

Cross-allergenicity of peanut and lupine: The risk of lupine allergy in patients allergic to peanuts

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Background: Peanut allergy is common, but cross-allergy between legumes is rare. Proteins from *Lupinus albus* are increasingly eaten in the form of seeds or additives to wheat flour. The risk of cross-allergenicity is still insufficiently known.

Objective: We sought to study the risk of cross-allergy to lupine in patients allergic to peanut and to study lupine allergenicity.

Methods: Twenty-four patients allergic to peanuts were studied by means of skin prick tests with native lupine flour from *Lupinus albus*. Double-blind oral challenge tests were performed with lupine flour and peanut in 8 of these patients. Specific IgEs were assayed for peanut, lupine flour, and pollen in 6 sera. RAST inhibition tests for lupine pollen by peanut were performed on 4 of these sera. Peanut and lupine flour immunoblots were carried out for 6 sera, and crossed immunoblot inhibitions for peanut by lupine flour and lupine flour by peanut were carried out for 2 sera.

Results: The skin prick test responses with lupine flour were positive in 11 (44%) subjects. The challenge test responses were positive in 7 of 8 subjects at the same doses as with peanut. The major lupine flour allergen (molecular mass, 43 kd) is present in peanuts. The RAST inhibition and immunoblot tests indicated cross-reactivity of peanut with the lupine flour and pollen.

Conclusions: The risk of crossed peanut-lupine allergy is high, contrary to the risk with other legumes. The inclusion of 10% lupine flour in wheat flour without mandatory labeling makes lupine a hidden allergen, presenting a major risk of cross-reaction in subjects already allergic to peanut products. A high sensitizing potential can also be postulated for this legume. (*J Allergy Clin Immunol* 1999;104:883-8.)

Key words: Peanut allergy; legumes; cross-reactivity; lupine allergenicity; double-blind, placebo-controlled food challenge; immunoblot

Peanut allergy is common and severe.¹⁻³ Sensitization rates have been evaluated to be 1.2% in a pediatric popu-

Abbreviation used

DBPCFC: Double-blind, placebo-controlled food challenge

lation, and allergy rates have been evaluated to be from 0.31% to 1.1%.^{4,5} The risk of lethal food anaphylaxis has been mainly related to peanut.⁶⁻⁹ A strict avoidance is currently the only way of preventing accidents. There is a considerable in vitro cross-reactivity between legumes. Cross-sensitivity investigated by means of skin prick testing is less frequent, and crossed food allergy appears to be rare (ie, 2.9% to 6% of subjects).¹⁰⁻¹³ However, one legume, the lupine, is beginning to be consumed frequently either in the form of seeds or as flour used to improve wheat flour.^{14,15} Several cases of lupine allergy have been published.¹⁶⁻¹⁸ This risk of crossed allergy with peanut has been documented in one case.¹⁶ Lupine proteins, which react with sera from patients allergic to peanuts, do not seem to correspond to the major peanut allergens Ara h 1 (65 kd), Ara h 2 (17 kd), and Ara h 3 (14 kd).¹⁹⁻²¹ Insofar as it is planned to increase the presence of lupine protein in human food because of its nutritional value, the possible risk of crossed peanut-lupine allergy should be investigated.

We present 8 observations of peanut allergy with a possible cross-allergy with lupine. Five of 6 double-blind, placebo-controlled food challenge (DBPCFC) responses were positive, although these subjects had probably not previously consumed these proteins. This leads us to emphasize the fact that the use of lupine proteins to improve flour without labeling presents a definite risk for patients allergic to peanuts and soya.

METHODS

Clinical study

Twenty-four subjects were given a diagnosis of peanut allergy by means of conventional criteria (ie, allergic sensitivity confirmed by skin prick tests and DBPCFCs).^{22,23} Eight subjects underwent challenge tests for lupine (Table I).

Skin prick tests. Skin prick tests were performed with natural foods (a grilled peanut) and other legumes (ie, soya, chickpea, pea, broad bean, and dried bean). The lupine flour (*Lupinus albus*) came from a regional flour mill. Negative (saline serum) and positive (9% codeine) controls were also included. The criterion for positivity of the test is a wheal diameter of 3 mm, a negative glycerosaline control result, and a codeine control result of at least 2 mm.²²

Oral challenge tests. Oral challenge tests were performed with

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Received for publication Nov 10, 1998; revised July 9, 1999; accepted for publication July 9, 1999.

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0091-6749/99 \$8.00 + 0 1/1/101515

TABLE I. Skin prick tests, specific IgE, and food challenges to peanut, lupine flour, and soy flour

Patients	Sex	Atopic history	Age (y)	Peanut			Lupine pollen	Lupine flour			Symptoms	Labial challenge
				Skin prick test response (mm)	RAST (kU/L)	DBPCFC (mg)	RAST (kU/L)	Skin prick test response (mm)	RIA (kU/L)	DBPCFC (mg)		
1	F	AD, AO	5	25	>100	+965	4.5	7	7.8	+1000	RC, A	
2	M	AD, A, AO	11	8.5	>100	+15	57	8.5	2.5	ND		Grade 2
3	F	U, AO	6	7.5	>100	+965	8.6	12	2.35	+765	RC, U	
4	F	AD	4	10	65	+760	5.7	14	3.5	ND		Grade 2
5	F	A, AO	6	6	>100	+5	4.4	10	1.45	+265	A, AP	
6	F	A	16	12	>100	+700	74	9	3	+965	A	
7	F	AD	1.5	6.5	15	+265	ND	—	ND	+265	DA ex	
8	F		5	5	70	LT+	ND	7	ND	−7110		

LT, Labial challenge; AD, Atopic dermatitis; AO, angioedema; RC, rhinoconjunctivitis; A, Asthma; U, urticaria; AP, abdominal; ex, exacerbation.

peanut on 24 patients and with lupine flour on 8 patients (ie, 7 with positive skin prick test responses and 1 with a negative response). Two techniques were used: the labial challenge test and the DBPCFC.²³⁻²⁵

The labial challenge test consists of placing a drop of the food in liquid solution on the outer edge of the lower lip. Criteria for positivity are the appearance of one of the following symptoms in under 15 minutes, according to graded intensity: 1, labial edema at the site of application; 2, erythema or urticaria extending onto the chin; 3, an extension of the urticaria to half the face, possibly with unilateral conjunctivitis or rhinitis; and 4, remote eruptions (ie, rash in flexion folds in patients with atopic dermatitis).^{24,25}

If the labial provocation test response was negative, an oral challenge test with the natural food and a placebo was performed in a single-blind manner if a small child was involved or a double-blind manner in the case of an adult, according to the method published.³

The natural food was mixed with a nonallergenic vehicle (ie, mashed potato or stewed apple). The amounts of lupine flour were administered in increasing doses in 3 progressions: first progression, 5, 10, 50, 200, and 700 mg (total dose, 965 mg); second progression, 10, 100, 500, 1500, and 5000 mg (total dose, 7110 mg); and third progression, 10 and 20 g.

The patient was monitored through the afternoon and the following night. Monitoring criteria were as follows: color of the skin and mucosa, observation of the uvula, pulse rate, blood pressure, pulmonary auscultation, peak expiratory flow rate, and FEV₁.

The objective skin symptoms taken into consideration were as follows: the appearance of angioedema, urticaria, rash in the flexion creases, and generalized rash or eczema reaction 24 to 48 hours after the test, as evaluated by the SCORAD index.²⁶

The only subjective symptom taken into consideration was the occurrence of abdominal pain. Indeed, it has been consistently observed that in this case, the continuing administration of doses leads to vomiting, diarrhea, asthma or cutaneous symptoms.

Observation of a clinical reaction led to the prescription of an intramuscular injection of methylprednisolone with an antihistamine and, in the event of asthma, an aerosol-administered dose of salbutamol. If there was no reaction, the progression was continued the next day.

Immunologic study

Six sera from patients with positive DBPCFC responses to lupine flour were studied.

Extracts. The defatted peanut protein and lupine flour were

extracted by a solution of 4% ammonium bicarbonate (1/5 wt/vol) for one night at 4°C with stirring. After centrifuging and sterilizing filtering through a 0.2-μm membrane, the concentration of protein was determined by using Bradford's method: 50 mg/mL for lupine flour and 4 mg/mL for peanut.²⁷

IgE specific to peanut, lupine flour, and lupine pollen. Determination of peanut- and lupine flour-specific IgE was performed by using the RAST method with Allerbio disks (Allerbio Laboratory, Varennes en Argonne, France). Peanut and lupine flour extracts were coupled to cellulose cyanogen bromide-activated disks. Fifty microliters of serum was used for the test. IgE was detected by iodine 125-labeled anti-IgE mAbs (Immunotech, Marseille, France).

Because of the frequent cross-reactivities between pollens and food vegetable allergens, the lupine pollen-specific IgE was searched by using the RAST method Phadebas Cap System. RAST inhibition assays were performed as described.²⁸ RAST inhibition to lupine pollen by a peanut solution in PBS was performed at doses of 1 to 10⁻⁶ g/mL (7 concentrations). An inhibiting mixture of 25 μL of serum and 25 μL of peanut solution was incubated for 30 minutes with stirring. A control mixture was prepared with 25 μL of serum and 25 μL of PBS. The percentage inhibition corresponded to ratio 1: cpm bound to the inhibiting mixture/cpm bound to the control mixture.

SDS-PAGE/immunoblotting. The SDS-PAGE test was performed on a 12% polyacrylamide gel with a 6% concentration gel in the Tris-Tricine system.²⁹ The samples were diluted in a sample buffer containing 1% SDS and heated for 3 minutes at 95°C. Twelve micrograms per centimeter of gel for the peanut and 24 μg/cm for the lupine flour were deposited on the gel. After migration (1 hour and 30 minutes at 50 mA and 13°C), the gel was subjected to a semidry transfer on cellulose nitrate membrane (BA 83 S; Schleicher and Schull, Dassel, Germany) in a Tris-glycine-methanol buffer for 1 hour at 0.8 mA/cm² of gel.³⁰ One part was colored with silver nitrate.³¹

Immunodetection. The membrane, cut into 3-mm strips, was saturated in a solution of PBS-0.1% Tween and 1% BSA for 1 hour.²⁰ The strips were then incubated with the diluted sera (1:20 for peanut and 1:5 for lupine flour) and a control buffer for one night with stirring. After 3 washes in PBS-0.1% Tween, 10,000 cpm/strips of ¹²⁵I-labeled mouse anti-IgE human mAbs (Immunotech, Marseille, France) were incubated for one night. After 3 washes, the strips were subjected to autoradiography at 10 days.

Immunoblot inhibition. The sera were preincubated volume for volume with extracts of peanut (2, 1, 0.5, and 0.25 mg/mL) or lupine flour (4, 2, 1, and 0.5 mg/mL). This mixture was then diluted with saturation buffer to obtain a serum dilution identical to that described above. IgEs were revealed as described previously.

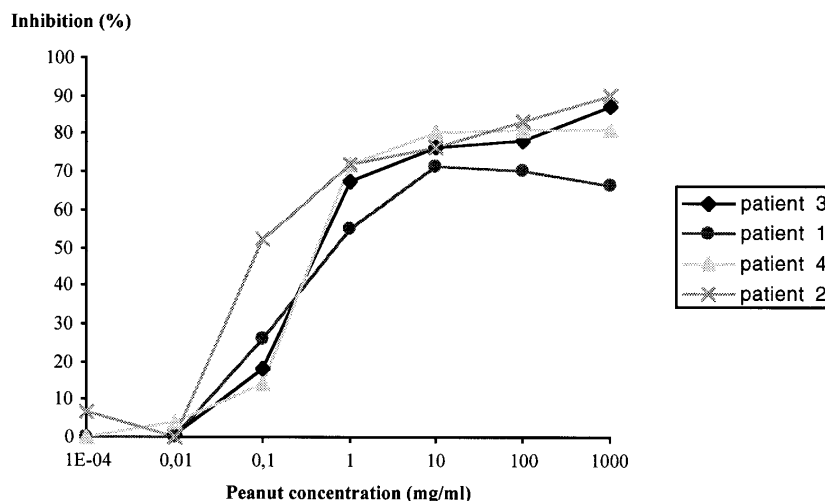


FIG 1. Inhibition of RAST to lupine pollen by peanut.

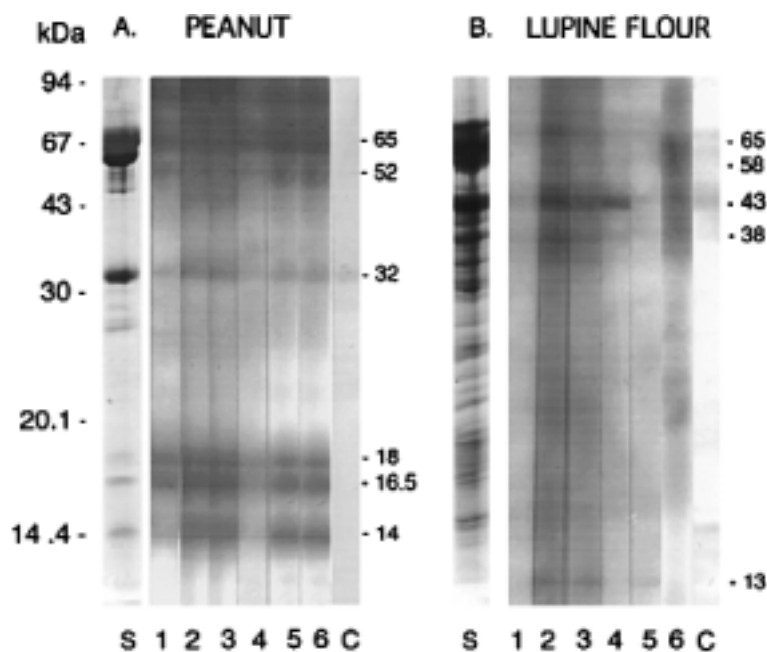


FIG 2. SDS-PAGE of peanut extract (A) and lupine extract (B), either silver stained (S) or immunoblotted. Sera from subjects allergic to peanut were diluted 1:20 for peanut strips and 1:5 for lupine blot. C, Blocking buffer.

RESULTS

Eleven of 24 (44%) skin prick test responses to lupine were positive. Specific IgEs were present in large quantities for peanut and low quantities for lupine pollen and flour. In 5 of 6 patients, the oral challenge test responses were positive, 6 times with an immediate reaction and once with a delayed reaction (reactivation of eczema). Two labial test responses were positive (grade 2).

The sera studied (patients 1 to 6) displayed IgE specific to lupine pollen and flour. Levels were higher for the pollen than for the flour, except in the case of patient

1. They were much lower than the levels of peanut-specific IgE (Table I).

RAST inhibition to lupine pollen by peanut was positive for the 4 sera from peanut-allergic subjects (Fig 1).

Immunoblots were performed to peanut and lupine flour. Fig 2, A, shows the IgE-binding pattern of the 6 patients allergic to peanuts on immunoblot of SDS-PAGE-separated peanut extract. Numerous bands are observed, with molecular masses of 78, 68, 65, 52, 45, 32, 18, 16.5, and 14 kd. The SDS-PAGE of *Lupinus albus* extract shows numerous proteins between 14 and 75 kd (Fig 2, B).

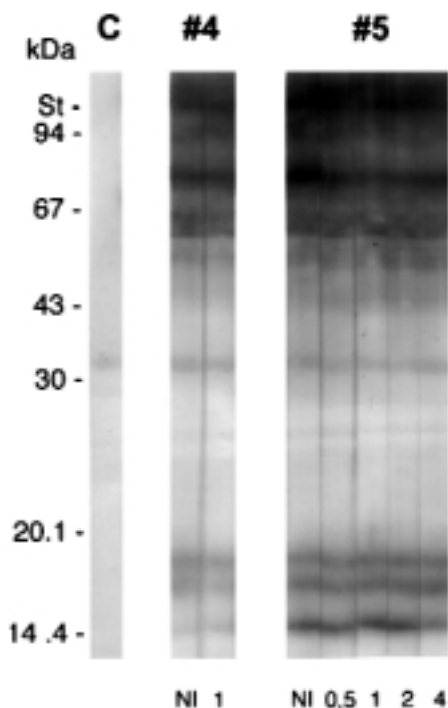


FIG 3. Immunoblot inhibition of peanut allergen recognition by 2 patient sera caused by lupine extract. Concentrations of lupine extracts ranging from 0.5 to 4 mg/mL were mixed volume to volume with serum. The mixtures were diluted further to obtain a final sera dilution of 1:20. *NI*, Noninhibited; *C*, control buffer.

Lupine immunoblots with sera from patients allergic to peanut revealed reactive IgE bands at 65, 43, 38, and 13 kD. All sera bind IgE at 43 kD. Sera 2, 3, and 6 reacted with an allergen located at 65 kD. A 38-kD band was recognized by sera 2, 3, 4, and 6. A light IgE-binding band could be seen at 13 kD with serum 5 (Fig 2, *B*).

The negative control showed slight nonspecific binding of radiolabeled anti-IgE between 45 and 65 kD, which may be due to the presence of lectin in lupine flour extracts.

There was no inhibition of the peanut immunoblots on sera 4 and 5 by extract of lupine flour. No reduction in intensity of reactive IgE bands could be seen (Fig 3). Inversely, the inhibition of the lupine immunoblot by peanut was observed increasingly for the 43-kD allergen with serum 4 (Fig 4).

DISCUSSION

The lupine is a legume belonging to the genus *Lupinus*, which includes 450 species. Four species are of agricultural interest: the white lupine (*Lupinus albus*), the blue lupine (*Lupinus angustifolius*), the yellow lupine (*Lupinus luteus*), and *Lupinus mutabilis*. A sulfur-rich transgenic variety is available for animal feed.³² Lupine flour is an excellent source of proteins, with 39% to 45% having a higher content of albumin (55.6%) than globulins (31.5%).^{14,33}

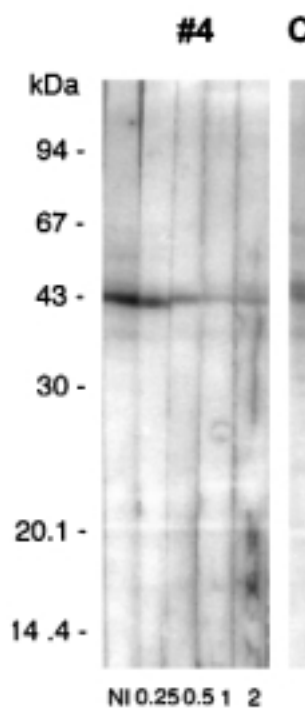


FIG 4. Immunoblot inhibition of lupine allergen recognition by one patient serum caused by peanut extract. Concentrations of peanut extracts ranging from 0.25 to 2 mg/mL were mixed volume to volume with serum. The mixtures were then diluted to obtain a final sera dilution of 1:5. *NI*, Noninhibited; *C*, control buffer.

The inclusion of lupine (*Lupinus albus*, variety Ares) in wheat flour was authorized in France at the end of 1997. Lupine flour is used in biscuits, pasta, sauces, dietetic products sold as milk, and soya substitutes.^{15,34} The seeds are served with aperitifs.

The cross-reaction between lupine and peanut, as investigated by means of skin prick testing, concerned 44% of patients (5 of 7 patients in the study by Hefle et al¹⁶). The risk of cross-allergy appears to be high because the challenge test to raw flour was positive at less than 1 g every time the skin prick test response was positive (patients 1-6) and also unexpectedly positive in patient 7, for whom the skin prick test response was negative. It will be necessary to find out whether heating modifies the allergenicity, as was shown for soya.³⁵

Food allergens produced by legumes are not totally different from aeroallergens represented by plant dust or pollens. Concerning soya and peanut, it has been shown that common allergens are present in the foods, soya seed dust, and peanut shells.^{13,36,37} A high similarity (80%) has been found between the lupine mature 11 S globulin and the soybean glycinins.³⁸ The lupine β -conglutinin is closely related to Ara h 2.³⁹

RAST values to lupine pollen are higher than those of RASTs to lupine seeds. Because lupine flour is very rich in proteins, in spite of an adjustment of rate to 5 mg/mL for the disk coupling, it can be postulated that the quantity of nonallergenic proteins prevents the coupling of

allergenic proteins and hence the fixation of specific IgE. Moreover, the hypothesis could be put forward that sensitivity to lupine pollen occurs first. Oral challenge tests frequently triggered conjunctival, nasal, or bronchial reactions (patients 1, 3, 5, and 6), indicating mucosal sensitivity and hence possible sensitivity to airborne allergens.

The RAST inhibition of lupine pollen by peanut shows a 50% inhibition by a concentration of 1 mg/mL of peanut on 4 peanut-allergic sera. This indicates closely related allergens in peanut, lupine flour, and lupine pollen extracts.

Well-characterized peanut allergens were detected by the 6 sera from patients allergic to peanut: Ara h 1 (65 kd), Ara h 2 isoforms (18 and 16.5 kd), and Ara h 3 (14 kd).^{19,21,39}

The immunoblots to lupine flour showed that the most distinctly reactive band had a molecular mass of 43 kd for 5 of 6 sera. The 43-kd band located could contain the major allergen because it was recognized by 5 of 6 sera. The 35- to 55-kd group of allergens indicated by Hefle et al¹⁶ was found in this study.

The total inhibition of immunoblot lupine flour by peanut confirmed the cross-reactivity of the 43-kd allergen. This corresponds to a protein not identifiable as an allergen described for peanut without using antibodies of known specificity. There is an interesting possibility that this allergen may be clinically relevant. The complete characterization of this molecule should be determined to understand the cross-reactivity of lupine with peanut allergens. The inhibition assays failed to demonstrate a cross-reactivity for the lupine allergens of 65, 38, and 13 kd. Although Ara h 2 is closely related to the β -conglutinin from lupine,³⁹ such a band was not observed in the lupine immunoblots.

The apparent absence of inhibition of the peanut immunoblot by lupine flour could be due to the large quantity and high affinity of peanut-specific IgE in the sera tested (65 and 100 kU/L), a small part of which was cross-reactive for lupine (3.5 and 1.45, respectively).

Although the levels of lupine-specific IgE are low compared with those of anti-peanut IgE, clinical sensitivity to lupine occurred at identical doses to those of peanut. This leads one to suspect that one of the common allergenic structures between peanut and lupine is particularly involved in the clinical reactivity. The frequency of positive challenge test responses also seems to indicate that the cross-allergy to lupine is a major risk for persons allergic to peanuts. The inclusion of lupine flour in wheat flour at an accepted proportion of 10% could provide a large quantity of allergens. This flour improver is not subject to labeling and can be considered as a dangerous hidden allergen. It could cause anaphylactic shock or continuous atopic dermatitis in spite of a strictly peanut-free diet.

The existence of cross-reaction between lupine pollen and peanut and the frequency of symptoms in the ocular, nasal, and bronchial mucosa raises suspicion that a primary sensitization to lupine pollen could evolve toward

sensitization to lupine flour and to cross-reactivity to peanut. The risk of lupine allergy in patients with peanut allergy could be much higher than cross-allergy to the other legumes. Modifications in allergenicity caused by heating or simulated digestive models should be studied for a better appreciation of the risk of introducing lupine into human daily food intake.^{40,41}

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