



Letter

Pollinosis to tree of heaven (*Ailanthus altissima*) and detection of allergenic proteins: a case report

The tree of heaven (*Ailanthus altissima*), native to China, belongs to the Simaroubaceae family. This tree has spread from a prized ornamental plant to a highly invasive species in many regions worldwide.¹ During the last 2 decades, this species was introduced in arid and semiarid regions of Iran as a cultivated species and is now present as an invasive plant in most parts of the country.² Despite several clinical reports of allergy to *A. altissima* pollen,^{3–5} the allergens responsible for allergic symptoms have not yet been detected. The identification of IgE-reactive proteins would be important for the development of allergen-based diagnostics and therapy of this pollinosis. In a recent animal study, an IgE-binding protein of 42 kDa was identified in *A. altissima* pollen collected from Kerman City, Iran.⁶ Here, we describe for the first time, to our knowledge, the IgE-binding pattern of an allergic patient with *A. altissima* pollinosis.

A 31-year-old woman, who sampled *A. altissima* pollen during a research project, had pollinosis symptoms, including shortness of breath, conjunctivitis, rhinitis, dry coughing, itching, and contact dermatitis, after direct exposure to *A. altissima* pollen and during the 2 successive pollination seasons after the sampling (from mid-April to mid-May). The patient's symptoms started with the beginning of the *A. altissima* blooming and intensified toward the end of the pollination season. During her allergy consultation at the Immunology, Asthma, and Allergy Research Institute, the patient filled out a detailed questionnaire and, because of the lack of allergy diagnostic tests for *A. altissima*, performed a regular skin prick test with common airborne allergens present in Tehran (including a panel of 32 trees, weeds, grasses, animals, and mites; Stallergenes, Antony, France). Histamine hydrochloride and phenolate glycerosaline were used as positive and negative controls, respectively.

After the patient signed an informed consent form, her serum was drawn at the time of the visit and stored at -20°C until specific IgE screening (RIDA Allergy Screen panel 2 for inhalant allergens; R-Biopharm, Darmstadt, Germany) and total IgE (enzyme-linked immunosorbent assay method). The RIDA Allergy Screen panel 2 includes common mites, animal danders, and mold allergens, as well as the following pollen allergens: *Alnus* species, *Birch* species, *Corylus* species, *Quercus* species, mixed grass, *Lolium* species, and *Plantago* species. For IgE immunoblotting experiments that used fresh pollen extracts, *A. altissima* pollen was collected from male trees planted in an urban green space of Tehran (western district near the Argentine Square) for 2 successive years (2014 and 2015) and stored at -20°C until use. The pollen grains were defatted

using acetone and extracted in phosphate-buffered saline (PBS). The total protein content of PBS pollen extracts was measured using the Bradford protein assay.⁷ The protein components of pollen extracts were then analyzed using electrophoresis (10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis) followed by IgE immunoblotting. Briefly, 40- μg proteins were loaded per well, and separated protein bands were visualized with Coomassie Brilliant blue R-250. Then, for immunoblotting, proteins were transferred to a polyvinylidene fluoride (PVDF; Bio-Rad Laboratories, Hercules, California) membrane. The PVDF membrane was blocked with 0.3% Tween 20 and incubated overnight with the patient's serum at 4°C under shaking. After several washing, the PVDF membrane was incubated during 1 hour with alkaline phosphatase (AP)–conjugated goat anti-human IgE (Sigma-Aldrich, St Louis, Missouri). Finally, the AP activity was visualized with 5-bromo-4-chloro-3-indolyl phosphate/Nitro blue tetrazolium (Sigma-Aldrich).

According to our clinical analyses, the patient had a normal level of serum total IgE (33.2 IU/mL) and was polysensitized to several inhalant allergens. Among the standard skin prick test panel of common inhalant allergens present in Tehran, *Acer negundo*, tree mix, *Olea europaea*, *Plantago lanceolata*, *Chenopodium album*, *Artemisia vulgaris*, *Amaranthus retroflexus*, *Salsola kali*, *Cynodon dactylon*, and *Dermatophagoides farinae* extracts produced positive results. The histamine positive control induced a wheal of 5 mm in diameter. Among the 20 inhalant allergens that constituted the RIDA Allergy Screen panel 2, only the *Artemisia* species extract produced positive results (2+), whereas the *Plantago* species and *D. farinae* extracts produced negative results, despite the positive reactions to the skin prick tests.

The total protein content of fresh PBS extracts reached 4 mg/mL for *A. altissima* pollen grains collected in 2014 and increased by 50% in 2015 samples (6 mg/mL). Therefore, the PBS extracts of *A. altissima* pollen appeared to be rich in protein when compared with other anemophilous allergenic pollen, such as cypress (approximately 200 μg of protein per milliliter of PBS extract⁸) and grasses (ranging from 924 to 1646 μg of protein per milliliter of extract⁹).

In both the 2014 and 2015 pollen extracts, Coomassie-stained sodium dodecyl sulfate–polyacrylamide gel electrophoresis patterns revealed several protein bands that ranged from 10 to 110 kDa (Fig 1). However, the major difference between the 2 extracts was the higher expression of a 52-kDa protein band in the 2014 samples compared with those collected in 2015. IgE immunoblotting using the serum of the allergic patient revealed 2 IgE-binding proteins of 42 and 52 kDa in both pollen extracts (Fig 1). This result contrasts with the findings of previous animal

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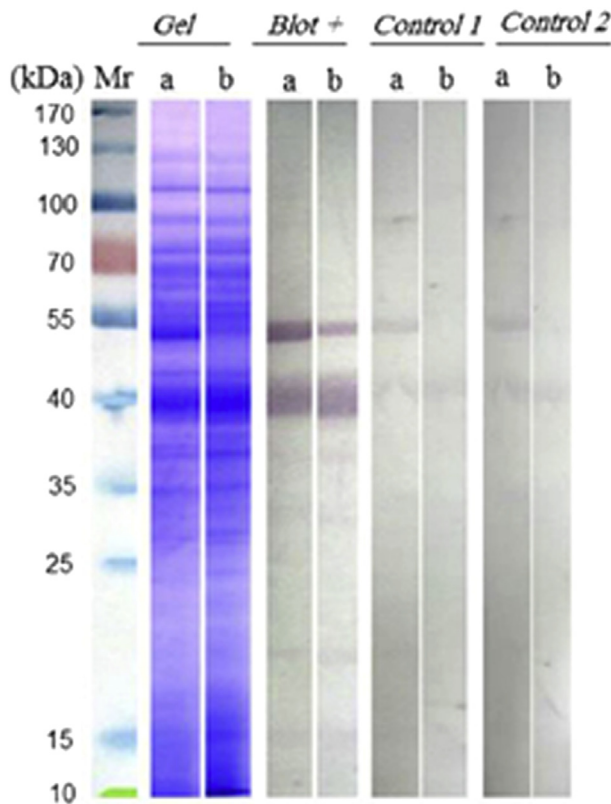


Figure 1. IgE immunoblots using fresh extracts of *A. altissima* pollen grains collected in 2014 (lines a) and 2015 (lines b) probed with the patient's serum (Blot +); without serum (Control 1), a non-allergic serum (Control 2).

studies in which no specific IgE reactivity to the 52-kDa protein has been detected. Moreover, this 52-kDa protein was present in variable concentrations in our 2014 and 2015 samples. These results seem to reflect the variability of *A. altissima* pollen IgE-reactive content from year to year. In this manner, a number of studies have already found that some environmental conditions, such as soil, shading, air pollution, and climate, may influence the pollen allergenic content.^{8,10}

Until now, several cases suggestive of allergy to *A. altissima* pollen have been reported in the United States³ and Italy⁴ without any molecular evidence. To our knowledge, this study represents the first case of IgE-mediated *A. altissima* pollinosis confirmed by an allergen-based molecular analysis. The prevalence of sensitization to these allergens should be explored by screening a larger number of serum samples from patients presenting with allergic symptoms during the *A. altissima* pollination. Additional experiments on these 2 IgE-binding components are in progress using proteomic devices

to determine their nature and to evaluate the risks of IgE cross-reactivity with other allergenic species.

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