Allergy 2006: 61: 1112–1116

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DOI: 10.1111/j.1398-9995.2006.01123.x

Original article

Allergic hypersensitivity to the lentil pest *Bruchus lentis*

Background: Lentils are the most common legume involved in allergic reactions in the Mediterranean area and India. Lentil crops could be attacked by a wide range of insect species. Occupational asthma caused by the lentil pest *Bruchus lentis* has been described in the present study.
Objective: We studied the possibility of immunoglobulin (Ig)E-mediated hypersensitivity to lentil pests in 16 patients who suffered from allergic symptoms (asthma and anaphylaxis) related to inhalation of lentil particles or ingestion of lentils, in which sensitization to legume proteins was not clear.
Methods: Extracts prepared either from noninfested and infested lentils, and from the lentil parasite *B. lentis* were used for skin prick testing (SPT), bronchial and oral challenges and *in vitro* determinations.
Results: Skin prick test were positive to infested lentils and *B. lentis* in all patients and negative to noninfested lentil extracts. Five asthmatic patients reacted in the bronchial challenge test with *Bruchus* extract. Oral challenges performed with boiled infested lentils were positive in six of seven patients. Immunoglobulin E immunoblotting with *Bruchus* and infested lentils extracts revealed protein bands that were reactive to serum IgE from six and four, respectively, out of 16 patients. These patients had no IgE to lentil-specific proteins as determined by immunoblotting and Len c 1-specific IgE test.
Conclusions: Lentil pest proteins can be a cause of IgE-mediated rhinoconjunctivitis and asthma in patients eating or inhaling infested lentil particles.

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Key words: legume allergy; lentil allergy; lentils pests; occupational asthma.

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Accepted for publication 26 February 2006

Lentils seem to be the most common legume implicated in paediatric allergic patients in the Mediterranean area and India (1–5). Allergy reactions to lentils start early in life, usually bellow 4 years of age. Oropharyngeal symptoms and acute urticaria are the most common symptoms through ingestion followed by anaphylaxis (1). Cases of bronchial asthma induced by inhaling vapours from cooking lentils have rarely been described (2).

Infestation of lentil with lentil pests, mainly *Bruchus lentis* in our area, is a very common event (3). We recently reported the case of a man who experienced asthmatic attacks when exposed to lentil dust during his work (agronomist) (4). These lentils were infested by adult specimens of *B. lentis* (Frolich, 1799) (5). Type I hypersensitivity to infested lentils was demonstrated by positive prick response, immunoglobulin (Ig)E and specific bronchial challenge.

Years ago, lentils were cleaned by hand before their consumption and these legumes were introduced for hours in water in order to collect the floating lentils, which were the infested ones. Their content had been eaten by the pest and they usually presented the hole through which the parasite went off (Fig. 1). At present, the manufacturer process has changed and lentils are frozen before storage or treated with toxic gasses that produce the paralysis of the parasite in the lentil. Thus, the lentil's external appearance looks normal, although 30% of the lentils are normally infested (3). Treated lentils are not capable of floating in water and these legumes infested with *Bruchus* are eaten with the pest.

Lentils are consumed after a heat treatment, usually boiling. Heating can produce inactivation, modification of both of the putative allergens. In the case of the patients with occupational asthma, raw lentils produced the disease. We now studied the possibility of IgE-mediated hypersensitivity to lentil pests in 16 patients who suffered from allergic symptoms related to inhalation or ingestion of boiled lentils, in which sensitization to legume proteins was not clear (negative *in vivo* and *in vitro* diagnostic tests with pure lentil extract).

Methods

Patients

We selected 16 patients with symptoms related with ingestion or inhalation of lentils in which sensitization to legume protein was not clear: negative pricks or low levels of specific IgE and negative oral and bronchial challenge with pure lentil extract. Nine patients suffered from anaphylaxis, two were cooks and presented asthma after

Figure 1. Freshly collected *Bruchus lentis* infested lentils.

inhalation of boiling vapours, thre were farmers, one agronomist engineer and five suffered also from sensitization to grass pollen. Two patients presented sensitization to Anisakis but denied symptoms after eating raw fish (see Table 1). We included six patients with sensitization to other legumes and negative tests to pure and infested lentils as control patients for the experiments (see Table 2).

Extracts

Noninfested lentils (*Lens culinaris* syn. *Esculenta* cv. Rubia Castellana) and infested lentils containing living insects were ground to a fine powder. Some living lentil parasites were collected and they were identified by an entomologist (Escuela de Ingeniería Agrícola de Palencia, Spain) as *B. lentils* (Frolich 1799), belonging to the Coleoptera family. The different extracts (*Bruchus* whole bodies, noninfested and infested lentils) were prepared in phosphate-buffered saline (PBS) at 5% w/v with a potter. They were centrifuged at 17 700 ***g*** for 30 min and, after dialyzing (3.5 kDa molecular weight cut-off), they were sterilized through a 0.22-μm pore diameter filter. Protein concentration was determined by the method of Lowry (6). Part of the extracts was 50% glycerinated for skin prick test (SPT). Another part was adjusted to 1 mg/ml with sterile 0.9% saline solution for the bronchial challenge test (BCT). The rest of the extracts were used for *in vitro* studies. In order to perform oral challenge, pure and infested lentils were boiled during 30 min.

Table 2. Clinical data of controls

Patient	Age (years)	Symptoms	Prick	IgE
2537	8	Anaphylaxis	Soy 8 × 8, Peanut 10 × 12	Soy 4.18, Peanut 8.1
3705	13	Anaphylaxis	Soy 8 × 10, Peanut 12 × 12	Soy 3.02, Peanut 4.8
2532	23	Anaphylaxis	Green pea 4 × 4	Green pea 20.3
5663	17	Anaphylaxis	Peanut 19 × 10	Peanut 35.8, Almond 12.3
266	25	Occupational asthma	Soy 13 × 13	Soy 2.3
252	10	Anaphylaxis	Soy 4 × 3	Soy 2.3

Prick: positive pricks with legumes extracts. Pricks with infested lentils and *Bruchus* were negative as oral challenge and bronchial challenge.
Ig, immunoglobulin.

Skin prick tests

Skin prick testing were performed with a standard battery from ALK-Abelló diagnostic extracts (Madrid, Spain; composed by

Table 1. Clinical data of the patients

Patient/sex	Age (years)	Job	Symptoms	Prick L (raw/boil)	Prick PL (raw/boil)	Prick *Bruchus*	IgE lentil (units/ml)	IgE (other allergens)	OC	BC	BCB
1487/M	32	Agron	Occupation asthma	2 × 2/3 × 3	15 × 10/15 × 10	15 × 15	<0.35		ND	+	+
4859/F	10	Study	Anaphylaxis	2 × 2/2 × 2	8 × 8/10 × 10	12 × 12	2.40		ND	ND	
5384/F	25	Cook	Occupation asthma	Negative	5 × 5/6 × 6	8 × 8	<0.35		–	+	+
4948/M	22	Farmer	Anaphylaxis	Negative	9 × 9/9 × 10	10 × 10	0.92		+	ND	
5224/M	50	Baker	Anaphylaxis	5 × 5/5 × 5	12 × 12/12 × 12	15 × 15	23.9	Bean 13, Wheat 3.4	+	ND	
3629/M	57	Baker	Anaphylaxis	2 × 2/2 × 2	14 × 14/15 × 15	16 × 16	19.80	Soy 32, Bean 35, Anisak 29	ND	ND	
4910/M	17	Study	Anaphylaxis	Negative	13 × 13/14 × 14	15 × 15	0.900	Cat 14	+	ND	
4824/F	10	Study	RC pollen, angioedema	Negative	5 × 5/5 × 5	8 × 10	0.96	Peanut 1.4, Lolium 32	+	ND	
5627/M	29	Farmer	RC pollen, occupation asthma	Negative	3 × 3/8 × 8	10 × 10	1.48	Soy 0.81, Lolium 13	ND	+	+
5498/F	12	Study	Anaphylaxis	Negative	5 × 5/9 × 9	9 × 9	0.48	Peanut 1.64	+	ND	
3539/F	14	Study	RC pollen, anaphylaxis	5 × 5/5 × 5	8 × 8/8 × 8	10 × 10	4.1	Lolium >100	ND	ND	
5052/M	17	Study	Anaphylaxis asthma	4 × 4/4 × 4	8 × 8/10 × 10	10 × 10	1.02	Lolium 5.2	ND	ND	
5778/M	29	Farmer	RC pollen, occupation asthma	2 × 2/3 × 3	5 × 8/8 × 8	9 × 9	2.21	Lolium 12.4	ND	+	+
5772/M	35	Baker	Occupation asthma	Negative	10 × 10/13 × 13	10 × 15	1.30	Wheat 2.3, Barley 5.3	ND	+	
568/M	30	Agron	Occupation asthma	2 × 2/2 × 2	8 × 8/8 × 8	10 × 10	16.90	Peanut12.3, Almond 4	ND	ND	
5714/F	43	Cook	RC pollen, anaphylaxis	Negative	5 × 10/10 × 10	10 × 12	14.4	Lolium 8, Anisakis 23	+	ND	

Prick L, prick with pure lentil extract (major diameters in mm); PL, prick with infested lentils; RC, rhinoconjunctivitis; OC, oral challenge; BC, bronchial challenge with infested lentil extract; BCB, bronchial challenge with *Bruchus*; ND, not done; +, positive, –, negative; Ig, immunoglobulin.

pollens, mites, animal dander, foods and moulds), infested and noninfested lentils, and *Bruchus* whole body extracts at a concentration of about 1 mg/ml of protein. We also included storage mites, *Bruchus* bean and *Eurygaster* extracts that were performed as previously described (7). Histamine phosphate (10 mg/ml) and sterile 0.9% saline were used as positive and negative controls respectively. A mean wheal area of 7 mm^2 or a major diameter >5 mm, 15 min after puncture, was considered a positive response. The control subjects and five healthy subjects were also tested with these extracts.

Bronchial challenge tests

The BCT were performed according to previous publications (4) starting with a concentration of extract of 0.01 mg/ml in sterile 0.9% saline solution, which was used as dilution buffer. We used the extract of infested lentils and the *Bruchus* whole bodies extract. Two atopic patients with mild asthma caused by *Eurygaster* were also challenged with the same extract as controls.

Oral challenge tests

Double-blind, placebo-controlled food challenges (DBPCFC) were performed following recently published criteria (8) only in patients with anaphylaxis I–II of Müller (9) and after informed consent. They were done after all suspected food have been strictly avoided and no symptomatic medications had been taken for at least 1 week. The patients came with an empty stomach to enhance absorption of the test food and to avoid suspicion about previously ingested foods. Measures for treating potential life-threatening reactions were taken. Infested lentils, ground to a fine powder, were introduced in empty opaque capsules. The initial challenge dose of lentils' powder depended on the previous response of each patient. In the most symptomatic anaphylaxis we began with 5 mg and the dose was increased until 500 mg in one capsule. If no symptoms occurred, the total cumulative dose administered was at least equivalent to the quantity the patient had eaten when the reaction appeared. We also include capsules prepared with sucrose powder as negative controls, randomly given to patients during the challenge tests. Four patients denied consent for oral challenge with infested lentils. All subjective symptoms and objective signs were recorded before each challenge dose.

Specific IgE determinations

Specific IgE to lentil was determined by CAP (Pharmacia, Upssala, Sweden). IgE to Len c 1 was determined using the ADVIA-Centaur platform from Bayer Diagnostics (Tarrytown, NY, USA). Len c 1 was purified (10), biotin-labelled and used in the Centaur equipment as indicated elsewhere (11).

IgE immunoblotting

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out in a 15% PAGE using nonreducing conditions. About 100 μg of extract protein were loaded per lane. The extract proteins, separated by SDS-PAGE, were electrotransferred onto nitrocellulose membranes (Hybond-ECL, Amersham Biosciences, Little Chalfon, UK). After blocking with 5% bovine serum albumin (BSA) in PBS for 1 h at room temperature, the membranes were incubated with 1.5 ml of a 1/5 dilution of patient's serum, and then with 1.5 ml of a 1/3000 dilution of mouse antihuman IgE monoclonal antibody (mAb) HE-2 ascitic fluid. After incubating with 1/5000 dilution of a rabbit antimouse IgG conjugated with peroxidase (Dako Diagnósticos, Barcelona, Spain), immunodetection of IgE-binding proteins was achieved by enhanced chemiluminiscence (ECL), following the manufacturer's instructions (Amersham Biosciences). As negative control, blots with extract proteins transferred were incubated with dilution buffer instead of with the patient's serum. We performed three immunoblotting experiments: at first, with no infested lentils, secondly with *B. lentils* and finally with infested lentils extracts.

Results

Clinical data and tests results obtained in our patients can be seen in Table 1. The mean age of our patients was 28.8 years. Six patients suffered from occupational asthma, nine suffered from anaphylaxis and one from oral syndrome. All patients showed positive SPT to whole body *B. lentis* and infested lentils extracts, and negative to other pests (storage mites, *Bruchus* bean, *Eurygaster*) and noninfested lentils. Of the five bronchial challenges that could be performed, five asthmatic patients reacted in the BCT with *Bruchus* extract and four patients (80%) with infested lentils extract too. Oral challenges performed with boiled infested lentils were positive in six patients (85%) who gave their consent and negative in one patient (15%). The bronchial challenges in the control patients were negative (Table 2).

The immunoblotting profile with noninfested lentils (Fig. 2) revealed IgE-binding bands, with different intensities, in 10 patients. Six patients had no IgE against lentil-specific proteins. These patients also had no IgE against Len c 1, which is the main lentil allergen. Figure 3 shows the result of the immunoblotting experiment carried out with *Bruchus* and infested lentil extracts and sera from the six patients with negative IgE-binding in the noninfested lentil immunoblotting. All six patients had IgE against *Bruchus*-specific proteins. Four patients had IgE against several bands of about 13, 19, 24, 27, 30 and >35 kDa. The other two patients had a much simple pattern. Only four patients also had IgE against protein bands that were present in the infested lentil extract (Fig. 3). However, only in two cases the IgE-binding pattern obtained was similar to the one obtained with *Bruchus* extract.

Discussion

Peanuts and soya bean are the major legumes involved in human food allergy. However, scarce data exist on adverse reactions to other temperate legumes such as lentils. An increasing number of legume proteins or glycoproteins have been characterized as food allergens (12), but limited data tend to indicate that they are possibly different from legume-inhalant allergens (13).

A common feature to legume allergens is their natural resistance to thermal, chemical and in some way protein

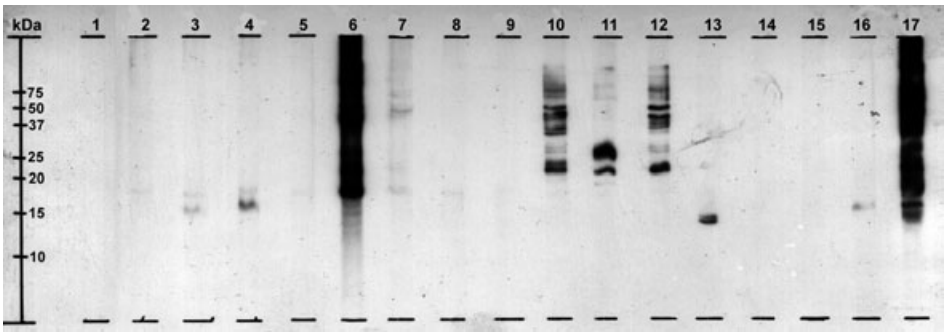

Figure 2. Immunoglobulin (Ig)E immunoblotting noninfested lentil extract. 1, negative control; 2, serum 1487; 3, serum 4859; 4, serum 5384; 5, serum 4948; 6, serum 5224; 7, serum 3629; 8, serum 4910; 9, serum 4824; 10, serum 5627; 11, serum 5498; 12, serum 3539; 13, serum 5052; 14, serum 5778; 15, serum 5772; 16, serum 5683; 17, serum 5714. The molecular weight of prestained markers, run in parallel, is indicated on the left.

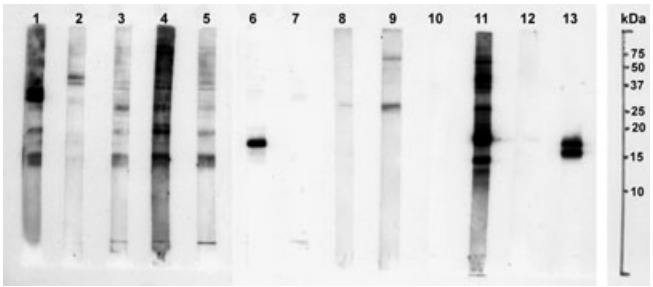

Figure 3. Immunoglobulin (Ig)E immunoblotting *Bruchus lentis* and infested lentil extracts. Lanes 1–6, *B. lentis* extract; lanes 8–13, infested lentil extract; lane 7, negative control. 1 and 8, serum 4948; 2 and 9, serum 4910; 3 and 10, serum 4824; 4 and 11, serum 5778; 5 and 12, serum 5772; 6 and 13, serum 1487. The molecular weight of prestained markers, run in parallel, is indicated on the right.

denaturation. Martinez et al. compared crude and boiled extracts on 36 patients with a history of allergic reactions after the ingestion of lentil. Of these, 13 patients had a negative oral challenge, but the heated extract identified best the clinically sensitive individuals (14). Nevertheless in other studies, the heating process caused a significant decrease in specific IgE-binding (15, 16). In another study with 22 subjects who had symptoms at the diagnosis, five sera recognized nine IgE-binding band ranging from 14 to 18 kDa (14). López-Torrejón et al. (10) has indicated that the main lentil allergen (IgE prevalence higher than 75%) has molecular weight of about 50 kDa and belongs to the vicilin family. Specific protein cleavage produces subunits of 12–35 kDa that are IgE reactive.

Allergy reactions to legumes through inhalation have rarely been described. In 1992, Martin et al. (2) reported the case of a 20-year-old man who experienced asthmatic attacks when exposed to the steam from cooking either chickpea or lentil. Type I hypersensitivity to the antigens in these legumes was demonstrated by means of immediate skin reactivity, histamine release tests, radioallergosorbent test (RAST) and RAST inhibition. Specific bronchial challenges with the heated extracts of these legumes elicited isolated immediate responses.

We have recently described a case of occupational asthma in an agronomist due to inhalation of *Bruchus* antigen. In that case, sensitization to lentil antigens was ruled out but protein antigens from pests living into the lentils was the cause of the allergic symptoms suffered from our patient. Only sera not reactive with noninfested lentils were selected for immunoblotting with *Bruchus* in order to evaluate a specific response. The IgE immunoblotting results suggested that the response may by specific against this pest (*B. lentis*): six patients had IgE against *Bruchus*-specific proteins and four had also IgE against protein bands that were present in the infested lentils extract (Fig. 3). In two cases (5778 and 1487) the IgE-binding patterns obtained with both extracts were similar indicating that the patients had developed IgE against *Bruchus*-specific proteins. In the other two cases (notably, serum 4910) there were conflicting findings in the sense that IgE-binding to bands with different molecular weight was observed in infested lentil and in *Bruchus* lentil extracts. These results raise the possibility of a specific IgE response to a new allergen developed by the relationship between the pest and legume proteins. In this way interaction of allergens from house dust mite and from cereal flours have been described (17). Another possibility is the induction of defense proteins in infested lentils (18).

Lentil crops are attacked by a wide range of insect species, but only some of these are economically important and require control measures. During storage, several species of seed beetles, the univoltine *Bruchus* spp. and multivoltine *Callosobruchus* spp. can cause severe damage. Bruchid larvae cause major losses of grain legume crops throughout the world. Some bruchid species, such as the Azuki bean beevil and the cowpea weevils are pests that damage stored seeds. Others, such as the pea weevil (*B. pisorum*) and *B. lentis* attack the crop growing in the field (3). In our area, lentils are freezing or treated with poison gasses before consumption in order to avoid that

the pests living into the lentils could go out through little holes made by the parasite that we can see sometimes in lentils. These procedures may also stimulate the protein defense from this legume.

In conclusion, our study suggests the possibility of anaphylaxis and asthma after inhalation of boiling vapours when cooking and after ingestion of infested lentils due to pest antigens. Six of 16 patients with allergic symptoms after ingestion or inhalation of lentils and with no specific IgE to the main lentil allergen, Len c 1, and to other lentil components, as determined by immunoblotting, were sensitized to different *B. lentis* proteins. Our results indicate that parasite proteins act as allergens in the lentil, and this may explain the negative oral challenge that were observed in other studies after lentil provocation in patients that complain for allergic symptoms after eating lentils or inhaled their emanations when cooking.

Furthermore, sensitization to lentil pests should be investigated in patients that refer allergic symptoms after cooking and/or eating lentils and have a negative or low skin test response and IgE to lentil extract.

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