

**Allergic Rhinitis to Thuja Pollen**

21908

B. Guerin<sup>a</sup>  
G. Kanny<sup>b</sup>  
G. Terrasse<sup>c</sup>  
J. L. Guyot<sup>d</sup>  
D. A. Moneret-Vautrin<sup>b</sup>

<sup>a</sup> Laboratoire Allerbio,  
Varennnes-en-Argonne,  
<sup>b</sup> Service de Médecine D, CHU Nancy,  
Hôpital de Brabois, Vandœuvre,  
<sup>c</sup> Montceau-les-Mines,  
<sup>d</sup> Service d'ORL CHU, Nancy  
Hôpital Central, Nancy, France

**Abstract**

Allergy to pollen of Cupressaceae has been linked to pollens of *Cupressus*, *Juniper* and *Cryptomeria*. The authors report 2 cases of rhinitis and conjunctivitis induced by thuja, another member of the Cupressaceae family. Monosensitization to thuja pollen has been identified as the causal agent: (1) of a long-standing springtime rhinitis in 1 patient with negative skin tests and specific IgE titers to the main inhalants (specificity of the prick test and nasal provocation with the thuja extract was confirmed by a positive RAST) and (2) in a 2nd patient without former history of allergy, who consulted for conjunctivitis following acquisition of a dog; the standard battery of skin tests, and Phadiotop<sup>®</sup> were all negative. After controls, only the thuja extract gave significant skin test response. The level of total IgE was low, and RAST was negative. Sensitization and pollen provocation were produced by the intermediary of the dog, carrying thuja pollens on its fur. The immunoprint and the crossed radioimmuno-electrophoresis revealed common antigenicity between cypress and thuja extracts.

**Key Words**

Rhinitis  
Thuja  
Conjunctivitis

**Introduction**

Allergy to pollen of Cupressaceae has developed quickly in the Mediterranean region in parallel with the spreading of cypress hedges around dwellings [1–6]. It is frequently characterized by monosensitization which is expressed always as a seasonal rhinitis, together with conjunctivitis and more rarely, asthma [7]. This tree does not exist north of the Loire as it does not tolerate the winter temperatures of the eastern regions. The 2 cases reported are the first indication of monosensitization to thuja pollen, another member of the Cupressaceae, being very close to the cypress, which is spreading quickly outside the Midi, also as a hedge tree. After confirming the etiology of this pollinosis, the antigenic and allergenic community between the two species is discussed.

**Patients and Methods***Case History*

*Case 1.* A 65-year-old woman was referred for long-standing rhinitis of imprecise occurrence, without other history except migraine and without family or personal atopic history. She lived in a house that was surrounded by a thuja hedge. In July, the rhinitis was a great nuisance, with a PAREO score of 6 (P for pruritis, A for anosmia, R for rhinorrhea, E for sneezing and O for obstruction). The symptoms abated the following week and ended at the end of July, which was confirmed by the score in October.

*Case 2.* A 36-year-old woman consulted in March for appearance of severe conjunctivitis following acquisition of a dog. All the skin tests, including dog epithelia were negative and Phadiotop<sup>®</sup> was also negative. The patient returned after exacerbation of the conjunctivitis that presented all the characteristics of a pollen conjunctivitis. The skin test was strongly positive to thuja extract. Hedges of thujas surrounded the house. The dog rubbed itself continually against them, before returning to its owner.

*Methods*

*Preparation of the Thuja Extract.* 20 g of thuja pollen (Allerbio, Varennes, France) were extracted with a Coca solution for 12 h at 4°C with stirring and at a weight/volume ratio of 1/5. The extract was then

concentrated 1:2 and then some was mixed with phenolated glycerol to obtain an extract at a final titer of 1/5 (w/v). The remainder of the initial extract was frozen and lyophilized for eventual production of disks used for RAST, for sensitization of rabbits and the various electrophoretic techniques.

**Skin Tests.** Prick tests were made with Allerbio extracts of the main inhalants (*Der pt*, *Der far*, *Penicillium*, *Aspergillus*, *Alternaria*, dog, cat, grass mix, birch, mugwort, plantago, latex, cypress and thuja), in comparison with a solution of phenolated glycerosaline as negative reference and glycerinated 9% solution of codeine phosphate as positive reference. The criterion of a positive test was a wheal diameter of 50% of that produced by the positive reference.

**In vivo Provocation Test.** A single blind nasal provocation test was made by anterior rhinomanometry, after spraying physiological saline solution.

**Nasal Lavage.** Before and 24 h after the nasal provocation test by the usual techniques, nasal secretory eosinophilia was evaluated by a cytometric method [4].

**In vitro Tests.** Measurement of total and specific IgE to the inhalants by the Pharmacia-CAP system RAST® and by MAST CLA® with 36 allergens. The measurement of anti-thuja-specific IgE was made by the RAST technique with Allerbio disks activated by CNBr [3].

**Anti-Thuja Antiserum.** Six white New Zealand rabbits were immunized. Each received an initial subcutaneous injection around the ganglions draining the four limbs of 0.25 ml of extract that contained 0.3 mg/ml total protein, mixed with an equal volume of complete Freund's adjuvant. Hyperimmunization was produced by weekly injection of the extracts mixed with the incomplete adjuvant and antibody titers were checked regularly by a semiquantitative immunodiffusion.

**RAST.** Allerbio RAST was done by the method already described [3] using the thuja extract bound to cellulose disks activated with CNBr and <sup>125</sup>I radiolabelled anti-IgE monoclonal antibody (Immunotech, Marseille, France). The results were expressed in R.U. (arbitrary RAST units) of which the value is very close to that of the international units (IU) as the same antibody is used for measurement of both total and specific IgE and calibrated against the Second WHO International IgE reference.

**SDS-PAGE.** Electrophoresis in gel was made by the technique of Laemmli [5] on a 12.5% gel of polyacrylamide, in a multipore LKB 2001 apparatus. The lyophilized extract was rehydrated in 60 µl distilled water, then 50 µl were mixed with 50 µl of a buffer that contained 2% SDS, 0.0625 M Tris, pH 6.8, 10% glycerol and 0.0001% bromophenol blue. Forty microliters containing 260 µg protein were placed in the wells. Electrophoresis was done for 150 min at 600 V, 60 mA, 30 W then the gel was stained overnight in Coomassie blue or silver salts. The extracts are compared to protein text mixtures (Serva) to evaluate the molecular mass of the components.

**Two-Dimensional Electrophoresis.** This was done by the method of Axelsen et al. [8]. In short, 10 µl of thuja extract were placed in wells cut in the surface of 1% agarose M (LKB) gel. The first migration was made under a voltage drop of 10 V/cm for 1 h at ambient temperature. The second migration of the proteins so separated was done at right angle to the first, into a gel deposited on Gelbond film that contained hyperimmune rabbit antiserum against the thuja or cypress extracts (Allerbio antibodies), diluted after the results of immunodiffusions, at a voltage drop of 2 V/cm for 18 h at 4°C. The gel so obtained was then stained with Coomassie blue to disclose the precipitation arcs that corresponded with the antigens recognized by the rabbit serum.

**Two-Dimensional Radioelectrophoresis.** This was done by the method of Axelsen et al. [8] under the following conditions. The first phase was identical with that for crossed immunoelectrophoresis, but without staining, then incubation of the dried gels with patient sera diluted in a phosphate buffer (0.05 M, pH 8.0) and incubation for 18 h at ambient temperature in a closed box of appropriate size. After three washes the gels were recovered for 18 h at ambient temperature with 10 ml of monoclonal anti-IgE radiolabelled with <sup>125</sup>I (Immunotech) diluted in phosphate buffer (0.1 M, 9% NaCl, 1% Tween) to obtain 8,000 cpm per plate. Finally, after new washings with the same buffer, the plates were dried and placed in contact with Kodak XOMat AR film for 5 days at -70°C, before being stained in parallel with the development of the film. If films did not show visible arcs, the plates were re-exposed for a further 5 days. No measure of radioactivity of the different arcs were made.

## Results

For the 1st patient, all the prick tests to the various pneumoallergens were negative in July, then in October. Only those with Cupressaceae pollen were positive, weak for cypress (d = 2 mm versus 4 mm for positive reference) but strong for thuja (d = 9 mm). These first results were confirmed in October during the course of a check.

The basal nasal state was normal, the PAREO score being 0. The nasal provocation was negative after spraying the physiological saline, with a resistance of 0.20 Pa/cm<sup>3</sup>/s. After 3–4 min, the instillation of thuja pollen extract produced a pruritus with rhinorrhea and nasal obstruction which improved after 6–7 min. These symptoms were accompanied by a doubling of the resistance at 10 min. It returned to normal after 30 min. Results of nasal lavage showed a basal eosinophilia of 58% (out of 28,000 leukocytes) which remained unchanged 24 h after the provocation test. The titer of total IgE was 392 I.U./ml. MAST CLA® was negative. The cypress RAST titre was 3.5 U.R. (class 2) and thuja 14.5 U.R. (class 3).

For the 2nd patient, all the skin tests to airborne allergens were negative except for thuja (d = 12 mm). Total IgE was very low with a titer of 4.5 I.U./ml, and RAST were negative for all allergens, including cypress and thuja.

Four sera from patients not allergic to thuja were tested and the results were negative with our RAST thuja disks. Patients considered as negative control were tested in skin test and no positive reaction was observed.

In SDS-PAGE (fig. 1) the cypress and thuja extracts showed three main bands at the same level, with the molecular weights around 15, 30 and 40 kD. Two-dimensional electrophoresis of the thuja extract against an anti-thuja rabbit serum created two precipitation arcs, more visible on the crossed radioimmunoelectrophoresis film after incubation

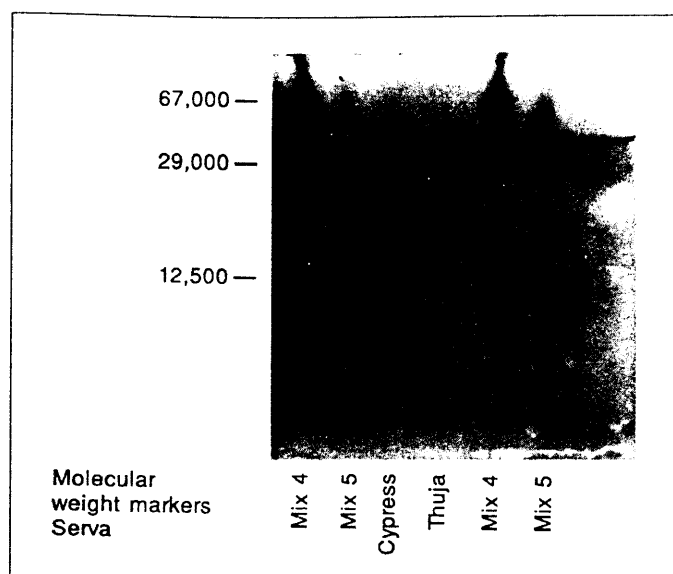


**Fig. 1.** SDS-PAGE of the cypress and thuja extracts.

with the serum from the 1st patient (fig. 2a). A second migration of the thuja extract in two superimposed gels that contained the rabbit antisera anti-thuja and anti-cypress showed a continuous arc of precipitation, which confirmed a common antigenicity between the two extracts (fig. 2b).

## Discussion

Presently pollinoses to Cupressaceae are well established with *Cupressus sempervirens* and *Juniper communis* in the Mediterranean countries and in Australia, *Juniperus ashei* in the USA and with *Cryptomeria japonica* in Japan. To our knowledge, no study has shown a possible involvement of thuja in Europe. This species is increasing in number progressively in the North of France as a hedge tree, without reaching the density of cypresses in the South. It pollinates in February-March in parallel with the first Betulaceae, before birch, which precedes the first junipers. The inhaled Cupressaceae pollen doses north of the Loire are, depending on the region, 10–100 times lower than in the South [9]. As there are no cypresses, they originate from thujas. They may be higher locally where thuja hedges are extending, and thuja has to be included in the possible species to consider for a rhinoconjunctivitis at the end of the winter. These pollinosis appear in older subjects in whom the total IgE is normal or low [7] and specific IgE is not measurable [10]. The natural spreading in the atmosphere

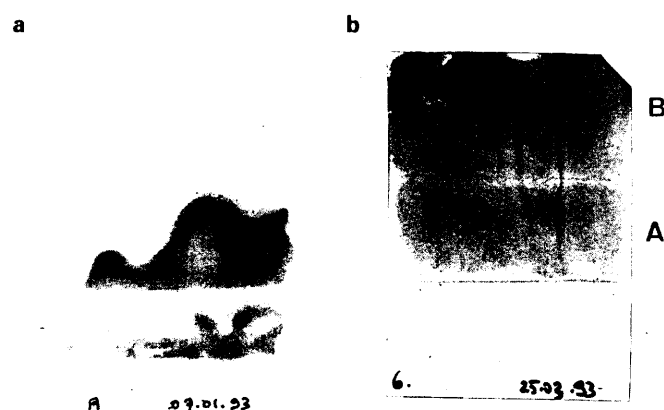


**Fig. 2.** Crossed radioimmuno-electrophoresis. **a** Thuja extract against rabbit anti-thuja serum. **b** Thuja extract against rabbit anti-thuja serum (A) and against rabbit anti-cypress serum (B).

could be low, but the possibility of natural vectors, such as the furs of domestic animals, as in the 2nd case, is worth being considered. The low IgE binding protein level and the viscosity of the extracts of Cupressaceae did not permit identification of the 17 IgE binding proteins in cypresses that have been shown by other manipulations. In the meantime the main band we have shown to be 40 kD could correspond to the major allergen defined as having a molecular mass of 42 kD and representing almost the total allergenic potency of the extract; we have shown in crossed radioimmuno-electrophoresis a cross-antigenicity between cypress and thuja by at least one dominant antigen [2]. In a few reports investigating the cross-reactivity in the Cupressaceae family, skin tests to *C. sempervirens* correlate well with skin tests to *J. communis* in 80% of the cases. The correlation would be lower (60%) with thuja orientalis [11]. A recent study proved a cross-reactivity between the two Cupressaceae *Callistris glaucophylla* and *C. sempervirens* [12]. In conclusion, crossed allergenicity between the various species of Cupressaceae should be considered for pollinosis found in February to May in the populations living in the North of Europe.

## References

- 1 Charpin D, Hugues B, Mallea M, et al: L'allergie au cyprès. *Rev Fr Allergol* 1990;30:21-26.
- 2 Ford SA, Baldo BA, Panzani R, Bass D: Cypress (*Cupressus sempervirens*) pollen allergens: Identification by protein blotting and improved detection of specific IgE antibodies. *Int Arch Allergy Appl Immunol* 1991;95:178-183.
- 3 Grassi J, Didierlaurent A, Stadler BM: Quantitative determination of total and specific IgE with the use of monoclonal antibodies. *J Allergy Clin Immunol* 1986;77:808-822.
- 4 Jankowski R, Foliguet B, Moneret-Vautrin DA, de Sousa Vieira A, Bounaas B, Wayoff M: Cytométrie de l'éosinophile sécrétoire nasale. *Ann Otolaryngol of Chir Cervicofac* 1992;109:47-51.
- 5 Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;227:680-685.
- 6 Panzani R, Centanni G, Brunel M: Increase of respiratory allergy to the pollens of cypresses in the south of France. *Ann Allergy* 1986;56:460-463.
- 7 Bousquet J, Knani J, Hedjjaoui A, Ferrando R, Cour P, Dhivert H, Michel FB: Heterogeneity of atopy. I. Clinical and immunological characteristics of patients allergic to cypress pollen. *Allergy* 1993;48:183-188.
- 8 Axelsen NH, Kroll J, Weeke B: Quantitative immunoelectrophoresis. *Scand J Immunol* 1973;2(suppl 1):manual.
- 9 Sell Y, Cour P, Evrard J, Guerin F: Cypress; in Guerin B (ed): *Pollen et allergie*. Varennes, Allerbio, 1993, p 32.
- 10 Spinozzi F, Cimignoli E, Grignani F: Immunotherapy: What are we administering to the allergic patient? *Lancet* 1993;341:386.
- 11 Panzani R, Zerboni R, Ariano R: Allergenic significance of Cupressaceae pollen in some parts of the Mediterranean area; in D'Amato G, Frits Th, Spiekma M, Bonini S (eds): *Allergenic Pollen and Pollinosis in Europe*. London, Blackwell Scientific, 1991, pp 81-86.
- 12 Pham NH, Baldo BA, Bass DJ: Cypress pollen allergy. Identification of allergens and cross reactivity between divergent species. *Clinical and experimental allergy*. 1994;24:558-565.