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Lupine Allergy: Not Simply Cross-Reactivity with Peanut or Soy

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Summary

Background: Reports of lupine allergy are increasing as its use in food products increases. Lupine allergy might be the consequence of cross-reactivity after sensitization to peanut or other legumes or de novo sensitization. Lupine allergens have not been completely characterized. *Objectives:* We sought to identify allergens associated with lupine allergy, evaluate potential cross-reactivity with peanut, and determine eliciting doses (EDs) for lupine allergy by using double-blind, placebo-controlled food challenges. *Methods:* Six patients with a history of allergic reactions to lupine flour were evaluated by using skin prick tests, CAP tests, and double-blind, placebo-controlled food challenges. Three of these patients were also allergic to peanut. Lupine allergens were characterized by means

of IgE immunoblotting and peptide sequencing. *Results:* In all 6 patients the ED for lupine flour was 3 mg or less for subjective symptoms and 300 mg or more for objective symptoms. The low ED and moderate-to-severe historical symptoms indicate significant allergenicity of lupine flour. Two patients allergic to lupine but not to peanut displayed IgE binding predominantly to approximately 66-kd proteins and weak binding to 14- and 24-kd proteins, whereas patients with peanut allergy and lupine allergy showed weak binding to lupine proteins of about 14 to 21 or 66 kd. Inhibition of binding was primarily species specific. *Conclusion:* Lupine allergy can occur either separately or together with peanut allergy, as demonstrated by 3 patients who are cosensitized to peanut and lupine. *Clinical implications:* Lupine flour is allergenic and potentially cross-reactive with peanut allergen, thus posing some risk if used as a replacement for soy flour.

Keywords: lupine allergy, cross-reactivity, legumes, allergens, IgE immunoblotting, amino acid sequencing, skin prick tests, doubleblind, placebo-controlled food challenge, eliciting dose, peanut

Abbreviations used
DBPCFC: Double-blind placebo-controlled food challenge
ED: Eliciting dose
pI: Isoelectric point
SPT: Skin prick test

Lupine (*Lupinus albus*), like peanut and soy, is a member of the Legume family, the second largest family of seed plants.[1] Lupine is cultivated globally, primarily for use as animal feed but also to be plowed under as a soil enhancer.[1] Inclusion of lupine in wheat flour was first permitted in the United Kingdom in 1996 and in France at the end of 1997 to add protein and fiber and improve food texture. Lupine flour was first considered as a supplement for bread[2] and cookies[3] more than 20 years ago and is now being used as an alternative to soy flour by companies seeking non–genetically modified food ingredients.

Because legumes, in particular peanut and to a lesser extent soybean, are well known as allergens, it is not surprising that allergy to lupine has been reported. The first reported case, in 1994, involved a girl with known peanut allergy who experienced urticaria and angioedema after ingesting a pasta fortified with sweet lupine seed flour.[1] Lupine allergy has been mainly reported in patients with allergies to other legumes, particularly peanut.[4–6] Sensitization can occur through the oral route but also through inhalation.[7–9] Isolated cases of lupine allergy have rarely been described.[9,10]

Serologic cross-reactivity between other members of the legume family and lupine is common.[11–13] However, clinical cross-reactivity is quite rare but has been identified with lupine, peanut, and pea.[1,4–6] Vicilins, also termed 7S globulins, are major storage proteins present in most legume seeds that might contribute to cross-reactivity between legumes.[14,15]

Characterization of the cross-reactive and unique allergens in lupine should facilitate the development of better diagnostic tools in legume allergy.[16] There is no consensus about the major allergens of lupine flour. We have partially characterized some of the proteins in lupine that are bound by IgE from sera of patients with lupine allergy, with or without concomitant peanut allergy, by means of immunoblotting and peptide sequencing.

Furthermore, we determined the eliciting dose (ED) in our group of patients by means of double-blind, placebo-controlled food challenge (DBPCFC) with lupine.

Methods

Patients

Six adult outpatients of the Department of Dermatology/Allergology of the University Medical Center Utrecht were investigated in this study. Detailed histories of legume allergies and atopy were obtained, with symptoms classified according to the Mueller method,[17] which is designed to classify allergic reactions to insect venom. Symptoms restricted to the oral cavity (oral allergy symptoms) were classified as Mueller grade 0, skin and mucous membrane symptoms (urticaria, angioedema, rhinitis, and conjunctivitis) were classified as grade 1, gastrointestinal symptoms (diarrhea, vomiting, nausea, and abdominal pain) were classified as grade 2, respiratory symptoms (asthma and hoarseness) were classified as grade 3, and hypotension was classified as grade 4. Ethical approval for the use of human subjects was obtained from the local ethics committee.

Sensitization

Skin prick tests (SPTs) were performed with commercial extracts of peanut, green pea, and soy (ALK-Abelló, Nieuwegein, The Netherlands) and with a research laboratory–made lupine extract. Lupine extract was prepared by suspending 20 g of lupine flour in 200 mL of PBS/0.1% phenol overnight at 4°C. After clarifying the suspension by means of filtration and centrifugation, the supernatant was mixed with an equal volume of glycerol and then sterilized by using filtration. The protein content of this extract was determined to be 10.2 mg/mL by using the Bradford method.[18]

Histamine dihydrochloride (10 mg/mL) and 50% glycerol diluent from ALK-Abelló served as positive and negative SPT controls. The SPTs were performed, and results were recorded as positive for wheal responses of at least 7 mm^2 (diameter, 3 mm) and greater than those elicited by the negative control.[19] The SPT reactions were recorded as 3+ when the wheal diameter was similar to the histamine wheal diameter, 2+ when it was 50% of the histamine-induced diameter, and 1+ when it was 25% of the histamine-induced diameter. Specific IgE levels to peanut, lupine, green pea, and soy were determined by using the CAP system FEIA (Pharmacia & Upjohn Diagnostics, Uppsala, Sweden). Sensitization to aeroallergens (mugwort, birch pollen, and grass pollen) was also determined by using SPTs and CAP-FEIA.

Clinical evaluation with DBPCFCs

Clinical reactivity to lupine was investigated by using DBPCFCs, as described previously.[19] A commercially available mild white lupine (*L. albus*) flour was purchased through Magenta Sales, England (product of CANA, Martigne-Ferchaud, France) with a protein content of 36.2%, as measured by means of a LECO FP-2000 Nitrogen/Protein Analyzer (LECO Corp., St. Joseph, Michigan). Increasing doses of lupine flour (1, 3, 10, 30, 100, 300, 1000, and 3000 mg) were hidden in mashed potatoes, and samples were randomly interspersed with 4 placebo doses with each patient. Challenges were discontinued when objective symptoms occurred or when subjective symptoms lasted for more than 45 minutes. The ED was defined as the lowest dose eliciting a convincing subjective allergic reaction. The challenges

were conducted in a hospital with careful medical monitoring of the patients and full emergency treatment readily available.

IgE immunoblotting

Green pea flour, white lupine flour, roasted Virginia peanut (ground), and raw Vinton soybean (ground) were extracted by mixing overnight at 4°C, 1:10 (wt/vol) in 0.01 mol/L PBS, pH 7.4. Extracts were clarified by means of centrifugation. Proteins in the extracts were denatured by using sample buffer containing 350 mmol/L electrophoresis purity dithiothreitol (Bio-Rad Laboratories, Inc., Hercules, California) and heating at 95°C for 5 minutes. Proteins were then separated by means of SDS-PAGE in precast 10% to 20% Tris-HCl Ready Gels (Bio-Rad Laboratories, Inc.) based on the method of Laemmli[20] by using the manufacturer's suggested protocol. Each well was loaded with 50 mg of protein, as determined by using the Lowry method.[21] In direct immunoblotting experiments broad-range molecular weight standards were used. In inhibition and 2-dimensional experiments Precision Plus molecular weight markers were used (Bio-Rad Laboratories, Inc.).

Separated proteins were transferred to Immun-Blot polyvinylidene difluoride membrane (Bio-Rad Laboratories, Inc.) based on the method of Towbin et al.[22] Then membranes were blocked with 0.01 mol/L PBS, pH 7.5, containing 0.2% BSA (Fraction V, RIA grade; USB Corp, Cleveland, Ohio) and 0.05% Tween 20 (Bio-Rad Laboratories, Inc.) for 2 hours and then incubated overnight with sera (1:20 vol/vol in blocking buffer) from individual allergic patients or a normal control serum pool. Membranes were washed 5 times in blocking buffer and then probed with iodine 125–labeled anti-human IgE (Diagnostic Products Corp, Los Angeles, California) diluted 1:20 in blocking buffer overnight. Probed blots were washed 5 times, dried, and used to expose X-OMAT LS film (Eastman Kodak Co., Rochester, New York) for 48 hours at –80°C immediately before developing. For immunoblotting inhibition experiments, all conditions were the same with the following exceptions: birch pollen extract was prepared as a 1:20 extract in distilled water, pH 8.0. The extract was defatted with Freon, clarified, and dialyzed against distilled water. The protein level was measured as 8.3 mg/mL by using a bicinchoninic acid assay kit (Pierce Biotechnology, Inc., Rockford, Illinois). Protein samples used in the inhibition experiment were prepared with Laemmli buffer but without reducing agent and without heating before separation in SDS-PAGE. The amount of peanut protein included in gels was 5 μg per lane, whereas the protein in the other samples was 50 μg because of the differential binding. Inhibitors (200 mg of protein in 1:20 dilutions of individual sera) were preincubated for 2 hours before addition to the previously blocked blots. Individual blots for patients 1, 2, and 3 were incubated with peanut, lupine, or birch pollen extracts as inhibitors, and blots for patients 4, 5, and 6 were incubated with green pea, lupine, birch, or soy extracts as inhibitors and compared with an uninhibited control.

Peptide sequencing

Lupine protein bands at approximately 50- to 66-kd bound IgE from 2 of the 3 patients with lupine allergy (patients 4 and 5) were isolated by means of 2-dimensional electrophoresis for peptide sequencing. Raw lupine flour extract in PBS (pH 7.4) representing 150 mg of protein was separated by means of 2-dimensional electrophoresis with an Invitrogen

Life Technologies (Carlsbad, Calif) nonlinear 4-7 isoelectric focusing IPG strip for the first dimension and a Novex 4% to 20% Zoom SDS-PAGE gel for the second dimension. Identical gels were stained with Coomassie blue or blotted on polyvinylidene difluoride membranes and incubated with individual patient sera (2, 4, or 5) diluted 1:20 in PBS with nonfat dry milk, followed by horseradish peroxidase–labeled monoclonal anti-IgE from Southern Biotech (Birmingham, Alabama). Bound IgE was detected by using electrochemiluminescence with ECL (Pierce Biotechnology) with a Kodak 1D-Imaging system. Coomassie blue–stained spots corresponding to IgE-bound proteins were excised from the gel and analyzed by Macromolecular Resources at Colorado State University (Fort Collins, Colorado) using matrix-assisted laser desorption/ionization–time of flight mass spectrometry for isolated spots or liquid chromatography–mass spectrometry–mass spectrometry of 2 partially separated proteins. Identified peptide sequences based on mass analysis were searched against the NCBInr database (http://www.protein.sdu.dk/gpmaw/GPMAW/Databases/NCBInr/ncbinr.html) using the Mascot search engine (version 2.1, Matrix Science, Boston, Massachusetts) to identify known lupine proteins. The lupine sequences were compared with all National Center for Biotechnology Information sequences by using the Basic Local Alignment Search Tool algorithm to identify homologous proteins from peanut and other legumes.

Results

Patient characteristics

Six patients with a suspected lupine allergy were included in this study. Patient characteristics are summarized in Table I. Of the 6 patients, 4 were sensitized to grass and birch pollen, 1 was sensitized to grass pollen, and 1 was not sensitized to grass or birch pollen. None were sensitized to mugwort. Three patients (nos. 1–3) had a convincing history of peanut allergy and IgE CAP results to peanut of greater than 100 kU/L, whereas CAP results to lupine ranged from 3.2 to 7.1 kU/L. All 3 were sensitized to pea and soy, and 2 reported symptoms after ingesting 1 or both legumes. The other 3 patients tolerated peanut without any restriction (nos. 4–6). Of the 3 patients without peanut allergy, only 1 (no. 5) was mildly sensitized to peanut by means of CAP (IgE, 1.5 kU/L), whereas another (no. 4) had only a mild positive SPT response to peanut. Specific IgE to lupine varied from 1.3 to 67 kU/L (Patients 4–6). None of the 3 patients reported symptoms to pea or soy, and only 1 was sensitized to these allergens by means of CAP (no. 5). The foods that elicited allergic reactions and the lupine-related symptoms are shown in Table II.

Table I. Sensitization and clinical reactivity to legumes of patients with lupine allergy (n = 6)

Patient no.	Sex	Age (y)	Atopic history	Sensitization to aeroallergens	Lupine		Peanut		Pea		Soy		Symptoms to other legumes (Mueller)
					CAP	SPT	CAP	SPT	CAP	SPT	CAP	SPT	
1	M	21	A, AD, AR	b, g	3.2	4+	> 100	4+	3.0	2+	1.8	NT	p (3)
2	M	26	AR	b, g	7.1	3+	> 100	NT	1.3	0	4.9	2+	p (4), s (0)
3	F	27	A, AR	b, g	6.8	2+	> 100	NT	4.3	2+	5.2	1+	p (3), s (2), pea (3)
4	F	42	—	—	10.3	3+	0	2+	0	1+	0	NT	—
5	M	48	AR	b, g	67	3+	1.5	0	1.2	2+	3.7	2+	—
6	F	32	A, AR	g	1.3	NT	0	0	0	0	0	1+	—

A, Asthma; AD, atopic dermatitis; AR, allergic rhinitis; b, birch pollen; g, grass pollen; NT, not tested; p, peanut; s, soy

Table II. Clinical reactivity to lupine flour by history and during DBPCFC (n = 6)

Patient no.	Eliciting food	Symptoms by history (Mueller)	DBPCFC dose (mg)								ED (mg flour)
			1	3	10	30	100	300	1000	3000	
1	A piece of bread	3	OAS	OAS	n	OAS	OAS	OAS, d (FEV_1 ↓)	NT	NT	≤ 1
2	A piece of bread, potato chips	3	OAS	OAS	OAS	OAS	OAS	OAS, n, ap	NT	NT	≤ 1
3	Croquette, cookie	2	—	OAS	OAS	OAS	OAS, n	OAS, n, d	NT	NT	3
4	A bite of a small round croquette	3	—	OAS	OAS	OAS	OAS	OAS	OAS, h	NT	3
5	A small round croquette	3	OAS	OAS	OAS	OAS	OAS, ap	OAS, rc, d	NT	NT	≤ 1
6	A bite of a waffle	3	OAS	OAS	OAS, ap	NT	NT	NT	NT	NT	≤ 1

OAS, oral allergy symptoms; n, nausea; d, dyspnea; NT, not tested; ap, abdominal pain; h, hoarseness; rc, rhinoconjunctivitis

Clinical reactivity to lupine and EDs

Positive DBPCFC responses with lupine flour confirmed the diagnosis of lupine allergy in all 6 patients (Table II). The EDs for subjective symptoms varied from 1 mg or less to 3 mg and were similar in both patients with and without peanut allergy. All but 1 had moderate-to-severe nausea or abdominal pain in addition to oral allergy symptoms at higher doses (10–300 mg). Doses inducing objective symptoms were established in 3 patients and varied from 300 to 1000 mg. Objective symptoms varied and included a 50% decrease in FEV_1, hoarseness, and rhinoconjunctivitis (Table II).

IgE immunoblotting

Figure 1 shows the India ink blot of the transferred proteins from extracts. Figure 2 shows the autoradiogram results of IgE immunoblotting in the patients allergic to both peanut and lupine. The difference in relative intensities of IgE binding to peanut and lupine protein bands is quite pronounced and parallels the relative CAP results in Table I. Patient 1 displayed IgE binding to peanut proteins only, even though his CAP score to lupine was 3.2 kU/L. This might be due to differences in presentation of epitopes. However, he experienced oral allergy symptoms on DBPCFC to 1 mg of lupine flour. Patient 2 (ED, ≤ 1 mg of lupine flour) displayed prominent IgE binding to peanut, with some binding to lupine bands at approximately 14 kd and to soybean at 36 kd, with minor bands at 21 and 14 kd. Patient 3, who had an ED of 3 mg of lupine flour, also had binding to peanut, as expected, but had very light IgE binding to a protein at 36 kd in green pea and very light binding to a series of bands in soy, with 1 minor band in lupine at approximately 24 kd. The results

of immunoblotting, along with the CAP results, show that in vitro IgE binding to lupine does not correlate well with the presence or severity of lupine allergy (Table II). Figure 3 shows the IgE-binding profiles of patients who are allergic to lupine but not peanut. No peanut IgE binding was evident from blots with these sera. Patient 4 (ED, 3 mg of lupine flour) had faint IgE binding to a green pea peptide at 31 kd and relatively strong binding to a 66-kd band in lupine, with minor binding to proteins of various molecular weights in soy. Patient 5 (lupine ED was ≤ 1 mg) had prominent binding to 2 lupine proteins at approximately 50 and 66 kd but also displayed moderately strong binding to a soy band at 60 kd. Serum from patient 6 bound lightly to a green pea protein at 31 kd. This patient also had slight binding to lupine proteins at 21 and 66 kd, with very faint binding to soy proteins between 16 and 97 kd. Because of the clinical observations of coreactivity to peanut and lupine, inhibition experiments were attempted to evaluate whether the allergic responses in those with allergies to both peanut and lupine might be due to cross-reactivity.

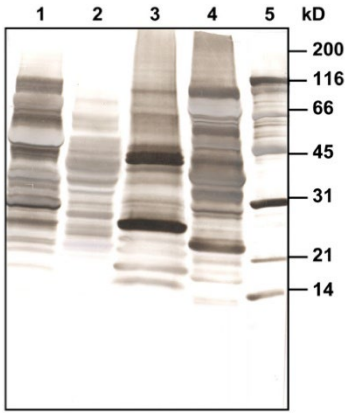

Figure 1. India ink–stained polyvinylidene difluoride blot. Lanes 1–4, Extracts (50 μg of protein) of green pea, lupine, peanut, and soybean, respectively; lane 5, molecular weight standards.

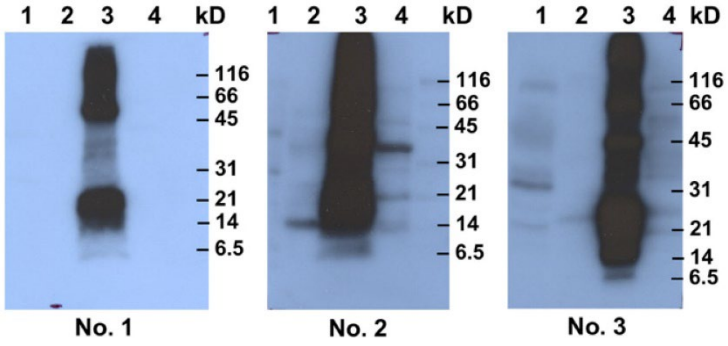

Figure 2. Autoradiograms of IgE immunoblots for patients 1 to 3 with allergies to both peanut and lupine. Lanes 1–4, Extracts of green pea, lupine, peanut, and soybean, respectively; kD, molecular weight standards.

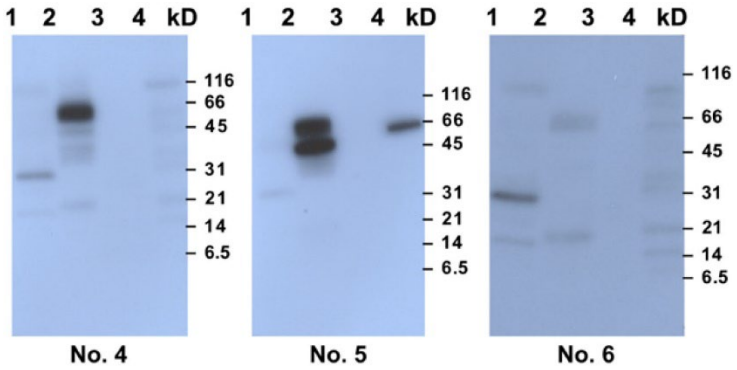

Figure 3. Autoradiograms of IgE immunoblots for patients with allergy to lupine but not to peanut. Lanes 1–4, Extracts of green pea, lupine, peanut, and soybean, respectively; kD, molecular weight standards.

Inhibition of IgE binding to proteins on nonreduced SDS-PAGE blots was accomplished by means of preincubation of sera with extracts of peanut flour, lupine flour, birch pollen, soy flour, and green pea flour compared with no inhibitor. Selected blots demonstrating inhibition are shown in Figure 4. Preincubation of sera from patients with peanut allergy with peanut extract prevented binding to peanut proteins on the blot (Fig. 4, 3P). Preincubation of serum from Patient 5 with lupine allergy with lupine extract inhibited binding to lupine protein (Fig. 4, 5L). However, the only 2 cases in which clear evidence of cross-reactivity between peanut and lupine was found were the inhibition of binding of IgE from serum 1 to the peanut band at approximately 150 kd by preincubating sera with lupine extract (Fig. 4, 1L) and inhibition of the faint lupine doublet bands from serum 3 (molecular weight, approximately 18–22 kd) when sera were preincubated with peanut extract (Fig. 4, 3P and 3L). It is important to note that the relative IgE band intensity differences between peanut and lupine made it difficult to evaluate the extent of cross-reactivity.

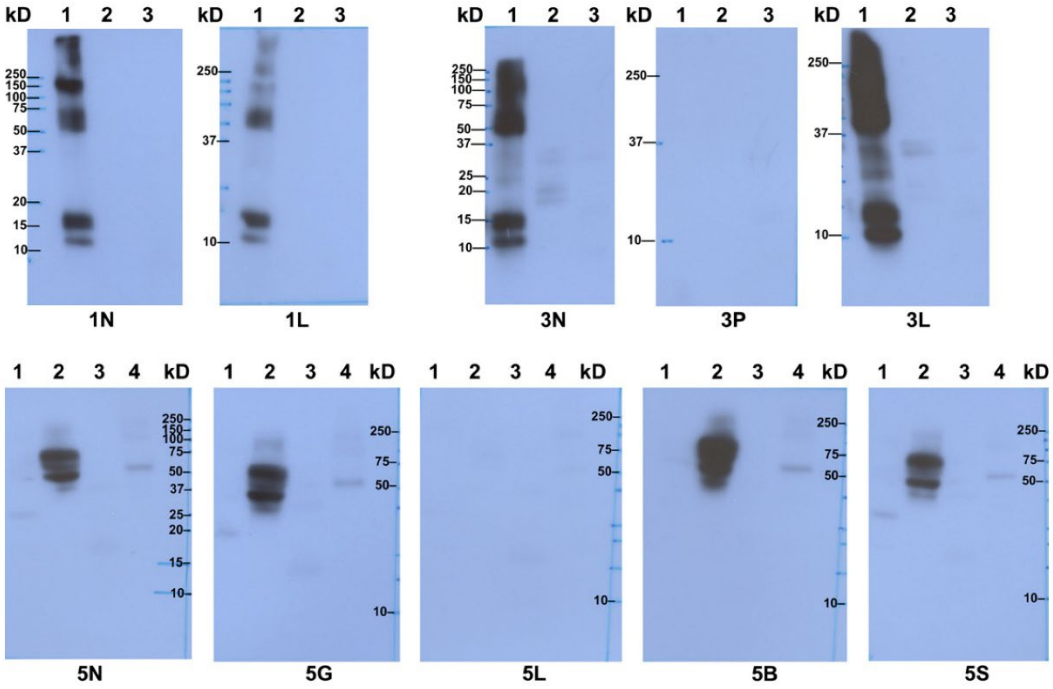

Figure 4. Autoradiograms of IgE immunoblot inhibition. *Upper panels:* lanes 1–3, extracts of peanut, lupine, and birch pollen, respectively, used as inhibitors. Patient sera and treatments: 1N, serum 1 with no inhibitor; 1L, serum 1 with lupine extract; 3N, serum 3 with no inhibitor; 3P, serum 3 with roasted peanut extract; 3L, serum 3 with lupine extract. *Lower panels:* lanes 1–4, extracts of green pea, lupine, birch pollen, and soybean, respectively, used as inhibitors. Patient sera and treatments: 5N, serum 5 with no inhibitor; 5G, serum 5 with green pea extract; 5L, serum 5 with lupine extract; 5B, serum 5 with birch pollen extract; 5S, serum 5 with soybean extract.

Peptide sequencing of a lupine allergy–associated IgE-binding protein

The 2-dimensional gel patterns and immunoblots demonstrated multiple spots in 2 close regions (50 to 66 kd) that represent a series of related IgE-binding proteins with sera from 2 patients (Fig. 5, B). Similar patterns were visible from blots incubated with sera from Patients 4 (data not shown) and 5, although the relative intensity of IgE binding from the serum of Patient 4 was markedly weaker. Although these spots would appear as bands of 1 or 2 molecular weights in 1-dimensional gels, at least eleven 2-dimensional spots were recognized between the 2 patients, with putative identities of spots labeled 5 through 15 listed in supplemental Table E1. Preliminary identification of the proteins bound by IgE from Patients 4 and 5 was accomplished by means of liquid chromatography–mass spectrometry–mass spectrometry and matrix-assisted laser desorption/ionization–time of flight mass spectrometry conducted on trypsin-digested peptides excised from the stained gel. The 11 spots most closely matched either of 2 closely related lupine proteins derived from cDNA sequences of *L. albus*. Identified peptide fragments covered more than 30% of the full-length proteins. Some spots more closely matched a 533-amino-acid β-conglutin

precursor (gi|46451223) with a nominal predicted mass of 62 kd and a calculated isoelectric point (pI) of 6.43. Others more closely matched a 531-amino-acid vicilin-like protein (gi|89994190) with a predicted nominal molecular weight of 62 kd and a calculated pI of 6.08. Both proteins were identified from *L. albus*. The 2 proteins are 94% identical based on Basic Local Alignment Search Tool alignments and are homologues of a number of legume seed storage proteins, some of which are known allergens. The closest nonlupine identity match is to soybean β-conglycinin α-prime, with approximately 52% identity in a full-length alignment. The peanut allergen Ara h 1 is approximately 47% identical to the lupine proteins. It is not clear whether the multiple protein spots identified in this study represent products of multiple genes, proteins from differentially spliced RNAs, or posttranslationally modified proteins. Interestingly, even though the sequence identities are so similar, these 2 patients with lupine allergy recognized spots with apparently different affinities or abundance of IgE based on qualitative spot intensities (data not shown). Even more interesting was the apparent lack of cross-reactivity of these proteins and the homologues of other legumes based on failure of soybean or pea to inhibit binding to these proteins for serum 5 (Fig. 4, 5S and 5G) and lack of IgE binding of serum 5 to any peanut proteins (Fig. 3). Serum from Patient 2 bound two 23-kd proteins, labeled as 1 and 2 in Figure 5, with different pI values that were closely associated with protein spots 3 and 4 (Fig. 5, A). These 4 spots were analyzed by means of peptide sequencing and had limited (approximately 10% to 18%) coverage of 2 legumin-like lupine proteins (see supplemental Table E1). The structural differences leading to IgE binding to spots 1 and 2, but not 3 and 4, are not known.

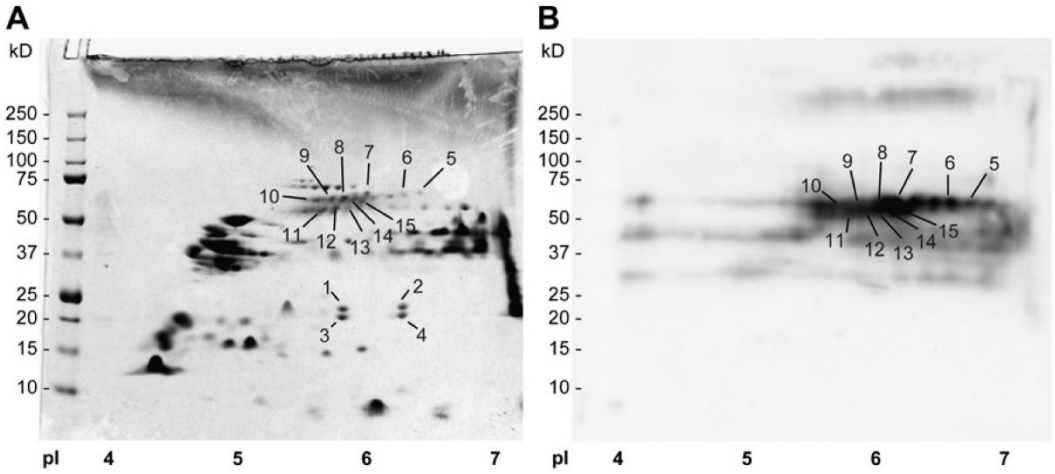

Figure 5. Two-dimensional gel protein stain and IgE binding. A, Coomassie blue–stained gel of lupine extract separated by means of isoelectric focusing (pI values estimated), followed by SDS-PAGE of separated proteins in the second dimension. B, Polyvinylidene difluoride membrane incubated with patient serum 5 and indicating IgE-binding proteins. Numbers correspond to supplemental Table E1.

Discussion

Allergy to legumes is well known, with peanut being the most prominent and soy often mentioned as the next most important allergenic legume. Recently, lupine flour has been introduced as a food alternative to soy flour. However, recent reports demonstrate a number of allergic reactions to lupine in food.[1,4–6] The United Kingdom–based Institute of Food Science and Technology has recommended that lupine flour should be added to the list of 12 allergenic ingredients requiring food labeling and that methods are needed for detecting lupine proteins in processed foods.[23]

The lowest ED reported previously was 200 mg for lupine, which induced asthma and abdominal pain.[4] Our data show that in 4 of 6 patients with lupine allergy, the ED was 1 mg or less for subjective symptoms (oral allergy symptoms) and 300 mg for objective symptoms, confirming the histories of modest-to-severe reactions to lupine in 5 of 6 patients. These doses are similar to those identified as EDs in peanut[24] and are an indication of the significant allergenicity of lupine flour. Allergy to lupine has been reported as a result of cross-reactivity with peanut.[1,4,6] However, our data clearly show that lupine allergy can occur as a separate entity, without evidence of clinical or laboratory cross-reactivity to other legumes (Patients 4–6). Hefle et al[1] reported that the IgE-binding proteins of a lupine extract appeared to have approximate molecular weights of 21 kd and 35 to 55 kd. Moneret-Vautrin et al[4] showed that the most IgE-reactive protein in lupine flour had a molecular weight of 43 kd for their patients with peanut allergy and also identified bands at 13, 38, and 65 kd that were not cross-reactive with peanut. Another study with serum of a patient without peanut allergy but with allergic symptoms to airborne lupine flour showed binding to proteins with molecular weights of 34, 59, and 71 kd as the most prominent IgE-binding proteins, with minor bands of 17 and 24 kd.[8] Immunoblot analysis with the serum of a child with airborne lupine flour allergy with a CAP class of 1 to peanut revealed an IgE-binding band with an approximate molecular weight of 45 kd.[9] Collectively, these reports indicate that a variety of lupine proteins are likely allergens, although identification of proteins by molecular weight in different experiments is not definitive.

In our study, patients with a combined lupine and peanut allergy showed weak IgE binding to protein bands of lupine at 14, 24, 30, or 66 kd. In contrast, 2 patients with lupine allergy without peanut allergy showed IgE binding to proteins of lupine at approximately 50 and 66 kd, and 1 patient bound two 23-kd proteins. This indicates that there are remarkable differences in allergen recognition between these 2 groups of patients.

Peptide sequencing of the 50- to 66-kd proteins showed that multiple spots represent 2 highly similar proteins that are approximately 47% identical to Ara h 1, a peanut vicilin, and 52% identical to β-conglycinins (or vicilin-like proteins) of soybean. Presumably, the shared 47% identity with Ara h 1 does not provide enough structural similarity for shared IgE binding because clinical reactivity to peanut was absent and specific IgE and SPT reactivity was low or absent. The predicted 61- to 62-kd proteins could be similar to the 65-kd band found by Moneret-Vautrin et al[4]; in fact, by means of 1-dimensional SDS-PAGE, we observed binding to a protein with an estimated molecular weight of between 50 and 66 kd. It is typically difficult to accurately predict the molecular weight of proteins based solely on migration distances in either 1- or 2-dimensional gels by using different

molecular weight standards. This study also demonstrates the complexity of identifying proteins that bind. The multiple spots on 2-dimensional gels and differing patterns of IgE recognition between subjects demonstrate the need for further structural studies. In addition, a larger population of subjects with lupine allergy will be needed to completely describe the allergens in lupine.

Together, the data indicate that lupine allergy might equal peanut allergy in its severity. Therefore lupine might be a less attractive replacement for soy. Moreover, lupine allergy is more complicated than previously thought because both cross-reactive and unique allergens are involved.

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Supplemental material

Table E1. Peptide sequencing of IgE-binding lupine proteins*

Lupine protein†	Lupine protein—best match		No. serum bound IgE	Matched sequence coverage (%)
	Accession no.	Description		
1	Gi|62816184	Legumin-like	2	10%
2	gi|85361412	Legumin-like	2	18%
3	gi|62816184	Legumin-like	None	10%
4	gi|85361412	Legumin-like	None	18%
5	gi|46451223	β-Conglutin precursor	4, 5	35
6	gi|46451223	β-Conglutin precursor	4, 5	35
7	gi|46451223	β-Conglutin precursor	4, 5	35
8	gi|89994190	Vicilin-like protein	4, 5	36
9	gi|89994190	Vicilin-like protein	4, 5	42
10	gi|89994190	Vicilin-like protein	4, 5	35
11	gi|89994190	Vicilin-like protein	4, 5	34
12	gi|89994190	Vicilin-like protein	4, 5	34
13	gi|89994190	Vicilin-like protein	4, 5	30
14	gi|89994190	Vicilin-like protein	4, 5	39
12	gi|89994190	Vicilin-like protein	4, 5	34
15	gi|46451223	β-Conglutin precursor	4, 5	37

*Peptide sequences were identified by means of matrix-assisted laser desorption/ionization–time of flight mass spectrometry or liquid chromatography–mass spectrometry–mass spectrometry of stained protein spots isolated from gels. Mass comparisons and peptide identities were performed by using Mascot (version 2.1) to search the NDBInr database by Macromolecular Resources at Colorado State University.
†Protein number refers to marked spots on the Coomassie-stained gel (Fig. 5).