

## Original article

# Clinical characteristics of soybean allergy in Europe: A double-blind, placebo-controlled food challenge study

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**Background:** Soybean is a relevant allergenic food, but little is known about individual threshold doses in soy allergy.

**Objective:** We sought to determine the clinical characteristics of soy allergy in Europe, including a dose-response curve.

**Methods:** Patients with a history of soy allergy underwent a titrated, double-blind, placebo-controlled food challenge. A statistical model was used to calculate the risk of allergic reactions to experience an allergic reaction to soy. Sera were analyzed for specific IgE to soy, peanut, Bet v 1, and Gly m 4. **Results:** All patients but one responded primarily with subjective symptoms to the challenge followed by objective symptoms in 11 subjects, ranging from rhinitis up to a decrease in blood pressure. Cumulative threshold doses for allergic reactions ranged from 10 mg to 50 g for subjective symptoms and from 454 mg to 50 g for objective symptoms. The pattern of IgE reactivity against proteins with molecular weights of between approximately 10 and 70 kd was highly individual among the patients and did not correlate with the severity of symptoms.

**Conclusions:** When data are fitted by using a normal distribution statistical model, they predict that 1% of patients with soy allergy would react subjectively and objectively with 0.21 and 37.2 mg of soy protein, respectively.

**Clinical implications:** Both the clinical and immunologic basis of soy allergy in Europe are highly complex, which affects the diagnosis of soy allergy and the advice given to patients with soy allergy in regard to risk management. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

**Key words:** Food allergy, double-blind, placebo-controlled food challenge, threshold dose, soy allergy, diagnosis, soy allergens

Soybean is the most important nutrient of the legume family. Soy products are nowadays widely used in the food industry.<sup>1</sup> Food allergy to soy has been described mainly in young children with atopic dermatitis, and there have been just a few studies in adult patients.<sup>2-5</sup> Recently, we observed a prevalence of 10% of soy allergy among Central European patients sensitized to birch pollen caused by IgE cross-reactivity between the major birch pollen allergen Bet v 1 and its homologous protein in soy, Gly m 4.<sup>5</sup> The prevalence of soybean allergy in the general population, however, is unknown, and few data exist on class I food allergy to soy in Europe. Soybean-induced allergic symptoms can range from skin, gastrointestinal, or respiratory tract reactions up to anaphylaxis.<sup>5-7</sup> The minimum amount of soybean inducing immediate-type reactions, however, has not been determined thus far. Knowledge of the severity of symptoms of soy allergy and the threshold dose of soy is most important and might have a major effect on food-labeling directives.

Soybean contains approximately 37% protein, of which 16 IgE-reactive proteins have been suggested as allergens, as reviewed elsewhere.<sup>8-11</sup> Of these, the hydrophobic soybean hull protein Gly m 1, the soybean hull protein Gly m 2, the soybean profilin Gly m 3, and the Bet v 1-homologous pathogenesis-related PR-10 family protein Gly m 4 are officially accepted as soybean allergens according to the criteria of the International Union of Immunological Societies Allergen Nomenclature Sub-Committee.<sup>5,12-14</sup> However, only the latter 2 allergens represent food allergens, whereas soy hull proteins have been described to be relevant in respiratory soy allergy acquired through inhalation of soy particles.<sup>12</sup>

Knowledge of the severity of soy allergy and the threshold level of soybean protein inducing allergic symptoms, information on the allergenic proteins responsible for clinical reactivity, and the tools for detecting soy in processed foods represent a whole package necessary to advise allergic consumers, regulatory agencies, and the soy-processing industry in a concise manner. Our present study is one important step in that direction. We performed,

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*Abbreviations used*

|         |                                                     |
|---------|-----------------------------------------------------|
| DBPCFC: | Double-blind, placebo-controlled food challenge     |
| EAACI:  | European Academy of Allergy and Clinical Immunology |
| LOAEL:  | Lowest observed adverse effect level                |
| NOAEL:  | No observed adverse effect level                    |
| OAS:    | Oral allergy syndrome                               |
| SPT:    | Skin prick test                                     |

for the first time, a clinical multicenter study on food allergy to soy in Europe, including double-blind, placebo-controlled food challenges (DBPCFCs) and determination of threshold levels for clinical reactivity to soy.

## METHODS

### Patients

Three allergy centers in Zurich, Odense, and Milan participated in a multicenter study on soy allergy within the European Union Project FAREDAT (5th Framework Programme). Patients with a suspected soy allergy were recruited between August 2002 and December 2003. The primary inclusion criterion was a positive case history of an allergic reaction to soy. The criterion for a definitive inclusion into the study was a positive food challenge result with soy or a convincing case history of anaphylactic reactions to soy. Patients with anaphylactic reactions to soy, in whom challenge was considered unethical, were discussed between all involved clinicians and were included only if the case history was considered convincing to soy intake and supported by positive *in vivo* tests, *in vitro* tests, or both.

Pregnancy, significant concurrent disease, or medication with glucocorticosteroids, H<sub>1</sub>-receptor antagonists, angiotensin-converting enzyme inhibitors, or  $\beta$ -blocking agents were exclusion criteria.

### Ethical considerations

The study was reviewed and approved by the local ethics committees of the 3 institutions. All subjects provided written informed consent before enrollment into the study.

### Soy material

The material used in this study was fine-ground soy flour that was made from dehulled and defatted whole soybeans (soy flakes). The product contained about 53% protein and all soy oligosaccharides, according to the manufacturer's information. The material had undergone a mild heating process to inactivate enzyme activities and allow for human consumption. This type of soy flour is primarily used in baked goods (Solae Company, Aarhus, Denmark).

### Skin tests

Skin prick tests (SPTs) were performed with the prick-to-prick-technique<sup>15</sup> for roasted peanuts (variety Peruvian Runner) and soy flour. Commercial extracts (Soluprick; ALK, Hørsholm, Denmark) from soy and peanut and pollen from birch, mugwort, and grass were tested, and results were evaluated according to the recommendations of the European Academy of Allergy and Clinical Immunology (EAACI).<sup>16</sup>

### Determination of specific IgE

Specific IgE to soy and peanut extract and to recombinant (r) Bet v 1 were measured by using the CAP system (Phadia, Uppsala, Sweden). For determining specific IgE to Gly m 4, biotinylated rGly m 4 coupled to streptavidin-coated CAPs (Phadia) were used.<sup>5</sup> For control of performance, an internal control serum from a patient

with a positive case history of soy allergy (CAP classes: soy, 3; rGly m 4, 4) was used (Table I).

### DBPCFC with soy

Two different meals identical in color, texture, and taste were prepared by using chocolate bars as vehicle. Lack of differences in active and placebo meals was assessed by using the triangle test.<sup>17,18</sup> Homogeneity of soy distribution in the active meal was controlled by histamine release (data not shown). The active chocolate bar consisted of 27 g of milk-free chocolate, 46 g of milk-free vegetable margarine, 1 pinch of salt, 8 to 9 drops of peppermint oil extract, 24 g of powdered sugar, 7 g of oat flakes, and 50 g of soy flour. The placebo meal contained the same ingredients but no soy flour. Apart from soy flour, all ingredients were known to be tolerated by each patient.

Active and placebo challenges were performed on 2 different days with 9 increasing doses containing 0.002, 0.008, 0.148, 0.296, 1.183, 2.367, 4.734, 9.47, and 31.8 g of soy flour, respectively, administered at an interval of 15 minutes (Table II). Challenges were discontinued after the dose leading to objective allergic symptoms or ingestion of the whole meal.

In case of a negative DBPCFC result with the chocolate bars, patients from Zurich underwent a second challenge with a milk shake with soy protein isolate, as described recently.<sup>5</sup> Patients less than 3 years of age were challenged openly according to the EAACI guidelines.<sup>18</sup>

### Statistical evaluation of dose-response data

A cumulative distribution function (plotting cumulative number of reacting patients against log dose) was used to model this type of dose-response data. A survival analysis approach was then taken to fit a mathematic equation to the log-transformed data, with a normal distribution function being used to model the shape of the curve. CIs were then calculated and subsequently added.<sup>19</sup> For further explanation, see Table III.

### IgE immunoblotting

Soy flour (Solae Company) was extracted in PBS, pH 7.4, (1:20 wt/vol) overnight; centrifuged at 20,000g; and filtrated through cellulose acetate membrane (1.2  $\mu$ m). The extracted protein was quantified (Coomassie Plus Bradford reagent; Pierce Perbio, Bonn, Germany). SDS-PAGE was performed under reducing conditions (sample load, 20  $\mu$ g of protein per centimeter of gel; 5% stacking and 12.5% resolving gels) according to the method of Laemmli, and separated proteins were transferred to nitrocellulose (Protran BA83; Schleicher & Schuell, Dassel, Germany) by means of semidry blotting. The membrane was blocked with 5% nonfat dry milk in Tris-buffered saline and incubated with patients' sera (diluted 1:12 vol/vol in Tris-buffered saline, 2.5% nonfat dry milk, and 0.05% Tween) overnight. Bound IgE was detected with biotinylated goat anti-human IgE (1:2000, 1 hour; KPL via Medac, Hamburg, Germany), horseradish peroxidase-labeled NeutrAvidin (1:10,000, 0.5 hours; Pierce Perbio), and chemiluminescent substrate (LumiGlo Reserve, KPL). Chemiluminescence was documented on Hyperfilm ECL (Amersham Biosciences, Munich, Germany). Incubation buffer and a serum pool of 3 nonallergic subjects were used as negative controls. Sensitivity of the immunodetection was controlled by using an internal control serum, as described for IgE determinations.

## RESULTS

### A wide variety of soy-based foods elicit clinical reactions

Thirty patients (19 female and 11 male patients) fulfilled the inclusion criteria. The mean age was  $26.4 \pm 13.7$

**TABLE I.** Clinical characteristics of the 30 soy allergen subjects

| No.                | Patient     |   | Symptoms to soy<br>Case history | Skin testing    |                   | CAP (kU/L) |         |         |         |
|--------------------|-------------|---|---------------------------------|-----------------|-------------------|------------|---------|---------|---------|
|                    | Nationality |   |                                 | Soy flour (mm)* | Soy extract (mm)* | Soy        | Gly m 4 | Bet v 1 | Peanut  |
| 1                  | CH          |   | OAS, AE, D,<br>Dph, E, BP       | —               | 4                 | 2.1        | <0.35   | <0.35   | 2.26    |
| 2                  | CH          |   | U, AE, D, V                     | ND              | 4                 | <0.35      | 3.23    | 21.4    | <0.35   |
| 3                  | CH          |   | N, V                            | 4               | —                 | 0.54       | 3.98    | 19.9    | 0.78    |
| 4                  | CH          |   | N, GIT                          | 4               | 4                 | <0.35      | 3.06    | 23      | <0.35   |
| 5                  | CH          |   | OAS, U, AE                      | 10              | 3                 | 0.43       | 3.95    | 37.9    | 1.55    |
| 6                  | CH          |   | OAS, AE, D, E                   | 8               | —                 | 6.38       | <0.35   | <0.35   | >100    |
| 7                  | CH          |   | OAS, Dyph,<br>Thi, D            | 10              | —                 | <0.35      | 7.18    | >100    | 0.46    |
| 8                  | CH          |   | AE, D                           | 7               | —                 | 15.9       | <0.35   | <0.35   | 2.26    |
| 9                  | CH          |   | OAS                             | 4               | —                 | <0.35      | 1.01    | 5.67    | <0.35   |
| 10                 | CH          |   | OAS, Bli                        | 4               | 4                 | 1.59       | 9.34    | 30.6    | 4.73    |
| 11                 | CH          |   | OAS, Thi, GIT                   | 8               | 7                 | 0.51       | 10.4    | 36.3    | 1.06    |
| 12                 | CH          |   | OAS, Thi, D                     | 6               | 3                 | 0.41       | 1.82    | 30.6    | 91.9    |
| 13                 | CH          |   | P, F                            | —               | —                 | <0.35      | 0.37    | 4.65    | <0.35   |
| 14                 | CH          |   | nk                              | —               | —                 | 1.54       | 6.08    | 61.7    | 62.9    |
| 15                 | CH          |   | OAS                             | 8               | 4                 | 1.34       | 15.43   | >100    | 3.17    |
| 16                 | CH          |   | OAS                             | 13              | 8                 | 2.12       | 77.7    | >100    | 6.52    |
| 17                 | CH          |   | OAS, AE, D                      | 6               | 4                 | 1.79       | 22.8    | 48.1    | 5.25    |
| 18                 | CH          |   | nk                              | —               | —                 | 9.97       | 4.05    | 30.8    | 21.1    |
| 19                 | DK          |   | OAS                             | 11              | ND                | 20.8       | 11.21   | >100    | 19      |
| 20                 | DK          |   | AD                              | 7               | ND                | 4.74       | <0.35   | <0.35   | 1.79    |
| 21                 | DK          |   | OAS, U, C, E                    | 4               | ND                | 18.1       | >100    | >100    | >100    |
| 22                 | I           |   | P, D, L, BP                     | ND              | 7                 | <0.35      | 17.24   | >100    | 4.4     |
| 23                 | I           |   | OAS                             | ND              | 3                 | 2.45       | <0.35   | <0.35   | 7.4     |
| 24                 | I           |   | OAS, AE                         | ND              | 5                 | 3.05       | <0.35   | <0.35   | <0.35   |
| 25                 | I           |   | OAS                             | ND              | 5                 | <0.35      | <0.35   | 8.23    | <0.35   |
| 26                 | I           |   | OAS                             | ND              | 3                 | 0.66       | 6.29    | 93.2    | 5.82    |
| 27                 | I           |   | U, AE, D,<br>GIT, BP            | ND              | 6                 | 2.94       | <0.35   | <0.35   | 8.61    |
| 28                 | CH          |   | OAS, U,<br>AE, D, BP            | ND              | ND                | 52.5       | 0.63    | <0.35   | >100    |
| 29                 | I           |   | GIT                             | ND              | 8                 | 1.22       | 2.37    | 34.67   | 1.50    |
| 30                 | I           |   | OAS                             | ND              | 10                | 64.89      | <0.35   | <0.35   | 20.63   |
| Total positives    |             | — | —                               | 16/20 (80)      | 18/26 (69)        | 23 (77)    | 21 (70) | 21 (70) | 24 (80) |
| per no. tested (%) |             |   |                                 |                 |                   |            |         |         |         |

CH, Swiss; OAS, oral allergy syndrome; AE, angioedema; D, dyspnea; Dph, dysphagia; E, emesis; BP, decrease in blood pressure; —, negative; U, urticaria; V, vertigo; ND, not done; N, nausea; GIT, gastrointestinal pain; Dyph, dysphonia; Thi, tightness of throat or chest; Bli, blisters of the oral mucosa; P, pruritus; F, flush; nk, not known; DK, Danish; C, conjunctivitis; I, Italian; L, laryngeal edema.

\*Wheal diameter in millimeters.

years (range, 1–69 years). All but 1 patient had at least 1 atopic disease. Ten patients had atopic dermatitis, 20 patients had bronchial asthma, and seasonal rhinoconjunctivitis was diagnosed in 21 patients. Case histories in respect to soy allergy are summarized in Table I. The first reaction to soy occurred at a mean age of  $19 \pm 10$  years (range, 3–44 years). Soy products eliciting reactions were soybean sprouts ( $n = 7$ ), soy milk, yogurt, desserts or drinks ( $n = 12$ ), flakes ( $n = 6$ ), or flour ( $n = 3$ ). Three patients reported allergic reactions after ingestion of meat substitute products, such as tofu and soy protein-based sausage ( $n = 3$ ). In 8 patients food allergy was restricted to members of the Leguminosae family. All other patients had multiple food allergies to fresh fruits, vegetables, and nuts. Sixty-seven percent of patients with soy allergy reported concomitant peanut allergy.

### Clinical manifestation and DBPCFC results

Five patients were included on the basis of a convincing history of anaphylaxis to soy. All but 1 of these patients reacted with multiple symptoms, such as skin, respiratory, and gastrointestinal tract manifestations, in addition to a decrease in blood pressure (Table I). The fifth patient responded on different occasions with severe laryngeal edema and dyspnea leading to emergency hospitalization. In one Danish child an open challenge elicited emesis. All other patients underwent DBPCFC with the chocolate bars. Twelve patients experienced mere subjective symptoms, such as itching and paresthesia of the oral mucosa and the lips (oral allergy syndrome [OAS]), nausea, gastrointestinal pain, or thoracic tightness. In those patients challenges were continued until a cumulative top dose of 50 g of soy was reached. Objective symptoms, such as

**TABLE II.** Symptoms under DBPCFC with soy flour and threshold doses

| Patient no. | Symptoms under ingestion of discrete doses (cumulative doses) of soy in grams |               |               |               |               |                |                |               |                      | Cumulative threshold dose (g) |                    |
|-------------|-------------------------------------------------------------------------------|---------------|---------------|---------------|---------------|----------------|----------------|---------------|----------------------|-------------------------------|--------------------|
|             | 0.002                                                                         | 0.008 (0.010) | 0.148 (0.158) | 0.296 (0.454) | 1.183 (1.637) | 2.367 (4.004)  | 4.734 (8.738)  | 9.47 (18.208) | 31.8 (50.0)          | Subjective symptoms           | Objective symptoms |
| 2           | —                                                                             | —             | —             | —             | P             | P              | P              | P             | R                    | 1.637                         | 50                 |
| 3           | —                                                                             | —             | —             | OAS           | OAS           | OAS, F, N      | OAS, F, BP     | ND            | ND                   | 0.454                         | 4.004              |
| 4           | —                                                                             | OAS           | OAS           | OAS, Dph      | D, N, R       | P, R, AE, F, U | ND             | ND            | ND                   | 0.01                          | 1.637              |
| 5           | —                                                                             | —             | —             | U             | ND            | ND             | ND             | ND            | ND                   | 0.454                         | 0.454              |
| 7           | —                                                                             | OAS           | OAS, Thi      | OAS, Thi      | OAS, Thi      | OAS, Thi       | OAS, Thi       | OAS, Thi      | OAS, Thi             | 0.01                          | —                  |
| 9           | —                                                                             | OAS           | OAS           | OAS           | OAS, N        | OAS, N         | OAS, F         | ND            | ND                   | 0.01                          | 8.738              |
| 10          | —                                                                             | OAS           | OAS           | OAS, Bli      | OAS, Bli      | OAS, C         | OAS, D, Bli, F | ND            | ND                   | 0.01                          | 0.454              |
| 12          | —                                                                             | —             | —             | OAS           | OAS, FE       | ND             | ND             | ND            | ND                   | 0.454                         | —                  |
| 13          | —                                                                             | —             | OAS           | OAS           | —             | OAS            | OAS, N         | OAS, Thi, N   | ND                   | 0.158                         | —                  |
| 14          | —                                                                             | —             | OAS           | OAS           | OAS, N        | OAS, N         | OAS, N         | OAS, N        | ND                   | 0.158                         | —                  |
| 15          | —                                                                             | —             | —             | —             | OAS           | OAS            | OAS            | OAS           | OAS, N               | 1.637                         | —                  |
| 16          | —                                                                             | —             | —             | —             | —             | OAS            | OAS            | OAS           | OAS                  | 4.004                         | —                  |
| 17          | —                                                                             | —             | —             | —             | —             | OAS            | N              | N             | N                    | 4.004                         | —                  |
| 18          | —                                                                             | —             | —             | —             | —             | —              | OAS            | OAS           | OAS, Dia             | 8.738                         | 50                 |
| 19          | —                                                                             | —             | —             | —             | —             | OAS            | OAS            | OAS           | OAS                  | 4.004                         | —                  |
| 20          | —                                                                             | —             | —             | —             | —             | —              | E*             | ND            | ND                   | —                             | 8.738              |
| 21          | —                                                                             | —             | —             | —             | —             | —              | OAS, GIT       | OAS, GIT      | OAS, GIT             | 8.738                         | —                  |
| 23          | —                                                                             | —             | —             | —             | —             | —              | —              | OAS, GIT      | OAS, F, U, GIT, R, C | 18.208                        | 50                 |
| 24          | —                                                                             | —             | —             | OAS           | OAS           | OAS            | OAS            | OAS           | OAS                  | 0.454                         | —                  |
| 25          | —                                                                             | —             | —             | —             | —             | —              | —              | OAS           | OAS, R, F, Thi       | 18.208                        | 50                 |
| 26          | —                                                                             | —             | —             | —             | —             | —              | —              | —             | GIT                  | 50                            | —                  |
| 29          | —                                                                             | —             | —             | —             | —             | GIT            | GIT            | GIT           | GIT                  | 4.004                         | —                  |
| 30          | —                                                                             | —             | —             | —             | OAS           | —              | P, D, BP       | ND            | ND                   | 1.637                         | 8.738              |
| Mean ± SD   | —                                                                             | —             | —             | —             | —             | 5.8 ± 11       | 21.2 ± 22      | —             | —                    | 5.8 ± 11                      | 21.2 ± 22          |

—, Negative; P, pruritus; R, rhinitis; F, flush; N, nausea; BP, decrease in blood pressure; ND, not done; Dph, dysphagia; D, dyspnea; AE, angioedema; U, urticaria; Thi, tightness of throat or chest; Bli, blisters of the oral mucosa; C, conjunctivitis; FE, decrease in FEV<sub>1</sub> of greater than 20%; Dia, diarrhea;

GIT, gastrointestinal pain.

\*Open challenge.

blistering of the oral mucosa, rhinoconjunctivitis, urticaria, flush, or diarrhea occurred in 11 patients. Two patients among those with objective symptoms experienced also a decrease in blood pressure during DBPCFC.

Two Swiss patients did not respond to the chocolate bar challenge but to the following DBPCFC with a liquid vehicle containing a soy protein isolate with a high content of Gly m 4.<sup>5</sup> One responded to the active challenge with OAS and thoracic tightness and the other with OAS, angioedema, and conjunctivitis. No reactions occurred during placebo challenges (Table II).

### Determination of clinical threshold levels by means of DBPCFC

In 23 of the 30 patients with soy allergy, threshold levels of soy-inducing symptoms could be determined (Table II). Because none of our patients reacted to the first serving of the active meal, the “no observed adverse effect level” (NOAEL) was 2 mg of soy (1.1 mg of soy protein).

The first subjective symptoms occurred at a cumulative dose of 10 mg of soy. Consequently, among our study population, the “lowest observed adverse effect level” (LOAEL) for subjective symptoms was 10 mg, corresponding to 5.3 mg of soy protein. The LOAEL for objective symptoms, however, was much higher (ie, 454 mg of soy flour; 240.6 mg of soy protein). The symptoms observed at each provocation step are summarized in Table II.

Fig 1 shows the correlation between the cumulative proportion of patients responding with either subjective (Fig 1, A) or objective (Fig 1, B) symptoms (as a threshold dose reaction) and the amount of soy inducing those reactions. In Table III predicted challenge threshold doses to soy (milligrams) to which a given proportion of patients would react and the predicted proportion (percentage) of patients with soy allergy experiencing a clinical reaction at given doses of soy are summarized and presented for subjective and objective reactions.

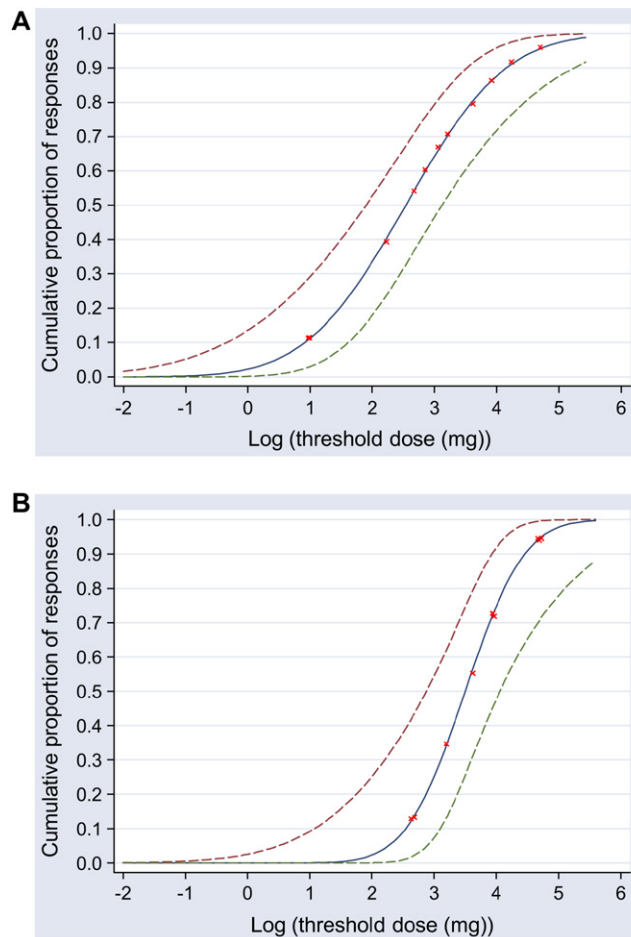


**FIG 1. A,** The cumulative proportion of subjective responses ( $n = 21$ ; Fig 1, A) and objective responses ( $n = 10$ ; Fig 1, B) against log doses of soy (milligrams). Each of the actually achieved challenge doses is marked with a cross on the predicted curve (—).

### Combination of various *in vivo* and *in vitro* tests required for high diagnostic sensitivity

Prick-to-prick test results with soy flour used for the DBPCFCs were positive in 16 of 20 tested patients (sensitivity, 80%). The sensitivity of the SPTs with commercial soy extract, however, was lower (69%). Results of skin tests with soy extract and flour are summarized in Table I. Sensitization to peanut was found in 48% of patients (13/27) by using the prick-to-prick test with roasted peanuts and in 83% by using the commercial peanut extract. Among 29 patients undergoing SPTs with pollen extracts, a sensitization to birch pollen extract was found in 76%, and a sensitization to mugwort and grass pollen was found in 69%.

CAP results for soy, rGly m 4, rBet v 1, and peanut are summarized in Table I. Twenty-three of 30 patients had positive soy extract CAP results, with a median CAP class of 2. Thus determination of specific IgE to soy by using the ImmunoCAP showed a sensitivity of 77%. Of the 30 patients with soy allergy, 24 had specific IgE to peanut, with a median CAP class of 3. Specific IgE to rBet v

1 and rGly m 4 was found each in 21 patients with a median CAP class of 4 and 2, respectively. All but one of the 7 patients with soy allergy with a negative soy extract CAP result were sensitized to rGly m 4. Of the 12 patients (nos. 7, 11-17, 19, 21, 24, and 29) who responded with pure subjective symptoms to the challenge, all but 1 (no. 24) were sensitized to rBet v 1 and rGly m 4. By contrast, among the 7 patients who experienced anaphylaxis either by means of history or during challenge (nos. 1, 3, 8, 22, 27, 28, and 30), just 2 were sensitized to Bet v 1 and Gly m 4, respectively (nos. 3 and 22). Thus a class I sensitization to soy protein is likely to result in systemic and even potentially life-threatening allergic reactions to soy. By contrast, severe systemic reactions appeared to be less likely in subjects primarily sensitized to Bet v 1 and showing IgE cross-reactivity to Gly m 4. No obvious correlation was observed between the kind and severity of symptoms and the level of specific IgE to soy extract.

Patients with birch pollen-associated soy allergy (ie, with IgE against Bet v 1/Gly m 4 without measurable soybean-specific IgE) were less likely to have a concomitant peanut

**TABLE III.** Estimated challenge threshold doses to soy based on subjective or objective reactions, respectively**1.1.** Estimated challenge threshold doses to soy (milligrams) based on subjective reactions

|        | <b>LCLs</b> | <b>Predicted</b> |
|--------|-------------|------------------|
| 1:29   | 0.20        | 1.8              |
| 1:100  | 0.02        | 0.4              |
| 1:1000 | 0.001       | 0.04             |

Predicted doses and lower confidence limits (*LCLs*) are calculated based on Fig 1, A.

**1.2.** Calculated proportion (percentage) of patients with soy allergy experiencing a subjective reaction at 10, 100, and 1000 mg of soy and higher confidence limits

|      | <b>HCL</b> | <b>Predicted</b> |
|------|------------|------------------|
| 10   | 22.8       | 10.8             |
| 100  | 49.3       | 32.2             |
| 1000 | 78.6       | 62.2             |

*HCL*, Higher confidence limit.

**2.1.** Estimated challenge threshold doses to soy (milligrams) based on objective reactions

|        | <b>LCLs</b> | <b>Predicted</b> |
|--------|-------------|------------------|
| 1:29   | 23.6        | 167.2            |
| 1:100  | 6.7         | 70.2             |
| 1:1000 | 1.0         | 19.0             |

Predicted doses and lower confidence limits (*LCLs*) are calculated on the basis of Fig 1, B.

**2.2.** The calculated proportion (percentage) of patients with soy allergy experiencing an objective reaction at 10, 100, and 1000 mg of soy and higher confidence limits

|      | <b>HCL</b> | <b>Predicted</b> |
|------|------------|------------------|
| 10   | 0.2        | 0.03             |
| 100  | 7.1        | 1.7              |
| 1000 | 44.2       | 22.0             |

*HCL*, Higher confidence limit.

In Table III, 1.1, and Table III, 2.1, the values are presented as predicted doses from Fig 1, A and B, together with lower confidence limits, which determines the worst-case scenario (ie, the lowest calculated dose based on the x-axis parallel confidence limit). Tables III, 1.2, and III, 2.2, present the predicted proportion of the patients reacting to a given dose of 10, 100, or 1000 mg of soy together with higher confidence limits, which in this case represent the worst-case scenario (ie, the lowest calculated dose based on the y-axis parallel confidence limit).

allergy (2/5 [40%]) than the remaining patients with soybean allergy (18/25 [72%]).

### High complexity of IgE response to soybean proteins

Immunoblotting analysis revealed that IgE of patients' sera reacted to various soybean proteins with molecular weights of between 10 and 70 kd (see Fig E1 in the Online Repository at [www.jacionline.org](http://www.jacionline.org)). The negative controls for the reagents and a serum pool of 3 nonallergic subjects gave no signals and thus proved the specificity of IgE detection. No differences in IgE-binding pattern were

observed between sera from subjects with subjective symptoms and sera from those with objective symptoms. Similarly, no clear differences in IgE reactivity toward soy protein were seen between subjects who had a convincing history of soybean anaphylaxis (nos. 1, 8, 22, 27, and 28) or experienced anaphylaxis on challenge (nos. 3 and 30) and subjects with subjective or mild objective symptoms during DBPCFC. In summary, the pattern of IgE reactivity was complex and highly individual. No correlation could be drawn from the severity of symptoms and the pattern of IgE reactivity detected by means of immunoblotting.

## DISCUSSION

Here we present data of the first European multicenter study on soy allergy including clinical characteristics and DBPCFC results. Both adults and children with histories of immediate reactions were included. Under challenge, we observed all kind of symptoms, ranging from isolated OAS to anaphylactic reactions. Our results underline the importance of soy as an allergenic food.

Both the United States and the European Union have introduced regulations for mandatory labeling of allergenic ingredients in compound food products according to the US Food and Drug Administration Food Allergen Labeling and Consumer Protection Act of 2004 (108-282) and EC directive 2003/89, respectively. However, because of limitations in food production and processing, compound food products might occasionally contain undeclared traces of allergens.<sup>20</sup> Knowledge on the severity of clinical reactions and threshold doses is of great concern to allow a risk assessment for allergic consumers, which might serve as guidance for regulators and the food industry for establishing allowance levels for allergenic foods.<sup>21</sup> A threshold dose for allergenic foods is defined as the LOAEL (ie, the lowest amount of the allergenic food inducing allergic symptoms), whereas the NOAEL represents the amount of the allergenic food not inducing allergic symptoms in individuals with a clinically relevant sensitization to that particular food. Titrated DBPCFCs starting with low doses of the investigated food is the standard procedure to determine threshold doses.

Even though 3 clinical centers from different geographic regions were involved, recruitment of patients with soy allergy was difficult because of the low prevalence of soy allergy in Europe, particularly in Scandinavia. After a recruitment period of 30 months that was predetermined by the funding period of the European Union Project FAREDAT, only 23 patients could be challenged according to the low-dose chocolate challenge protocol. Five patients with anaphylaxis were excluded from DBPCFCs, according to recommendations of the EAACI.<sup>18</sup> In future low-dose studies, such patients, who might be the most sensitive subjects, should be considered. However, as a prerequisite, adapted safety measures would be required to receive ethical permission issues. Furthermore, 2 of the Swiss patients with a clear history

of soy allergy and a sensitization to Gly m 4 had a negative challenge response to the chocolate meal. Analysis performed with a rabbit anti-Gly m 4 serum revealed that the Gly m 4 concentration in the soy flour used in our current challenge meal was approximately 10-fold lower compared with the soy protein isolate used previously.<sup>5</sup> As already shown by Mittag et al,<sup>5</sup> Gly m 4 content is highly variable in soy products, ranging from 0 to 70 ppm (milligrams per kilogram), and strongly depends on the degree of food processing. Accordingly, challenges were repeated in the 2 subjects with negative results by using a soy source with a higher content of the allergen Gly m 4, resulting in clear reactivity. In addition, this difference of clinical reactivity might be also due to the relatively high fat content in the challenge material used in this study because Grimshaw et al<sup>22</sup> and Van Odjik et al<sup>23</sup> have demonstrated the influence of fat content on allergen availability and thereby threshold doses.

As a consequence, the number of subjects with threshold levels was less than the “magic” 29 subjects to be included in a low-dose challenge study for predicting with 95% probability a soy dose that is safe for 90% of allergic subjects. The statistical model applied, however, renders this number less important because the model uses the actual threshold levels in a computer-based model.<sup>19</sup> Moreover, to our knowledge, our investigation represents the largest threshold dose study performed in soy allergy.

None of our patients with soy allergy reacted to the starting dose of 2 mg of soy (1 mg of soy protein), which thus was the NOAEL for our study population. Four patients reacted with an OAS at a discrete dose of 8 mg of soy (ie, a cumulative dose of 10 mg [5.3 mg of soy protein]), which was the LOAEL for subjective symptoms. The first objective symptoms occurred at a discrete dose of 296 mg and a cumulative dose of 454 mg (240.6 mg of soy protein; LOAEL for objective symptoms). One patient responded to that dose with urticaria, and one responded with blistering of the oral mucosa, flush, conjunctivitis, and dyspnea. Population-based risk assessment is mainly based on the NOAEL for objective symptoms. From a clinical point of view, however, the broad gap between the NOAEL for objective and subjective symptoms highlights the significance of subjective symptoms as important warning signs preceding, in a major portion of patients, more severe and systemic allergic reactions.

Dose-related challenges in 3 other studies were exclusively performed in children, whereas our study mainly involved adults. Those studies revealed a LOAEL for objective symptoms of 406 mg of soy protein in a 19-month-old child responding with nausea and urticaria and of 88 mg of soy protein in a child reacting with vomiting and collapse, respectively.<sup>24,25</sup> The study by Sicherer et al<sup>4</sup> retrospectively analyzed data assessed by means of DBPCFC in children with atopic dermatitis. Twenty-eight percent of children with soy allergy reacted at the first dose of 500 mg of soy (ie, 200 mg of soy protein; the LOAEL for objective symptoms in this study is somewhere less than 200 mg of soy protein). Overall, these data are in good accordance with our results.

Interestingly, 2 patients without a history of anaphylactic reactions responded with a decrease in blood pressure during soy challenge. Those 2 subjects were, however, not those with the lowest threshold doses. Thus we could not observe a clear correlation between the severity of symptoms and the individual threshold dose, as described for peanut.<sup>26</sup> In 2 recent studies the lowest threshold dose for peanut observed for subjective symptoms was 100 µg of protein (ie, more than 1 order of magnitude lower than observed in our population with soy allergy).<sup>26,27</sup>

On the basis of a statistical analysis of our experimental data, we calculated that 10.8% of patients with soy allergy are predicted to react with subjective symptoms at a dose of 5.3 mg of soy protein (corresponding to 10 mg of soy flour), 0.03% with objective symptoms, and 9.8% with either subjective or objective symptoms to the same dose, respectively.<sup>19</sup> or 1% of patients with soy allergy are predicted to react with subjective symptoms to a dose of 0.21 mg of soy protein (0.4 mg of soy flour), with objective symptoms to 37.2 mg (70.2 mg of soy flour), and with either subjective or objective symptoms to 0.37 mg (0.5 mg of soy flour).

A recent study retrospectively determined threshold levels for allergenic foods by means of statistical analysis of pooled published data from the literature.<sup>19</sup> For soy, just 2 studies provided sufficient dose-response data to be considered.<sup>24,25</sup> On the basis of the pooled data of these 2 studies, the calculated threshold value was 0.3 mg of protein (ie, 0.75 mg of whole soy). Furthermore, the soy threshold dose eliciting a reaction in 1 of 100 patients was calculated as 2.2 mg, meaning 1 order of magnitude higher than that calculated in our study population. As mentioned previously, the fat content of the challenge material varies, and direct comparisons can thus be difficult.

IgE immunoblotting revealed that patients' sera reacted to various soybean proteins with molecular weights of between approximately 10 and 70 kd. The pattern of IgE reactivity was complex and highly individual between the patients and did not correlate with the severity of symptoms, indicating that both the clinical and immunologic basis of soy allergy might be very complex. Despite the fact that 70% of the subjects had a positive CAP result to Gly m 4 (Table I), an allergen of 17 kd was not recognized by the majority of subjects on soy extract Western blots. This result is most likely due to the low amount of Gly m 4 present in the soy flake material but not in other processed soy products.<sup>5</sup>

In conclusion, threshold doses, as assessed in 23 European patients with soy allergy, are more than 1 order of magnitude higher than observed in peanut allergy. They were highly variable, ranging from cumulative doses of 10 mg up to 50 g of soy for subjective symptoms and from 454 mg up to 50 g for objective symptoms. No correlation was found between the level of individual threshold doses and the severity of induced symptoms.

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