

In vitro and in vivo Cross-Reactivity Studies of Legume Allergy in a Mediterranean Population

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Key Words

Legume allergy · Legume cross-reactivity · Legume allergens

Abstract

Background: Legume allergy, mainly to lentils and chickpeas, is the fifth most common cause of food allergy in Spanish children. Serological cross-reactivity among legumes is frequent, but its clinical relevance is controversial. The aim of this study was to investigate the cross-reactivity among lentils, chickpeas, peas, white beans and peanuts and its clinical relevance in pediatric patients. **Methods:** Fifty-four children with clinical allergy to legumes were included. Cross-reactivity was evaluated by ELISA inhibition experiments and oral food challenges to legumes. SDS-PAGE immunoblots were conducted with raw and boiled legume extracts. **Results:** ELISA inhibition experiments demonstrated more than 80% inhibition with lentil, chickpea and pea extracts. Immunoblots performed with raw legume extracts (lentil, chickpea and pea) and individual sera revealed that more than 50% of the sera identified an allergen with approximately 50 kDa in all three legume extracts. In all three boiled extracts an intense band at approximately 50 kDa was visualized using a serum pool. The oral legume challenges demonstrated that 37 children (69%) were allergic to 2 or more legumes (median 3 legumes). The most frequent associa-

tions were allergy to lentils and chickpeas (57%), allergy to lentils and peas (54%) and allergy to lentils, chickpeas and peas (43%). **Conclusions:** In vitro inhibition experiments demonstrated a high degree of cross-reactivity among lentils, chickpeas and peas. Food challenges confirmed that clinical allergy to all three legumes is frequent in our cohort of Spanish children.

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Introduction

Food allergy is a common cause for medical consultation. Although its true prevalence is unknown, food allergy may affect 1.5–2.5% of adults [1, 2] and 6–8% of children [3]. Any food can induce an allergic reaction, but only a few are common causes of food allergy. In children, milk, egg and fish are the foods most commonly involved in allergic reactions [4]. In the USA and in some European countries, peanuts (PN) are one of the main causes of food allergy among children and adults. In Spain, allergy to legumes such as lentils (L), chickpeas (CP) and peas (P) is the fifth most common cause of food allergy in children [5]. Skin or in vitro testing, such as RAST or SDS-PAGE immunoblotting, is frequently positive to several legume extracts [6, 7]. Clinical reactivity to more than one legume has been described as very rare

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among North American children [8]. However, in the Mediterranean area and in India, clinical reactivity to several legumes has been reported as common [9–11]. It seems that the clinical relevance of legume cross-reactivity is dependent on the dietary habits in different countries.

PN or soybean allergens have been studied extensively, but allergens of other legumes, such as L, CP and P, have been investigated less. Recently, major L and P allergens have been identified. They belong to the storage proteins of the vicilin family. These allergens are proteins with approximately 50 kDa (Len c 1 of 48 kDa and Pis s 1 of 47 kDa) and an amino acid sequence homology of 90% [12, 13].

The aim of this study was to investigate the in vitro and in vivo cross-reactivity among legumes in a pediatric Mediterranean population and its clinical relevance.

Material and Methods

Patients

This study was carried out at the Allergy Department of the Niño Jesús Hospital in Madrid, Spain. In order to be included in the study, patients had to fulfill the following inclusion criteria: (1) age between 1 and 15 years; (2) a history of allergic reactions to at least one legume: L, CP, P, white beans (WB) and PN; (3) positive skin tests and/or serum-specific IgE to at least one legume, and (4) a positive legume challenge or recent history of severe reaction. Fifty-four children allergic to legumes were included in this study.

At the initial visit, a complete clinical history and physical exam were performed. All patients were skin tested with commercial legume extracts including L, CP, P, WB, green bean, soybean and PN (Laboratorios LETI, SL, Madrid, Spain). Serum samples were collected from each child and frozen in small aliquots until use. As a control group, sera from 5 atopic children with good tolerance to legumes were used.

This study was performed with the approval from the Ethics Committee of the Niño Jesús Hospital in Madrid.

Legume Extracts

One hundred grams of L, CP, P and WB were ground in a blender for 3 min and extracted in 2 liters of a 0.85% NaCl solution in a shaker for 16 h at 4°C. Afterwards, two types of extracts of each legume were prepared. One half remained raw and the other half was boiled for 60 min. All legume extracts were centrifuged individually at 10,000 rpm for 15 min and the supernatants were filtered under sterile condition. Finally, the legume extracts were lyophilized and kept at 4°C until use. A PN extract (Laboratorios LETI, SL) was used in ELISA inhibition experiments.

Specific Serum IgE to Legumes and Inhibitions

Specific serum IgE to legumes were determined by enzymatic immunoassay CAP-FEIA (Pharmacia Diagnostics, Uppsala, Sweden) performed according to the manufacturer's instructions.

Specific IgE inhibition experiments were conducted by ELISA inhibition experiments, as previously described [14]. Briefly, plastic microtiter plates (Immulon IV, Dynex Technologies, Chantilly, Va., USA) were coated with L, CP, P and WB extracts (20 µg of freeze-dried material/ml) overnight. Several dilutions were made from each extract. Each dilution was incubated with a serum pool (diluted 1/40) containing high titers of specific IgE to legumes (L, CP and P) for 2 h at room temperature. Afterwards, 100 µl was transferred to the coated plates and incubated overnight. After washing, 100 µl of anti-human peroxidase-labeled IgE were added and left to stand for 30 min at room temperature. After washing, the plates were developed for 30 min and stopped with sulfuric acid 1 N.

SDS-PAGE and Immunoblotting

The protein profile of the raw and boiled extracts (L, CP, P and WB) was evaluated by SDS-PAGE. Samples were run in a gel with 2.67% C, 15% T acrylamide/bisacrylamide. A total of 100 µg from each freeze-dried extract in nonreducing conditions was loaded onto the gels. The gel was stained in 0.1% Coomassie blue (R-250).

After electrophoresis, the gels were electrotransferred onto Immobilon membrane (Millipore, Bedford, Va., USA). Membranes were incubated overnight with individual sera, diluted 1/3 (when raw extracts were used) and the serum pool, diluted 1/40 (when boiled extracts were used) in PBS-Tween 0.1%. After washing, membranes were incubated with peroxidase-conjugated monoclonal α-human IgE (Ingenasa, Madrid, Spain).

Oral Food Challenges

Oral food challenges were carried out with legumes in an open fashion or a double-blind, placebo-controlled oral food challenge when the results of the open food challenges were not conclusive, as previously described [15]. Previously, informed consent was obtained from the parents. Children were challenged with the following legumes: L, CP, P, WB and PN, if (1) they had a history of adverse reactions with one or more of these legumes (no recent severe reactions) or if (2) their tolerance was unknown. Twelve children were challenged with only one legume because they had a recent history of severe reactions with the other legumes or reported good tolerance. Five children were challenged with 2 legumes, 17 children with 3 legumes, 15 children with 4 legumes and 2 children with 5 legumes. Three children were not challenged with any legume because they had a recent history of severe reactions with 1 legume (2 children with PN and 1 with L) and reported good tolerance to the other legumes. The legumes were given in increasing amounts, which were doubled every 30 min until the children presented convincing symptoms, or until the standard dietary dose for their ages was reached. A positive challenge was defined as cutaneous (urticaria and/or angioedema) and/or respiratory symptoms (rhinitis, conjunctivitis and/or asthma) as single manifestations, or associated with other signs and symptoms of anaphylaxis. When the results of the open food challenges were not conclusive, a double-blind, placebo-controlled oral food challenge was performed. Up to 8 g of a boiled and lyophilized legume extract were administered in orange juice. As a placebo, only orange juice was used, since its taste and texture were not altered with the lyophilized food.

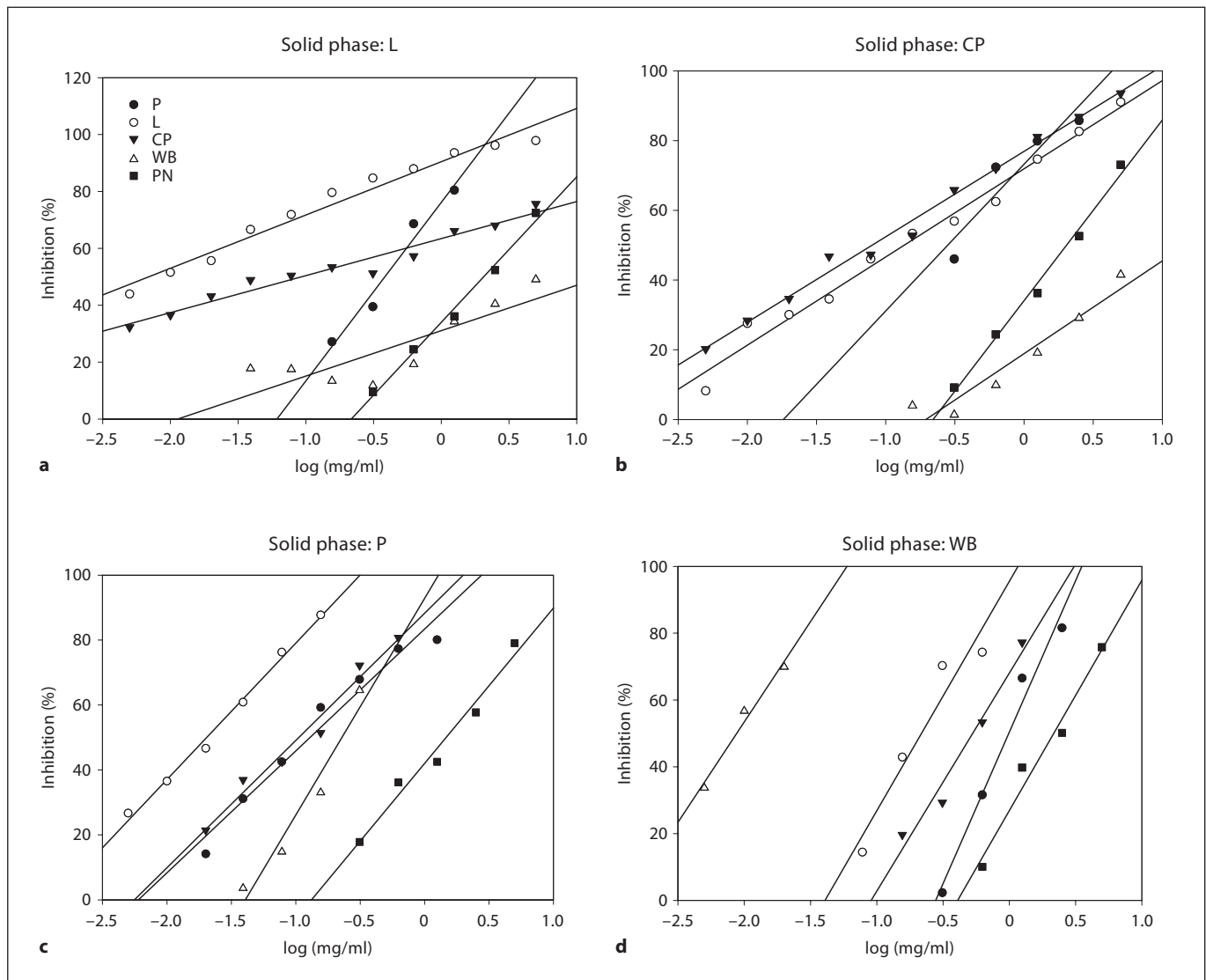


Fig. 1. ELISA inhibition regression curves of legumes with other legumes. **a** Specific IgE inhibition of L with L, CP, P, WB and PN. **b** Specific IgE inhibition of CP with CP, L, P, WB and PN. **c** Specific IgE inhibition of P with P, L, CP, WB and PN. **d** Specific IgE inhibition of WB with WB, P, L, CP and PN.

Results

Specific Serum IgE to Legumes and Inhibitions

In each patient specific IgE to legumes was positive for several legumes (table 1). ELISA inhibition experiments demonstrated more than 80% inhibition among L, CP and P, more than 70% between these legumes and PN, but less than 50% inhibition between WB, L and CP. Table 2 shows the 50% inhibition points for each legume extract. The concentration of inhibitor needed to obtain

the 50% inhibition point using L, CP and P in the solid phase was higher with WB and PN than with L, CP and P extracts.

SDS-PAGE Immunoblotting

SDS-PAGE of raw extracts showed a higher number of protein bands than boiled extracts. In all legume extracts, a band with approximately 50 kDa was observed. Immunoblots performed with raw extracts (L, CP and P) and individual sera from legume-allergic patients revealed multiple protein bands in all extracts (fig. 2). In L extract,

Table 1. Symptoms and specific IgE in 39 children who underwent oral challenges with 2 or more legumes

No.	Age years	Legumes	Symptoms	Specific IgE to legumes, kU/l	No.	Age years	Legumes	Symptoms	Specific IgE to legumes, kU/l
1 ^a	15.6	L CP WB P	OAS, U, A, Ds U, Ds NS AE	>100 >100 2.0 >100	15	3.4	L CP P WB	OAS, A OAS, A, V OAS, C NS	10.5 7.4 11.3 0.63
2	15	L CP P	U, AE, C OAS, R, C, A NS	5.28 1.48 4.02	16	3.2	CP P	NS OAS, U	3.37 9.52
3 ^a	15.6	L CP WB P PN	OAS, AE, C OAS, R NS OAS, R R, C, A	>100 93.7 27.1 >100 65.2	17	4	L CP P	U U, R, C U	>100 68.1 93.7
4 ^a	15.5	L CP P	OAS, Ds OAS, C, A U, R	13.7 8.61 12.9	18	9.7	L CP P	R, C, LE NS NS	10.1 16.3 9.17
5	11.8	L CP	OAS OAS	6.73 7.75	19	3.2	L CP P WB	OAS R, C NS NS	2.06 1.64 2.48 <0.35
6	9.5	L CP PN	OAS OAS OAS	5.37 4.81 6.14	20	3.5	L WB P PN	OAS, U NS OAS, U, R, A OAS, A	28.0 2.0 26.9 12.5
7 ^a	7	CP P PN	U, C AE, C NS	51.3 80.1 16.0	21 ^a	15.6	L CP P PN	OAS, Ds OAS, U, A, R OAS, U, Ds, C OAS, C, V	42.9 25.9 47.3 17.7
8	5.6	L CP P	OAS, AE, LE NS NS	16.0 12.7 12.6	22	3	L CP	OAS NS	2.16 2.07
9 ^a	10.2	L CP P	OAS, R, A OAS, U, A OAS, Ds	99.2 >100 >100	23	7.6	L CP P WB	OAS OAS OAS, V OAS	>100 >100 79.2 72.0
10	14	L CP WB	NS OAS, R, C NS	1.9 1.86 1.2	24	3.6	L CP WB PN	OAS, U, R, A NS NS NS	16.5 11.7 1.38 23.6
11	7.1	L CP P PN	U, AP OAS, C NS NS	39.6 47.7 57.8 32.2	25 ^a	13.2	L CP WB P PN	OAS OAS, R, C, A NS OAS, C, AP NS	11.4 8.72 1.26 8.77 6.06
12 ^a	5.6	L CP P	OAS, U, R, Ds, V OAS OAS, Ds	>100 95.1 >100	26	3	L CP P WB	OAS, AP U, V NS NS	15.8 16.7 18.1 3.64
13	4.7	L CP P	OAS OAS, U, R OAS, U, A	16.8 9.8 9.03	27	2.2	L P	OAS OAS	<0.35 <0.35
14	11.1	L CP P	OAS, U, AE NS U, R	18.8 12.6 23.4					

Table 1 (continued)

No.	Age years	Legumes	Symptoms	Specific IgE to legumes, kU/l
28 ^a	5	L	OAS	13.8
		CP	U, R, C, A, AP	13.4
		P	OAS	12.5
29 ^a	3.3	L	U, Ds, A	11.3
		CP	OAS, U, R	10.0
		P	OAS	12.0
		PN	U, V, A, R, C	>100
30	2.4	L	OAS	3.2
		CP	NS	2.49
		P	NS	3.27
31	1.9	L	OAS	6.3
		CP	NS	4.33
		P	NS	6.61
32	1.5	L	OAS	32.6
		CP	U	22.2
		P	U	33.9
33 ^a	2.6	L	OAS	2.33
		CP	U	1.17
		P	R, C, A, LE	2.67
		WB	NS	<0.35
34 ^a	2.1	L	OAS, C	36.0
		CP	OAS, Ds, A, LE	21.3
		P	U, A	40.1
		WB	NS	<0.35
35	5.5	L	U, R, C	5.1
		CP	R, C	4.24
36	2	L	OAS	10.4
		CP	NS	1.93
		P	U	10.9
		WB	U	1.48
37	8.8	L	R, C	5.1
		CP	OAS, AE, R, LE	4.32
		P	U, R, C	5.58
		WB	NS	<0.35
38	3.4	L	OAS	6.61
		CP	U	3.82
		WB	OAS	15.5
39	1.8	L	OAS	1.45
		CP	U, V	1.11
		P	OAS, V	1.22
		WB	U	2.41

OAS = Oral allergy syndrome; U = urticaria; A = asthma; Ds = dysphagia; AE = angioedema; C = conjunctivitis; R = rhinitis; LE = laryngeal edema; AP = abdominal pain; V = vomiting; NS = no symptoms.

^a Sera from these patients were used as serum pool in ELISA inhibition experiments and immunoblots with boiled extracts.

Table 2. Results of ELISA inhibition of legumes (solid phase) with legume extracts

Legume extracts	Legume (solid phase)			
	L	CP	P	WB
L	98 (0.007)	91 (0.136)	88 (0.02)	74 (0.22)
CP	76 (0.093)	93 (0.079)	81 (0.10)	77 (0.53)
P	80 (0.385)	85 (0.33)	80 (0.13)	82 (0.99)
WB	49 (4.983)	41 (9.944)	64 (0.22)	70 (0.009)
PN	72 (2.048)	73 (2.02)	79 (1.46)	76 (2.15)

Values represent maximum percentage of inhibition and concentration (mg/ml) needed to obtain the 50% inhibition (in parentheses) for each legume extract.

2 bands with approximately 50 and 67 kDa were detected by 84 and 69% of the sera, respectively. In P extract, 2 protein bands with 50 and 30 kDa were detected by 67 and 71% of the sera, respectively. In CP extract, 3 protein bands with approximate molecular weights of 50, 67 and 34 kDa were recognized by 79, 67 and 67% of the sera, respectively. In WB extract, 1 protein band with 30 kDa was identified by 56% of the sera and another one with 54 kDa by 44% (fig. 2).

Immunoblots were also performed with boiled legume extracts and a serum pool of legume-allergic children (L, CP and P); 1 intense band at approximately 50 kDa was identified in L, CP and P extracts. No protein bands were detected in boiled WB extract (fig. 3). No bands were detected with the control serum pool.

Oral Food Challenges

One hundred forty-three food challenges were performed in 51 children; 105 were positive (73%). Children with a recent history of severe reaction to a legume (defined as urticaria, wheezing and vomiting, and/or laryngeal edema after ingestion, contact or inhalation) were not challenged with this legume. They included 6 to L, 3 to CP, 3 to P and 9 to PN. All these children had clear positive skin prick tests and high specific IgE levels to these legumes. After oral challenges, 43 children (80%) were allergic to L, 32 children (59%) to CP, 27 children (50%) to P, 18 children (33%) to PN and 6 children (11%) to WB (table 3). All food challenges in the 12 children challenged with 1 legume were positive, except 1 with PN in a child with a recent history of severe reactions to L, CP and P (5 with PN, 2 with L, 2 with CP and 2 with WB).

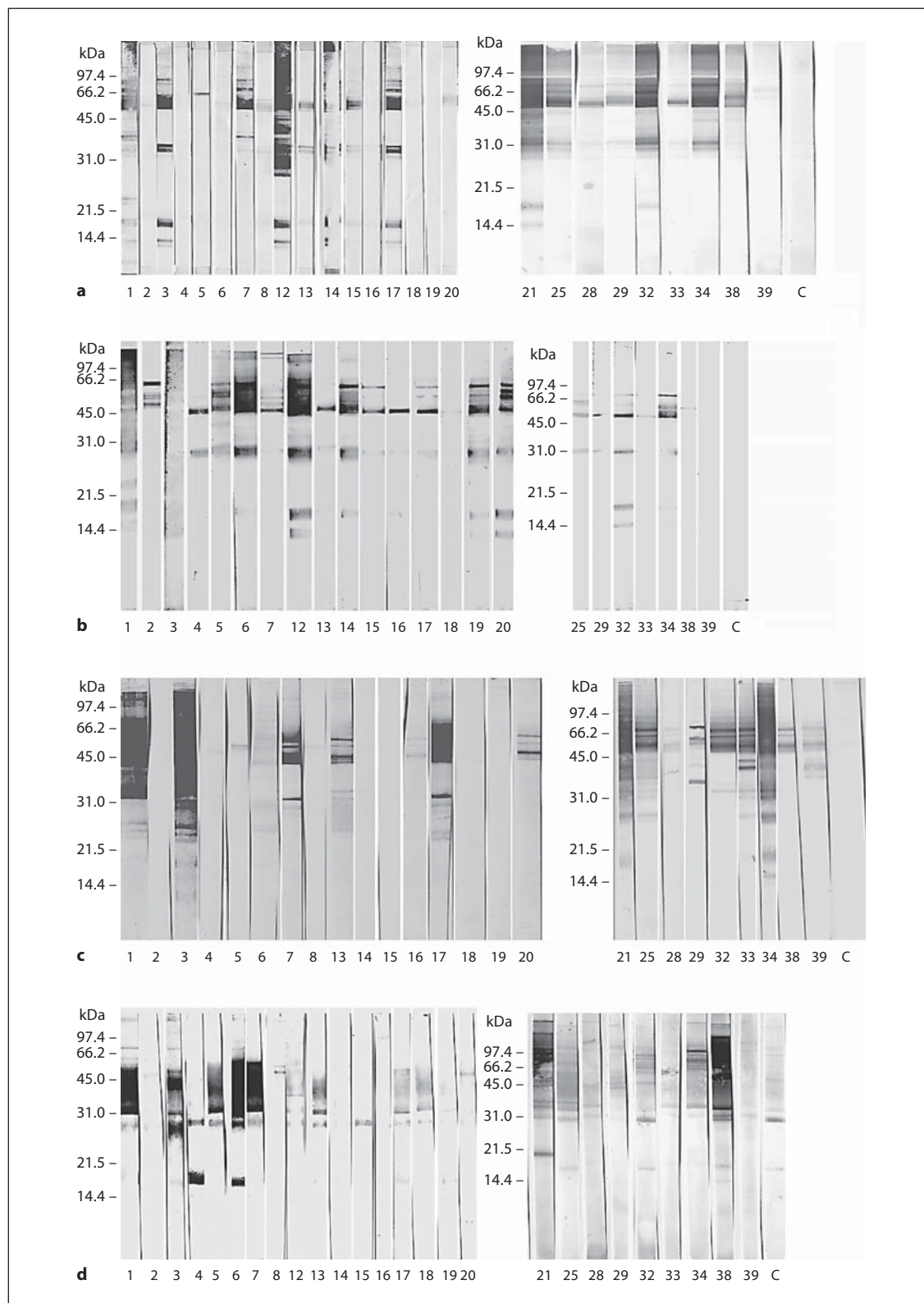


Fig. 2. SDS-PAGE immunoblots of raw legume extracts under nonreduced conditions with sera of patients allergic to legume and control group (C). **a** L extract. **b** P extract. **c** CP extract. **d** WB extract. kDa represents the molecular weight standard.

Table 3. Clinical characteristics in a cohort of children allergic to legumes most commonly consumed in Spain

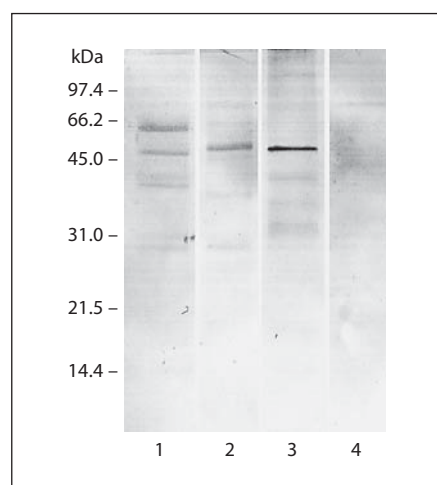
	L (n = 43)	CP (n = 32)	P (n = 27)	WB (n = 6)	PN (n = 18)
Sex (M/F)	36/7	28/4	23/4	5/1	11/7
Age ^a , years	4 (1–15)	7 (1–15)	5 (1–15)	2.8 (1–9)	6 (2–15)
Age of onset ^a , months	15 (4–84)	18 (8–108)	36 (9–168)	28 (18–60)	36 (12–192)
Unknown previous tolerance	4	0	9	1	1
Recent history of severe reaction	6	3	3	0	9
Oral challenges (P/N), n	37/2	29/10	24/8	6/12	9/6
Other clinical legume allergy ^{b, c}	35 (81)	31 (97)	27 (100)	5 (83)	11 (61)
Symptoms on oral challenges ^c					
Cutaneous	14 (38)	15 (52)	13 (54)	2 (33)	3 (33)
Gastrointestinal	4 (11)	3 (10)	3 (13)	2 (33)	2 (22)
Respiratory	12 (32)	17 (59)	11 (46)	0	4 (44)
OAS	16 (43)	5 (17)	3 (13)	2 (33)	2 (22)
Severe reaction	3 (8)	4 (14)	2 (8)	0	2 (22)

P = Positive; N = negative; OAS = oral allergy syndrome.

^a Figures represent median with the range in parentheses.

^b Confirmed by oral food challenges.

^c Figures in parentheses represent percentage.

**Fig. 3.** Immunoblots performed with a serum pool of patients allergic to legumes (L, CP and P) and boiled legume extracts: lane 1: L; lane 2: CP; lane 3: P, and lane 4: WB. kDa represents the molecular weight standard.

Thirty-nine children were challenged with 2 or more legumes (median 3 legumes, range 2–5). Seven of them (18%) had a positive result with 1 legume (6 with L and 1 with CP); the other 32 children (82%) had positive reactions with 2 or more legumes (median 3 legumes) (table 1). Patient 7 had a convincing history of severe reac-

tions to L and challenges with CP and P were positive. Patient 16 had a convincing history of severe reactions to L and the challenge with P was positive. Patient 20 had a convincing history of severe reactions to CP and challenges with L, P and PN were positive. Overall, 17 children (31%) were allergic only to 1 legume (8 to L, 7 to PN, 1 to WB and 1 to CP) and 37 children (69%) were allergic to 2 or more legumes (median 3 legumes): 11 children (20%) to 2 legumes, 19 children (35%) to 3 legumes, 6 (11%) to 4 legumes, and 1 (2%) to 5 legumes. The most frequent associations were allergy to L and CP (31 of 54 children, 57%), allergy to L and P (29 of 54 children, 54%), and allergy to L, CP and P (23 of 54 children, 43%). Allergy to L and PN was present in 9 children (17%) (table 1).

Discussion

In this study, we have demonstrated that serological and clinical reactivity to more than 1 legume is frequent among this cohort of Spanish children. During the school age, Spanish children eat legumes once or more times at week, in particular L, CP and beans. Consumption takes places at school or at home. In a previous report, we described that sensitization to L and CP was the most frequent cause of legume allergy in Spanish children. We

also established that P allergy is common, and that clinical allergy to WB, green beans and soybean is rare [9].

In our study, specific serum IgE to legumes was positive for several species in the majority of the children, even to those showing good tolerance, suggesting the CAP shows a very low specificity. ELISA inhibition experiments demonstrated a high degree of cross-reactivity among legumes, mainly L, CP and P. However, the cross-reactivity between L, CP and P with WB and PN was lower. Our findings are in accordance with those of Barnett et al. [7], who also demonstrated extensive cross-reactivity among legume extracts (PN, garden pea, soybean and CP) using RAST and RAST inhibition tests.

Immunoblot experiments revealed the existence of a band with a molecular weight of approximately 50 kDa in L, CP and P raw extracts identified by more than 50% of the sera. A protein band with intense specific IgE binding at approximately 50 kDa was identified in the homologous boiled legume (L, CP and P) extracts. This fact confirms that this allergen is thermostable and remains active in cooked legumes. In previous studies, we described that an allergen with approximately 53 kDa was recognized by 92% of children allergic to L in a raw L extract [16]. In a raw CP extract, 1 protein band with approximately 50 kDa was identified by 60% of children allergic to CP [17]. These data are in agreement with the results of other investigators, who isolated several proteins in boiled L and P extracts. These authors characterized and sequenced the major allergens of L and P, *Len c 1* [12] and *Pis s 1* [13], of 48 and 47 kDa, respectively, belonging to the vicilin family. A high degree of homology between them and with other legume allergens of the vicilin family, such as *Ara h 1*, was demonstrated [13]. Wensing et al. [18] described cross-reactivity between P and PN, and demonstrated that the molecular basis for this cross-reactivity was the homology between P and PN vicilins. In our study, we found that more than 50% of sera of patients allergic to legumes identified a protein of approximately 30 kDa in raw WB extract, but less than 50% recognized a protein band of 54 kDa. These data may explain the lower cross-reactivity between WB, L, CP and P demonstrated by ELISA inhibition experiments. No protein bands were recognized in the WB boiled extract. The serum pool used in these immunoblots did not contain any sera of WB-allergic children.

Using oral food challenges, we confirmed that clinical allergy to L, CP and P is very frequent. Thirty-seven children (69%) were allergic to 2 or more legumes (median 3 legumes). The most frequent association was allergy to L and CP (57%), followed by allergy to L and P (54%), and

allergy to L, CP and P (43%). In our study, we have also established that the 60% of children allergic to PN were allergic to other legumes, mainly to L. Other studies reported clinical reactivity to various legumes. Martín et al. [10] using bronchial provocations demonstrated clinical allergy to L, CP and P in a woman who reported asthma after inhaling steam from these cooked legumes. Other authors using double-blind, placebo-controlled food challenge (DBPCFC) demonstrated that clinical allergy to lupine and PN is frequent [19, 20]. Moneret-Vautrin et al. [19] confirmed clinical reactivity to lupine in 7 out of 8 patients allergic to PN. Peeters et al. [20] also observed clinical cross-reactivity between lupine and PN; 3 of 6 patients allergic to lupine were also allergic to PN. Recently, Patil et al. [11] studied 59 Indian patients who reported adverse reactions to CP; 31 of them had a positive DBPCFC to this legume, and 7 of these patients (23%) had positive challenges to other legumes. In contrast, Bernhisel-Broadbent and Sampson [8] carried out DBPCFC to 5 legumes (PN, soybean, P, lima bean and green bean) in 69 children sensitized to legumes; only 2 (5%) of 41 children with positive results had positive challenges to 2 legumes (both to PN and soybean). In this latter study, the majority of children included were allergic to PN. We consider that the different dietary habits of both communities, and the fact that most children were sensitized to PN, might explain the differences in this study compared to ours. In Spain, L and CP are introduced earlier in the diet than PN. PN are mostly consumed as dry fruits, and not as PN butter, and therefore, introduced later in life. In general, in our population, when PN-allergic children are evaluated in an allergy clinic, if the ingestion of other legumes is tolerated, the parents are advised that the child should not avoid the ingestion of these legumes if possible.

In conclusion, this study demonstrates a high cross-reactivity among L, CP and P, using ELISA inhibition experiments. Food challenges confirmed that clinical allergy to all three legumes is frequent among our cohort of Spanish children.

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References

- 1 Young E, Stoneham MD, Petrukevitch A, Barton J, Rona R: A population study of food intolerance. *Lancet* 1994;343:1127–1129.
- 2 Niestijl JJ, Kardinaal AF, Huijbers G, Vlieg-Boerstra BJ, Martens BP, Ockhuizen T: Prevalence of food allergy and intolerance in adult Dutch population. *J Allergy Clin Immunol* 1994;93:446–456.
- 3 Sampson HA: Clinical practice. Peanut allergy. *N Engl J Med* 2002;346:1294–1299.
- 4 Sampson HA: Adverse reactions to foods; in *Allergy Principles and Practice*, ed 5. Mosby Year Book, St. Louis, 1998.
- 5 Crespo JF, Pascual C, Burks AW, Helm RM, Esteban MM: Frequency of food allergy in a pediatric population from Spain. *Pediatr Allergy Immunol* 1995;6:39–43.
- 6 Bernhisel-Broadbent J, Taylor S, Sampson HA: Cross-allergenicity in the legume botanical family in children with food hypersensitivity. II. Laboratory correlates. *J Allergy Clin Immunol* 1989;84:701–709.
- 7 Barnett D, Bonham B, Howden ME: Allergic cross-reactions among legume food. An in vitro study. *J Allergy Clin Immunol* 1987;79:433–438.
- 8 Bernhisel-Broadbent J, Sampson HA: Cross-allergenicity in the legume botanical family in children with food hypersensitivity. *J Allergy Clin Immunol* 1989;83:435–440.
- 9 Ibáñez MD, Martínez M, Sánchez JJ, Fernández-Caldas E: Legume: cross-reactivity. *Allergol Immunopathol* 2003;31:151–161.
- 10 Martín JA, Compaired JA, de la Hoz B, et al: Bronchial asthma induced by chick pea and lentil. *Allergy* 1992;47:185–187.
- 11 Patil SP, Niphadkar PV, Bapat MM: Chick-pea: a major food allergen in the Indian sub-continent and its clinical and immunochemical correlation. *Ann Allergy Asthma Immunol* 2001;87:140–145.
- 12 López-Torrejon G, Salcedo G, Martin-Esteban M, Diaz-Perales A, Pascual CY, Sanchez-Monge R: Len c 1, a major allergen and vicilin from lentil seeds: protein isolation and cDNA cloning. *J Allergy Clin Immunol* 2003;112:1208–1215.
- 13 Sanchez-Monge R, Lopez-Torrejon G, Pascual CY, Valera J, Martin-Esteban M, Salcedo G: Vicilin and convicilin are potential major allergens from pea. *Clin Exp Allergy* 2004;34:1747–1753.
- 14 Boluda L, Casanovas M, Prieto JL, Fernandez-Caldas E: Determinations of Par j1 by a competitive enzyme immunoassay using human specific IgE and IgG. Validation by skin prick testing. *J Invest Allergy Clin Immunol* 1998;8:207–213.
- 15 Martínez M, Ibáñez MD, Fernández-Caldas E, Marañón F, Muñoz MC, Laso MT: The diagnostic value of crude and boiled extracts to identify tolerant versus nontolerant lentil sensitive children. *Ann Allergy Asthma Immunol* 2001;86:686–690.
- 16 Ibáñez Sandín MD, Martínez San Ireneo M, Marañón Lizana F, Fernández-Caldas E, Alonso Lebrero E, Laso Borrego T: Specific IgE determinations to crude and boiled lentil (*Lens culinaris*) extracts in lentil-sensitive children and controls. *Allergy* 1999;54:1209–1214.
- 17 Martínez San Ireneo M, Ibáñez Sandín MD, Fernández-Caldas E, Marañón Lizana F, Rosales Fletes MJ, Laso Borrego MT: Specific IgE levels to *Cicer arietinum* (chick pea) in tolerant and nontolerant children: evaluation of boiled and raw extracts. *Int Arch Allergy Immunol* 2000;121:137–143.
- 18 Wensing M, Knulst AC, Piersma S, O’Kane F, Knol EF, Koppelman SJ: Patients with anaphylaxis to pea can have peanut allergy caused by cross-reactive IgE to vicilin (Ara h 1). *J Allergy Clin Immunol* 2003;111:420–424.
- 19 Moneret-Vautrin DA, Guerin L, Kanny G, Flabbee J, Fremont S, Morisset M: Cross-allergenicity of peanut and lupine: the risk of lupine allergy in patients allergic to peanuts. *J Allergy Clin Immunol* 1999;104:883–888.
- 20 Peeters KA, Nordlee JA, Penninks AH, et al: Lupine allergy: not simply cross-reactivity with peanut or soy. *J Allergy Clin Immunol* 2007;120:647–653.