

Immunoglobulin E reactivity to Arizona cypress pollen extracts: evidence for a 35-kDa allergen

Y. Shahali*, Z. Pourpak, M. Moin, A. Mari, A. Majd

Key words: allergens; Arizona cypress; immunoblotting; pollen.

Allergy to Cupressaceae pollen is a worldwide pollinosis caused by several species (1–3). In this study, we evaluated the immunoglobulin E (IgE) reactivity to Arizona cypress (*Cupressus arizonica*, CA) pollen extracts in 15 allergic subjects living in Tehran.

A 35-kDa allergen in Arizona cypress pollen.

A total of 15 allergic patients according to their clinical history of seasonal rhino-conjunctivitis and bronchial asthma, correlating with the typical pollination period of CA (mid-wintertime), have been selected for skin prick testing (SPT), ImmunoCAP and immunoblotting. After obtaining an informed consent from the patient or the patient's parents, blood samples were drawn at the time of the visit and stored at -20°C until use. Recorded data of each patient are given in Table 1.

The SPT was performed with a commercial cypress pollen extract supplied by Stallergenes (Antony, France). Serum specific IgE to CA was evaluated using the ImmunoCAP system, testing the Arizona cypress allergenic extract (code: t222; Phadia AB, Uppsala, Sweden). Specific IgE antibodies were measured according to the manufacturer's instruc-

tions and a positive ImmunoCAP result was defined as a value >0.35 kU/l.

For the preparation of pollen extracts, pollens collected from trees growing in Tehran were extracted in 0.01 M phosphate buffered saline (5% w/v), pH 7.4, for 14 h at 4°C under stirring. The suspensions were centrifuged at 14 000 g for 1 h at 4°C and the supernatant was dialysed against distilled water. Extracted proteins were separated by 10% SDS-polyacrylamide gel electrophoresis (90 μl of each pollen extract per well) and detected by Coomassie blue staining. After electrophoresis, proteins were transferred from the gel onto polyvinylidene difluoride membrane (4) and saturated by bovine serum albumin prior to overnight incubation with 1 : 15 diluted sera of allergic subjects. The membrane was washed and incubated for 1 h with 1 : 20 000 dilution of goat anti-human IgE conjugated with peroxidase (A9667;

Table 1. Recorded data of allergic patients

Patient no.	Gender	Age	Clinical symptoms	Skin prick test WD (mm)		CAP	Other sensitizations
				Cypress extract	Histamine		
1	M	13	AA, AR	0	7	–	Grasses, <i>A. alternata</i>
2	F	29	AA, AR, AC	4	6	2+ (1.86 kU/l)	Trees, grasses, weeds, mites
3	F	27	AR	0	5	–	–
4	F	10	AR, AC	0	5	–	Trees, grasses, mites (DF)
5	M	12	AR, AD	0	4	–	Grasses
6	F	25	AR	3.2	5	–	Trees, grasses, weeds, cockroach
7	M	14	AA	0	6	–	Trees, weeds, tomato
8	M	11	AA, AR, AC	0	4	–	Trees, grasses, weeds
9	M	52	AR, AD	0	6	–	<i>A. alternata</i>
10	M	6	AR, AC	0	8	–	Trees, grasses, weeds
11	M	6	AR, AD	0	6	–	–
12	F	20	AR, AC	0	8	–	Trees, <i>A. alternata</i> , cat fur
13	M	30	AA, AR, AC	0	5	–	–
14	M	20	AR	0	6	–	Grasses
15	F	22	AR	0	5	–	Trees, grasses, weeds
6F, 9M 19.8 $\pm$ 11.96				0.48 $\pm$ 1.27	5.73 $\pm$ 1.22		12 poly, 3 mono

AA, allergic asthma; AR, allergic rhinitis; AC, allergic conjunctivitis; AD, atopic dermatitis; WD, wheal diameter; DF, *Dermatophagoides farinae*; mono, monosensitized; poly, polysensitized.

Negative CAP (–), represent patients with a specific IgE binding of less than 0.35 kU/l.

Data in the last row are mean $\pm$ SD for age and wheals (histamine, cypress extract).

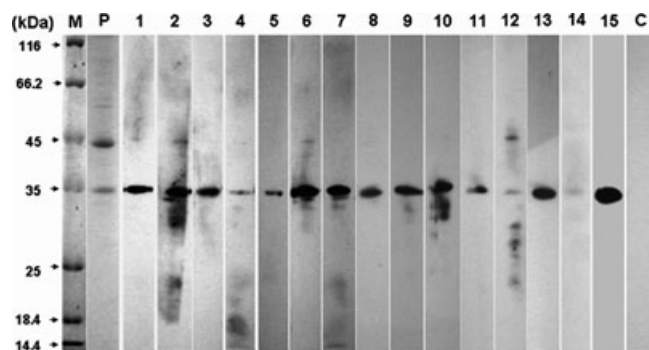


Figure 1. Immunoblotting: M represents the marker; P, the SDS-PAGE profile of a *Cupressus arizonica* (CA) pollen extract; lanes 1–15, specific IgE-binding protein bands to CA pollen extracts probed with the sera of 15 CA allergic subjects; C, immunoblotting using a control serum.

Sigma, Munich, Germany) in PBS. The membrane was washed again and then treated by the Amersham ECL kit (GE Healthcare, Munich, Germany). The allergens were revealed after 1 min contact with X-OMAT Kodak film.

Among 15 patients, 13 were negative to SPT performed with the commercial cypress pollen extract and the other two reacted with wheals of 3.2 and 4 mm in diameter. Fourteen of 15 patients were also ImmunoCAP negative. Among all selected patients, only one was positive to both SPT and ImmunoCAP (Table 1).

Western blotting, performed by using extracts of mature pollen collected from Tehran, revealed that sera-specific IgE of all allergic subjects reacted to the 35-kDa protein and only three patients showed a weak IgE binding to the band at about 45 kDa, most probably Cup a 1, present in our pollen extracts (Fig. 1).

No IgE binding was observed in the Western blot performed with the control serum.

Immunoblots revealed that 100% of CA tested subjects showed a specific IgE binding to the 35-kDa protein, while only 20% of them had a weak IgE reaction to Cup a 1. No specific difference appeared between the sensitization patterns of selected patients, according to their gender, age, symptoms and the presence of other sources of sensitization. The high prevalence of this major 35-kDa allergenic protein in CA allergic subjects living in Tehran reflects the need to complete by the purified natural or recombinant 35-kDa CA pollen allergen the panel of cypress-related molecules for a more comprehensive allergen-based diagnosis and therapy. This study also suggests that to optimize standard allergen preparations, the variability of pollen

allergenic components has to be considered for different regions.

We thank Maryam Haftlang and Raheleh Shokouhi for their excellent technical assistance.

*ESPCI-PECSA
UMR CNRS 7195

Paris

France

Tel.: +33 (1) 40 79 46 68

Fax.: +33 (1) 40 79 46 54

E-mail: youcefshahali@espci.fr and
yshahali@yahoo.com

Accepted for publication 9 April 2009
Allergy 2009

© 2009 John Wiley & Sons A/S

DOI: 10.1111/j.1398-9995.2009.02107.x

References

1. Charpin D, Calleja M, Lahoz C, Pichot C, Waisel Y. Allergy to cypress pollen. *Allergy* 2005;**60**:293–301.
2. Di Felice G, Barletta B, Tinghino R, Pini C. Cupressaceae pollinosis: identification, purification and cloning of relevant allergens. *Int Arch Allergy Immunol* 2001;**125**:280–289.
3. Mari A, Di Felice G, Afferni C, Barletta B, Tinghino R, Pini C. Cypress allergy: an under estimated pollinosis. *Allergy* 1997;**52**:355–356.
4. Towbin H, Staehelin I, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA* 1979;**76**:4350–4354.