

Soy allergy in perspective

Barbara K. Ballmer-Weber^a and Stefan Vieths^b

^aAllergy Unit, Department of Dermatology, University Hospital Zurich, Zurich, Switzerland and ^bDepartment of Allergology, Paul-Ehrlich-Institut, Langen, Germany

Correspondence to Barbara Ballmer-Weber, Allergy Unit, Department of Dermatology, University Hospital Zurich, Gloriastr. 31, CH-8091 Zurich, Switzerland
Tel: +41 44 2553079; fax: +41 44 2554431;
e-mail: barbara.ballmer@usz.ch

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Purpose of review

The purpose of this paper is to review and discuss studies on soy allergy.

Recent findings

In Central Europe soy is a clinically relevant birch pollen-related allergenic food. Crossreaction is mediated by a Bet v 1 homologous protein, Gly m 4. Additionally, birch pollen allergic patients might acquire through Bet v 1 sensitization allergies to mungbean or peanut, in which Vig r 1 and Ara h 8 are the main cross-reactive allergens. Threshold doses in soy allergic individuals range from 10 mg to 50 g of soy and are more than one order of magnitude higher than in peanut allergy. No evidence was found for increased allergenicity of genetically modified soybeans.

Summary

In Europe, both primary and pollen-related food allergy exist. The diagnosis of legume allergy in birch pollen-sensitized patients should not be excluded on a negative IgE testing to legume extracts. Bet v 1 related allergens are often underrepresented in extracts. Gly m 4 from soy and Ara h 8 from peanut are nowadays commercially available and are recommended in birch pollen allergic patients with suspicion of soy or peanut allergy, but negative extract-based diagnostic tests to screen for IgE specific to these recombinant allergens.

Keywords

birch pollen-related food allergy, genetically modified soy, mungbean allergy, soy allergy, threshold doses

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Introduction

Soybean (*glycine max*) is a member of the legume family. Soybeans are particularly rich in proteins of high-biological value and are therefore an important nutrient. Allergenicity of soybean proteins has been documented since the 1980s. Soybean exposure is widespread in Asia and the USA but its consumption has increased in Europe too during the past years, particularly in vegetarian cuisine and because of food technological benefits achieved by adding soy proteins to various processed food products. Soy products are nowadays widely used in the food industry, in particular as texturizer, emulsifier and protein filler. Soy is consumed as soy oil, soy flour, soy milk, soy drinks, soy flakes or as fermented soybean products such as Miso, Okara, soy sauce (Tamari, Shoyu), Tempeh or tofu. As soy is a good and cheap protein source, it may be part of a wide variety of processed foods such as meat products, sausages, bakery goods, chocolate or breakfast cereals. Therefore, undeclared traces of soy may represent a hidden allergen in compound food products. In this context, knowledge on the minimum

doses of soy capable of inducing an allergic reaction is highly important.

Food allergy to soy has been described to date primarily in young children with atopic dermatitis [1–3]. Nevertheless, the prevalence of soybean allergy in children is largely unknown. Moreover, epidemiologic data on soybean allergy in adults is virtually lacking [4,5].

Despite the fact that soy is an important nutrient worldwide, the general belief that soy would be one of the most important allergenic foods in infancy and the degree of concern regarding the allergenicity of genetically modified soy, the overall information on soy allergy is scarce. Just a few papers on clinical aspects of soy allergy have been published within the recent years.

Allergens in soybean

Soybean contains approximately 37% protein of which at least 16 IgE-reactive proteins have been suggested as allergens, as reviewed in the Allergome Database

(www.allergome.org). The soybean Kunitz trypsin inhibitor [6–8], the thiol-protease Gly m Bd 30k, published as a major soybean allergen [9–11], the α subunit of the major storage protein β -conglycinin (BCG) [12], the acidic chain of the major storage protein glycinin G1 subunit [13], an agglutinin/lectin precursor [14] and the basic chain of the glycinin G2 subunit [15] have been described to date. A recent publication [16[•]] that provides additionally a summary on allergenic soy proteins was targeted to identify soy allergens by proteomic approach and to compare IgE recognition patterns to transgenic and non-transgenic soy. The authors identified two new potential soybean allergens – a maturation associated protein and a cysteine proteinase inhibitor.

Unfortunately, the so far identified soy allergens have – with few exceptions – mainly been assessed in a patient population that was not characterized well enough and in which a clinically relevant sensitization to soy has not been proven by positive food challenges. For instance, IgE reactivity of the A5B3 glycinin was detected in children, with cow's milk allergy but without confirmed soybean allergy [17] or the soybean allergen 2S albumin [8] was not IgE-binding in 23 European soybean allergic subjects [18]. Gly m Bd 30k was reported to be a major allergen in soy sensitive patients with atopic dermatitis without providing further clinical details [11]. Despite the considerable amount of IgE reactive soy proteins described so far, just a few allergens have been thoroughly characterized, and there is still a considerable lack of knowledge. To date, only the hydrophobic soybean hull protein Gly m 1, the soybean hull protein Gly m 2, soybean profilin Gly m 3 and the Bet v 1-homologous pathogenesis-related PR-10 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 (IUIS) [19–22]. However, only the latter two allergens represent food allergens, whereas soy hull proteins have been described to be relevant in respiratory soy allergy acquired through inhalation of soy particles.

Soybean allergy as an inhalant allergy

Sensitization and allergic reactions to soy flour or dust might occur by inhalation. Asthmatic reactions triggered in the surrounding of a soybean mill were first described by Duke *et al.* in 1934. The most extensive series of inhalant soy allergy were described in Spain, in the city of Barcelona. Unloading of soybean in the harbour area induced multiple epidemic outbreaks of asthma [23]. Allergens responsible for allergic reactions following inhalant exposure differ from those inducing allergic reactions by ingestion of soy. Low molecular weight (LMW) allergens from the soybean hull Gly m 1 and Gly m 2 have been identified as the main allergens responsible for the epidemic asthma outbreaks in Barcelona.

To which extent soybean is responsible for occupational asthma, for example, in bakeries or food-processing plants is largely unknown. Gomez-Olles and coauthors [24[•]] have recently investigated the soy aeroallergen levels in different work environments, that is, soy unloading and/or processing plants in Spain, the Netherlands and Germany using different assays measuring either soy flour or soy hull proteins. The levels of LMW soy hull proteins were highest at sites where whole soybeans were unloaded and stored. In work environments, however, where soy flour and processed soy were used, that is, feed and food industry, airborne soy allergens might be to a higher extent derived from soy flour.

Food allergy to soybean in Europe

In a recent international multicenter study, we investigated the clinical characteristics of soybean allergy in Europe in a group of 30 subjects [25^{••}]. Apart from the determination of threshold levels, we had the objective to investigate the IgE binding pattern of our patients to soy proteins. It was evident that a very diverse pattern of allergens, apparently including storage proteins expressed at high levels, was recognized by IgE from the patients' sera, with no obvious major allergen being detected. Sixty-seven percent of the subjects had concomitant peanut allergy, and more than two-thirds of the subjects were sensitized to a Bet v 1-related food allergen in soy, Gly m 4. In summary, the study results suggest that a relevant proportion of soy allergy in Europe is associated with peanut allergy, but peanut-independent soy allergy also exists. In addition, there is a strong association between soybean and birch pollen allergy. Further studies are required for identifying the classical soy allergens causing clinical symptoms in our study population.

Soybean as a birch pollen-related allergenic food

Allergic reactions to legumes are generally thought to be acquired by a primary sensitization through the gastrointestinal tract. Recently, however, two publications have demonstrated that in Central Europe soy allergy is another clinically relevant birch pollen-related allergenic food [19,22]. Birch pollen-related food allergies are mainly mediated by crossreactions between the PR-10-protein Bet v 1 or the profilin Bet v 2 and homologous proteins in plant food [26]. Crowell *et al.* [27] described in 1992 a stress-induced, developmentally regulated gene family from soybean called SAM22 encoding a disease resistance response protein, with structural homology to Bet v 1. We have cloned and produced the Bet v 1 homologous soy protein named Gly m 4 in recombinant form and characterized its physico-chemical and allergenic properties [19]. Furthermore, Rihs and colleagues [21] cloned, sequenced and characterized Gly m 3, the profilin from

soybean. Twenty-one out of 22 Swiss birch pollen-allergic patients with soy allergy were sensitized to Gly m 4 and six patients to Gly m 3. Thus, Gly m 4 was clearly identified as the major soy allergen in this study population, accounting for more than 70% of IgE-binding capacity of soybean extract.

Most importantly, just 45% of the patients with a confirmed soy and birch allergy were positive to the commercially available soy extract Immuno-CAP (Phadia, Uppsala, Sweden) but 96% to Gly m 4, partly even with very high specific IgE concentrations. Thus, these results indicate that Gly m 4 is underrepresented in the total protein extracts from soybean. It is a well known fact that in Bet v 1-mediated food allergy diagnostic tools using commercial food extracts are often affected by a low sensitivity, in particular due to degradation of the Bet v 1-related food allergens during the extraction procedures [28]. Therefore, we strongly recommend in birch pollen-sensitized patients with suspicion for soy allergy, but negative IgE testing to soy extract to determine specific IgE to the nowadays commercially available rGly m 4 (Phadia, Uppsala, Sweden). Accordingly, in the EU-Project food allergy risk evaluation based on improved diagnosis, allergens and test methods (FARDEAT), where soy allergic patients were included irrespective of a concomitant birch pollen sensitization, all but one of the seven soy allergic patients with a negative soy extract CAP were sensitized to rGly m 4 [25^{••}].

An assessment of the clinical relevance of Gly m 4 in 94 birch pollen sensitized patients revealed that 71% were sensitized to Gly m 4 and that 10% of the investigated population suffered concomitantly from a soy food allergy.

Even though birch pollen-related food allergies are generally regarded to be accompanied by mild allergic symptoms, that is, mainly local oral itching (OAS), in the studies by Kleine-Tebbe *et al.* [22] and by Mittag *et al.* [19], a considerable percentage of patients responded with systemic symptoms including anaphylaxis.

The determination of Gly m 4 content in commercially available foods with soy as a main constituent revealed that the highest amounts of Gly m 4 were present in dietary powders or soy drinks based on soy protein isolate [19]. This explains the phenomenon that first cases of birch pollen-related soy allergy have been observed in patients ingesting soy containing dietary powder [22]. No Gly m 4 was detected in fermented products such as soy sauce or miso and in strongly heated products such as roasted soybeans or soybeans cooked for 4 h. Textured soy protein, tofu and soy flakes, which are usually treated by limited heating, however, contained low amounts of Gly m 4 [19].

Gubesch *et al.* [29^{••}] recently investigated the allergenicity of three novel vegetables, that is, water spinach, hyacinth bean and Ethiopian eggplant by applying a three-step strategy. First, the authors screened the vegetable extracts for the presence of panallergens, thereafter they investigated the IgE-binding of the vegetable extracts by using sera of food allergic patients and finally they assessed the clinical relevance of in-vitro IgE binding by skin prick testing and open food challenges with the investigated vegetables. IgE binding of sera derived from soybean allergic patients was frequent and strong for all three novel foods and in particular for hyacinth beans. Most of the included soy allergic patients were sensitized to Gly m 4 making crossreaction between Bet v 1 homologues in legumes to be the most likely for causing cross-reactive food allergies in that group of soybean allergic patients. Also in the study of Mittag *et al.* [19], patients with a Gly m 4-mediated soy allergy were particularly prone to be allergic to multiple other pollen-related allergenic foods. Furthermore, 50% of those patients reported allergy to the so-called raw soybean sprouts and 55% to peanut. However, soybean seedlings are not marketed for ingestion in raw form, as they cause non-allergic intolerance reactions due to their content of toxic lectins and the Bowman-Birk enzyme inhibitor. The latter affects the human digestion by inhibition of trypsin and chymotrypsin. Both have to be primarily inactivated, for example, by heat processing, before ingestion [30]. From further investigations it became evident that those patients ingested actually mungbean seedlings, which are often mislabelled as soybean seedlings. The allergic reactions of those patients pointed towards the existence of another Bet v 1-homologous allergen in mungbean, which was identified as Vig r 1 [31]. Similarly, in Swiss and Dutch patients with birch pollen and peanut allergy, we could demonstrate that Ara h 8, the Bet v 1-related allergen was the major allergen in peanut [32].

In summary, apart from primary sensitization, allergy to legumes might be acquired in birch pollen allergic patients also by crossreaction mediated by PR-10 proteins present in birch pollen (Bet v 1) and legumes (i.e. Gly m 4, Vig r 1 or Ara h 8). In particular, in Gly m 4-induced soy allergy, the clinical reaction might be severe and even anaphylactic reactions have been observed.

Threshold doses for elicitation of soy allergy

Both the USA and the EU have introduced regulations for mandatory labelling of allergenic ingredients in compound food products according to *FDA Food Allergen Labeling and Consumer Protection Act of 2004 (108–282)* and *EC-Directive 2003/89*, respectively. However, due to limitations in food production and processing, compound food products may occasionally contain undeclared traces of allergens [33].

In a recent retrospective assessment in Spain, 22% of 530 allergic reactions reported by 436 food allergic patients over a period of 5 years were considered to be induced by so-called hidden allergens. Thirty-nine percent of soy allergic patients had a history of an allergic reaction due to ingestion of hidden soy. Sources of hidden soy allergens were identified in boiled ham, sausages, cheese puffs, precooked dishes, desserts and gravy [34•].

Recently, we have published the first allergen-dose-response study [25••] in soy allergic subjects. Patients were challenged by double-blind, placebo-controlled, food challenge (DBPCFC) using chocolate bars with doses from 2 mg to 50 g of soy. The material used for the challenges was soy flour from decapsulated and defatted whole soybeans with a protein content of 53%. 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 [i.e. the lowest observed adverse effect level (LOAEL) for subjective symptoms was 10 mg soy or 5.3 mg of soy protein, LOAEL for objective symptoms was 454 mg soy or 240.6 mg of soy protein]. When data were fitted by a normal distribution statistical model it predicted that 1% of soy allergic patients would react subjectively and objectively with 0.21 and 37.2 mg soy protein, respectively. As none of the included 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 soy protein).

Dose-related challenges in three other studies revealed a LOAEL for objective symptoms ranging from 88 mg to 406 mg soy protein [35–37].

In the European threshold study about soy allergy, a clear correlation between the severity of symptoms and the individual threshold level dose, as described for peanut [37], could not be observed [25••]. In two studies [37,38], the lowest threshold dose for peanut observed for subjective symptoms was 100 µg of protein, that is, more than one order of magnitude lower than observed in the soy allergic population. This observation leads to the conclusion that soy allergic patients are less endangered for allergic reactions to traces in processed foods than peanut allergic patients.

Allergenicity of genetically modified soybeans

In 2005, the genetically modified Roundup Ready soybean was planted on approximately 89% of the US and 60% of the global soybean areas [39]. It is the most cultivated biotechnology product to date. Roundup Ready soybeans contain the *CP4 EPSPS* gene from *Agrobacterium* sp. strain CP4, a common soil-borne bacterium that produces the

CP4-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) protein [40]. The CP4 EPSPS protein produced in Roundup Ready plants is functionally identical to endogenous plant EPSPS enzymes with the exception that CP4 EPSPS naturally displays reduced affinity for glyphosate relative to endogenous plant EPSPSs [40]. Glyphosate, the active ingredient in Roundup herbicides, inhibits the enzyme EPSPS which is essential for the biosynthesis of aromatic amino acids in plants and microorganisms. The introduction of CP4 EPSPS protein in this crop restores the enzyme function and makes the plant insensitive for the Roundup herbicides.

All proteins introduced into genetically engineered plants have to undergo a rigorous safety assessment to ascertain that no transfer of a known allergen or cross-reacting allergen into a food crop occurs, that there is no potential for increasing the endogenous allergenicity of an already allergenic crop and to exclude expression of novel proteins that may be allergens in man.

In a recent study, Hoff *et al.* [41•] determined the potential for developing allergic sensitization to the CP4 EPSPS protein in two different patient populations: children with atopic dermatitis and adult individuals with documented soy allergy and control groups. CP4 EPSPS protein did not bind significant quantities of IgE in soy-sensitive patients and there was no evidence for an increased allergenic potential of this biotech protein [41•]. IgE binding pattern to extracts from genetically modified and wild type soybeans were found to be identical in western blotting experiments. Similar conclusions have been published by Batista *et al.* [16•,42]. However, as only single extracts of GMO soy and parental lines have been investigated [16•,41•], general conclusions on changes of endogenous allergen levels resulting from the genetic modification of soy are not possible. To address this question, natural biovariability (e.g. caused by nutritional status, climatic conditions etc.) has to be considered as an important factor and a sampling plan with a thorough statistical basis is required.

Conclusion

Even though in the most recent multicenter study on soy threshold doses [25••] three clinical centers from different geographic regions were involved, recruitment of soy allergies was difficult. This might reflect that the prevalence of soy allergy is lower than generally assumed. Epidemiological data on the real prevalence of soy are virtually lacking. In Central Europe soy allergy seems to be mainly encountered in birch pollen allergic patients, but subjects with primary soy allergy have been identified as well. In how many cases this finding can be confirmed in other geographical areas in which allergy to tree pollen of the Fagales order are clinically relevant, has to be

investigated. Another important aspect of future research activity will be the analysis of the allergen pattern recognized by subjects with confirmed clinically relevant soy allergy. Once identified and cloned, a panel of purified recombinant soy allergens may help to identify those soy allergens that cause severe or persistent soy allergy and can be subsequently used for component resolved diagnosis of soy allergy.

References and recommended reading

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Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 283).

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