

Letter to the Editor

Sensitization to *Cannabis sativa* caused by a novel allergenic lipid transfer protein, Can s 3

To the Editor:

The plant nonspecific lipid transfer protein (LTP) family includes a high number of 9-kd allergens, mainly from foods. These have been well-characterized as mediators of sensitization by ingestion.¹ However, there are also LTPs associated with pollinosis, with some of them responsible for cross-sensitization between pollen (ie, mugwort, plane tree) and plant foods (ie, peach, cabbage, chestnut).² Furthermore, sensitization by the respiratory route has been recently reported for food LTPs, such as those from rice³ and wheat Tri a 14 linked to baker's asthma.⁴

Allergy to marijuana (*Cannabis sativa*) is rare, but several cases of allergic disorders caused by inhalation during smoking, injection, or even ingestion of *Cannabis* have been reported,⁵⁻⁸ and cross-reactivity with plant foods, such as tomato, was suggested.⁶ Interestingly, *Cannabis* is used not only for recreational activity but also as a medical drug to improve the quality of life in some illnesses. No marijuana allergen has ever been isolated and characterized to date. We describe the identification of the most probable allergen causing allergy to marijuana and several plant foods.

A 28-year-old man without any previous allergic symptoms initially reported contact urticaria with the marijuana plant. Subsequently, after smoking the plant, the patient presented sneezes, watery rhinorrhea, palpebral edema, itching, and eye redness, thus stopping the consumption. He presented the same symptoms in places where marijuana was being smoked. Some months later (no allergic reaction to food was previously shown), the patient had anaphylaxis (urticaria-angioedema, hypotension, dyspnea, and hypoxemia) after the ingestion of tomato and pepper, contact urticaria with peach peel, anaphylaxis by fig, and oral allergy syndrome by apple, almond, eggplant, and chestnut. Rubbing skin test with peach peel and marijuana leaves were positive, as well as skin prick tests with commercial extracts (ALK-Abelló, Madrid, Spain) of apple, peach, and tomato and with a crude extract from marijuana buds (0.5 mol/L NaCl, pH 3.0, 1:10 wt:vol, 1 hour at room temperature, 100 µg protein/mL) and purified Pru p 3 (the major LTP and allergen from peach fruit; 20 µg/mL). The serum of the patient was obtained for further evaluation (with written informed consent and approval from the Hospital Ethics Committee). Total IgE was 235 kU/L, and specific IgE to peach, 3.05 kU/L; apple, 1.96 kU/L; tomato, 1.51 kU/L; fig, 1.18 kU/L; almond, 0.77 kU/L; and hazelnut, 3.93 kU/L (CAP system FEIA; Phadia, Uppsala, Sweden).

To search for potential allergens, the crude extract from marijuana was separated by SDS-PAGE and IgE immunodetected with the patient's serum (Fig 1). IgE-binding components of approximately 9 kd, 14 kd, and 35 to 100 kd were detected, being the same blot also recognized

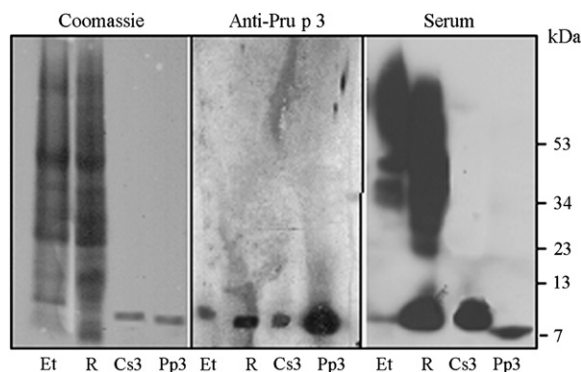


FIG 1. Protein staining (Coomassie) and immunodetection with polyclonal antibodies against Pru p 3 (Anti-Pru p 3) or with the patient's serum (Serum) of the following samples separated by SDS-PAGE: marijuana extract (Et; 20 µg), its retained (R; 15 µg) fraction after cation exchange chromatography, and purified allergens Can s 3 (Cs3) and Pru p 3 (Pp3; 5 µg each).

by rabbit polyclonal antibodies to peach Pru p 3 (Fig 1). This peach allergen weakly reacted with IgE from the patient's serum (Fig 1). All these results suggested that a LTP was involved in *Cannabis* sensitization.

To support this hypothesis, the 9-kd IgE-binding protein was purified from the marijuana extract by means of a 2-step chromatographic procedure: cation-exchange chromatography on an Acell Plus CM Waters SepPak Cartridge (Waters, Milford, Mass; retain fraction shown in Fig 1) followed by reverse-phase HPLC separation of the retained material on a Nucleosil 300-C4 column (8 × 250 mm; particle size, 5 µm; Scharlau Science, Barcelona, Spain). The purified protein, hereafter named Can s 3, showed a single band on SDS-PAGE, bound IgE from the patient's serum (Fig 1), and was identified as LTP according to its N-terminal amino acid sequence (XITCGQVASS), molecular size (9226 d as determined by matrix-assisted laser desorption/ionization analysis), and recognition by anti-Pru p 3 antibodies (Fig 1). *In vitro* tests (specific IgE determination, ELISA inhibition and basophil activation test) performed as previously described⁹ supported the relevance of the purified protein as *Cannabis* allergen (Fig 2). IgE to Can s 3, as well as to Pru p 3, was found in the patient's serum, and inhibition values of 50% and 30% of the IgE-binding to the marijuana extract were recorded for Can s 3 and Pru p 3, respectively. Cross-inhibition between both LTP allergens was also observed (Fig 2). Furthermore, Can s 3 exerted a strong biological activity in basophil activation test (61.8%; 0.33 µg/mL; basal response, 3.2%; anti-IgE positive control, 55.6%), higher than that found for the marijuana extract (36.7%; 0.01 mg/mL) or Pru p 3 (38.2%; 0.33 µg/mL). Finally, an *in vivo* response (skin prick test; 7 × 7 mm of wheal) to Can s 3 (20 µg/mL) was obtained in the patient.

The different lines of evidence detailed strongly indicated that Can s 3 is a relevant allergen of *Cannabis sativa*,

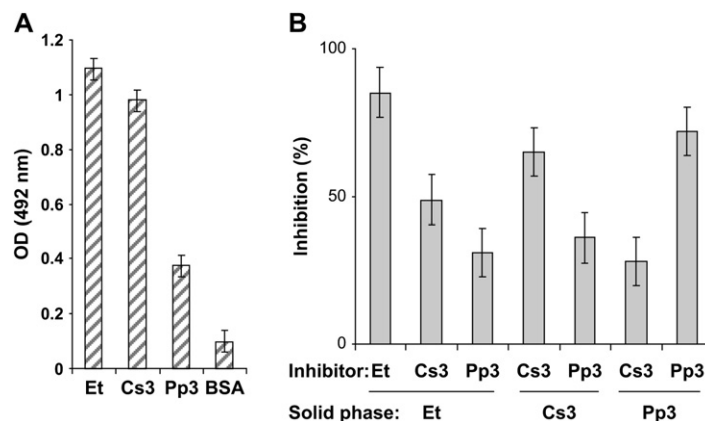


FIG 2. A, Specific IgE of marijuana extract (Et; 20 μ g/mL in solid phase) and purified allergens Can s 3 (Cs3) and Pru p 3 (Pp3; 5 μ g/mL of each in solid phase). The patient's serum (1:3 dilution) and BSA (5 μ g/mL) as negative control was used. B, ELISA-inhibition assay using marijuana extract and purified Can s 3 in solid phase, and both samples and purified Pru p 3 as inhibitors. Means ($n = 3$) and SDs (bars) are represented.

being the first allergenic protein characterized in this plant species. A 10-kd IgE-binding band, which most probably corresponds to Can s 3, has been detected in other cases of marijuana allergy,^{6,7,10} thus suggesting a common role of LTP in patients with *Cannabis* allergy. On the other hand, the cross-reactivity with peach Pru p 3 helps explain the cosensitization between marijuana and plant foods showed in this and other studies.⁶

It can be speculated that contact with the marijuana bud was probably the initial cause of Can s 3 sensitization in our patient according to his clinical history. Inhalation of incomplete-burning marijuana buds could be a complementary route of sensitization. Interestingly, respiratory allergy to peach leaves, mediated by LTP Pru p 3, had been reported.¹¹ Therefore, LTP allergens can induce symptoms by ingestion, inhalation, or even contact. This versatility together with the unusual stability point to the importance of the LTP panallergen family.

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Supported by Ministerio de Educación y Ciencia, DGI (Project BIO 2006-07473).

Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

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doi:10.1016/j.jaci.2007.07.052