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Letters

Allergy to orange with cystatine-like protein as one of its allergens

Orange (*Citrus sinensis*) belongs to the family Rutaceae. First originated in China, orange is nowadays a worldwide-distributed fruit. Even though its consumption is very common and it is used both as a food and as a condiment or flavoring, there are few cases of orange allergy described.[1] In fact, orange allergy is estimated to account for only 3% of food allergy.[2] Among the scarce number of orange allergy cases described, Cit s 1 (germin-like protein) and Cit s 2 (profilin) have been identified as major allergens.[3] To date, Cit s 3 (lipid transfer protein), Cit s 7 (giberellin-regulated protein), and Cit s isoflavone reductase have also been described as orange allergens.

We report the case of a 13-year-old girl with a history of eyelids and uvula edema, dysphonia, nasal congestion, and shortness of breath within minutes of consuming freshly squeezed orange juice. This patient reported seasonal rhinoconjunctivitis caused by grass and olive pollen and had also been diagnosed as having egg allergy (with successful desensitization), peanut and pine seed allergy, food-dependent exercise-induced anaphylaxis secondary to peach nsLTP (nonspecific lipid transfer protein), and atopic dermatitis.

Skin prick tests to commercial extracts of common aeroallergens, including pollens, dust mites, molds, animal dander, and pan-allergens, were performed revealing positive (≥3 mm) results to pollens (*Phleum pratense, Cynodon dactylon, Olea europaea, Cupressus arizonica, Platanus acerifolia, Artemisia vulgaris, Plantago lanceolata*), animal dander (dog and cat), molds (*Alternaria alternata* and *Aspergillus fumigatus*), and peach nsLTP (Pru p 3). Skin prick test revealed negative results to house dust mites and pollen profilin (Pho d 2). Saline was 0 mm and histamine was 6 mm. Prick-by-prick skin test with the orange juice involved in the reaction was 6 mm. The result of prick-by-prick skin test with orange seeds was negative. This test was performed also in control subjects with a negative result. Serum-specific immunoglobulin E (sIgE) (Thermo Fisher Scientific Inc, Phadia, AB, Uppsala, Sweden) to *P pratense* was 25.6 kU/L, 2.09 kU/L to *O europaea*, 20.3 kU/L to *C arizonica*, 2.03 kU/L to orange, 0.9 kU/L to peanut, 0 kU/L to Ara h 9, 0.43 kU/L to pine seed, and 0.69 kU/L to Pru p 3, of a total IgE of 486 kU/L.

Orange extracts (OE), both peel and pulp, were prepared by homogenization in phosphate-buffered saline (20% wt/vol), dialysis, and lyophilization (Roxall Laboratory, Bilbao, Spain). Protein content of extracts from orange pulp and peel was 20% and 24% (wt/wt) respectively, according to Bradford. The OE were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) as described by Laemmli,[4] revealing protein bands ranging from 90 kDa to 10 kDa. SDS-PAGE IgE immunoblotting assay (without 2-mercaptoethanol–nonreducing conditions) with the patient sera revealed an IgE-binding zone between 97 and 40 kDa and IgE-reactive bands with molecular weights (MW) of approximately 25, 21, 19, and 11 kDa (Fig 1). Cit s 1 is an allergen of approximately 25 kDa[3]; Cit s 3 and Cit s 7 are 2 allergens involved in severe allergic reactions with MW of 9 and 7 kDa, respectively. Owing to the patient's clinical history after orange intake, her background of food-dependent exercise-induced anaphylaxis and pollen allergy, and the similarity of the molecular weight of the smallest IgE-reactive band with the one

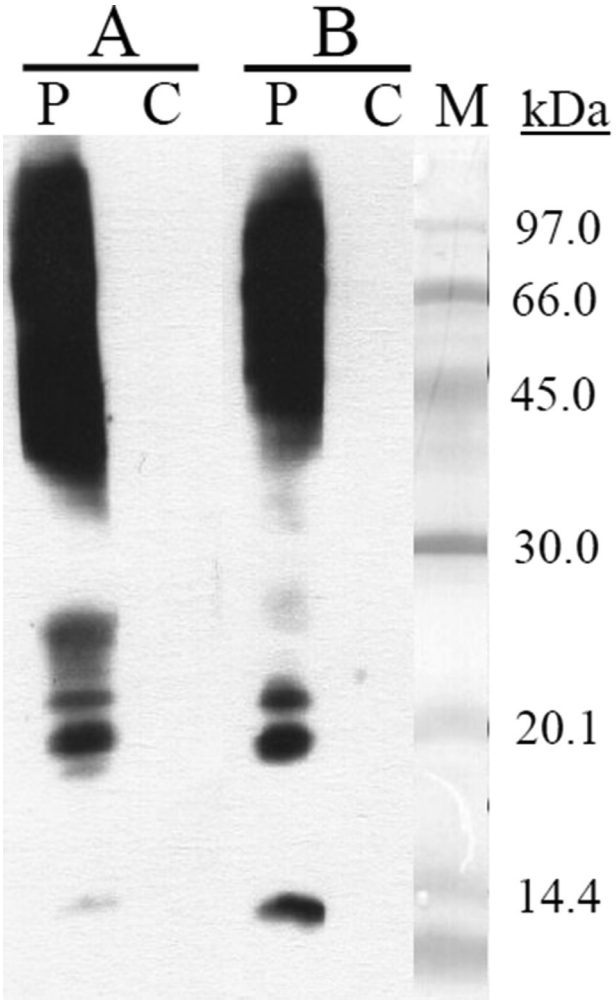

Figure 1. SDS-PAGE IgE immunoblotting with orange extracts in nonreducing conditions (without 2-mercaptoethanol). A, orange peel extract. B, orange pulp extract. Lane P, patient's serum. Lane C, control serum (pool of sera from non-atopic subjects). Lane M, molecular weight marker. IgE, immunoglobulin E; kDa, kiloDalton; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

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of nsLTP, a new SDS-PAGE immunoblotting was performed with 16% acrylamide-tricine gel as described by Shagger et al[5] using the orange pulp extract, the patient serum and an anti–Pru p 3 rabbit serum. Subsequently, an SDS-PAGE immunoblot inhibition assay (without 2-mercaptoethanol–nonreducing conditions) with orange pulp extract in solid phase and Pru p 3 as inhibitor revealed no inhibition on the 11 kDa-orange band. To identify this protein, the 11 kDa band from the orange pulp extract was manually excised from the gel, digested with trypsin, and analyzed by matrix-assisted laser desorption-ionization time of flight and liquid chromatography coupled to tandem mass spectrometry (MS/MS), after the methods of Pastor et al.[6] Protein identification was performed by searching a nonredundant protein sequence database (National Center for Biotechnology Information), using the Mascot program (http://www.matrixscience.com). When compared with the databases, the analysis of the resulting peptides by MS or MS/MS corresponded to a cystatin-like protein for the 11 kDa band.

Cystatins are a family of proteins that inhibit the activity of cysteine proteinases,[7] which are enzymes that play a major role in intracellular and extracellular processes, such as development and ripening of fruits[8] and protein degradation.[9] Cystatins are present in microorganisms, animals, and plant species.[10] They have been described as allergens in several allergenic sources and may behave as either ingested (egg white Gal d, anisakis Ani s 4, kiwi Act d 4, potato Sola t 3, soybean Gly m CPI) or inhaled allergens (cat Fel d 3) according to Allergome Database.

We report the case of a patient with IgE-mediated allergy to orange with a cystatin-like protein as one of its allergens, not previously described. It is important to always keep in mind that even food considered as uncommon allergens, such as oranges, can cause a potentially lethal reaction. In addition, even if a patient is sensitized to common—and hazardous—food allergens, such as LTP, further study is necessary to reach a correct diagnosis and to provide appropriate treatment and avoidance measures.

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