

Lettuce-Induced Anaphylaxis. Identification of the Allergen Involved

O Bascones,¹ R Rodríguez-Pérez,² S Juste,¹ I Moneo,² ML Caballero²

¹ Department of Allergology, Hospital General Yagüe, Burgos, Spain

² Department of Immunology, Hospital Carlos III, Madrid, Spain

The first two authors have contributed equally to this article

■ Abstract

Background: Only 2 allergenic proteins have been described in lettuce allergy: a 16-kDa protein (putative profilin) and a lipid transfer protein (LTP) named Lac s 1.

Objective: Our aim was to identify the allergens involved in the anaphylactic reactions of 2 patients who had eaten lettuce.

Methods: The study was performed by Ig (immunoglobulin)-E immunodetection and immunodetection-inhibition assays.

Results: Both patients' sera showed specific IgE binding to a single protein from the crude lettuce extract (apparent molecular weight of 14 kDa). To characterize the allergen detected, the lettuce extract underwent proteolytic digestion and heat treatment and was highly resistant to both. The patients' sera also recognized the major peach allergen Pru p 3 by immunodetection. When the lettuce allergen was incubated with both Pru p 3 from peach peel and recombinant Pru p 3, the immunodetection-inhibition assay indicated that patients were sensitized to the lettuce LTP Lac s 1.

Conclusions: The allergen involved in the lettuce-induced anaphylaxis of our patients was the LTP Lac s 1.

Key words: Allergy. Anaphylaxis. Lettuce allergy. Lipid transfer protein.

■ Resumen

Antecedentes: Hasta el momento se han descrito dos alérgenos de lechuga, una proteína de 16 kDa, presumiblemente una profilina, y una proteína transportadora de lípidos denominada Lac s 1.

Objetivos: Identificar los alérgenos implicados en dos casos de anafilaxia tras la ingesta de lechuga.

Métodos: El estudio se realizó mediante inmunodetección de IgE específica y ensayos de inhibición.

Resultados: Los sueros de los 2 pacientes estudiados reconocieron una única proteína del extracto crudo de lechuga, de un peso molecular aparente de 14 kDa en SDS-PAGE. Para caracterizar el alérgeno detectado, el extracto de lechuga se sometió a tratamientos térmicos y a digestión proteolítica. El alérgeno resultó ser altamente resistente al calor y a la digestión con pepsina. Los sueros de los pacientes reconocieron además el alérgeno principal de melocotón Pru p 3 mediante inmunodetección. El ensayo de inhibición del reconocimiento del alérgeno de lechuga con Pru p 3 nativo y con Pru p 3 recombinante, indicó que los pacientes estaban sensibilizados al alérgeno de lechuga Lac s 1.

Conclusión: El alérgeno implicado en los 2 casos de anafilaxia inducida por lechuga es Lac s 1.

Palabras clave: Alergia. Anafilaxia. Lechuga. Proteína transportadora de lípidos.

Introduction

Lettuce (*Lactuca sativa*) is a vegetable of the *Compositae* family. Previous studies have reported systemic reactions (including anaphylaxis) after lettuce ingestion [1-4] and have described several immunoglobulin (Ig)-E binding proteins involved in lettuce allergy [5-8].

Vila et al [5] detected 4 allergens (molecular weights of 50, 43, 39, and 16 kDa) in the serum of a patient who presented mucocutaneous manifestations after eating lettuce, and suggested that the 16-kDa allergen corresponded to profilin, a panallergen responsible for allergic cross-reactivity between pollens and vegetables [9].

San Miguel-Moncin et al [1,3] described Lac s 1, a 9-kDa

lipid transfer protein (LTP) and a major allergen of lettuce, and reported cross-reactivity between Lac s 1 and *Platanus* and mugwort pollen LTPs, and between LTPs from the *Rosaceae* family and nuts. LTPs have also been described as the predominant allergen in the Mediterranean area, both in plant foods and in several pollens [10].

Our aim was to identify the allergens involved in the anaphylactic reactions of 2 patients who had eaten lettuce.

Methods

Patients and Sera

We studied 2 patients who experienced anaphylaxis after eating lettuce. Both patients had also presented symptoms after eating fruits from the *Rosaceae* family (apple and peach). The clinical data of these patients are presented in the Table. Two nonallergic subjects were used as negative controls.

Skin prick tests (SPTs) were performed with commercial inhalant allergens (Bial-Aristegui, Vitoria, Spain): *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*; *Alternaria alternata*, *Cladosporium herbarum*, *Penicillium notatum*, and *Aspergillus fumigatus*; dog, cat, horse and cow dander; and grass, weeds, and tree pollens (including Cupressaceae). Concentrations of histamine dihydrochloride and saline solution were used, respectively, as positive and negative controls. Skin prick-by-prick tests (SPPTs) with fresh apple, peach, and lettuce were performed. Specific IgE determinations to peach and lettuce were performed using the CAP System (Phadia, Uppsala, Sweden).

Crude Extract Preparation

Crude extract from the fresh green lettuce used for SPPT was prepared as described by Vieths et al [11] with some modifications.

Briefly, 200 g of lettuce was homogenized in 100 mL of acetone at -60°C and stored overnight in dry ice. The extract was then centrifuged at 4500g for 15 min, and the pellet was washed 3 times with acetone at -60°C . After the last wash, the pellet was dried and lyophilized. The dried acetone powder was stored at -20°C .

Approximately 3 g of the dried acetone powder was dissolved in 110 mL of 0.01 M potassium phosphate buffer (pH 7.4) containing 0.15 M NH_4Cl and extracted by shaking for 2 hours. It was then centrifuged at 20 000g for 45 min at 5°C . The pellet was discarded and the supernatant was lyophilized using a Cryodos freeze dryer (Telstar, Barcelona, Spain).

A peach peel extract of enriched Pru p 3 was prepared as described elsewhere [12]. After extraction with Tris-HCl buffer (0.1 M Tris pH 7.5, 10 mM ethylene diamine tetraacetic acid; 1:5 [w/v], 1h 4°C), the remaining material was washed with water and re-extracted with 1.5 M LiCl (1:5 [w/v], 1 h, 4°C). The LiCl extract was dialyzed against water and freeze-dried.

Treatment of the Crude Extract

Aliquots of the crude extract underwent 2 different treatments: heating at 100°C for 15 min and digestion with simulated gastric fluid as described elsewhere [13]. Briefly, 25 μg of the extract was dissolved in 50 μL of pre-warmed 100 mM HCl, pH 1.2, and 30 mM NaCl, or in the same solution (150 μL) containing 0.32% (w/v) of pepsin A (Sigma, St. Louis, Missouri, USA). The extract was digested with continuous shaking for 30 min at 37°C . Samples were then analyzed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), as set out below.

Tris Tricine Gel Electrophoresis

Fifteen micrograms of protein of each sample was loaded in a 16% Tris-tricine SDS-PAGE gel under nonreducing

Table. Clinical Data of Patients

Patient	Age	Sex	Symptoms	SPT (Inhalant Allergens), mm	SPPT (Peach and Apple), mm	CAP (Peach), kU _A /L	SPPT (Lettuce), mm	CAP (Lettuce), kU _A /L
1	40	F	Facial angioedema, wheals, dyspnea, vomiting, and tachycardia after lettuce ingestion Similar symptoms with chicory and fruits (apple, peach, plum, cherry, and raspberry) Seasonal rhinitis	Grass (6×7) Weeds (6×6) Trees (5×6) Cat/dog dander (3×4)	Peach (6×6) Apple (5×4)	5.04	4×3	0.97
2	22	F	Systemic urticaria, lip angioedema, dyspnea and bronchospasm after lettuce ingestion Wheals and lip angioedema with fruits (apple, peach, and melon)	Negative	Peach (5×6) Apple (6×5)	9.89	(5×5)	0.86

Abbreviations: CAP, serum-specific IgE determination by the CAP method; SPPT, skin prick-by-prick testing; SPT, skin prick testing.

conditions and subjected to a constant 20-mA current for 2-3 hours as described elsewhere [14].

Specific IgE Immunodetection

Separated proteins were transferred onto nitrocellulose membranes and incubated overnight in a 1:20 dilution of the individual sera, a pool of sera from the 2 lettuce allergic patients studied, or with nonallergic subjects' sera as a negative control. IgE-binding proteins were detected as described elsewhere [15].

To perform the immunodetection-inhibition assays, a pool of sera from the patients was incubated for 3 hours at room temperature with bovine serum albumin (30 μ g) as a negative control, peach peel extract (15 μ g), or recombinant Pru p 3 (5 μ g) (provided by Drs. G. Salcedo and A. Diaz-Perales from E.T.S Ingenieros Agrónomos, Universidad Politécnica, Madrid, Spain).

Results

For both patients, the results of SPPT and serum-specific IgE determination by CAP were positive. The Table contains the results of the SPT, SPPT, and specific IgE determination by the CAP system.

Figure 1A shows the result of protein separation by SDS-PAGE performed with the crude lettuce extract. The patients' sera showed specific IgE binding to a unique protein with an apparent molecular weight of approximately 14 kDa (Figure 1B).

To characterize the allergen detected, the crude lettuce extract underwent different treatments and a study by IgE

immunodetection was performed with the patients' sera pool.

Digestion and heat treatment did not affect the IgE-binding capacity of the protein (Figure 1C). These results indicate that the allergen involved could correspond to Lac s 1.

To confirm the identification of the reactive band as LTP, we prepared a peach peel extract enriched in Pru p 3, the major peach allergen. As Figure 1D shows, the patients' sera recognized this LTP. We then performed an immunodetection-inhibition assay with the sera pool pre-incubated with Pru p 3. The inhibition assay showed that the allergen found in the crude lettuce extract recognized by the patients was strongly inhibited both by LTP peach peel extract and by rPru p 3 (Figure 1E).

Discussion

The patients studied experienced anaphylaxis after eating lettuce and similar symptoms with *Rosaceae* fruits. When the sera were studied with a crude lettuce extract, both recognized a unique protein band with a molecular weight that indicated profilins. However, the clinical manifestations suggested that an LTP could be involved. LTPs have been reported to induce severe systemic reactions [9,10].

Profilins and LTPs are easily differentiated. LTPs are resistant both to heat and digestion; profilins are resistant to heat, but are quickly digested in simulated gastric fluid [16].

The major allergen described from lettuce is a lipid transfer protein named Lac s 1. Lac s 1 and Pru p 3 share 66% of the amino acid sequence [3]. In fact, our patients experienced urticaria and anaphylaxis with both peach and apple, whose major allergens in the Mediterranean area are LTPs.

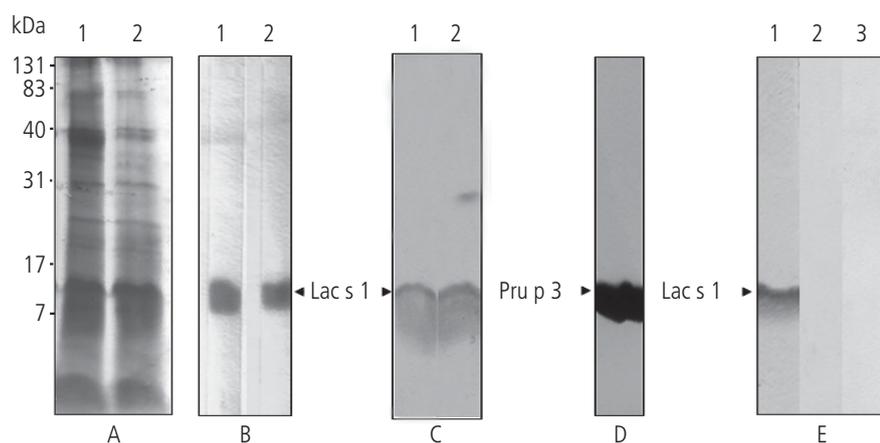


Figure. Electrophoretic analysis of lettuce extract. A, Protein staining after SDS-PAGE performed with extracts from lettuce: lane 1, crude extract; lane 2, extract heated at 100°C for 15 min. B, IgE immunodetection performed with the individual sera of patients using the crude lettuce extract. C, IgE-immunodetection performed with a pool of sera from both patients: lane 1, crude lettuce extract digested with simulated gastric fluid; lane 2, lettuce extract heated at 100°C for 15 min. D, IgE immunodetection performed with the pool of sera with peach peel extract. E, IgE immunodetection inhibition assay: lane 1, lettuce extract inhibited with bovine serum albumin (negative control); lane 2, lettuce extract inhibited with peach peel extract; and lane 3, lettuce extract inhibited with recombinant Pru p 3. Ig indicates immunoglobulin; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis.

Both natural Pru p 3 from peach peel and purified rPru p 3 inhibited IgE binding to the reactive band, indicating that both patients were sensitized to the lettuce LTP Lac s 1.

To our knowledge, this is the first report of sensitization to lettuce alone. All previous reports describe cosensitization to *Rosaceae* fruits or nuts. Severe clinical manifestations involving LTPs have been reported from southern Europe. Patients with lettuce allergy should be monitored for the presence of IgE to LTPs in order to prevent further sensitizations.

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■ **María Luisa Caballero, PhD**

Hospital Carlos III
C/ Sinesio Delgado, 10
28029 Madrid, Spain
E-mail: mlcsoto@hotmail.com