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## CROSS-REACTIVITY BETWEEN THE POLLENS OF *CUPRESSUS SEMPERVIRENS* (COMMON CYPRESS) AND OF *CRYPTOMERIA JAPONICA* (JAPANESE CEDAR)

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*A clinical and immunologic study was performed comparing a group of French patients allergic to the pollens of cypress (Cupressus sempervirens, which belongs to the Cupressaceae family) and a group of Japanese patients allergic to the pollens of Sugi (Cryptomeria japonica, which belongs to the Taxodiaceae family). By skin testing, RAST, and RAST inhibition, clear cross-reactivity was detected between the two pollens. No cross-reactivity was detected between the pollens of Cupressus sempervirens and Sugi and the Pinaceae family. In addition, one can speculate that an antigen in Cupressus sempervirens is cross-reactive with SBP, the major allergen of Sugi, suggesting that there is a closer relationship between the Taxodiaceae family and the Cupressaceae family than between these two families and the other families of the gymnosperms. This finding throws new light on the taxonomy of the gymnosperms.*

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### Abbreviations

|          |                                              |
|----------|----------------------------------------------|
| CE,      | crude extract                                |
| CPM (O), | counts per minute using buffer as inhibitor  |
| CPM (X), | counts per minute using antigen as inhibitor |
| CPM (N), | counts per minute using normal serum pool    |
| SBP,     | major allergen of Cryptomeria                |

### Introduction

CROSS-REACTIVITY is a well known biologic phenomenon in the plant and animal kingdoms. Patients allergic to pollens generally react to almost all the species within a given family (eg, grasses, Chenopodiaceae etc . . . ).<sup>1-3</sup> Previously, we have shown cross-reactivity among the species of the Euphorbiaceae, which contain the castor bean and among various arthropods.<sup>4-6</sup>

In plant-pollen or seed-related allergies a cross-reaction is often observed between species of different genera, but these genera are always within a clearly defined botanical family. With insect allergies however, and in particular arthropod allergy, it seems that the classes of Arthropoda (Hexapoda and Arachnida) are more closely related than are the different families of plants.

Cross-reactivity or secondary reactivity is a well-

recognized problem in the diagnosis and treatment of atopic respiratory allergy. Furthermore, a change in botanical nomenclature has altered the naming of many allergenic plants (Figure 1). A study of cross-reactivity can throw new light on plant taxonomy.

A joint study was conducted to further examine the relationship between the allergens from the pollen of *Cryptomeria japonica* (commonly designated "sugi" in Japan)—which belongs to the Taxodiaceae family and is a major allergen in Japan—and the allergens from the pollen of *Cupressus sempervirens*, which we have recently found to be an increasing cause of allergy in France<sup>7</sup> and *Cupressus sempervirens* belongs to the Cupressaceae family.

One of us (Yasueda) has already isolated the major allergen of Cryptomeria, which has been designated SBP.<sup>8,9</sup> We have demonstrated a cross-allergenicity between *Cryptomeria japonica* and *Chamaecyparis obtusa*, a widely pollinating member of the Cupressaceae family in Japan. This is in contrast to the work of Yoo<sup>10</sup> who suggested a lack of cross-reactivity between the Cupressaceae family, the redwood (*Sequoia sempervirens*), and the Japanese cedar.

### Materials and Methods

Lyophilized sera from 40 patients (Table 1) allergic to *Cupressus sempervirens* and with positive skin tests

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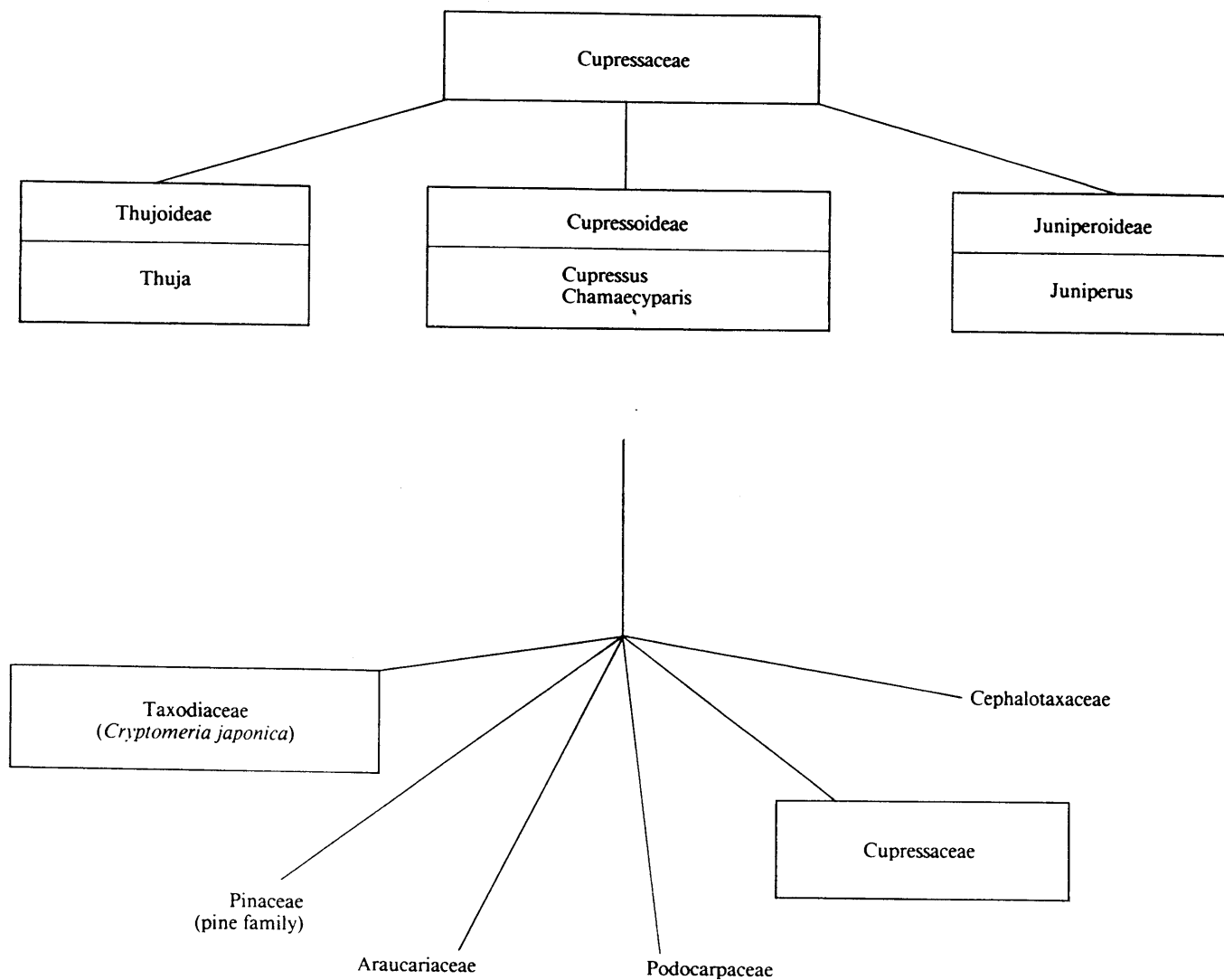


Figure 1. Revised botanical nomenclature. Reproduced from Relations botaniques et réactions croisées immunologiques dans l'allergie aux pollens. (1982; pp 7 and 24) Copyright © 1982 by Pharmacia, France.

Table 1. Clinical Data on 40 French Patients

| Sex | Age      |       |         | Symptoms |                 |                    | Other Airborne Allergens |            |            | Skin Test to <i>Cupressus sempervirens</i> |          |
|-----|----------|-------|---------|----------|-----------------|--------------------|--------------------------|------------|------------|--------------------------------------------|----------|
|     | Under 15 | 15-50 | Over 50 | Asthma   | Con-junctivitis | Al-lergic Rhinitis | Grass Pollens            | Plane Tree | House Dust | Positive                                   | Doubtful |
| F   | 2        | 14    | 4       | 4        | 1               | 15                 | 4                        | 0          | 8          | 18                                         | 2        |
| M   | 2        | 17    | 1       | 2        | 0               | 18                 | 10                       | 2          | 8          | 19                                         | 1        |

Table 2. Results of Skin Testing 30 French Patients with Japanese Cedar Antigens

| Clinical Data |                   | Skin Tests Positive to <i>Cupressus sempervirens</i> | CE       |          | SBP      |          |
|---------------|-------------------|------------------------------------------------------|----------|----------|----------|----------|
| Asthma        | Allergic Rhinitis |                                                      | Positive | Doubtful | Positive | Doubtful |
| 5             | 25                | 30                                                   | 22       | 4        | 19       | 6        |

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Table 3. Results of Skin Testing 13 Japanese Patients

| Allergen                        | Concentration                                      | No of Negative | No of Positive |
|---------------------------------|----------------------------------------------------|----------------|----------------|
| Cupressoideae                   |                                                    |                |                |
| <i>Cupressus arizonica</i> *    | 1:20                                               | 8              | 5              |
| <i>Cupressus macrocarpa</i> *   | 1:20                                               | 8              | 5              |
| <i>Cupressus sempervirens</i> * | 1:20                                               | 9              | 4              |
| <i>Cupressus sempervirens</i> † | 1:20                                               | 7              | 6              |
| <i>Cupressus sempervirens</i> ‡ | 20,000 PNU                                         | 12             | 1              |
| Juniperoideae                   |                                                    |                |                |
| <i>Juniperus sabinoidea</i> *   | 1:20                                               | 8              | 5              |
| <i>Juniperus virginiana</i> *   | 1:20                                               | 6              | 7              |
| <i>Juniperus communis</i> †     | 1:20                                               | 5              | 8              |
| Pinaceae                        |                                                    |                |                |
| <i>Pinus maritima</i> †         | 1:20                                               | 12             | 1              |
| <i>Pseudotsuga taxifolia</i> †  |                                                    | 13             | 0              |
| <i>Abies alba</i> ‡             | 20,000 PNU                                         | 13             | 0              |
| <i>Cedrus libani</i> ‡          | 20,000 PNU                                         | 13             | 0              |
| <i>Pinus sylvestris</i> ‡       | 20,000 PNU                                         | 13             | 0              |
| Thuyoidaeae                     |                                                    |                |                |
| <i>Thuja orientalis</i> ‡       | 20,000 PNU                                         | 12             | 1              |
| Taxodiaceae                     |                                                    |                |                |
| <i>Chamaecyparis obtusa</i> §   | 1:20 (coca)                                        | 6              | 7              |
| <i>Cryptomeria japonica</i> §   | 1:20                                               | 4              | 9              |
| SBP§                            | 20 µg/mL (phenol saline 0.03/ human serum albumin) | 5              | 8              |

\* Berkeley Biologicals (USA).

† Labo des Stallergènes (France).

‡ Labo Farmaceutico Lofarma (Italy).

§ Sugamihara Hospital (Japan).

Table 4. End Point Titre

| No             | <i>Cryptomeria japonica</i> | <i>Chamaecyparis obtusa</i> | <i>Juniperus sabinoidea</i> | <i>Cupressus sempervirens</i> |
|----------------|-----------------------------|-----------------------------|-----------------------------|-------------------------------|
| 1              | 10 <sup>-6.5</sup>          | 10 <sup>-6</sup>            | 10 <sup>-6.5</sup>          | 10 <sup>-5.5</sup>            |
| 2              | 10 <sup>-7</sup>            | 10 <sup>-6</sup>            | 10 <sup>-7</sup>            | 10 <sup>-6</sup>              |
| 3              | 10 <sup>-6</sup>            | 10 <sup>-5</sup>            | 10 <sup>-5.5</sup>          | 10 <sup>-4.5</sup>            |
| 4              | 10 <sup>-7</sup>            | 10 <sup>-6</sup>            | 10 <sup>-6</sup>            | 10 <sup>-5</sup>              |
| 5              | 10 <sup>-6</sup>            | 10 <sup>-5.5</sup>          | 10 <sup>-6</sup>            | 10 <sup>-5</sup>              |
| 6              | 10 <sup>-7</sup>            | 10 <sup>-6</sup>            | 10 <sup>-7</sup>            | 10 <sup>-5.5</sup>            |
| 7              | 10 <sup>-7</sup>            | 10 <sup>-5</sup>            | 10 <sup>-5.5</sup>          | 10 <sup>-4.5</sup>            |
| 8              | 10 <sup>-7</sup>            | 10 <sup>-6.5</sup>          | 10 <sup>-6.5</sup>          | 10 <sup>-5.5</sup>            |
| 9              | 10 <sup>-7</sup>            | 10 <sup>-6.5</sup>          | 10 <sup>-6</sup>            | 10 <sup>-5.5</sup>            |
| 10             | 10 <sup>-7</sup>            | 10 <sup>-5.5</sup>          | 10 <sup>-7</sup>            | 10 <sup>-5</sup>              |
| Geometric mean | 10 <sup>-6.8</sup>          | 10 <sup>-5.8</sup>          | 10 <sup>-6.3</sup>          | 10 <sup>-5.2</sup>            |

to this allergen, were sent by air mail from Marseilles to Japan. The serum samples were reconstituted to their initial volume by addition of distilled water, and were kept at -20 °C during the period of analysis. It has been shown that IgE and IgG are not altered by the process of lyophilization. Samples of CE and SBP of the pollens of *Cryptomeria japonica* were sent for skin testing in 30 French patients allergic to *Cupressus sempervirens*. In these studies, skin testing was done by scratch test and the average diameter of the wheal and flare was recorded in mm. A wheal of more than 5 mm and a flare of more than 10 mm were considered a positive response (Table 2).

Thirteen Japanese patients allergic to *Cryptomeria japonica* were studied to determine their reactivity to

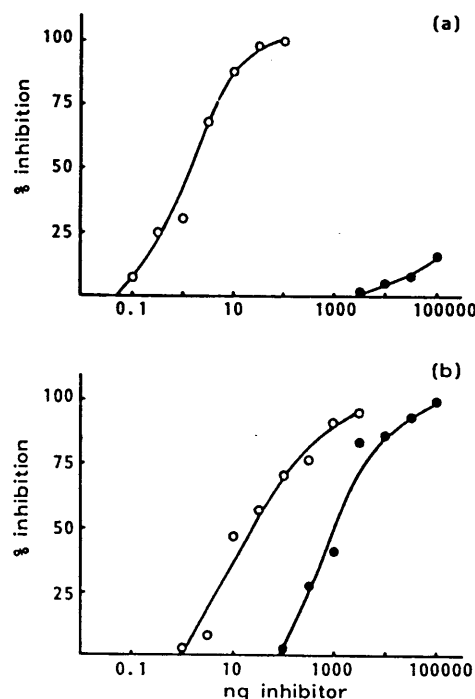


Figure 2. (a) Inhibition of the binding of IgE antibodies found using the Japanese serum pool and (b) inhibition obtained using the French serum pool when using SBP (○) and *Cupressus sempervirens* crude extract (●) and SBP-coupled disks.

different pollen extracts of the Cupressaceae family. The extracts were kindly supplied by different European and American laboratories. In ten more patients, an "end point titre" was determined. In these studies, each pollen was extracted with Coca's solution (1:20), and diluted with phenol saline containing 0.03% (wt/vol) human serum albumin (Table 3).

Intradermal skin tests were carried out by using 0.02 mL of serial 10-fold dilutions of extracts. A wheal of 8 × 8 mm was considered a positive response. Values were extrapolated to the nearest 1/2 log, when the lower dilution gave a strongly positive reaction (Table 4).

RAST assays were carried out as recommended by Pharmacia, the results being expressed as a RAST score. RAST solid phases were prepared by coupling either dialysed or lyophilized crude pollen extracts (2 mg/mL or purified SBP, 100 µg) to cyanogen bromide-activated paper disks.

RAST inhibition experiments were performed as described in a previous paper,<sup>9</sup> and the results were expressed as % inhibition.

$$\% \text{ inhibition} = \frac{\text{CPM (O)} - \text{CPM (X)}}{\text{CPM (O)} - \text{CPM (N)}}$$

CPM (O) and CPM (X) are the counts per minute measured with the use of buffer and antigen preparation as inhibitors, respectively. CPM (N) was measured in a normal serum pool instead of the patient serum pool (Figure 2).

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Table 5. Results of RAST on Sera from 40 French Patients

|                      | <i>Cupressus sempervirens</i> | <i>Juniperus saminoides</i> | <i>Chamaecyparis obtusa</i> | <i>Cryptomeria japonica</i> | Ag SBP |
|----------------------|-------------------------------|-----------------------------|-----------------------------|-----------------------------|--------|
| Phadebas reference A | 1                             | 1                           | 0                           | 0                           | 3      |
| Phadebas reference B | 14                            | 22                          | 9                           | 3                           | 14     |
| Phadebas reference C | 14                            | 10                          | 11                          | 12                          | 13     |
| Phadebas reference D | 3                             | 2                           | 5                           | 5                           | 1      |
| Phadebas negative    | 8                             | 5                           | 15                          | 20                          | 9      |
| Total positive       | 32                            | 35                          | 25                          | 20                          | 31     |

Table 6. Results of RAST on Sera from 13 Japanese Patients

|           | <i>C. sempervirens</i> | <i>J. sabinoides</i> | <i>C. obtusa</i> | <i>C. japonica</i> | Ag SBP |
|-----------|------------------------|----------------------|------------------|--------------------|--------|
| Negative  | 0                      | 0                    | 0                | 0                  | 0      |
| Class I   | 0                      | 0                    | 0                | 0                  | 0      |
| Class II  | 8                      | 7                    | 3                | 1                  | 1      |
| Class III | 4                      | 5                    | 5                | 4                  | 3      |
| Class IV  | 1                      | 1                    | 5                | 8                  | 9      |

## Results

The results of skin tests and RAST assays in both Japanese and French patients are shown in Tables 1 to 6. It can be seen that skin tests, performed by scratch tests on 30 French patients were positive in 73% of cases to CE and 63% of cases to SBP (Table 2). The average diameter of the wheal and flare recorded in mm revealed a greater percentage of positivity with the CE than with the purified antigen of *Cryptomeria japonica*. The results of skin tests performed on Japanese patients using the prick test on 13 patients are shown in Table 3. Employing end point titration on ten patients, the skin tests were positive over a dilution ranging from  $10^{-4}$  to  $10^{-7}$  in all the cases (Table 4). The dilution was higher with *Cryptomeria japonica* than with *Cupressus sempervirens*. In almost all the cases (French and Japanese patients) skin tests to Pinaceae:cedar (*Cedrus libani*) pine tree were negative.

The results of RAST assays on 40 French patients using different extracts (Table 5) indicated positivity to *Cupressus sempervirens* in 32 cases, to *Cryptomeria japonica* in 20 cases, and to SBP in 31 cases. Further, RAST assays were consistently positive against all the extracts (*Cupressus sempervirens*, *Juniperus sabinoides*, *Chamaecyparis obtusa*, *Cryptomeria japonica*, SBP) using the sera from the 13 Japanese patients.

RAST inhibition, a useful tool to study cross-reactivity, was performed on two pools of sera; one being a pool of 15 Sugi pollinosis patients' sera (Japanese pool) and the other a pool of seven French sera (French pool) (Figure 2).

## Discussion

The results of the present studies with the French patients indicate a good correlation between skin tests and RAST using crude extracts. These results are striking since French patients have never been exposed to Sugi pollen.

Skin tests and RAST testing with the Japanese patients using cypress pollen extracts from different

laboratories in Europe and USA also revealed a high prevalence of positivity in both series. As we have already shown in French patients, Pinaceae pollens were negative and this was again demonstrated in the Japanese patients.

In RAST inhibition experiments, the ability of SBP and *Cupressus sempervirens* to inhibit the binding of IgE antibodies to SBP disk was influenced markedly by the use of two types of serum pool. When a Japanese pool was used, small amounts of SBP (1.6 ng) and large amounts of *Cupressus sempervirens* (more than 100 µg) were required for 50% inhibition. On the other hand, when French pool was used, larger amounts of SBP (16 ng) and smaller amounts of *Cupressus sempervirens* (1,000 ng) compared to when the Japanese pool was used, were required for 50% inhibition. These results indicate that IgE antibody has high affinity for SBP in the Japanese serum pool and, conversely, SBP cross-reacting allergen in *Cupressus sempervirens* has a high affinity for IgE antibody in the French pool and low affinity for IgE antibody in the Japanese pool.

From the results of skin testing and RAST we can draw a number of conclusions. (1) An antigen in *Cupressus sempervirens*, which is cross-reactive with SBP, is the major allergen of *Cupressus sempervirens*, ie, the major allergen of Sugi (SBP) and that of *Cupressus sempervirens* (unknown) cross react. This is supported by the strong binding of IgE antibodies in the French sera to SBP discs. (2) Isolation of the major allergen from *Cupressus sempervirens* pollen and RAST and/or RAST inhibition analyses, using the purified major allergen of *Cupressus sempervirens*, are necessary to confirm this speculation. (3) It is suggested by the data obtained up to this time that there is clear cross-allergenicity between the Cupressaceae and Taxodiaceae families.

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## TRUE SCIENCE

"When we begin to base our opinions on medical tact, or inspiration or on more or less vague intuitions about things, we are outside of science and offer an example of that fanciful method which may involve the greatest dangers, by surrendering the health and life of the sick to the whims of an inspired ignoramus. True science teaches us to doubt and, in ignorance, to refrain."

Claude Bernard  
*An Introduction to the Study of  
Experimental Medicine. Pt. I,  
Ch. 2, Sect. vii*