Severe oral allergy syndrome and anaphylactic reactions caused by a Bet v 1–related PR-10 protein in soybean, SAM22

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Background: Anaphylactic reactions to soy products have been attributed to stable class 1 food allergens.
Objective: IgE-mediated reactions to a soy-containing dietary food product in patients allergic to birch pollen were investigated.
Methods: Detailed case histories were taken from 20 patients. Their sera were analyzed for IgE (UniCAP) specific for birch, grass, mugwort, the recombinant birch allergens rBet v 1 and rBet v2, and soy protein. Extracts from birch pollen, soy isolate, rBet v 1, and the recombinant PR-10 soy protein rSAM22 were coupled to paper disks or nitrocellulose for IgE measurements (enzyme allergosorbent test) or Western blot analysis. Enzyme allergosorbent testing, Western blot inhibition, and histamine release studies were performed with the same allergens.
Results: Most patients (17/20) experienced facial, oropharyngeal, and/or systemic allergic symptoms within 20 minutes after ingesting the soy product for the first time. Birch pollen allergy (16/20) was common, along with oral allergy syndrome to apple (12/20) or hazelnut (11/20). IgE levels to birch and Bet v 1 but not to other inhalants were high in 18 of 20 patients. Significant IgE binding to rSAM22 occurred in 17 of 20 patients. Blot experiments with the soy isolate revealed IgE-binding bands at 17 kd (15/20), 22 kd (1/20), and 35 to 38 kd (2/20); the former was inhibited by preincubation of the sera with rBet v 1 or rSAM22. Birch extract and soy isolate, rBet v 1, and rSAM22 induced dose-dependent histamine release in the nanomolar range.
Conclusion: Immediate-type allergic symptoms in patients with birch pollen allergy after ingestion of soy protein–containing food items can result from cross-reactivity of Bet v 1–specific IgE to homologous pathogenesis-related proteins, particularly the PR-10 protein SAM22. (J Allergy Clin Immunol 2002;110: 797-804.)

Key words: *Food allergy, soy protein, SAM22, pathogenesis-related protein, birch pollen allergy, Bet v 1, IgE*

Abbreviations used
DBPCFC: Double-blind, placebo-controlled food challenge
EAST: Enzyme allergosorbent test
OAS: Oral allergy syndrome

Soy-derived proteins are considered one of the most important nutrients of the legume family. Clinically relevant immediate-type and late-phase allergic reactions can occur in atopic children younger than 3 years of age[1,2] when milk is substituted by soy products in patients with cow's milk allergy.[3] Allergic reactions as a result of primary gastrointestinal sensitization to soy products in adults are rare events, and the prevalence of soybean allergy has been estimated to be less than 0.5% of the general population.[4] Cutaneous, respiratory, and gastrointestinal symptoms caused by soy protein allergy are common, but severe systemic reactions, including fatal anaphylaxis, have been reported also.[5,6]

Epidemic exacerbations of asthma have been attributed to inhalant sensitizations and subsequent reactions after high exposure to soybean dust as a result of large-scale shipment and handling in sea harbors.[7-9] IgE-mediated allergy to soy products might be the result of primary sensitization but could also result from cross-reactivity to a variety of legumes (eg, peanut, pea, and bean) and cereals (eg, wheat and barley).[10,11] Cross-reactivity between soy and peanut proteins represents a particular clinical and therapeutic challenge: both share common antigens, are widely used in food products, and account for a growing number of allergic reactions. Because IgE cross-reactions between peanut and soy appear to be clinically irrelevant in many patients with peanut allergy,[12] controlled food challenges have been emphasized to demonstrate the clinical relevance of IgE-mediated sensitization to soy proteins.[13]

At least 16 soybean allergens have been described in the literature.[4] The major ingestive allergens appear to be Gly m Bd 30 k (thiol-protease P34), the storage proteins glycinin and β-conglycinin, and profilin (Gly m 3). Recently, the acidic subunit of glycinin G1[14] and the basic subunit of glycinin G2[15] have been reported to be important allergens in patients with food allergy to soybeans. Because most double-blind, placebo-controlled food challenge (DBPCFC) studies have been performed in pediatric populations in the United States,[13] the relevance of soybean allergy and allergen recognition in adults and adolescents is not clear.

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TABLE I. Clinical symptoms and treatment of patients reacting to a soy protein–containing dietary supplement food product

Patient no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	n
Allergic symptoms after ingestion of a soy-containing food product																					
Face swelling		×	×	×	×	×	×	×	×	×	×	×		×	×	×	×	×	×	×	17
OAS		×	×	×			×	×		×	×			×	×	×	×	×	×	×	14
Throat tightness, swallowing discomfort							×	×	×	×	×				×	×	×	×	×	×	11
Dyspnea (chest tightness, wheezing)	×				×	×			×					×			×				6
Hives, urticaria	×		×		×										×	×			×		6
Drowsiness, vascular dysregulation		×	×				×							×		×					5
Gastrointestinal discomfort, vomiting							×						×	×					×		4
Nasal secretion, nasal congestion				×										×							2
Treatment because of the allergic reaction																					
Emergency treatment	×	×	×	×	×	×			×					×	×	×	×	×		×	13
Intravenous injection	×	×			×	×			×					×	×	×	×	×		×	11
Intravenous infusion		×				×			×					×		×	×	×			7
Hospital									×							×	×	×			4

A case of severe oral allergy syndrome (OAS) and anaphylactic symptoms after ingestion of a soy protein–containing food supplement in a patient with birch pollen allergy without any signs of a classical soy allergy[16] prompted us to study a number of similar patients with severe reactions after ingestion of this processed soy product in greater detail.

METHODS

Soy protein–containing supplementary food products and other allergens

Allergic reactions occurred after ingestion of a supplementary food product (Almased; Vitalkost, Bienenbüttel, Germany) consisting of 50% wt/vol soy protein (Protein technologies, Du Pont & Dow, Bad Homburg, Germany), 25% wt/vol spray-dried yogurt (Uelzena Milchwerke, Uelzen, Germany), and 25% wt/vol bee honey (raw honey from different sources and countries). Minute amounts of different vitamins and colloid silica had been added to the dry powder, which was subsequently marketed as a dietary and health-promoting food supplement diluted in water by the consumer.

On the basis of the information provided by the manufacturer, the soy protein isolate was produced by extraction at pH 8 to 8.5 of defatted soybean flakes that were produced by using a crusher process (ie, soybean oil preparation) and purchased from different companies. After neutralization, the protein solution was spray-dried, resulting in a standard soy protein isolate.

Patients

After clinical and laboratory evaluation of the initial individual who experienced a severe anaphylactic reaction after ingestion of Almased,[16] the developer and the manufacturers were informed. Patients with similar reactions were identified and asked for their cooperation. After taking their individual case histories, all patients (n = 20, 15 female and 5 male patients) were informed about the purpose of the study. Blood was drawn from all patients by their local physicians, and sera were sent to the investigators and stored at –20°C. For histamine release studies, we selected additional patients with birch pollen allergy (n = 8) with a high basophilic response to birch pollen. Two of 8 patients had a history consistent with soy allergy, and one of them (patient 20, Table I) reacted to the indicated product.

Case histories

Detailed case histories of each patient were collected by the same allergologist. Questions were designed to elucidate family-related and self-reported atopic status, respiratory symptoms, seasonal variations and the general course of the disease or diseases, allergen-specific sensitizations to atopic allergens, self-reported diagnostic results of previous allergy tests (particularly to seasonal inhalants and food-related IgE-mediated sensitization), or clinical reactions, including OAS. The interview was focused on circumstances and the amount of ingested soy product, onset and time course of each symptom, emergency treatment, and general time course of the reaction.

Preparation of allergen extracts

The constituents of the dietary product (eg, soy isolate, yogurt, honey, and vitamin mixture) were extracted with 0.05 mol/L PBS. In all extracts, the protein concentration was measured with a commercial dye binding assay (Pierce, Rockford, Ill). All extracts were stored at –20°C until used.

Generation of recombinant SAM22

The cDNA encoding for SAM22 protein was prepared as previously described[17] and originally cloned into the pBluesript SKII plasmid through *Eco*RI (5′) and *Not*I (3′) restriction sites. For ligation into the pET-11a expression vector (Novagen, Schwalbach, Germany) restriction sites (5′-*Nde*I and 3′-*Bam*HI) were added to the coding cDNAs of the allergen by means of PCR with the primers 5′-Nde-Sam-pET11(+) (5′-GCA GCC *CAT ATG* GGT GTT TTC ACA TTC GAG GAT G-3′) and 3′-Sam-Bam-pET11(–) (5′-CCA AAC *GGA TCC* TTA GTT GTA ATC GGG ATG GGC C-3′; restriction sites and stop codons are underlined). Primers were purchased from ARK (Darmstadt, Germany). The PCR conditions were as follows: hot start (95°C for 2 minutes), 35 cycles each of 0.5 minutes of denaturation at 95°C, 1 minute of annealing at 54°C, 1.5 minutes of polymerization at 72°C, and a final extension for 10 minutes at 72°C. After digestion with the appropriate enzymes, the product was ligated into pET11a and initially established in *Escherichia coli* strain Nova Blue (Novagen). Positive clones were selected by means of PCR screening with the vector-specific T7 promoter primer and the T7 terminator primer and by sequencing. For gene expression, the plasmid was transformed into *E coli*

TABLE II. Reported allergic sensitizations of patients reacting to a soy protein–containing dietary supplement food product

Patient no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	n
Clinically relevant pollen allergens																					
Birch	×	×	×	×	×		×	×		×	×			×	×	×	×	×	×	×	16
Alder			×	×	×			×			×			×	×	×	×	×	×	×	12
Hazel			×	×	×			×			×			×		×	×	×	×	×	11
Other (grasses, mugwort)			×													×	×				3
Associated food allergens																					
Apple	×	×	×		×		×	×			×			×		×	×		×	×	12
Hazelnut	×		×					×		×	×			×	×	×	×	×		×	11
Peach				×											×		×		×	×	5
Carrot				×				×													2
Other (cherry, nectarine, kiwi)		×					×	×					×	×	×		×	×	×	×	10

BL21(DE3) cells. Protein synthesis was induced with 1 mmol/L isopropyl-β-D-thiogalactoside for 5 hours at 37°C and then overnight at room temperature. The cells were harvested by means of centrifugation (20 minutes at 5000*g*), washed once with LB medium, and resuspended in 25 mmol/L imidazole (pH 7.4, 4 mL of buffer per gram of bacterial pellet) containing protease inhibitors (complete protease inhibitor cocktail tablet; Boehringer, Mannheim, Germany). The cell suspension was freeze-thawed 3 times in liquid nitrogen and subsequently centrifuged at 10,000*g* for 30 minutes. The supernatant was stirred for 1 hour at 4°C with 1% streptomycin sulfate and 0.1% polyethylene imine to remove DNA. Finally, the mixture was centrifuged at 12,000*g* for 1 hour. Purification of SAM22 from the supernatant was performed by means of chromatofocusing with an ÄKTA-FPLC system (Amersham Biosciences, Freiburg, Germany) by using a Mono P HR 5/5 column. After SDS-PAGE analysis, fractions containing SAM22 (molecular weight, 17 kd; eluted at an isoelectric point of 4.4) were pooled, dialyzed against PBS (diluted 1:10), and concentrated by means of ultrafiltration with Centricon filter units (Millipore, Eschborn, Germany) with a molecular weight cut-off point of 3000 d. Recovery was about 25% to 50% of the total protein content with a purity of greater than 98%, as estimated from silver-stained gels.

Determination of specific IgE levels

The sera were analyzed for total IgE and allergen-specific IgE by using the UniCAP system (Pharmacia Diagnostics, Uppsala, Sweden) according to the manufacturer's instructions, with birch (t3), timothy grass (g6), and mugwort (w6) pollen; the recombinant birch pollen major allergen Bet v 1 (Rt215); the recombinant birch pollen profilin Bet v 2 (Rt216); and soybean protein (f14) as antigens. Results were expressed in kilounits of antigen per liter and as CAP classes (0-6).

For additional IgE determinations, rSAM22 and soy protein isolate extract were coupled to CNBr–activated filter-paper disks (Hycor, Kassel, Germany) at optimized concentrations depending on the source (5 μg per disk of soy protein and 0.25 μg per disk of rSAM22), according to the method originally described by Ceska and Lundkvist.[18] The enzyme allergosorbent test (EAST) was performed with "Allergopharma Spez. IgE ELISA," according to the instructions of the manufacturer (Allergopharma, Reinbek, Germany). Dose-related EAST inhibition studies were performed as previously described.[19,20] Concentrations of inhibitors and dilutions of sera are given in the legends of the corresponding figures.

Western blotting

SDS-PAGE was carried out according to the method of Laemmli[21] and performed as previously described.[22,23] For blotting inhibition, sera were preincubated with inhibitors (15 μg of recombinant allergen or 100 μg of total protein from allergen extracts) and added to blot strips with rSAM22. Thereafter, immunodetection was continued according to the standard procedure.

Histamine release assay

Histamine release from basophils was performed with washed leukocytes, as previously described,[24,25] by using an automated fluorometric assay.[26] Cells were stimulated with increasing concentrations of birch pollen extract (MAST Diagnostica, Reinfeld, Germany), rBet v 1 (Biomay, Vienna, Austria), soy isolate extract, rSAM22, and anti-IgE (Behring, Germany).

RESULTS

Case histories

After ingestion of 4 to 8 spoons of a soy protein–containing food supplement dissolved in 0.5 to 1 glass of tap water, 18 of 20 patients who never had hypersensitivity reactions to soy products before experienced severe allergic symptoms (Table I) within 10 to 30 minutes after the first intake. Only 2 individuals had symptoms without immediate onset after several hours (patient 13) or after 1 day (patient 12). Facial symptoms, including swelling and oropharyngeal reactions, were most common. Some patients had additional or exclusively (patient 1) systemic symptoms at sites far from the mucosal exposure. The majority of patients required immediate emergency treatment (antihistamines and corticosteroids administered by means of intravenous injection). Some individuals were hospitalized and monitored in emergency units because of the life-threatening character of the reaction (Table I). Symptoms declined within 0.5 to 4 hours after reaching their maximal severity.

The majority of patients reported symptoms during the tree pollen season (Table II). More than half of them described oropharyngeal symptoms to apple, hazelnut, peach, or a variety of other fruits, such as apricot, cherry, kiwi, nectarine, pear, or plum. Only a minority (4/20) of patients (Table II, see data of patients 6, 9, 12, and 13) neither reported on previous birch pollen–related airway symptoms nor experienced any oropharyngeal symptoms to apple, hazelnut, or other biologically and allergenically related fruits. Two of these individuals (patients 6 and

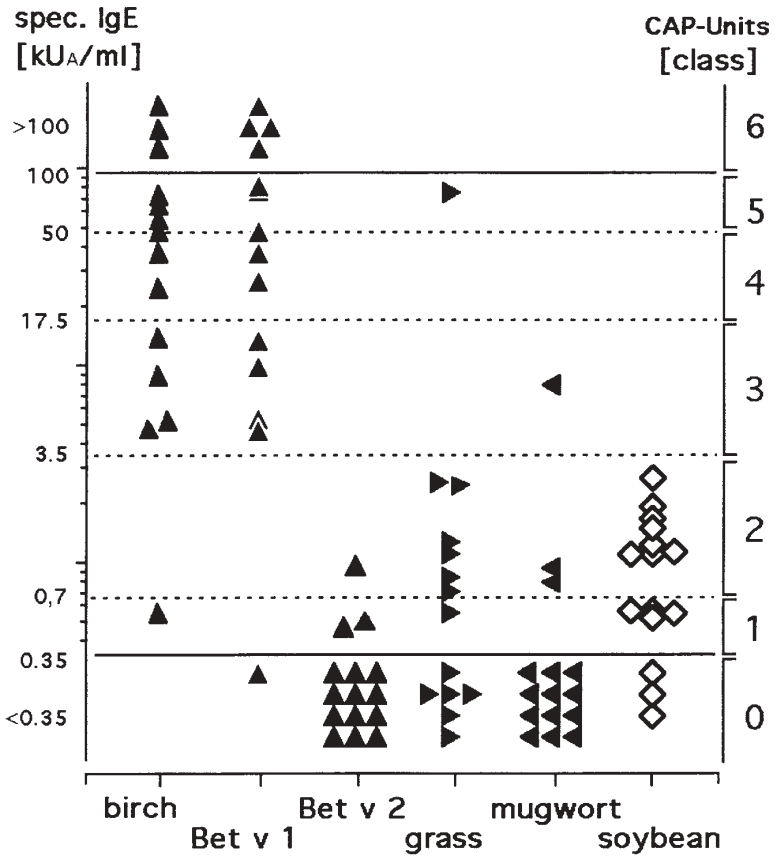

FIG 1. High allergen-specific IgE levels (*left y-axis,* quantitative levels; *right y-axis,* corresponding CAP classes) to birch pollen extract and the major allergen rBet v 1 of 20 patients with anaphylactic reactions to a soy product.

9) with a negative case history of birch pollen allergy, OAS, or both had severe symptoms requiring emergency treatment with intravenous drugs and fluid administration and further monitoring in a hospital setting (patient 9).

High IgE levels to birch pollen are associated with moderate titers to soybean

Total serum IgE levels ranged between 12.7 and 458 kU/mL (geometric mean, 117 kU/mL; median, 134 kU/mL). Most patients (18/20) had increased allergen-specific IgE levels to birch pollen extract and to rBet v 1 (Fig 1). The ratio of birch pollen–specific IgE levels and total IgE levels revealed values between 12% and 50.5% (average ± SEM, 29% ± 2.8%). In other words, almost one third of the individual's total IgE was specific for rBet v 1, indicating a birch pollen–dominated allergen-specific immune response in this group of patients. Only a minority of patients (3/20) showed slightly increased IgE levels (<1.0 kU/mL) to the birch pollen profilin Bet v 2 (Fig 1). A few patients demonstrated allergen-specific IgE to other seasonal inhalant allergens, such as grass or mugwort pollen. IgE measurements with commercially available total soy protein revealed moderate IgE titers in 55% (11/20) of the patients, with values ranging from 0.56 to 2.7 kU/mL, corresponding to CAP class 1 to 2.

IgE reactivity to the soy protein isolate in the supplementary food product

Extracts were prepared from all the main constituents of the supplementary food product (soy isolate, yogurt, honey, and vitamin mixture) and coupled to cyanogen bromide–activated filter paper disks to identify the allergen source of the severe reactions of the patients. In the subsequent EAST, increased IgE levels were found to the soy isolate in 12 of 20 of the sera, whereas no IgE reactivity to the other ingredients was observed (data not shown). Thus milk proteins and pollen allergens in the honey were convincingly excluded as the allergen source. In western blotting 14 of 20 patients showed IgE binding to a 17-kd band of the soy isolate extract. Two sera reacted to a band at approximately 35 to 38 kd, and one reacted to a 22-kd protein. Four sera did not show any IgE binding to soy isolate immunoblots (data not shown).

Identification of the PR-10 protein SAM22 as the allergenic component in soy isolate

Considering the strong sensitization of most patients to Bet v 1 and the fact that a stress-induced PR-10 protein in soybean[17] presented 53% amino acid sequence

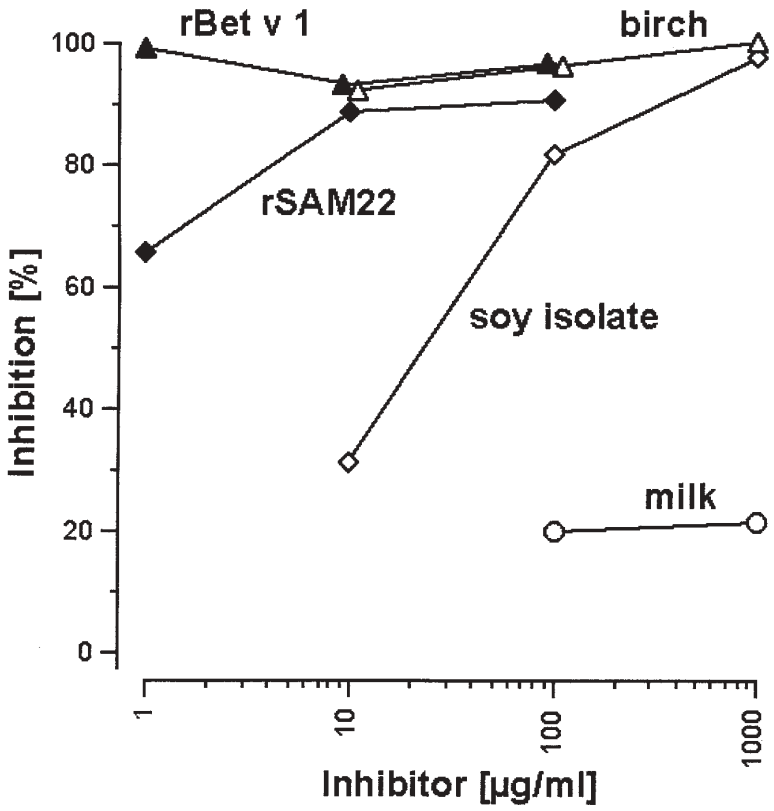

FIG 2. EAST inhibition. Allergen disks with soy isolate extract were incubated with pooled serum from patients 5, 6, 7, 8, 16, 18, and 20 (diluted 1:5) and increasing amounts of inhibitors.

identity with Bet v 1, the major birch pollen allergen,[27] we decided to produce a recombinant SAM22 molecule for further immunologic characterization. The coding region of the SAM22 cDNA was cloned into the prokaryotic expression vector pET-11a and expressed in *E coli* BL21 (DE3) cells as a nonfusion protein. After purification by means of chromatofocusing, the protein appeared to be greater than 98% pure in a Coomassie-stained SDS-PAGE gel. Recombinant SAM22 was coupled to cyanogen bromide–activated filter disks and tested for IgE binding by means of EAST. Seventeen of 20 patients presented specific IgE levels to rSAM22 corresponding to EAST classes of between 1 and 4. Quantitative IgE levels to rBet v 1 and rSAM22 were significantly ($P <$ .01) associated (Spearman rho = 0.68, data not shown).

An EAST inhibition assay was carried out with disks coated with soy isolate extract and a serum pool from 7 patients with EAST classes of at least 2 to rSAM22 (patients 5-8, 16, 18, and 20; Fig 2). Extracts from birch pollen and soy isolate, as well as rSAM2 2 and rBet v 1, were used as inhibitors. Maximal inhibitions were as follows: birch pollen, 100%; Bet v 1, 97%; soy isolate, 97%; and rSAM22, 90%. The estimated 50% inhibition concentrations were less than 1 µg/mL for birch pollen, rBet v 1, and rSAM22 and 50 µg/mL for soy isolate extract. No inhibition was observed with 1 mg/mL milk protein, which served as a negative control. These data demonstrated that greater than 90% of the soybean-specific IgE in the serum pool was directed against the PR-10 protein SAM22 and that these antibodies are highly cross-reactive with Bet v 1. In concordance with the immunoblotting and EAST results, the relatively high 50% inhibition concentration of soy isolate extract indicated a low abundance of SAM22 in the total soy isolate proteins. Immunoblot inhibition experiments confirmed that SAM22 was the predominant IgE-binding component in the soy isolate. Using a pool of sera from patients 7 and 10, IgE binding to natural SAM22 in soy isolate extract was completely inhibited by soy isolate, birch pollen extract, Bet v 1, and rSAM22 but not by the minor birch pollen allergen rBet v 6[28] and milk protein, which were applied as negative controls (Fig 3).

Histamine release assays demonstrate biologic activity of rSAM22

Dose-dependent basophilic histamine release demonstrated cross-linking of cell-bound IgE by all stimuli, with strong variability in sensitivity among individuals (Fig 4, data of 2 representative experiments). Birch pollen extract and rBet v 1, the latter being up to one log more sensitive, induced basophilic histamine release at extremely low concentrations (10^{-15}-10^{-12} g/mL). The soy isolate extract was far less potent, triggering substantial histamine release (>30%) at higher concentrations (nanograms to micrograms per milliliter). The same individuals showed histamine release to variable concentrations (10^{-15}-10^{-9} g/mL) of rSAM22. Two individuals

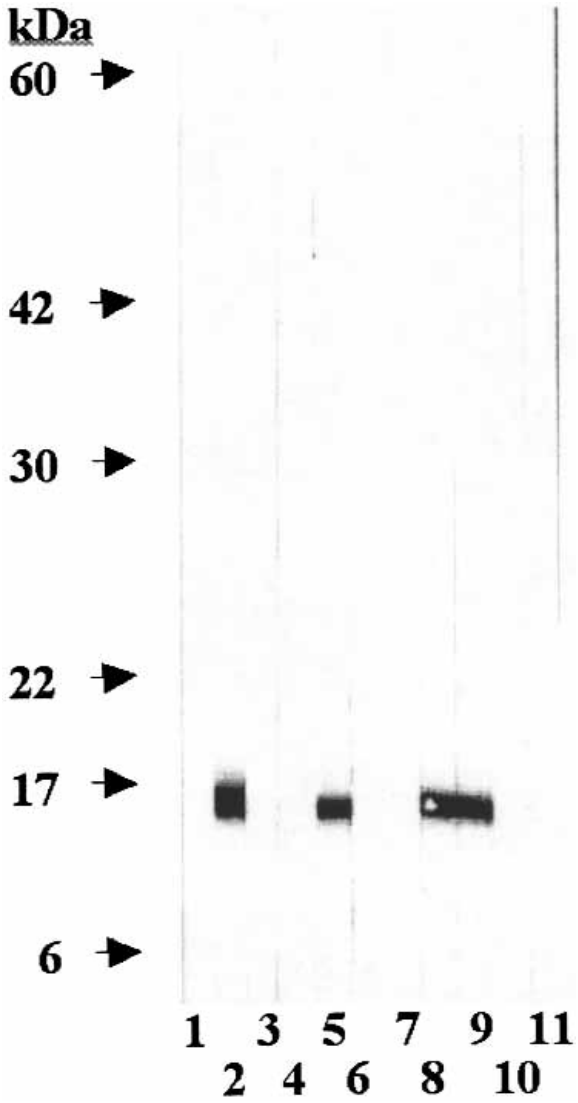

FIG 3. Immunoblotting inhibition. Soy protein isolate extract (30 μg of protein per centimeter) was separated by means of SDS-PAGE and transferred onto a nitrocellulose membrane. *Lane 1* indicates buffer control. Pooled serum from patients 7 and 10 (130 μL, diluted 1:7.5) was added to the blot strips without inhibitor *(lane 2)* and after preincubation with 100 μg of soy isolate protein *(lane 3)*, 100 μg of birch pollen protein *(lane 4)*, 100 μg of skim milk protein *(lane 5)*, 15 μg of rBet v 1 *(lane 6)*, 50 μg of rBet v 1 *(lane 7)*, 15 μg of rBet v 6 *(lane 8)*, 50 μg of rBet v 6 *(lane 9)*, 100 μg of Almased extract *(lane 10)*, and 15 μg of rSAM22 *(lane 11)*.

experienced anaphylactic reactions after ingesting soy products (patient 20 to the soy isolate investigated). However, mediator release to rSAM22 also occurred in some patients with birch pollen allergy (ie, 21) who had no history of soy allergy.

DISCUSSION

The allergic reactions after ingestion of a soy-containing supplementary food product in 20 patients displayed striking similarities. First, none of the individuals was aware of an allergic sensitization or a previous reaction to soy. Second, most patients experienced a severe, systemic reaction after their first ingestion of the soy-containing product, indicating a previous sensitization. Third, the reported symptom pattern affecting the head and throat (ie, itchy eyes, angioedema, face and ear swelling, nasal secretion and congestion, various oromucosal symptoms, throat tightness, and swallowing discomfort) indicated rapid oropharyngeal adsorption of the allergens, resembling an OAS of extraordinary severity that was probably caused by high amounts of locally released inflammatory mediators (ie, histamine). Fourth, most individuals reported previous conjunctival and nasal symptoms during the birch pollen season and had experienced oral allergy symptoms after ingestion of apple, hazelnut, peach, carrot, and/or other fruits (ie, cherry, nectarine, and kiwi).

The in vitro analysis of IgE antibody reactivities to common inhalants revealed additional parallels. Fifth, most patients had high birch pollen–specific IgE titers. Sixth, on average, up to one third of the total serum IgE was directed toward birch pollen allergens. Seventh, high absolute and relative levels of Bet v 1–specific IgE and low levels of IgE to birch pollen profilin (Bet v 2) indicated a predominant role of the birch pollen major allergen as the sensitizing allergen in these patients. Eighth, low and in some cases negative levels of allergen-specific IgE to soybean, determined by using a commercial immunoassay, did not correspond to the severe clinical reactions of most individuals.

Thus the majority of allergic reactions were unlikely to have been the result of a primary gastrointestinal IgE-mediated sensitization to one of the classical, stable soy allergens designated class 1 food allergens[29] and described in previous studies.[4] Because of the peculiar nature of the reported clinical reactions and the frequent and strong sensitizations to the birch pollen major allergen Bet v 1, a pollen-related food allergy to a Bet v 1–homologous protein in soy was assumed. Interestingly, several stress-related mRNAS, so-called starvation-associated messages (ie, SAM22, SAM26, and SAM46), had been identified in soy.[30,31] SAM22 (accession no. X60043) encodes a disease resistance response protein[30] structurally related to Bet v 1–homologous proteins and belonging to the PR-10 family of pathogenesis-related proteins. A BLAST search (http://www.ncbi.nlm.nih.gov/blast) revealed a high degree (>50%) of amino acid sequence identity of SAM22 with members of the PR-10 family: 53% with Bet v 1,[27] 58% with the major hazelnut allergen Cor a 1.0401,[32] 53% with the major apple allergen Mal d 1,[33] and 54% with the major cherry allergen Pru av 1.[34]

To prove the hypothesis that a pollen-related allergen in soybean was responsible for the allergic reactions to the supplementary food product, we produced a highly pure recombinant SAM22 protein. Increased IgE levels to rSAM22 occurred in most patients. Both rSAM22 and rBet v 1 fully inhibited IgE binding to the soy protein isolate in EAST (Fig 2) and immunoblotting inhibition assays (Fig 3). Considering the absence of IgE binding to

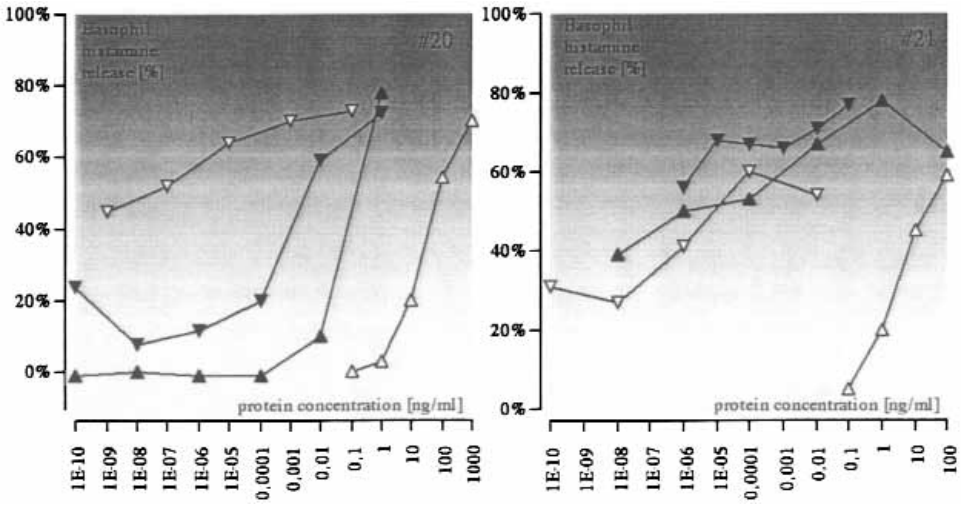

FIG 4. Dose-dependent histamine release from washed leukocytes of patients with birch allergy (2 examples of 8 experiments) to birch pollen extract, rBet v 1, soy isolate extract, and rSAM22 (patient 20 with and patient 21 without a case history of soy allergy).

other constituents of the food product, such as honey or milk, these results support the view that SAM22 was the elicitor of the adverse food reactions in our patient panel. This conclusion is further supported by the capacity of rSAM22 to induce mediator release from basophils of patients with birch pollen allergy (Fig 4). Interestingly, some patients with birch pollen allergy without a history of soy-induced allergy also demonstrated soy extract–mediated and rSAM22 histamine release, suggesting IgE-mediated cross-reactivity without clinical relevance in these cases.[24]

In addition to SAM22, non–pollen-related allergens in the soy isolate were also detected. Two patients showed IgE binding to a 35- to 38-kd allergen in the isolate, and one patient reacted to a 22-kd soy allergen. Because all 3 patients also had IgE specific for rSAM22, it is not possible to assess the clinical relevance of pollen-related versus pollen-independent soy sensitization in these patients. Sera from 4 patients did not show IgE binding to both soy isolate extract and rSAM22 in immunoblotting. Two of these patients (patients 12 and 13) were lacking not only true immediate-type symptoms (face swelling of patient 12 was not immediate) to the soy product but also high IgE levels to birch pollen, rBet v 1, or rSAM22, questioning an underlying IgE-mediated hypersensitivity. Therefore the proposed relationship between birch and soy sensitization seems to be unlikely in these 2 individuals. Apart from questioning an IgE-mediated mechanism or considering insufficient sensitivity of the immunodetection of IgE, there is no obvious reason to explain this observation in at least 2 other patients. However, a similar phenomenon has been described in 4 of 22 patients with pollen-related allergy to celery confirmed by means of DBPCFCs.[35] Four other individuals (patients 1, 5, 6, and 9) did not report on oral symptoms after soy ingestion; 3 of them (patients 5, 6, and 9) had systemic symptoms plus eye, ear, or face swellings instead, possibly another effect of significant amounts of locally released and spread mediators.

In conclusion, we found strong evidence that a birch pollen–related protein from soy, SAM22, might cause adverse reactions to soy in patients with high IgE titers to Bet v 1. Further studies are required to assess the risk of adverse reactions to the soy isolate in patients with birch pollen allergy, high IgE levels to Bet v 1, and no history of adverse reactions to soy by means of DBPCFCs. Moreover, the abundance of SAM22 in other soybean-derived food products is currently under investigation.

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