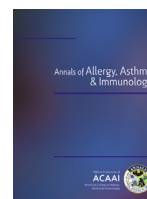




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Letter

Indoor, long-term persistence of cypress pollen allergenic potency: a 10-month study

People living in industrialized countries spend 80% to 95% of their time indoors, and allergic disease triggered by indoor aeroallergens may cause disabilities that affect the quality of life in predisposed patients.¹ It is now well documented that various pollen airborne allergens may occur in the air of the home,² hospitals,³ or work sites,⁴ and a considerable amount of pollen allergens may remain indoors, even after the end of the pollen season.⁵ In many regions worldwide, Cupressaceae trees release a large amount of allergenic pollen grains, some of which may penetrate indoors through ventilation systems, windows, or adherence to clothing.^{5,6} Furthermore, Cupressaceae pollen-derived aerosols may be present in habitats as particles of smaller diameter than pollen grains.⁷ In an indoor environment, areas that are more out of site, such as those below or above certain home furniture, are often miscleaned for a long period. In the present study, we aimed to investigate whether cypress pollen grains that have been placed several months in an indoor environment may preserve their allergenic potency.

Cypress pollen allergic patients were selected on the basis of seasonal symptoms, positive skin prick test results to cypress pollen, and their IgE binding patterns studied by immunoblotting on fresh cypress pollen extract. Serum samples were drawn after obtaining informed consent and pooled according to the 2 reactivity profiles previously described⁸: a first pool included 6 patients' serum samples with a heterogeneous IgE reactivity to high-molecular-weight (HMW; >30 kDa) allergenic glycoproteins, whereas specific IgE of the second set of 6 pooled serum samples mainly bound to a 14-kDa basic protein (BP14).

Pollen grains were freshly collected from *Cupressus sempervirens* trees growing in Southern France. Quadruplicates of 3 g of cypress pollen grains were deposited for 2, 6, 8, and 10 months in gauze-covered petri dishes (10 cm) placed on shelves at a height of 190 cm in a living room in the north of Marseille, France. For pollen protein extraction, 100 mg (1/10 wt/vol) of each pollen sample was incubated overnight under rotation in 1 mL of a 4% sodium dodecyl sulfate solution and centrifuged, and the collected supernatants were stored as aliquots at –20°C until use. Phosphate-buffered saline extracts were prepared as previously described by our group.⁸

Extracted proteins, obtained with similar yield for all samples, were separated by SDS-PAGE (8%–18% gradient polyacrylamide gel; ExcelGel; GE Healthcare, Uppsala, Sweden) as previously reported.⁸ The separated proteins in gel were then transferred to a cyanogen bromide-activated nitrocellulose membrane for immunoblotting,

and another part was stained by silver nitrate for protein detection. After electroblotting, the membrane was dried, blocked, and incubated with pooled sera as previously described.⁸

An inhibition enzyme-linked immunosorbent assay (ELISA) was also performed to quantitatively confirm the immunoblotting experiments. Microtiter plates were coated with a phosphate-buffered saline extract from fresh pollen (FPE; control not submitted to the indoor environment). Then patients' serum pool (1:20 dilution) pre-incubated without or with FPE (homologous inhibition) or the 16 (4 × 4) different indoor-exposed pollen extracts (heterologous inhibition) were applied to the FPE-coated plates. Bound IgEs were detected using an enzyme-conjugated goat anti-human IgE antibody followed by substrate. Heterologous inhibition curves were compared with the homologous inhibition obtained with FPE.

As shown in Figure 1, immunoblot experiments performed using either anti-HMW– or anti-BP14– serum pool revealed no obvious differences between IgE binding patterns to allergen extracts from unexposed fresh and exposed (2, 6, 8, and 10 m) cypress pollen grains to the indoor environment. The few minor qualitative differences observed were not meaningful because they also occurred between quadruplicates belonging to a same exposure group. The persistence of IgE reactivity was observed not only toward HMW allergens, which include the major allergen Cup s 1 at 45 kDa (Fig 1), but also toward a BP14 allergen, a marker of *C sempervirens* sensitization.⁸

ELISA inhibition experiments (not shown) confirmed immunoprint experiments. All experimental series (2, 6, 8, and 10 months of exposure to indoor environment) exhibited overlapping inhibition curves, and inhibition values were similar (±15%) to those obtained in the homologous inhibition using FPE.

In consequence, using ELISA, a quantitative technique measuring a global IgE reactivity, and immunoblot, a semiquantitative technique evaluating IgE reactivity to separated allergens, we found that a fresh pollen extract and an up to 10-month home-stored pollen could not be differentiated on the basis of the quantity and quality of the collection of IgE reactive allergens. Therefore, these home-stored pollen grains might potentially trigger the same allergenic symptoms than the fresh one when in contact with patient's mucosa.

In some regions cypress pollen grains could be detected in house dust sedimentations practically year-round. The present findings demonstrate that there is no change in the in vitro allergenic potency of *C sempervirens* pollen grains left for 10 months in an indoor environment representative of a home site. During this period, approximately from one pollinating season to another, the pollen was submitted to temperature, light, and humidity variations found in a dwelling house (ie, in uncontrolled conditions of storage). Ariano et al⁹ reported that *Cupressus arizonica* pollen grains stored at room temperature in paper envelopes in a department at a university conserve their allergenic properties despite a loss of

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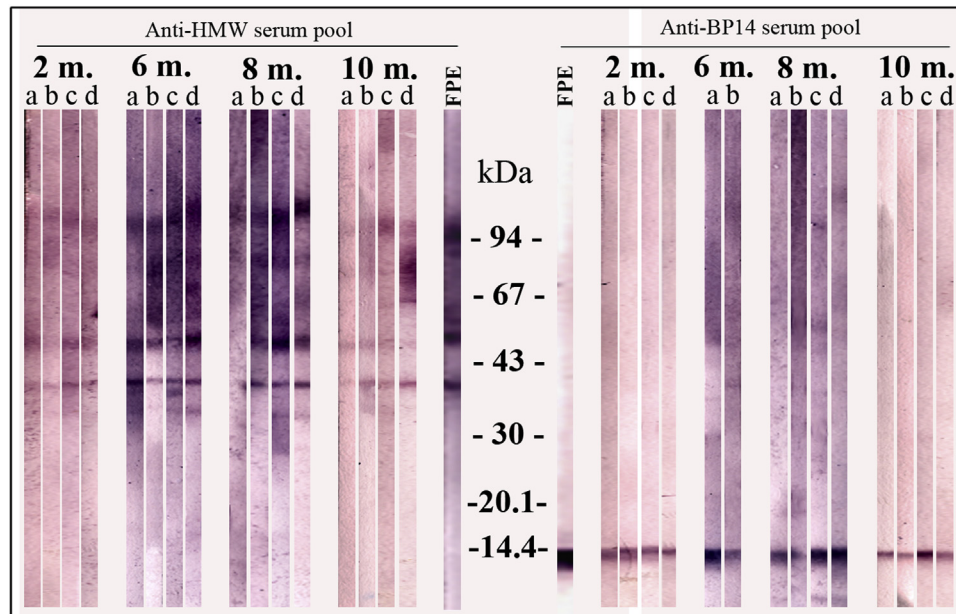


Figure 1. IgE immunoblots of quadruplicates (a, b, c, d) of 4 home-stored cypress pollen sodium dodecyl sulfate extracts separated by an 8% to 18% sodium dodecyl sulfate–polyacrylamide gel electrophoresis. 2 m., 6 m., 8 m., and 10 m. indicate months of indoor exposure. Strips were incubated with either an anti–high-molecular-weight (HMW) serum pool or an anti-BP14 serum pool as indicated; the 6-month sample was performed in duplicate. FPE indicates fresh pollen extract (control extract not submitted to indoor exposure). Relative molecular masses in kilodaltons are indicated.

their viability. This finding means that exposure of individuals to cypress pollen allergens may continue indoors even after the end of pollen season in agreement with the pollen counts that were in situ measured (data not shown). These results provide further support for the observation that some patients sensitized to a single pollen species have perennial symptoms.¹⁰ Complementary experimental investigation is required to determine whether indoor air pollution (25%–62% greater than outside levels; <http://www.arb.ca.gov/>) could interact with cypress pollen allergens and might exacerbate the respiratory diseases as demonstrated for other inhaled allergens.¹

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