serum IgE and microarray assay. For definitive

diagnoses of immediate allergy, both clinical symptoms and positive SPT results are necessary. However, in the patients who experience anaphylaxis, such as those examined in this study, there is always a risk of inducing similar symptoms by performing SPT. Therefore, we consider it useful to be able to diagnose such cases using a microarray assay in addition to the routine ImmunoCAP, in order to measure specific serum IgE antibody. In particular, a microarray assay has the advantage of measuring IgE antibodies to a large number of allergens using

a small sample. It is therefore useful for investigating sensitization of the components of allergens in a screening test.

We obtained an interesting result in the specific IgE antibodies that are generally measured. Case 1 showed oral allergy syndrome symptoms to several fruits, but case 2 did not. We considered the possibility that the difference in these results depended on the values of rBet v 1-specific IgE (case 1, 47.1 UA/mL; case 2, 18.5 UA/mL), and that this difference was reflected in their clinical symptoms.

Pollinosis affects an increasing number of people each year, while consumption of soy milk is growing. Patients with PFAS, including soy allergy, are expected to increase in number. Because it is not rare that PFAS induces anaphylaxis, we consider it desirable to establish safer and diagnostically valuable testing methods for patients with PFAS by combining methods such as ImmunoCAP and a microarray assay, which allow serological examinations.

ACKNOWLEDGMENTS

The authors thank the following people at Phadia who have been involved in the cloning and/or purification of the allergenic components: Dr Robert Movérare and co-workers (glycinin, β -conglycinin), Dr Jonas Lidholm and co-workers (Gly m 4, Ara h 1-3, Ara h 8, Bet v 1 and Phl p 12) and Dr Erik Unger (cross-reactive carbohydrate determinants).

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