

Allergy to lentils in Mediterranean pediatric patients

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Background: Peanuts and soybeans are the major legumes involved in human food allergy; however, scarce data exist on adverse reactions to other temperate legumes, such as lentils. **Objective:** The purpose of this study was to identify patients who are allergic to lentils, to assess clinical features and other associated food allergies, and to characterize allergens in lentil extract.

Methods: Twenty-two children each with a history of adverse reactions to lentils were enrolled in the study. The diagnosis of lentil allergy was based on food challenges or a convincing history of anaphylaxis, with positive skin tests and/or specific serum IgE to lentils. Lentil components were characterized by SDS-PAGE immunoblotting.

Results: Twenty of 22 subjects had symptomatic allergy to lentils at the diagnostic time. The most frequent symptoms were oropharyngeal symptoms (40%) and acute urticaria (30%); 3 patients also reported symptoms when they were exposed to steam from cooked lentils. In 18 patients, symptoms after lentil ingestion started under 4 years of age (median, 2.7 years). Nine patients had allergic reactions to other legumes: chick peas (6 patients), peas (2 patients), and green beans (1 patient). Immunoblotting patterns obtained with patients' sera showed IgE-binding bands ranging from 14 to 84 kd. Five sera recognized 9 or more IgE-binding bands, and more than 50% of patients who were tested have specific IgE antibodies to 7 components in lentil extract.

Conclusion: Allergic reactions to lentils started early in life, usually below 4 years of age; oropharyngeal symptoms and acute urticaria were the most common symptoms through ingestion, and symptomatic reactivity to chick peas is frequently associated. (*J Allergy Clin Immunol* 1999;103:154-8.)

Key words: Food hypersensitivity, legumes, lentils, chick peas, allergens

Legumes are among the most common antigens causing hypersensitivity reactions in children who are allergic to foods. Most studies have been focused on 2 members of the legume food family, peanuts and soybeans, which are considered the major legumes involved in human food allergy in the United States¹ and the United Kingdom.² However, our previous studies have shown that in Spain, with a typical Mediterranean diet, lentils are the

Abbreviation used

PDVF: Polyvinylidene fluoride
TBS: Tris-buffered saline

legumes most commonly implicated in IgE-mediated food hypersensitivity. In a series of 355 patients diagnosed as having food hypersensitivity, on the basis of an open food challenge or a convincing history of anaphylaxis, lentil allergy was found in 36 patients (10.1%), although peanuts caused allergic reactions in 24 patients (6.7%).³ Lentils (*Lens esculenta*), although probably originated in the Near East and Mediterranean, are now cultivated in most of the temperate and subtropical areas of the world. It is an annual plant with flattened edible seeds. These seeds are used in soups, flour, and mixed with cereal and in cakes. It is a basic food in the Near East, India, Pakistan (where as Khitchri [lentils plus rice] it is the weaning food), and in the Mediterranean area. Despite its wide consumption, not many cases of IgE-mediated lentil hypersensitivity have been previously reported.^{4,5} In this study we examined 22 patients with immediate adverse lentil reactions that could be objectively evaluated. The age of onset of allergic reactions, clinical manifestations, and other associated food allergies were evaluated. In addition, IgE antibody responses to lentil allergens were detected by immunoblotting.

METHODS

Subjects

From September 1994 to March 1996, 22 patients with histories of adverse reactions to lentils were enrolled in the study. Diagnosis of lentil allergy was based on food challenges or a convincing history of anaphylaxis after lentil ingestion, with positive skin prick test results and/or specific serum IgE to lentils. Initially, an open food challenge was carried out with the parent's consent, as described elsewhere.⁶ Briefly, the patients had to be symptom-free and had to have discontinued the use of any antihistamines or corticosteroids at least 1 week before the challenge. Patients were given increasing amounts of food every half hour, if no symptoms were elicited, until the standard diet amounts for their ages were reached. If the open food challenge was accompanied by an oral allergy syndrome or immediate (less than 2 hours) generalized objective signs and symptoms, a diagnosis of lentil-adverse reaction was made. If the open challenge was accompanied by mild and/or equivocal symptoms, a single-blind placebo-controlled food challenge was performed. For this purpose, dehydrated lentils were administered, with mashed potatoes as a vehicle. The order of administration of food antigen and placebo (mashed potatoes) was randomized, and

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TABLE I. Clinical features in 22 patients with histories of adverse reactions to lentils

Patient	Age of onset (y)	Symptoms	Lentil-sIgE (kU _A /L)	Other food allergies	Other diseases
1*	2.5	U	6.64	Chick peas	Pollinosis
2	3	U	4.75	Chick peas	Pollinosis
3	4	OAS	18.2		Perennial asthma
4	5	NS	44.9	Fish	
5	12	U,C,W	13.1		Pollinosis
6*	3	OAS/R	17.5	Green beans	Pollinosis
7	3	U,V,LE	5.61	Chick peas, peaches, fish	Pollinosis
8	3	V,AP,D	8.7	Fish, milk	Pollinosis
9	2	AD	54.6		
10	2	U/A	7.16	Peas	Pollinosis
11	3	U/A	16.7	Peas, fish	Perennial asthma
12*	3	OAS/R	4.72	Chick peas	
13	3	OAS	4.86		Pollinosis
14	12	U/A,C,W	20.3	Peaches	Pollinosis
15	6	NS	8.9	Chestnut	
16	1.5	U,C,W	>100		
17	1.5	OAS	25.9	Fish	
18	1.5	OAS	13.4	Chick peas	
19	1.7	U/A	5.43		
20	2.1	OAS	43.6		
21	1.8	OAS	5.87	Chick peas	
22	1.2	U/A	44.4		Perennial asthma

sIgE, Serum specific IgE; U, generalized urticaria; OAS, oral allergy syndrome; NS, no symptoms; C, cough; W, wheezing; R, rhinitis; V, vomiting; LE, laryngeal edema; AP, abdominal pain; D, diarrhea; AD, atopic dermatitis; A, facial angioedema.

*Also, symptoms after exposure to steam from cooked lentils.

an equal number of doses were given in 2 separate days. Skin tests with common inhalants (mites, molds, pollen, and animal danders; Bencard-Bayer, Germany; and Abello-ALK, Madrid, Spain), legume extracts (Leti Laboratories, Madrid, Spain), histamine control substance (1 mg/mL), and saline solution control substance were applied to the forearms by the prick method. The mean diameters were recorded; a mean wheal at least 3 mm larger than a wheal of the saline solution control substance was considered positive. Specific serum IgE to lentils and other suspected allergens were determined by the enzymatic immunoassay CAP-FEIA (Pharmacia, Diagnostics, Uppsala, Sweden), performed according to the manufacturer's instructions.

Preparation of lentils extract

Lentils of the *Rubia Castellana* variety (the most widely consumed in Spain) were purchased at a local market. One hundred grams of lentils were washed carefully, soaked for 1 hour in 500 mL of PBS, and then ground; 10 mmol/L ascorbic acid was added as an antioxidant. The mixture was stirred 18 hours at 4°C and centrifuged at 15000g for 30 minutes at 4°C. The supernatant was dialyzed (molecular weight cut-off, >3500 d; Spectrapor; Spectrum Medical Industries, Inc, Los Angeles, Calif) at 4°C during 24 hours against 100 vol/vol of 10 mmol/L glycine, centrifuged at 30000g for 30 minutes at 4°C, and filtered through a 0.45-μm filter (Millipore Corp, Bedford, Mass). Finally the material was lyophilized. The protein content was determined by the Lowry method.⁷ The lyophilized allergen was stored at -20°C in desecator until needed. This allergen was used at 1 mg/mL final dilution for immunoblotting studies.

SDS-PAGE and immunoblotting

The electrophoresis procedure was a modification of the method of Sutton et al.⁸ SDS-PAGE was performed with a 12% polyacrylamide separating gel and a stacking gel of 4%. Twenty microliters

of a 1 mg/mL solution of lentil extract was applied to each well. Electrophoresis was performed for 1 hour at 130 V per gel for the 8 cm × 7.5 cm gels (Mini-Protean II system; Bio-Rad Laboratories, Hercules, Calif). To assure proper protein separation and visualization, Coomassie brilliant blue (Sigma Chemical Co, St Louis, Mo) stains were done on gels. Proteins were electrophoretically transferred from the separating gel to a polyvinylidene fluoride (PDVF) (Millipore Corp) membrane in a transfer buffer (Tris-glycine) with 20% methanol. This procedure was performed in a minitransfer apparatus (Bio-Rad Laboratories). After being removed from the transblot apparatus, the molecular weight standard lane and a lentil lane were dyed with amido black (1% in ethanol acetic acid solution) as control substances. The rest of the PDVF membrane was placed in blocking solution (1% gelatin/tris-buffered saline [TBS] Tween) for 1 hour at room temperature. The PDVF blot was then washed 4 times with TBS with 0.1% Tween 20 and incubated with the sera (dilution, 1:3 to 1:10 vol/vol) for 3 hours at room temperature with rocking. The PDVF blot was again washed with TBS with 0.1% Tween 20 four times; alkaline-phosphatase-conjugated goat anti-human IgE (1:2000 vol/vol of TBS; Caltag Laboratory, San Francisco, Calif) was added and incubated at room temperature with rocking for 3 hours. After an additional wash with TBS 3 times, the blot was developed with an alkaline-phosphatase-conjugate substrate kit (Bio-Rad Laboratories) of nitro-blue tetrazolium and 5-bromo-4-chloro-3-indolyl-phosphate at room temperature for 15 minutes. The reaction was then stopped by decanting the developing solution and incubating the PDVF blot for 10 minutes with distilled water. The blot was then air-dried. The lentil allergen blot was also incubated with negative pool sera from patients who are allergic to fish and patients who are allergic to pollens.

RESULTS

The clinical features of the studied subjects are enumerated in Table I. Twenty of 22 studied patients (91%)

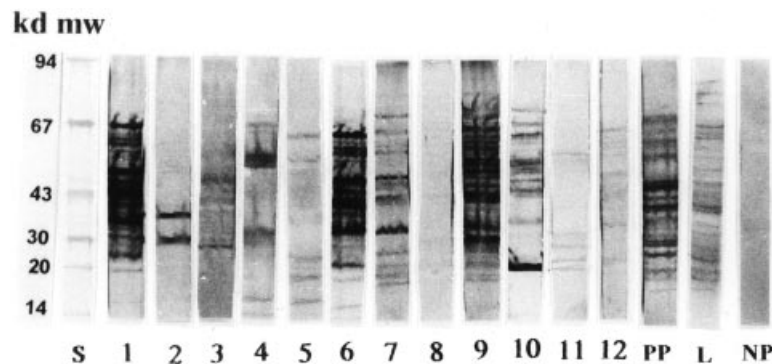


FIG 1. IgE antibody reactivity to lentil (*Rubia Castellana* variety) allergens by immunoblotting. Lentil proteins were resolved by means of SDS-PAGE and transferred to PDVF membranes. Five-millimeter strips from each blot were tested for reactivities to serum IgE from children who were allergic to lentils. *Lane S* is the molecular weight standard. *Lanes 1* through *12* represent sera from patient 1 to 12, respectively. *Lane PP* represents a pool sera from 22 patients who were allergic to lentils. *Lane L* represents staining for proteins by Amido Black. *Lane NP* represents a negative control of a pool serum from patients with pollinosis.

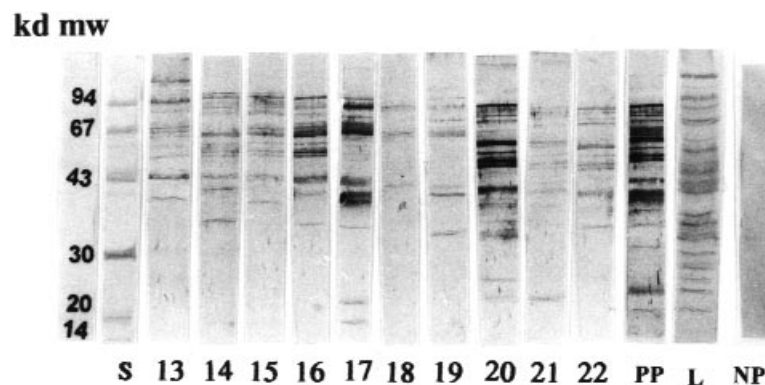


FIG 2. IgE antibody reactivity to lentil (*Rubia Castellana* variety) allergens by immunoblotting. Lentil proteins were resolved by means of SDS-PAGE and transferred to PDVF membranes. Five-millimeter strips from each blot were tested for reactivities to serum IgE from children who were allergic to lentils. *Lane S*, molecular weight standard. *Lanes 13* through *22* represent sera from patient 13 to 22, respectively. *Lane PP* represents a pool sera from 22 patients who were allergic to lentils. *Lane L* represents staining for proteins by Amido Black. *Lane NP* represents a negative control of a pool serum from patients with pollinosis.

had actual clinical reactivity to lentils. Patients 4 and 15 reported isolated mild oral pruritus after lentil ingestion, but oral challenges were negative. Eight of 20 patients (40%) had oropharyngeal symptoms after ingesting lentils, including tingling, pruritus, angioedema of the lips, or throat tightness (compatible with the oral allergy syndrome). Six patients (30%) had only cutaneous reactions, acute generalized urticaria, and/or angioedema. Four patients (20%) had severe acute reactions after ingesting lentils, including laryngeal edema or significant wheezing. Only one patient had isolated gastrointestinal symptoms (vomiting, abdominal pain, and diarrhea) within minutes after lentils ingestion. In addition, patients 1, 6, and 12 reported immediate symptoms after exposure to steam from cooked lentils (no specific challenges were performed to confirm symptoms through inhalation). In 18 patients, symptoms after lentil ingestion started at less than 4 years of age (median, 2.7 years). The male/female ratio was 4.5:1. Nine patients

had allergic reactions to other legumes: chick peas (6 patients), peas (2 patients), and green beans (1 patient).

SDS-PAGE-immunoprint patterns obtained with the 22 patients' sera demonstrated IgE-binding bands at 14, 17, 20, 22, 28, 30, 38, 40, 50, 54, 60, 68, 70, 72, 80, and 84 kD (Figs 1 and 2). The more recognized were 38 kD (81% of 22 sera tested reacted), 54 kD (77%), 68 kD (72%), 30 kD (59%), 40 kD (54%), 70 to 72 kD (54%), and 60 kD (42%; Fig 3). Most patients (64%) reacted to 7 or more allergens in lentil extract. The 9 patients with pollinosis did not show special band patterns. Control immunoblot experiments with a serum pool from patients with pollinosis (Figs 1 and 2, *lane NP*) and from children who are allergic to fish did not show nonspecific binding to lentil allergens.

DISCUSSION

Foods from the Leguminosae family, such as peanut and soybean, have been reported as a major cause of IgE-mediated food hypersensitivity. Peanut allergy is com-

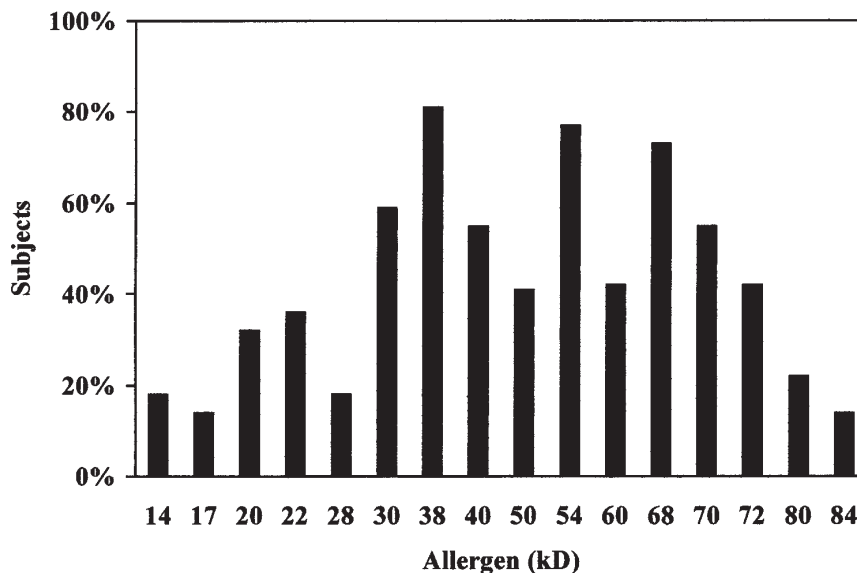


FIG 3. Individual IgE reactivities of 22 children who were allergic to lentils by SDS-PAGE immunoblotting to lentil (*Rubia Castellana* variety) extract. The most reactive band is the 38-kD protein to which 18 of 22 sera (81%) reacted. The second most reactive band was the 54-kD protein (17 of 22 sera; 77%).

mon in North America; generally, sensitivity is life long and is often associated with severe, life-threatening reactions after accidental ingestion. In fact, peanut is the food most frequently associated with fatal food anaphylaxis.⁹ Soybeans are the major crop of the legume family, which is used mostly as animal feed. Soy proteins have also been reported as a frequent cause of adverse reactions in infants, largely because of the exposure to soy-based infant formulas.¹⁰ In addition, peanut and soybean allergens have been isolated and extensively studied with the classic biochemical methods and more recently the molecular biology techniques.¹¹⁻¹⁷ However, there is scarce information on the role of other temperate legumes including peas, green beans, sweet lupin, chick peas, and particularly, lentils. Most reports come from Spain and Greece, where green beans, lentils, and chick peas are included in the Mediterranean diet. Martin et al⁵ reported a case of bronchial asthma induced by exposure to the steam from cooking either chick peas or lentils. Garcia Ortiz et al¹⁸ reported a 54-year-old female patient who experienced episodes of asthma when exposed to vapors from cooking some kinds of legumes (peas, chick peas, beans, and lentils). Recently, Kalogeromitros et al⁴ described an 8-year-old girl who had episodes of anaphylaxis related with the ingestion of cooked lentils and chick peas and with inhalation exposure to cooking lentil soup. Our previous studies showed that lentils ranked fourth, after hen eggs, fish, and cow's milk, as a cause of food allergy in children from Spain.³ Legumes represent an illustrative example of the effect of the staple diet on the development of food allergies; high consumption of peanuts and soybeans in North America correlates well with the importance of these foods in allergic reactions, although green bean, chick pea, and lentil allergy is

found in Mediterranean countries where these foods are an important source of fiber and inexpensive proteins. In fact, lentils, chick peas, and beans contribute to 4.9% of the protein in the diet in Spain. These findings induced us to focus on patients who reported adverse reactions to lentils. Clinical reactivity was evaluated initially by open food challenges and followed, if they were accompanied by mild and/or equivocal symptoms, by single-blind placebo-controlled food challenges. Double-blind placebo-controlled food challenges have been labeled as the gold standard for the diagnosis of food allergies.¹⁹ However, we based the clinical diagnosis on open and single-blind placebo-controlled food challenges because most subjects under challenges were small children, who had immediate classic allergic symptoms after the initial test and because chronic disorders such as eczema or chronic urticaria were uncommon in our patients.

In our study, lentil allergy started in early childhood (median, 2.7 years), most frequently with oropharyngeal symptoms or acute urticaria and/or angioedema, although severe anaphylaxis was found in 20% of our patients. Three of our patients also reported symptoms after inhalative exposure to steam from cooked lentils, which concurs with previous reports. A common feature of most legume allergens is their natural resistance to thermal, chemical, and in some way, proteolytic denaturation.²⁰ In addition, 6 patients also showed allergic reactions to chick peas, suggesting some degree of clinical cross-reactivity. Clinical allergy to more than one member of the Leguminosae food family is considered rare. Bernhisel-Broadbent and Sampson²¹ found only 2 of 41 patients who had symptomatic hypersensitivity to 2 legumes. However, neither chick peas nor lentils were evaluated in this study. All reports on lentil allergy

included not only symptomatic reactivity to this food but also to chick peas. Our study shows that lentils have multiple allergens, 5 sera recognized 9 or more IgE-binding bands, and greater than 50% of patients tested to have specific IgE antibodies to 7 components in lentil extract. No significant differences with respect to IgE binding were found in 2 patients who had no current clinical reactivity to lentils (negative oral challenges).

In summary, IgE-mediated lentil hypersensitivity could not be rare in the Mediterranean area, although insufficient attention had been brought to this matter. Allergic reactions to lentils started early in life, usually younger than 4 years of age; oropharyngeal symptoms and acute urticaria were the most common symptoms through ingestion, and symptomatic reactivity to chick peas is frequently associated. Further studies are needed to isolate and characterize the major allergens of lentils and their role in allergic reactions through ingestion and/or inhalation.

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