

Lead Review Article

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Biochemical Features of Grain Legume Allergens in Humans and Animals

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Peanuts and soybeans are the major legumes involved in human food allergy, although some data exist on adverse reactions to temperate legumes including pea, green bean, sweet lupin, and lentil. An increasing number of legume proteins or glycoproteins have been characterized as food allergens. Limited data tend to indicate that they are usually different from legume inhalent allergens. Cross-recognition among legume allergens is immunochemically frequent but clinically less common. A common feature to most legume allergens is their natural resistance to thermal, chemical, and in some way, proteolytic denaturation. Finally, other mammals including preruminant calves, and piglets at the time of weaning, are prone to gut immune-mediated reactions to soybean and pea proteins.

Although children become tolerant to most food allergens after a few years of age, peanut allergy is rarely outgrown.² The factors determining the allergenicity of molecules and therefore the wide variability among individuals are still obscure.

Proteins from legumes, particularly soybean, are increasingly incorporated into milk replacers and solid feeds for young farm animals. However, preruminant calves and piglets at the time of weaning are prone to developing gut immune-mediated hypersensitivity reactions.³ Unlike piglets that become tolerant after a few weeks, calves remain hypersensitive for months.

The goal of this review is to gather the information currently available about the biochemical characterization of allergens from legume grains, including soybean, peanut, and temperate legumes, and to highlight the common features and particularities of these molecules.

Introduction

Grains from the botanical family of legumes are important protein sources for both humans and animals. These legume grains contain primarily globular proteins, which are usually multimeric and heterogeneous at the molecular level.¹ Water-soluble proteins, also called albumins, include a large array of metabolically important proteins such as enzymes and enzyme inhibitors. Variable, but usually high amino acid sequence homologies exist among legume species for a given protein. And finally, the oil fraction of legumes also contains small amounts of proteins with hydrophobic properties.

Legume grains such as peanut and soybean are increasingly consumed by humans, a phenomenon probably responsible for an increased development of food allergies and fatal anaphylactic reactions.²

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Soybean and Peanut Allergens

Soybean Allergens

Numerous proteins from soybean are allergenic to humans. In a study of young patients who presented with eczema after soybean flour ingestion, Shibasaki et al.⁴ indicated that the three globulin fractions, 11S, 7S, and 2S, all specifically bound to IgE. Although the authors noted a high cross-reactivity among these fractions, the 2S globulin had the highest allergenic capacity to inhibit the radio-allergo-sorbent test (RAST). This molecule probably corresponded to Kunitz soybean trypsin inhibitor (KSTI), reported to be a major allergen in food anaphylaxis.⁵ Burks et al.⁶ reported IgE to both the 7S and 11S fractions in eight patients with atopic dermatitis. More recently, they indicated that KSTI was a minor allergen in these children.⁷ In a study by Herian et al.,⁸ four out of seven American patients with food allergy to both soybean and peanut presented an IgE binding pattern toward high molecular weight (Mr[50,000 to 60,000]) soybean proteins, which were probably subunits of β -congly-

cinin; two patients sensitive only to soybean had a strong reactivity to a protein of Mr 20,000 that was neither KSTI nor the basic polypeptide of glycinin. Finally, IgE from one patient who was allergic only to soybean bound strongly to a major allergen with Mr of 14,000, as yet unidentified.

In contrast, in a Japanese study involving 69 infant to young adult patients suffering from atopic dermatitis, Ogawa et al.⁹ recorded approximately 16 IgE-specific protein bands. The frequency of sera with IgE binding to soybean proteins was in a decreasing order at Mrs 30,000 (frequency 65.2%), 70,000–68,000 and 28,000 (23.2% each), 67,000–63,000 (18.8%), 35,000–33,000 (15.9%), 55,000–52,000 (14.5%), 50,000–40,000 (13% to 10% in three distinct groups of protein bands), and below 3% for molecules of Mr < 21,000. The most frequent allergen was then isolated from the crude 7S globulin fraction; the monomeric allergen obtained after reduction had a Mr of approximately 32,000. It presented with an *N*-terminal amino acid sequence and composition identical with those of the soybean seed 34-kD oil-body-associated protein or the soybean vacuolar protein P34, and with close homology to papain-like thiol proteinases.¹⁰ According to the international nomenclature recommendations,¹¹ the proposed name was *Gly m Bd 30K*.

The second important allergenic protein in the soybean 7S globulin fraction was identified as the α -subunit of β -conglycinin.¹² There was no IgE cross-recognition with both the α - and β -subunits of β -conglycinin, known to bear high amino acid sequence homologies. The IgE binding sites are located on the peptide 232–383 of the α -subunit, a fragment devoid of sugar moieties.¹² A Danish study attempting to determine the relative allergenic activity among glycinin polypeptides came to the conclusion that no specific chain was entirely responsible for the observed adverse reactions to soybean proteins, even though the A4 acidic chain was usually more reactive.^{13,14}

In a study of children less than a year old suspected of having soybean protein intolerance with vomiting and/or chronic diarrhea, Burks et al.¹⁶ were unable to find particular patterns of IgE-bound soybean proteins.

Peanut Allergens

Various aspects of peanut allergy have been reviewed recently.^{2,16} Among the numerous peanut allergens, the proteins of mean Mrs 63,500 and 17,000 and isoelectric points (pIs) of 4.55 and 5.2 are considered to be the major allergens^{17,18} and have been named *Ara h I* and *Ara h II*, respectively. The former was similar to the concanavalin A-reactive

glycoprotein allergen described by Barnett and Howden;¹⁹ it presented at least four different antigenic sites out of which three also bound to IgE.^{20,21} The latter had two antigenic epitopes and two similar IgE-binding sites.²² Other allergens included a dimeric protein called peanut-1 with subunits of Mrs 20,000 and 30,000 and respective pIs of 5.25 and 5.75,²³ a protein of Mr 14,000,²⁴ peanut agglutinin (Mr of 30,000),⁷ and proteins of Mrs 55,000 and 34,000.²⁵ Moutete et al.²⁶ largely confirmed these observations, identifying six allergens of Mrs 15,000, 18,000, 28,000, 34,000, 41,000, and 67,000 in the nut, and two of Mrs of 18,000 and 41,000 in the hull.

Barnett et al.²⁷ found at least 16 peanut allergens, but many more allergens were revealed by 2D electrophoresis and immunoblotting (G Peltre et al., unpublished, 1995). Many of these allergens are not yet described in detail and need further physico-chemical characterization. The nature of allergens in peanut oil has been questioned in a number of earlier studies.^{2,16} It is now clear that peanut oil contains many proteins in the Mr range of 13,000 to 92,000, with one allergen common to the nut and the hull as well.¹⁶ These proteins, which await further characterization, are hydrophobic, amphiphatic, and associated with lipids in reverse micelles.¹⁷

Pea, Beans, and Lupin Allergies

Based on direct skin reactivity and Prausnitz-Küster tests, allergy to green pea has been reported in 10 patients from Oregon.²⁸ Unfortunately, the clinical signs were not described. The allergenic constituents were shown to belong to the albumin fraction. Partial purification from that fraction revealed one allergenic peak, which was separated further by polyacrylamide-gel electrophoresis (PAGE) into five distinct bands of “widely differing electrophoretic mobility.”²⁸ A dialysate of the crude pea extract was able to inhibit allergen-induced histamine release in vitro in a dose-dependent manner. The allergen purified from the dialysate had a molecular weight of 1800 ± 250 , as determined by ultracentrifugation.²⁹ It was a glycoprotein containing approximately 30% sugar moieties and was devoid of sulfur amino acids. The allergenic constituents of the albumin fraction were neither purified nor characterized.

A pea-sensitive patient as defined by positive food challenge and positive skin-prick test was also noted in a study by Bernhisel-Broadbent et al.³⁰ Immunoblotting following SDS-PAGE electrophoresis revealed two bands with a strong staining at Mrs between 20,000 and 14,000, and a large number of other stained bands at Mrs above 25,000. In a study by Hefle et al.,³¹ five patients with peanut sensitivity

also had a history of adverse reactions to green pea. Finally, a case of sensitivity to pea and to other legumes, including chick-pea and lentil, was recently reported by Huertas et al.³²

One patient in a study by Parker et al.³³ was allergic to green bean, as demonstrated by positive skin tests and food challenge and signs of urticaria and itchy eyes. Huertas et al.³² reported the case of a patient sensitive to haricot bean, among a large array of sensitivities to legumes. Sweet lupin seed flour incorporated into spaghetti-like pasta provoked urticaria and angioedema in a young girl with peanut sensitivity.³¹ Skin-prick tests and RAST with extracts of this lupin-fortified pasta were positive in five out of seven peanut-sensitive patients. Immunoblotting studies with patients' sera revealed lupin protein bands at Mrs of approximately 21,000 and in the range of 35,000 to 55,000. Interestingly, the band at 21,000 was a major allergen in three patients, but only a minor one in three other patients.³¹ Skin-prick tests in Peruvian children were not indicative of a higher risk of allergic reactions associated with the consumption of lupin grain or its derivatives in a study by Feldheim et al.³⁴ Although it had been suspected earlier in one patient,³ allergy to lentil has been reported in two recent communications by Huertas et al.³² and Sainza et al.³⁵

In India, recent data indicate that legumes are the predominant food allergen sources, with chick-pea (*Cicer arietinum*) being the major allergenic legume.³⁶ In Spain, Sainza et al.³⁵ reported two cases of patients sensitive to the cooked flour of *Lathyrus sativus* (chickling), an uncommon legume of the *Vicia* genus.

Are Legume Food Allergens Different from Legume Inhalent Allergens?

Soybean aeroallergens have been studied in detail following several outbreaks of asthma that occurred in Spain during the 1980s. Assays of urban aerosols collected during these periods indicated that soybean dust originating from harbor activities was the causal agent for these epidemics.³⁷ At least 90% of the patients had specific IgE to shell components of Mr of approximately 8000, and only 13% showed specific IgE to shell-depleted soybean grains.³⁸ A strong IgE recognition was noted for glycoprotein bands with Mr < 14,400 and pI < 6, which were therefore considered to be the major allergens.³⁹ These allergens were present in all parts of the plant at all the stages of growth, but the hulls and pods were by far the richest source.⁴⁰ Gonzalez et al.⁴¹ purified two glycoproteins (S1 and S2) with low Mrs (7000 and 7500, respectively). Isoelectrofocalization revealed two bands at pI 6.1 and 6.2 for S1, and a single band at pI 6.8 for S2. These two

proteins have been recently sequenced and have a strong amino acid sequence homology. They also contain a high percentage of hydrophobic residues, primarily leucine and isoleucine.⁴² They were recognized by the IgE from 95% of the patients with soybean-induced asthma. The S2 molecule seemed to be slightly more active in all the immunochemical tests employed. The authors have suggested that these isoallergens be named *Gly m* IA (protein S2) and *Gly m* IB (protein S1). IgE of patients with asthma-like response to soybean reacted weakly to two protein bands of Mrs 42,000 and 21,000;³⁹ whereas the former could correspond to acidic polypeptides of glycinin, the latter could be basic polypeptides of glycinin or Kunitz soybean trypsin inhibitor (KSTI). Soybean hulls were also implicated in a case of hypersensitivity pneumonitis noted by Zubeldia et al.⁴³

Bush et al.⁴⁴ identified nine major IgE-specific proteins with Mrs of 54,500, 52,000, 48,000, 47,500, 38,250, 22,750, 21,250, 17,000, and 14,875 in one patient with asthma. Although not discussed in that paper, some of these bands could correspond to the basic subunits of the 7S globulin β -conglycinin (Mr approximately 53,000), the acidic (Mr 40,000) and the basic (Mr 20,000) polypeptides of the 11S globulin glycinin, and KSTI (Mr 21,000), respectively. The same asthma-like symptoms may also occasionally appear in peanut-sensitive individuals by inhalation of peanut butter.⁴⁵

A case of allergy to green bean was reported recently by Igea et al.⁴⁶ This patient experienced rhinoconjunctivitis, attacks of asthma, and contact urticaria while trimming raw green beans or inhaling vapor from boiling green beans. However, she could touch and eat cooked green beans without any ill effect, and she showed no reactivity to any other foods. Skin-prick tests to raw green beans or extracts were positive. This patient was also allergic to grass pollen.

Cross-allergenicity between Legume Allergens and Other Allergens

Malley et al.²⁸ observed that patients sensitive to other members of the legume family but without a clinical history of pea sensitivity gave positive skin reactions to the allergenic pea albumin fraction. This fraction was able to inhibit the Prausnitz-Küstner test allergen-reagin interactions of other legumes, including string bean, soybean, and lucerne. One woman who was primarily sensitive to soybean had angioedema and urticaria after eating other legumes including pea, lentil, kidney, lima, and navy beans.⁵ Igea et al.⁴⁶ reported that one patient sensitive to raw green bean also displayed positive skin-prick tests to chickling pea.

Cross-reactivities or cosensitivities were observed between chickling pea, chick-pea, and lentil.³⁵ Huertas et al.³² indicated that skin-prick tests performed on a patient with different legumes (chick-pea, beans, lentil, pea) could usually induce urticaria and severe reactions.

Swanson et al.⁴⁰ reported that IgE to the major aeroallergens involved in soybean epidemic asthma cross-reacted with low Mr molecules from maize, wheat, oat, barley, but not rapeseed. It is known that the major cereal allergens include most numbers of the α -amylase/trypsin inhibitor family with Mrs of 12,000 to 15,000.⁴⁷ In another study, Bush et al.⁴⁴ were able to demonstrate apparent immunologic identity between a component in green pea extract and a soybean protein with an Mr of 17,000. Hefle et al.³¹ concluded that some patients with peanut allergy, particularly those with a history of sensitivity to green pea, might also experience adverse reactions to lupin after ingestion.

Bernhisel-Broadbent and coworkers⁴⁸ observed many cross-allergenicity reactions to legumes, including peanut, soybean, green bean, pea, and lima bean. Based on oral food challenge, they concluded that clinically important cross-reactivity to legumes in children was very rare. However, Zimmerman et al.⁴⁹ indicated that highly atopic infants are at special risk for sensitization to particular foods (peanut) even when they have never ingested them. Finally, it is thought that cross-reactivity is less common among grain legumes than cereal grains, for which approximately 20% of patients are clinically reactive to more than one cereal grain.⁵⁰ Ogawa et al.¹⁰ observed a 30% amino acid sequence homology between *Gly m Bd 30K*, the major soybean allergen in atopic dermatitis, and *Der p I*, a house dust mite allergen that is a thiol proteinase from *Dermatophagoides pteronissus*.

In the case of allergies to oil, Guéant et al.¹⁶ mentioned the existence of homologous allergenic proteins in oils from peanut, soybean, rapeseed, and sunflower. It has been shown that cross-reactivities among apparently unrelated allergen sources, such as birch pollen and apple, may be via a ubiquitous family of proteins called profilins,⁵¹ as well as through other cross-reacting major allergens (*Bet v 1* and *Mal d 1*). It is not known if some grain legume allergens share epitopes with particular molecules of these plant pan-allergens.

Decreasing the Allergenicity of Legumes

Herian et al.⁸ showed that although roasting soybeans led to largely reduced recognition of high Mr allergens by IgE from patients allergic to both soybean and peanut, it strongly enhanced binding of IgE to the low Mr allergens in patients sensitive

only to soybean. Heating soybean up to 100 °C for 1 hour kept virtually all of their binding capacity to IgE of pooled patients' sera.⁵² Soybean proteolysis in vitro using immobilized pepsin and intestinal enzymes sequentially was able to introduce a tenfold reduction in allergenicity.⁵² Likewise, various fermented soybean preparations including miso, tempeh, tofu, and mold-hydrolyzed soy sauce were shown by RAST to be much less allergenic than raw soybean.⁵³ However, selective destruction of epitopes was seen for a number of these products. A proportion of soybean allergens in rather native configuration was still found in hydrolyzed vegetable proteins and sprouts.⁵³

Contrasting with these observations, Tsuji et al.⁵⁴ indicated that the *Gly m I Bd 30K* protein allergen was largely insensitive to trypsin, chymotrypsin, and heat treatment. This allergenicity was consistently reduced in fermented foods such as miso, shoyu, and natto. In vitro, the concanavalin A-reactive protein from peanut was allergenically stable at temperatures up to 100°C and over a pH range of 2.8 to 10,¹⁹ and the sugar moieties did not appear as central to allergenicity. Keating et al.⁵³ mentioned that the roasting process did not destroy peanut allergens. Although Burks et al.⁵² noted no apparent effect of thermal treatment on peanut allergenicity, pepsin plus intestinal enzyme hydrolysis in vitro reduced the allergenicity by 100 times.

Allergens from green pea in both the raw extracts and purified form were reported to retain all their allergenic activity when either heated at 60 °C for 30 minutes or boiled at 100 °C for 5 minutes.²⁸ However, autoclaving at 120 °C for 15 minutes significantly reduced its allergenic activity. Igea et al.⁴⁶ showed that while allergens from green bean were partly sensitive to heat, boiling completely abolished skin-prick reactions. The 11S, 7S, and 2S soybean fractions were identified as allergens in patients with asthma or eczema;⁴ the RAST reactivity of the 2S globulin was slightly enhanced when heated at 80 °C for 30 minutes and decreased by 15% to 20% between 100 °C and 120 °C. The RAST reactivity of the other globulins remained above 50% of the activity of their native forms at temperatures of 80 °C to 120 °C.⁴ Allergens from lupin were reported to be heat stable.³¹

Legume Allergens in Farm and Laboratory Animals

Gut immune-mediated reactions to soybean and pea occur especially in young preruminant calves and in piglets at the time of weaning.³ Although specific reaginins (IgE or IgE-like) have been demonstrated by passive cutaneous anaphylaxis in the serum of some soy-sensitive calves,⁵⁶ this does not seem to

be a constant feature associated with intestinal disorders. To our knowledge, IgE to dietary allergens has not been characterized so far in piglets. Therefore, data obtained from analysis of animal sera deal with food antigens rather than allergens, except when direct skin testing has been carried out.^{57,58} As already reviewed,³ calves had an antibody response that was strong to soybean β -conglycinin subunits and weaker to proteins in the Mr range of 30,000–37,000, 20,000–25,000, and 15,000–20,000. Using purified proteins in antibody analysis, we came to the conclusion that soybean globulins, and to a lesser extent lectins and Bowman-Birk inhibitors (BBI), are antigenic in calves.⁵⁹ Skin tests at 1 hour post-injection were positive to glycinin, α -conglycinin, and β -conglycinin in young calves orally sensitized to heated soybean flour.⁵⁸ In contrast, lectins and BBI reacted mainly at 8 hours postinjection. Finally, β -conglycinin was found to induce long-lasting (>5 days) skin thickening, suggestive of cell-mediated, delayed-type immune mechanisms.⁵⁸ That was confirmed by specific in vitro proliferation of peripheral leukocytes from sensitive calves. In piglets, a strong binding was noted at Mr of 37,000 and 20,000 (including KSTI) and a weak binding to β -conglycinin subunits and low Mr proteins.⁶⁰ Direct skin testing indicated an immediate reaction only to glycinin in piglets.⁵⁷

Preruminant calves also experienced allergic reactions to raw pea flour.⁶¹ Many pea proteins were recognized by sera from pea-fed animals, but the most reactive bands had Mrs of 45,000 and 32,000.⁶⁰ The latter band may correspond to the pea protein partially purified by Gruppen et al.⁶² with a Mr of 29,000 and pI of 5.0. An isoform with pI of 5.2 was not recognized by pigs' sera in that study.

Steam heating of defatted soybean flour with or without prior aqueous extraction of oligosaccharides was insufficient to reduce immune reactions in young farm animals or to improve digestive utilization of soy products.³ In contrast, hot alcohol denaturation and industrial hydrolysis of protein provided suitable ingredients for feeds. Of interest is that residual immunoreactive β -conglycinin was found to be a good predictor of soy protein digestibility in calves.⁶³ Intraperitoneal immunization of BALB/c mice to soy protein revealed the strong allergenicity of KSTI.^{4,64}

Conclusions

Significant improvements have been made during the last decades in the knowledge of grain legume allergens. They include particular individual (glyco)proteins, many legume family-related molecules, and probably also many preserved structural or biologically active molecules. This may explain the

usually wide immunochemical cross-recognition observed among legume allergens. Although clinically relevant cross-allergenicity among or multiple allergy to legumes has been occasionally mentioned,^{5,31,32} its occurrence appears to be low, at least in children.^{30,48}

Many food allergens are naturally resistant to most conventional technological treatments, suggesting the importance of sequential allergenic epitopes. This resistance indicates the need to develop more efficient treatments to decrease allergen concentrations and to better inform consumers about the actual composition of foods.

In this regard, legume protein allergens present in oil must not be disregarded. Such information is of particular importance because grain legumes are destined for greater consumption, owing to their beneficial role in contributing to the prevention and treatment of chronic diseases in humans, such as heart disease and cancer.⁶⁵ Young farm animals also experience allergic reactions to legume proteins and may therefore represent naturally occurring models for studying oral sensitization and gut immune-mediated disorders in humans.

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