

Heterogeneity of atopy. I. Clinical and immunologic characteristics of patients allergic to cypress pollen

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The heterogeneity of pollen-allergic persons is well known but poorly characterized. Cypress is one of the major pollen-producing plants of the Mediterranean area. A study was undertaken to characterize the symptoms presented by patients allergic to cypress pollen and the heterogeneity of the IgE immune response between patients allergic only to cypress pollen and those who are polysensitized. Eighty-nine patients allergic to cypress pollen were studied, 26 being allergic only to cypress pollen. The IgE response was assessed by skin prick tests and the titration of serum total IgE and cypress-specific IgE by RAST. Clinical reactivity was assessed by symptom scores during the cypress pollen season and skin tests. Pollen counts were obtained. The clinical reactivity was similar in both patient groups. Rhinitis was present in all patients, conjunctivitis in 73.7–88.5%, and asthma in only 7.4–19.2%. The age of onset of symptoms caused by cypress pollen allergy was significantly greater in patients allergic to cypress pollen only. Total serum IgE was within the normal range in the cypress pollen group and significantly lower than in the polysensitized groups. Cypress pollen RAST was higher in the polysensitized group. We concluded that conjunctivitis is particularly common in cypress pollen allergy. Patients allergic only to cypress pollen may be unique in their way of expressing serum total IgE levels.

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The term “atopy” was introduced in 1923 by Coca & Cooke (9), but it has acquired various meanings. Broadly, it is a group of allergic diseases having some characteristics in common and associated with a genetic predisposition to develop an IgE immune response to low concentrations of common environmental allergens as a result of natural exposure (23). There are at least two main genetic mechanisms involved in the atopic status: the specific immune response genes associated with the histocompatibility complex (16–18), and a second genetic control regulating the IgE serum levels (high and low “responders”) not associated with this complex (1, 10). The heterogeneity of the IgE-mediated immune response to grass pollen has been observed by means of IgE-immunoblots (7, 13, 22). When compared with patients sensitized to grass pollen only, polysensitized patients had significantly increased levels of serum total and specific IgE and greater heterogeneity of IgE immunoblots, suggesting an enhanced qualitative and quantitative IgE immune response (7).

Members of the Cupressaceae family are important pollen-producing trees in the Mediterranean area (*Cupressus sempervirens* [common cypress] and *Juniperus communis* [common juniper] (2, 8, 12, 21, 26), North America (*Juniperus ashei* [mountain cedar] (24, 25), and Japan (*Cryptomeria japonica* [Japanese cedar]) (20). The amount of Cupressaceae pollen released in the atmosphere is often far greater than that of grass pollen, and the incidence of allergy to Cupressaceae pollen has probably increased dramatically within the past three decades because of interaction with air pollution (14). In the United States, it has been shown that patients allergic to mountain cedar have a heterogeneous IgE immune response, but it is not known whether patients allergic only to *Cupressus sempervirens* pollen and those also allergic to many allergen species, including cypress pollen, react similarly in terms of IgE immune response and clinical status.

A study was undertaken in 89 unselected patients allergic to cypress pollen attending the allergy unit of Montpellier University Hospital, in order to char-

acterize their symptoms during the pollen season and their immune reactivity, to assess symptoms elicited by cypress pollen, and to determine whether there is heterogeneity of the IgE immune response in patients allergic only to cypress pollen and in those allergic to cypress pollen and other allergen species.

Material and methods

Patients

Eighty-nine patients (48 men, aged 10–70 years) were studied after we had obtained their informed consent. They were selected from among all patients that consecutively attended the clinic on the following criteria:

- All patients had spent most or all of their life in the Montpellier area.
- All patients had rhinitis between January and March. The duration of symptoms ranged from 1 to 37 years.
- All patients had a positive prick test to a 1/10 (w/v) cypress (*Cupressus sempervirens*) pollen extract (Stallergènes Laboratories, Fresnes, France).
- None of the patients had received any form of specific immunotherapy to pollen extracts.
- Patients allergic to ash and *Phillyrea* pollen (*Oleaceae* species pollinating in the winter) had been screened to avoid difficulties in the diagnosis of cypress pollen allergy.

Thirty-seven nonallergic persons (17 men, aged 15–60 years) were used as a control group. None had any history of allergic diseases, and the skin prick tests used with the battery of allergens in our clinic were negative.

Characterization of patient sensitivity to allergens

An investigation of the patients' sensitivity to a variety of pollen species, chosen as a result of pollen counts in the Montpellier area (23), was performed.

Skin tests. Standardized extracts from house-dust mites, cat and dog dander, the molds *Alternaria* and *Cladosporium*, and pollen of the flowering plants orchard grass (*Dactylis glomerata*), olive (*Olea europaea*), plane tree (*Platanus occidentalis*), mugwort (*Artemisia vulgaris*), and *Parietaria officinalis* were prepared, according to the proposals of the Allergen Subcommittee of the International Union of Immunologic Societies, by the Laboratoires des Stallergènes (Fresnes, France). The preparation of the extracts has been described previously (4). The freeze-dried extracts were stored at 4°C. These extracts were used for skin tests.

Nonstandardized extracts (1/20 w/v) were prepared by the same extraction methods for cypress (*Cupressus sempervirens*), juniper (*Juniperus*

communis), nettle (*Urtica dioica*), ribwort plantain (*Plantago lanceolata*), pigweed (*Amaranthus pratensis*), goosefoot (*Chenopodium album*), golden rod (*Solidago virgaurea*), beech (*Fagus sylvatica*), alder (*Alnus glutinosa*), walnut (*Juglans regia*), mulberry (*Broussonetia papyrifera*), willow (*Salix nigra*), and common oak (*Quercus robur*). Extracts of ash and privet pollen were not tested because of their cross-reactivity with olive pollen (5).

Modified skin prick tests (SPT) were performed by a method previously described in detail (3). None of the patients had any factor that might have modified the performance of skin tests, and drugs that might have modified the performance of the tests were carefully avoided. All patients were tested with all the pollen extracts listed above, and a 9% codeine phosphate solution was used as a positive control. Mean wheal diameters were measured 15 min after the performance of the test. Skin tests were considered to be positive when allergen-induced wheals were identical with or greater than those of the codeine phosphate solution.

Serum specific IgE. Serum specific IgE was titrated by the Phadebas® RAST (Pharmacia Diagnostics AB, Uppsala, Sweden), following the instructions of the package insert. All patients had a RAST to cypress pollen. Other pollen specificities titrated on undiluted sera included orchard grass, *Parietaria*, olive, and plane tree.

Total serum IgE. Total serum IgE was measured by the Phadebas® PRIST kit (Pharmacia Diagnostics AB, Uppsala, Sweden), according to the package insert. When IgE levels were over 500 kU/l, the serum was diluted 1/3.

Symptom scores. When the patients attended the clinic, they filled in a questionnaire reporting nasal, bronchial, and ocular symptoms with all the medications that had been used. Symptom scores were quoted according to previous studies (6).

Pollen counts. Pollen counts were obtained by the method of Cour (11). The sampler was placed outdoors 2 m above the ground, and counts were available between 1974 and 1988.

Design of the study

Since some patients with cypress pollen allergy show a decreased skin reactivity to cypress pollen allergens just before the pollen season, all subjects were investigated within 3 months after the cypress pollen season. The study was performed over 5 years, 1984–8. Only patients with a clear-cut history of allergic symptoms during the cypress pollen season were studied. Patients were tested by skin prick test

Table 1. Global results

	Cypress	Multiple pollen allergy	Polysensitized	Normal subjects
Number of subjects	26	25	38	37
Age (mean $\pm$ SD yr)				
When studied	43.6 $\pm$ 13.4	33.5 $\pm$ 14.1	33.9 $\pm$ 17.6	30.6 $\pm$ 16.7
Onset of cypress pollen allergy	35.9 $\pm$ 13.7	23.2 $\pm$ 11.4	24.0 $\pm$ 15.5	
Sex (M/F ratio)	42.4	65.5	55.1	45.9
Total IgE (median $\pm$ SD kU/l)	45.0 $\pm$ 133.6	200.7 $\pm$ 160.8	321.1 $\pm$ 741.3	25.1 $\pm$ 36.4
Skin tests				
Codeine phosphate (m $\pm$ SD)	3.5 $\pm$ 1.1	3.8 $\pm$ 1.3	3.6 $\pm$ 1.1	3.5 $\pm$ 1.2
Cypress allergen (m $\pm$ SD)	3.6 $\pm$ 1.7	3.6 $\pm$ 1.3	3.5 $\pm$ 1.1	0
Specific IgE (median $\pm$ SD PRU/ml)*	1.08 $\pm$ 1.41	2.92 $\pm$ 4.1	2.79 $\pm$ 2.2	ND
Negative RAST	11	6	12	
Symptoms				
Rhinitis (%)	100.0	100.0	100.0	0
Conjunctivitis (%)	88.5	76.0	73.7	0
Asthma (%)	19.2	7.4	18.4	0

* Patients with positive RAST.

for all allergen species listed above and placed in the cypress pollen group when symptoms occurred only during the cypress pollen season and the skin prick test was positive for cypress pollen extract only. They were placed in the multiple pollen group when symptoms were also present during the grass or weed pollen season, and skin tests and RAST were positive for other pollen species. Patients were placed in the polysensitized group when perennial symptoms increased during the cypress pollen season and skin tests were positive for other allergen species. All patients underwent the same investigations, including measurement of serum total IgE and cypress-pollen-specific IgE and clinical investigations.

Statistical analysis was performed by means of nonparametric tests.

Results

Demographic characteristics of the subjects

Demographic characteristics of the subjects are presented in Table 1. The mean ages of polysensitized patients, patients allergic to multiple pollen species, and normal subjects were similar, ranging from 30.6 to 33.9 years. On the other hand, patients monosensitized only to cypress pollen were significantly older (43.6 $\pm$ 13.4 years, $P<0.02$, Mann-Whitney U test). The sex ratio ranged from 42.4% of males in the cypress pollen group to 65.5% males in the multiple pollen group.

Clinical characteristics of the patients

All patients presented nasal symptoms during the pollen season (Table 1). Conjunctivitis was often severe and was present in 73.7–88.5% of patients. Asthma, on the other hand, was never severe and was present in only 7.4–19.2% of patients. The age

of onset of allergic symptoms during the cypress pollen season was significantly ($P<0.005$, Mann-Whitney U test) greater in patients allergic only to cypress pollen (35.9 $\pm$ 13.7 years) than in the other two groups (23.2 $\pm$ 11.4 and 24.0 $\pm$ 15.5 years, respectively) (Fig. 1). Five patients allergic only to cypress pollen and three polysensitized patients developed cypress-pollen-related symptoms after the age of 50 years.

Skin prick tests with codeine phosphate or cy-

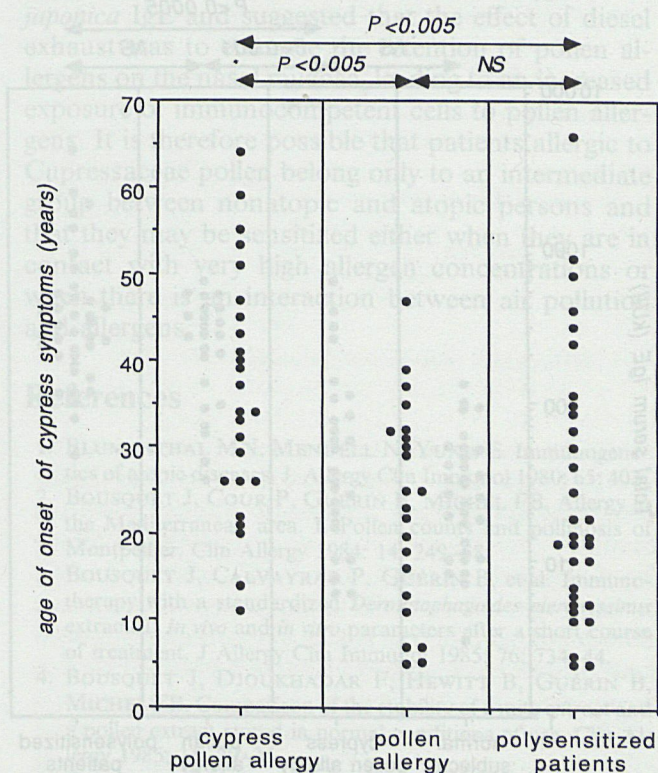


Fig. 1. Age of patients. Statistical analysis by Mann-Whitney U test.

press pollen extract were similar in all three groups of patients.

Serum IgE of the patients

There was a significant increase in IgE levels from the normal subjects to the polysensitized patients ($P < 0.0001$, Kruskal-Wallis test) (Table 1 and Fig. 2). More specifically, median levels of serum total IgE were slightly and nonsignificantly increased in the cypress pollen group, as compared with normal nonallergic subjects. On the other hand, patients allergic to multiple pollen species or polysensitized had a significantly higher median level of IgE than patients allergic to cypress pollen only ($P < 0.001$ and $P < 0.0005$, respectively; Mann-Whitney U test).

Serum cypress-pollen-specific IgE was detectable in 57.6% of patients allergic to cypress pollen only, 78% of pollen-sensitized patients, and 68.4% of polysensitized patients. The higher RAST value was 8.2 PRU/ml. In patients with a positive RAST, the level of IgE was significantly ($P < 0.05$, Mann-Whitney U test) lower in the cypress pollen group.

Pollen counts

Cypress and grass pollen counts are presented in Figs. 3 and 4. Cypress pollinates between January

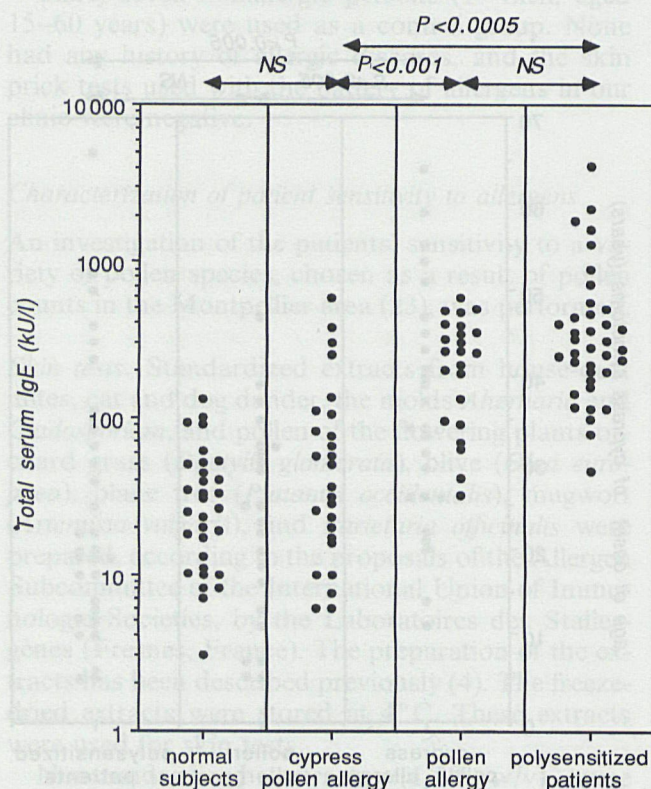


Fig. 2. Total serum IgE levels in the four groups of patients. Statistical analysis by Mann-Whitney U test.

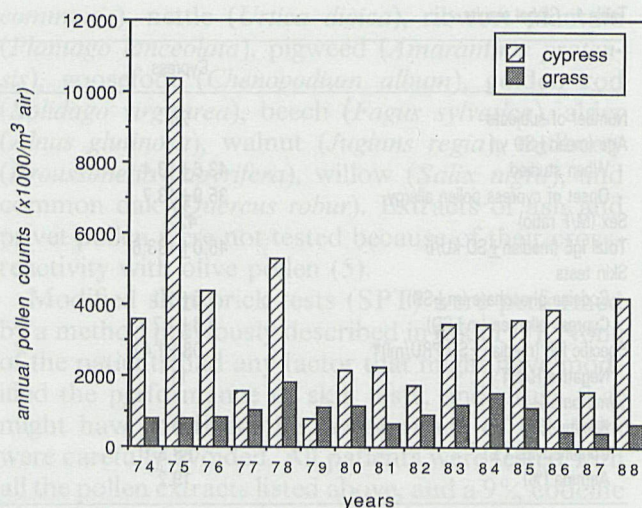


Fig. 3. Annual counts of Cupressaceae and grass pollen.

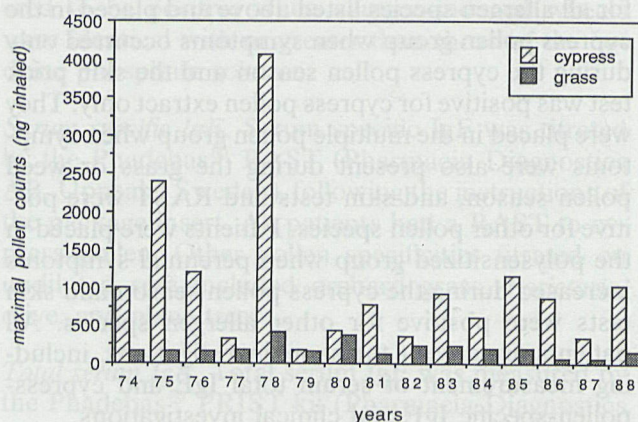


Fig. 4. Maximal weekly counts of Cupressaceae and grass pollen.

and March. It can be seen that the greatest counts of cypress pollen were 1.2–12.3-fold greater than those of grass pollen. The annual counts of cypress pollen were 1.9–13.3-fold greater than those of grass pollen.

Discussion

The results of this study indicate that patients sensitized to cypress pollen present similar clinical features during the cypress pollen season whether or not they are allergic to other allergen species. They present a high rate of conjunctivitis and a low rate of asthma. Patients allergic only to cypress pollen are immunologically different from those allergic to multiple pollen species or multiple allergen species. They have a normal or subnormal total serum IgE level and a low RAST titer, and, as a mean, they develop symptoms 10 years after those who are allergic to other allergen species.

The diagnosis of cypress pollen allergy is easy in the northern Mediterranean area since these trees pollinate in winter when usually no other plant is blooming. Ash and *Phillyrea* are Oleaceae species pollinating in winter (2), but their pollen season is distinct enough from that of cypress to allow us to ascertain the diagnosis of cypress pollen allergy. The methods of skin tests and symptom scores performed in this experiment have been widely used in our clinic. However, cypress pollen extracts are not available in a standardized form for the following reasons: 1) it is difficult to find appropriate sera with a high titer in specific IgE levels, making RAST-inhibition experiments difficult to perform; 2) the amounts of proteins released by the pollen grains are low; and 3) it is not possible to increase the strength of the extract because of the physicochemical properties of the cypress pollen grain (Guérin B, personal communication). Thus, *Cupressus sempervirens* pollen extracts are usually of low potency. Moreover, in our area, many patients with clear-cut cypress pollen allergy have low or negative cypress pollen IgE in their serum, the level of specific IgE decreases at times distant from the pollen season, and the performance of IgE immunoprints is almost impossible. These considerations do not apply to *Cryptomeria japonica* pollen, since RAST titers may be very high and IgE immunoprints can be performed (11). Moreover, in areas, such as southern Spain, where cypress pollen counts are higher than in the northern Mediterranean area, RAST titers may be higher. For these reasons, we performed the study purposely within 3 months after the pollen season, but the wheals of the skin prick tests were usually small, and only two-thirds of patients with a clear-cut history of cypress pollen allergy and positive skin prick tests had a positive RAST.

Patients allergic to cypress pollen have a similar clinical reactivity whether or not they are sensitized to other allergens. The incidence of conjunctivitis is very high, ranging from 73.7 to 88.5%. This is unusually high since, in the present area, grass-pollen-allergy-induced conjunctivitis is present in 50–60% of the patients (2). Moreover, conjunctivitis caused by cypress pollen is usually more severe. These differences may be due to the very high cypress pollen counts being counterbalanced by low allergenicity. On the other hand, the incidence of asthma is low in patients allergic to cypress pollen (7.4–19.2%), as compared with those allergic to grass pollen (2), and, these differences cannot be explained by the size of cypress or grass pollen counts.

Patients allergic to cypress pollen are heterogeneous in terms of immune reactivity. As compared with polysensitized patients, those allergic only to cypress pollen have normal or subnormal total serum IgE levels and lower serum specific IgE, and develop

symptoms during the cypress pollen season at an older age. This contrasts with the natural history of pollen-allergic patients of the northern Mediterranean area, in which most grass-pollen-allergic persons develop symptoms at 20 years of age. The differences between grass- and cypress-pollen-allergic patients may be related to the very high amounts of cypress pollen released in the atmosphere, and it is possible that patients allergic to cypress pollen are not atopic in essence but become sensitized as a result of the very high concentration of allergens in the atmosphere. Our data accord with those observed with another Cupressaceae pollen, mountain cedar, concerning which Ramirez (24) and Reid et al. (25) suggest that patients allergic only to mountain cedar pollen are not atopic. Thus, this study seems to indicate that Cupressaceae pollen allergy differs from allergy to pollen of other plant families. The sensitization of persons that might not have developed an IgE sensitization with low pollen counts may be due either to very high pollen counts or to interactions between allergens and pollution. It was observed in Japanese studies on *Cryptomeria japonica*, another member of the Cupressaceae family, that the incidence of allergy to pollen of this species has increased greatly within the past 20–30 years (20) and that this was related to pollution from automobile exhausts. Using animal experiments, Muranaka et al. (19) showed that diesel exhaust was a potent adjuvant for the synthesis of *Cryptomeria japonica* IgE and suggested that the effect of diesel exhaust was to enhance the retention of pollen allergens on the nasal mucosa, leading to an increased exposure of immunocompetent cells to pollen allergens. It is therefore possible that patients allergic to Cupressaceae pollen belong only to an intermediate group between nonatopic and atopic persons and that they may be sensitized either when they are in contact with very high allergen concentrations or when there is an interaction between air pollution and allergens.

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