

Soybean allergy in patients allergic to birch pollen: Clinical investigation and molecular characterization of allergens

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Background: Allergic reactions to legumes are generally thought to be acquired by means of primary sensitization through the gastrointestinal tract. Recently, Gly m 4 (starvation-associated message 22), a Bet v 1-related pathogenesis-related protein 10 from soy, was suggested to be an allergen in patients with allergic reactions to a dietary product containing a soy protein isolate.

Objective: We sought to evaluate the clinical relevance of Gly m 4 in subjects allergic to birch pollen with soy allergy and to assess the risk for subjects allergic to birch pollen to acquire soy allergy. **Methods:** Twenty-two patients allergic to birch pollen with soy allergy confirmed by means of positive double-blind, placebo-controlled food challenge results ($n = 16$) or a convincing history ($n = 6$) were investigated for IgE reactivity to birch pollen and soy allergens by using the Pharmacia CAP system and immunoblot analysis. Cross-reactivity was assessed by means of enzyme allergosorbent test inhibition. Ninety-four patients with birch pollen allergy were interviewed to assess soy tolerance and screened for IgE reactivity to Gly m 4 by means of immunoblotting. The Gly m 4 content in soy foods and soybean varieties was investigated by means of quantitative evaluation of immunoblots.

Results: During double-blind, placebo-controlled food challenge, 10 patients experienced symptoms localized to the oral cavity, and 6 patients had a more severe reaction. CAP analysis revealed Gly m 4-specific IgE in 96% (21/22) of the patients. All patients had Bet v 1-specific IgE antibodies, and 23% (5/22) had positive Bet v 2 results. In IgE immunoblotting 25% (6/22) of the patients recognized soy profilin (Gly m 3), and 64% (14/22) recognized other soy proteins. IgE binding to soy was at least 80% inhibited by birch pollen and 60% inhibited by rGly m 4 in 9 of 11 sera tested. Seventy-one percent

(67/94) of highly Bet v 1-sensitized patients with birch pollen allergy were sensitized to Gly m 4, and 9 (9.6%) of those patients reported soy allergy. The Gly m 4 content in soy products ranged between 0 and 70 ppm (milligrams per kilogram). **Conclusions:** Our results confirm that soybean is another birch pollen-related allergenic food. Gly m 4 is the major soy allergen for patients allergic to birch pollen with soy allergy. The content of Gly m 4 in soy food products strongly depends on the degree of food processing. (*J Allergy Clin Immunol* 2004;113:148-54.)

Key words: Double-blind, placebo-controlled food challenge; soy allergy; birch pollen-related food allergy; starvation-associated message 22; Gly m 4; birch pollen allergy; soy food products

To date, food allergy to soy proteins has been described mainly in young children with atopic dermatitis, who often outgrow their soy allergy after 1 to 2 years of dietary elimination.^{1,2} In adults soybean dust elicited respiratory allergy symptoms because of a sensitization to the soybean hull proteins Gly m 1 and Gly m 2.³⁻⁸ Allergic reactions to soy-derived foods in adults,⁹⁻¹³ however, are less frequent. The Kunitz trypsin inhibitor^{14,15} glycinin,¹⁶ the α -subunit of β -conglycinin,¹⁷ and a 50-kd protein with homology to chlorophyll A-B binding protein¹⁸ were identified as binding IgE of subjects with soy allergy.

In 2002, Kleine-Tebbe et al¹¹ reported 20 subjects with allergic reactions to a dietary powder containing 50% of a soy protein isolate. Eighty-five percent of those patients had specific IgE against the pathogenesis-related protein (PR-10) starvation-associated message 22 (SAM22) from soy, now designated as the allergen Gly m 4, as well as to the major birch pollen allergen Bet v 1, suggesting an association of birch pollen and soy food allergy.

The purpose of this study was (1) to confirm soy allergy in patients allergic to birch pollen by means of double-blind, placebo-controlled food challenge (DBPCFC); (2) to assess cross-reactivity between birch pollen and soy; (3) to evaluate the clinical relevance of sensitization to Gly m 4; and (4) to assess the risk for soy allergy in patients allergic to birch pollen by means of telephone interviews and analysis of the Gly m 4 content in soy-derived foods.

METHODS

Patients

Group 1 comprised 18 patients with a history of allergic reactions after ingestion of soy who were recruited at the Allergy Unit of University Hospital Zurich between February 2002 and March

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Abbreviations used

CD:	Circular dichroism
DBPCFC:	Double-blind, placebo-controlled food challenge
EAST:	Enzyme allergosorbent test
OAS:	Oral allergy syndrome
SAM22:	Starvation-associated message 22
SPT:	Skin prick test

2003. Furthermore, sera and case histories of 3 patients were provided from the serum bank of the Paul-Ehrlich-Institut, Langen, Germany. Pregnancy, history of a severe life-threatening anaphylactic reaction after soy consumption, significant concurrent disease or treatment with glucocorticosteroids, H₁-receptor antagonists, angiotensin-converting enzyme inhibitor, or β -blocking agents were exclusion criteria for DBPCFC. The symptoms and time course of pollinosis were assessed in each patient. The onset of soy allergy was at 21 ± 11 years of age (range, 5–52 years).

Group 2 comprised 94 patients with birch pollen allergy and specific IgE reactivity to Bet v 1 of at least 17.5 kU/L in CAP testing. All patients were interviewed by telephone for allergic reactions after consuming soy to investigate the prevalence of soy allergy in subjects with birch pollen allergy (prevalence group). Furthermore, they were investigated for IgE reactivity against Gly m 4. The serum of a subject without pollen and food allergy served as the control.

Ethical considerations

The study was reviewed and approved by the local ethical committee. Subjects provided informed consent before enrollment in the study.

DBPCFC

DBPCFC was performed by means of a 2-step spit (local mucosal challenge) and swallow procedure, as previously described for celery.¹⁹ Two different drinks, identical in color, texture, and taste, were prepared. The active drink contained 15 g of soy protein isolate (soy protein content, 90%; Protein technologies, Du Pont & Dow, Bad Homburg, Germany), 150 mL of milk, 50 mL of cream, 9 g of Sinlac (containing rice flour, and carob flour; Nestle, Vevey, Switzerland), 25 g of sugar, 7 g of cocoa, and 6 drops of banana flavor (Givaudan, Duebendorf, Switzerland). The soy protein isolate is a mildly heated soy product that was produced through extraction of defatted soybean flakes at pH 8 to 8.5, neutralization, and spray drying.¹¹ The composition of the placebo drink was identical but did not contain soy isolate. Apart from soy, all ingredients were known to be tolerated by each patient. For the local mucosal challenge, 4 doses containing 0.3, 0.5, 1, and 2 g of soy protein, respectively, were administered to the patients, whereas for the systemic challenge, 4 doses containing 0.5, 1, 2, and 4 g of soy protein, respectively, were used.

Skin prick tests

Skin prick tests (SPTs) were performed as previously described.²⁰ Patients were tested with pollen extracts from alder, birch, hazel, grass, mugwort (ALK Abelló, Horsholm, Denmark), soybean, peanut, pea, bean, wheat (Stallergènes, Antony, France). Raw lupine flour was tested by using the prick-to-prick-technique. Areas of wheal-and-flare reactions were recorded after 15 minutes. A wheal diameter of more than 3 mm was regarded as positive.

Determination of specific IgE

Specific IgE to rBet v 1, rBet v 2, rBet v 6, birch pollen, and soybean were analyzed by using the CAP FEIA system (Pharmacia Diagnostics, Uppsala, Sweden). For determining specific IgE to Gly

m 4, biotinylated rGly m 4 (5 μ g per CAP) was coupled to streptavidin-coated CAPs (Pharmacia Diagnostics). Biotinylation was performed by using 10 μ g of Biotin-X-NHS (Calbiochem, San Diego, Calif) per 100 μ g of protein at room temperature for 4 hours. Before coupling to activated CAPs, excessive Biotin-X-NHS was inactivated with ammonium chloride, and the sample was dialyzed against PBS at pH 7.4. As a control, the same procedure was performed with rBet v 1 and compared with the measurements of commercially available rBet v 1 CAPs (Pharmacia Diagnostics).

Soybeans, soy foods, protein extracts, and recombinant allergens

Ten different soybean varieties, crossbreeds of Swedish, North American, Chinese, and Swiss soy varieties, were provided by Arnold Schori (Swiss Federal Research Station for Plant Production, Changins, Switzerland) and Thomas Hebeisen (Swiss Federal Research Station for Agroecology and Agriculture, Zurich-Reckenholz, Switzerland). Soy-derived foods were bought in supermarkets (soy flakes), pharmacies (dietary powder), or health food shops (soy sauce, soy drink, miso, tofu, roasted soybeans, textured soy protein, and chocolate bar containing soy). Food and soybean protein extracts were prepared by freezing food with liquid nitrogen, followed by milling and stirring for 3 hours at 4°C with PBS. The resulting suspension was centrifuged at 20,000g for 20 minutes at 4°C. Finally, the supernatant was filtered through a nitrocellulose membrane of 0.45- μ m pore size (Sartorius, Goettingen, Germany). Extracts placed in aliquots were stored at –80°C.

Nonfusion rGly m 4 and rGly m 3 fused to maltose-binding protein were produced and purified to homogeneity, as previously described.^{11,21} Maltose-binding protein alone served as a negative control.

Enzyme allergosorbent test inhibition

Enzyme allergosorbent test (EAST) inhibition was performed to study cross-reactivity between birch pollen and soy allergens. Soy protein isolate extract was coupled to cyanogen bromide-activated filter-paper disks (Hycor, Kassel, Germany) at 25 μ g of soy protein per disc according to the method originally described by Ceska and Lundkvist.²² Six hundred paper disks were incubated with 100 mL of extract dilution in 0.1 mol/L NaHCO₃ containing 15 mg of soy protein overnight at 4°C. Excessive active sites were deactivated by incubating with 0.1 mol/L ethanolamine, and the disks were washed with acetate buffer, pH 4.0, and PBS, pH 7.4. Thereafter, they were freeze-dried and stored at –80°C.

Inhibition of IgE binding to soy protein isolate extract was studied with sera of patients with positive results to soy protein isolate in DBPCFCs by using rGly m 4 and birch pollen extract as inhibitors. Ovalbumin was used as a negative control. Diluted (1:2) patient sera (n = 11) were incubated with serial dilutions (1:10, n = 7 or 4 depending on the amount of serum available) of birch pollen extract (starting at 0.85 mg/mL) and rGly m 4 (starting at 0.1 or 1 mg/mL) and soy isolate allergen disks overnight. IgE binding to the solid phase in relation to an uninhibited control was detected with an EAST kit (Spez. IgE ELISA; Allergopharma, Reinbek, Germany). The results were calculated as the percentage of inhibition.

Electrophoresis and immunoblotting

SDS-PAGE was performed according to the method of Laemmli²³ by using a 15% separation gel and a 5% stacking gel. Proteins were reduced by heating with 1,4-dithiothreitol (Sigma-Aldrich, Deisenhofen, Germany) loaded onto the gel at a concentration of 1 μ g/cm for rGly m 4 and rGly m 3 and 30 μ g/cm for soy isolate extract, respectively, and transferred onto 0.2- μ m pore-sized nitrocellulose membranes by means of semidry blotting with a Novablot apparatus

(Amersham Pharmacia Biotech, Uppsala, Sweden). Remaining binding sites were blocked twice by means of incubation with 0.3% Tween 20 in Tris-buffered saline (50 mmol/L Tris/HCl and 150 mmol/L NaCl, pH 7.4). The nitrocellulose strips were incubated overnight with 130 μ L of patient serum diluted to 1 mL with incubation buffer (TBS containing 0.05% Tween 20 and 0.1% BSA).

Determination of immune-reactive Gly m 4 in soybeans and in soy food

Food extracts were loaded at 7 μ g of protein per centimeter together with 6 standard solutions of rGly m 4, ranging from 1 to 50 ng/cm, onto the same gel. Blots were incubated with a rabbit anti-serum raised against rGly m 4 (1:20,000) to determine extracted immune-reactive Gly m 4. Signals of standards and samples were recorded with the Scanning System LumiImager. Standard curve and sample concentrations were calculated by using the associated software LumiAnalyst (Roche Diagnostics, Basel, Switzerland). The results were expressed as parts per million (ppm = milligram per kilogram) of detectable Gly m 4.

Circular dichroism spectroscopy

A circular dichroism (CD) spectrum of Gly m 4 was recorded after dialysis of Gly m 4 with 10 mmol/L potassium phosphate buffer at pH 7.4 on a Jasco J-810 spectropolarimeter with a step width of 0.2 nm and a band width of 1 nm and within a spectral range from 185 to 255 nm. The Gly m 4 CD spectrum was measured 10 times at a concentration of 6 μ mol/L at 20°C.

RESULTS

Clinical characteristics and case histories of patient group 1

Twenty-two (18 female and 4 male) patients with allergic reactions to soy entered the study. The mean age of the study population was 31.1 ± 11.5 years (range, 18–57 years). All patients had a case history of nonpediatric soy allergy causing oral allergy syndrome (OAS) and, in some patients, more severe symptoms, such as urticaria (6/22) or drop of blood pressure (2/22). Case histories with respect to soy allergy are summarized in Table E1, which can be seen in the Journal's Online Repository at www.mosby.com/jaci.

All patients reported pollinosis symptoms during the flowering season of birch. The most frequent offending foods were raw soybean sprouts ($n = 11$), soy milk, or drinks containing soy protein isolate ($n = 9$). Two patients reported allergic reactions after ingestion of meat-substitute products, such as tofu and soy protein-based sausage, and 2 further patients reported allergic reactions after ingestion of bakery products containing soy. All patients had multiple food allergies to fresh fruits, vegetables, legumes, and nuts. Apple (91%) and hazelnut (82%) were most frequently mentioned, followed by carrot (73%), celery (59%), almond (64%), and fresh fruits, such as peach (64%), kiwi (64%), pear (50%), and cherry (50%). Allergic reactions to other legumes were reported for peanut (55%), pea (9%), and bean (5%).

Ten percent of highly sensitized patients with birch pollen allergy have soy allergy

Ninety-four patients with birch pollen allergy and

with specific IgE to Bet v 1 of at least 17.5 kU/L on the Pharmacia CAP test who visited the Allergy Unit Zurich between 1998 and 2001 were contacted by telephone. All these patients agreed to give an interview about their allergic reactions to foods. Sixty-six patients (70.2%) had knowingly consumed soy foods, and 9 (9.6%) experienced allergic reactions to such foods. Symptoms were restricted to the oral mucosa and complied with the OAS, as described in other Bet v 1-related food allergies. The eliciting foods were soybean sprouts, tofu, and soy milk. Two of the patients with soy allergy reported symptoms after ingestion of raw soybean sprouts, but cooked soy products were tolerated. Allergic reactions to other legumes were rare: one of the patients with soy allergy and 3 of the other patients did not tolerate peanut, and 2 patients reported reactions to bean, whereas no reactions to pea or other legumes were observed. Serologic screening by means of immunoblotting revealed that 67 (71%) of 94 patients with birch pollen allergy showed IgE binding to rGly m 4.

Clinical and serologic investigations in patients with soy allergy strongly suggest birch pollen-related food allergy to soybean

Soy allergy was confirmed by means of DBPCFC with soy protein isolate in 16 patients allergic to birch pollen. All of them had oropharyngeal symptoms (OAS), such as itching and swelling. In 4 patients more severe reactions occurred (see Table E1, On-Line Repository). Symptoms were elicited during mucosal challenge in 12 patients and after systemic challenge in 4 patients.

No DBPCFCs were performed in 6 patients with positive case histories of soy allergy who either refused to undergo DBPCFCs ($n = 5$) or had severe anaphylactic reactions after consuming soy foods ($n = 1$).

In 17 patients SPTs with pollen and food allergens were performed. All patients had positive SPT responses to birch and hazel pollen, 16 to alder, 14 to grass, and 5 to mugwort pollen. Positive skin reactions to soy (commercial extract) were observed in 13 patients, and positive skin reactions to peanut were observed in 12 patients. Positive SPT results for other legumes were observed in 6 patients to lupine ($n = 5$) and pea ($n = 1$).

CAP test and immunoblot results are summarized in Table E1 (On-Line Repository). Only 10 patients showed specific IgE in the commercially available CAP test for soy (>0.35 –17.5 kU/L). However, specific IgE to self-prepared rGly m 4 CAPs was clearly detectable in 96% of patients, ranging between 0.71 and greater than 100 kU/L (Table E1, On-Line Repository). In immunoblotting rGly m 4 bound IgE antibodies from 19 (86.4%) sera (Table E1, On-Line Repository; original blotting results not shown). IgE binding to rGly m 3 occurred in 6 patients, including 2 patients without IgE binding to rGly m 4 in immunoblots. Fourteen patients showed IgE binding to other soy proteins in the range of 8 to 10, 18, 20, 35, and 50 kd.

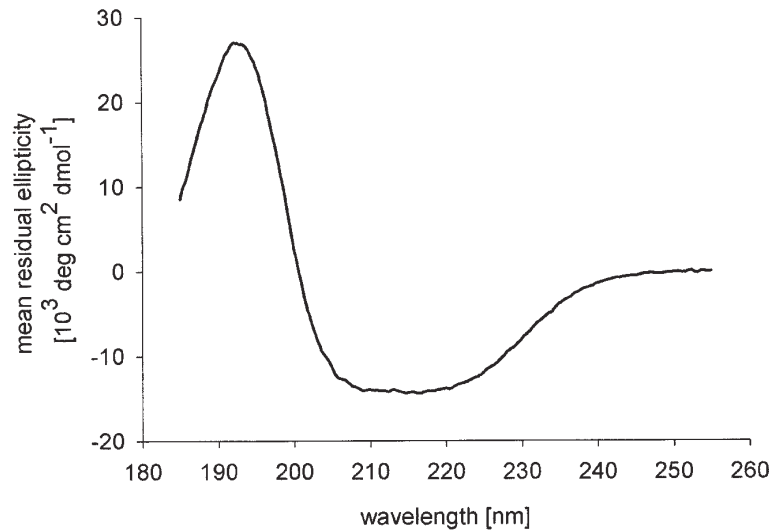


FIG 1. CD spectrum of rGly m 4.

Cross-reaction between birch pollen and soy allergens confirmed by means of EAST inhibition

Cross-reactivity between birch pollen and soy allergens was assayed in the sera of 11 patients with soy allergy by means of EAST inhibition (Table I). Birch pollen significantly inhibited IgE binding to soy protein isolate of all tested sera, with a minimum of 45% in one patient and maximum inhibitions of more than 80% in 9 of 11 patients. The maximum inhibition of IgE binding by rGly m 4 ranged between 36% and 100%, with 9 of 11 sera showing at least 60% inhibition. The 50% inhibition concentrations of rGly m 4 varied between 0.01 and 1 $\mu\text{g/mL}$, and only in 2 patients was 50% inhibition not reached. For birch pollen protein, 50% inhibition concentrations ranged between 0.1 and 100 $\mu\text{g/mL}$. The maximum inhibition obtained with the negative control protein OVA was 6%.

CD spectrum of rGly m 4

The CD spectrum of rGly m 4 (Fig 1) was found to be similar to the CD spectrum of Bet v 1 published by Scheurer et al,²⁴ and this indicates that rGly m 4 is a folded protein. An analysis with the software Jasco Secondary Structure Estimation revealed the following results: α -helix, 32.4%; β -sheet, 41.8%; turn, 0.0%; and random coil, 25.8%. These data indicate that Gly m 4 and Bet v 1 (27.6% α -helix, 40.3% β -sheet, 0.0% turn, and 32.1% random coil determined by means of x-ray and nuclear magnetic resonance spectroscopy)²⁵ share a similar distribution of secondary structure elements.

Gly m 4 content increases during ripening of soybeans and depends on the degree of processing of soy-derived foods

Ten soybean varieties were analyzed for their Gly m 4

TABLE I. EAST inhibition of IgE binding to soy protein isolate extract with sera of 4 patients, with recombinant Gly m 4 and birch pollen extract as inhibitors

Inhibitor	Patient	Maximum inhibition (%)	Inhibitor concentration at 50% inhibition ($\mu\text{g/mL}$)
rGly m 4	1	91	1
	2	79	0.01
	3	78	0.1
	4	72	1
	5	100	0.1
	16	72	0.1
	17	36	—
	14	60	0.1
	13	72	0.5
	22	66	1
	19	45	—
Birch pollen	1	100	1
	2	100	0.1
	3	100	1
	4	98	1
	5	88	1
	16	93	<1
	17	80	10
	14	50	100
	13	88	<1
	22	80	<100
	19	45	—

contents. The mean content of extractable and immune-reactive Gly m 4 was 256 ± 30 ppm. Different ripening states of the variety paradis were compared, and the Gly m 4 content was found to increase during ripening and storage. Unripe green soybeans contained approximately 8 ppm, ripe fresh soybeans contained 96 ppm, and ripe soybeans after 3 years of storage contained 224 ppm. On the basis of total protein, there was no difference between freshly harvested or stored ripe soybeans, but their Gly m



FIG 2. Quantification of Gly m 4 in soy foods: lane 1 to 6, rGly m 4 standards (50, 25, 10, 5, 2, and 1 ng/cm); lane 7, miso (0 ppm); lane 8, soy sauce (0 ppm); lane 9, roasted soybean (0 ppm); lane 10, choco bar with soy (1 ppm); lane 11, textured soy protein (2 ppm); lane 12, soya drink (6 ppm); lane 13, tofu (9 ppm); lane 14, soy flakes (11 ppm); lane 15 and 16, dietary powders I and II (69 ppm); lane 17, untreated soybean (260 ppm).

4 content on the basis of total protein amount was 6 times higher than that in unripe soybeans.

Ten soy-based foods and soybeans heated for between 0.5 and 4 hours were analyzed for Gly m 4 (Fig 2). In highly fermented soy foods (soy sauce and miso) and in roasted soybeans, no Gly m 4 could be detected. Textured soy protein contained 2 ppm Gly m 4, and tofu and soy flakes contained 9 and 11 ppm, respectively. In complex foods, such as candy bars and dietary powders, the Gly m 4 content is dependent on total soy protein content. Candy bars with soy contained approximately 1 ppm Gly m 4, and a tested soy drink contained 6 ppm. The highest amounts of Gly m 4 (70 ppm) were found in dietary powders containing soy. The estimated Gly m 4 content of the soy protein isolate we used for DBPCFC was 140 ppm. The Gly m 4 content of soybeans was reduced after 30 minutes of cooking, and after 4 hours of cooking, no Gly m 4 was detectable.

DISCUSSION

Allergic reactions to legumes are generally thought to be acquired by means of a primary sensitization through the gastrointestinal tract.²⁶ In the present study, however, we found strong evidence that in Central Europe soy allergy is another clinically relevant birch pollen-related allergenic food.

Birch pollen-related food allergies are typically mediated by cross-reactions of food allergens with birch pollen allergens, especially Bet v 1, Bet v 2 (profilin), and eventually Bet v 6.²⁷

In 1992, Crowell et al²⁸ described a stress-induced, developmentally regulated gene family from soybean. In particular, he characterized SAM22 encoding a disease-resistance response protein with structural homology to Bet v 1 that belongs to the pathogenesis-related protein family 10.²⁹ A recently published study by Kleine-Tebbe et al¹¹ described patients with birch pollen allergy with allergic reactions to a soy-containing dietary product and suggested for the first time an association between birch and soy allergy. Furthermore, Rihs et al²¹ cloned, sequenced, and characterized Gly m 3, the profilin from soybean.

To further evaluate the clinical relevance of Gly m 4 (SAM22) for patients with birch pollen allergy, 2 different populations of patients were recruited. Group one consisted of 22 patients allergic to birch pollen with non-pediatric soy allergy confirmed either by means of DBPCFC with soy protein isolate or a clear-cut case history. Group 2, however, consisted of 94 unselected patients with birch pollen allergy (apart from a positive

CAP result to Bet v 1 of at least 17.5 kU/L), in whom the prevalence of a sensitization to Gly m 4 and the risk of a clinically significant allergic reaction to soy was assessed.

It is a common belief that patients with pollen-related food allergies always have mild oropharyngeal symptoms. In the current study, however, approximately 50% of the patients in group 1 experienced systemic and even anaphylactic reactions after soy consumption according to the case history, and up to 50% of the patients had systemic symptoms during challenge, even when the DBPCFC was performed with a stepwise spit-and-swallow protocol that was abrogated after mucosal challenge in case of subjective symptoms. Consequently, no systemic challenge was performed in those patients. Furthermore, we have observed comparably high rates of systemic reactions in pollen-related food allergy during our former DBPCFC studies, particularly in celery and carrot allergy.^{19,30-32}

Twenty-one of 22 patients allergic to birch pollen with soy allergy were sensitized to Gly m 4, as determined by using the CAP method. Furthermore, in EAST-inhibition experiments, Gly m 4 accounted for at least 60% of the IgE-binding capacity of soybean extract in 9 of the 11 tested patients. These results are in agreement with those of Kleine-Tebbe et al,¹¹ who furthermore showed a high biologic activity of Gly m 4 by histamine release. Thus Gly m 4 was clearly identified as a major soy allergen in our study population. In addition, this hypothesis is in agreement with the CD spectrum of Gly m 4 measured in the present study, which suggests high structural similarity between Gly m 4 and Bet v 1.

Birch pollen extract inhibited IgE binding to soy protein isolate by 80% or more in 9 of 11 patient sera that were suitable for this test, suggesting that birch pollen allergens are the primary sensitizing agents in these patients, leading to soy allergy by means of cross-reaction with soy proteins, especially Gly m 4. Six patients showed soy profilin (rGly m 3)-specific IgE in Western blots, and 5 of them showed also birch pollen profilin (Bet v 2)-specific IgE reactivity in the CAP system. Because inhibition with Gly m 4 was lower than that with birch pollen extract, other allergens, such as Gly m 3, seem also to be involved in the cross-reaction between birch pollen and soy. In 2 of the 11 tested sera, birch pollen extract inhibited 45% and 50% of IgE binding to soy protein isolate, suggesting that apart from Bet v 1 and other birch pollen allergens, some of the patients in this study were also primary-sensitized to soy allergens.

Just 45% of patients with soy allergy had positive responses to the commercially available soy CAPs, but

96% had positive responses to the Gly m 4 CAPs prepared in our laboratory, and a subgroup of them had very high titers. Our results indicate that the low sensitivity of commercially available soy CAPs is at least in part caused by the lack or low amounts of Gly m 4 in the total protein extracts from soybean. The fact that diagnostic tools with commercial food extracts are often affected by a low sensitivity, in particular because of the lability of Bet v 1-related allergens, has been shown in previous studies.^{19,31}

The 22 patients with soy allergy included in the study had multiple other food allergies to fruits, nuts, and vegetables in a typical rank order, as previously described in pollen-related food allergy. The frequency of peanut allergy ($n = 12$, 55%) was noticeably higher in patients selected for soy allergy than in the group 2 patients allergic to birch pollen but not selected for soy allergy (results not shown). A high incidence of peanut allergy in patients with clinically relevant sensitization to Gly m 4 was also observed by Kleine-Tebbe et al.¹¹ Initial unpublished data from an ongoing investigation indicate that peanut allergy in these patients is also related to birch pollen.

Although 71% of patients allergic to birch pollen (group 2) with high titers of Bet v 1-specific IgE were sensitized to Gly m 4 (67/94), only 9.6% described allergic symptoms after soy food consumption in the telephone interviews. A similar lack of correlation between food-specific IgE and clinical relevance has already been observed with other Bet v 1-related food allergens.^{33,34}

The content of extractable Gly m 4 was measured in 10 commercially available soy foods to determine the stability of this allergen under conditions of different food-processing methods. The highest amounts of Gly m 4 were measured in dietary powders or soy drinks on the basis of soy protein isolate. No Gly m 4 was detected in fermented products, such as soy sauce and miso. Strong heating completely destroyed the antibody-binding capacity of Gly m 4 because we could not detect Gly m 4 in roasted soybeans and in soybeans cooked for 4 hours. Low amounts of Gly m 4 were found in textured soy protein, tofu, and soy flakes, which are usually treated with limited heating, other processing procedures, or both. From our results, we can conclude that Gly m 4 depicts certain stability to moderate heating because it was still detectable in soybeans after 2 hours of cooking. Accordingly, 3 of 22 soy-sensitive patients reported allergic reactions against heated soy foods, such as tofu, soy sausage, and soy-containing bread; 6 of 22 patients reported such reactions to soy milk that was briefly heated for pasteurization. Similarly, recent DBPCFC studies confirmed that both cooked celery and roasted hazelnut can cause allergic symptoms in subjects with pollen allergy,^{27,30} even if they were monosensitized to the Bet v 1-related allergen in celery, Api g 1, or hazelnut, Cor a 1, respectively.

Eleven soybean varieties and different stages of ripening of the soybean variety paradis were analyzed by means of immunoblotting for their Gly m 4 content. These data revealed a Gly m 4 content similar in all soybean varieties tested after at least 3 years of storage and are in good agreement with the results of Herian et al,³⁵

who also described no major differences in IgE binding between soybean varieties. In other birch pollen-related allergenic foods, varying contents of the Bet v 1-homologous proteins were described, for instance, in different apple varieties (Mal d 1)^{36,37} but not for celery tuber or hazelnut.³⁸ Interestingly, the mean Gly m 4 content is significantly lower in unripe soybeans than in freshly harvested soybeans. After storage of soybeans for 3 years, the Gly m 4 content was 2 times higher, which might be due to an increase of whole protein content by drying. For Mal d 1, however, no increase during storage of apples under ideal conditions, such as a controlled atmosphere, was described.³⁹

Soy foods are no common dietary component in Central Europe,⁴⁰ according to our study population, but its importance is rising because there is a trend toward Asian foods, as well as because of the promoted health benefits of soy proteins and other substances in soybean.⁴¹ As a result of the increasing importance of such foods, an increase in soy food allergy within the next years is very likely, especially in patients with birch pollen allergy. Our data indicate that these patients are at risk of experiencing allergic reactions to soy if they consume dietary products containing soy protein isolate. Probably because of mild technological treatment, soy protein isolates were found to contain the highest Gly m 4 levels, and such products contain up to 50% soy protein isolate and are consumed without further heat treatment. Food manufacturers might be able to reduce this risk by including a further heat-processing step of the source material.

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TABLE E1. Specific IgE to birch pollen, rBet v 1, rBet v 2, rBet v 6, soy, and recombinantGly m 4 (rGly m 4) as determined by CAP method and results of IgE-reactivity to rGly m 4 and recombinant soy profilin (rGly m 3) as detected on immunoblot in patients with positive DBPCFC or positive clinical history of soy allergy

Patient	Case history of soy allergy	DBPCFC symptoms	CAP (kU/L)						Western blot		SPT
			Birch pollen	Bet v 1	Bet v 2	Bet v 6	Soy	rGly m 4	rGly m 4	rGly m 3	Soy
Positive DBPCFC result											
1	OAS, Th	OAS, Th	78.90	81.7	<0.35	<0.35	<0.35	7.74	+	–	+
2	OAS	OAS	>100.00	>100	30.40	<0.35	2.12	77.7	+	+	++
3	OAS	OAS	77.10	89.3	<0.35	<0.35	<0.35	14.8	+	–	+-
4	OAS, U, BP	OAS, F, C	>100.00	>100	<0.35	<0.35	5.05	>100	+	–	++
5	OAS, U, LE	OAS, F	34.10	37.9	<0.35	<0.35	0.35	3.95	+	–	+
6	OAS	OAS, R, Th, H	32.30	37	<0.35	<0.35	<0.35	22.6	+	–	+
7	OAS, U	OAS	10.20	11.7	1.35	<0.35	0.65	0.92	–	+	+-
8	OAS, U	OAS	40.40	52.8	<0.35	<0.35	<0.35	3.84	+	–	+
9	OAS	OAS	2.90	2.45	<0.35	<0.35	<0.35	<0.35	–	+	–
10	OAS, N, D	OAS	35.30	38.1	<0.35	<0.35	<0.35	15.2	+	–	+
11	OAS, U, Dy, AE, BP	OAS	20.30	19.8	<0.35	<0.35	<0.35	3.23	+	–	+
12	OAS, U, N	OAS	27.50	21.1	<0.35	<0.35	<0.35	3.06	+	–	+
13	OAS, Th, Cr, N, R	OAS, H, Th	27.4	36.1	<0.35	<0.35	0.51	10.4	+	–	+++
14	OAS	OAS	7.71	5.16	<0.35	<0.35	<0.35	1.01	–	–	+
15	OAS, Th	OAS, Th	15.7	16.1	2.55	<0.35	<0.35	0.72	+	+	+
16	OAS, Bl	OAS, Bl, F	37.2	34.9	7.76	<0.35	1.59	9.34	+	+	++
Positive history											
17	OAS, N, F, BP	ND	16.20	20	<0.35	<0.35	0.54	3.98	+	–	–
18	OAS	ND	60.0	54	<0.35	3.85	1.85	16.6	+	–	ND
19	OAS	ND	69.7	80.7	0.52	<0.35	2.42	3.41	+	+	ND
20	OAS	ND	77.1	54.9	<0.35	<0.35	<0.35	12.8	+	–	ND
21	OAS	ND	81.4	68.7	<0.35	<0.35	<0.35	16.9	+	–	ND
22	OAS	ND	>100	89.6	<0.35	<0.35	1.25	68.8	+	–	ND
Total			100%	100%	23%	4.6%	45%	96%	86%	27%	77%

Th, Tightness of throat or chest; U, urticaria; BP, drop of blood pressure; F, flush; C, conjunctivitis; LE, larynx edema; R, rhinitis; H, hoarseness; N, nausea; D, diarrhea; Dy, dyspnea; AE, angioedema; Bl, blisters of the oral mucosa; ND, not determined.