

Existence of exine-free airborne allergen particles of Japanese cedar (*Cryptomeria japonica*) pollen

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We investigated whether exine-free pollen allergen particles exist together with the intact pollen grains of Japanese cedar (*Cryptomeria japonica*) in the air during the pollen season in Yamagata City. First, we separated the allergen particles in an Andersen multi-stage air-sampler according to their aerodynamic diameters. The amount of major allergen (*Cry j* I) on each stage of the sampler was determined by a sensitive fluorometric sandwich ELISA, and the pollen count of the same samples was done by light microscopy after Carberla staining. *Cry j* I was found in stages 1 to 6, whereas most of intact and ruptured pollen grains were microscopically observed only in stages 1 and 2. Second, we suctioned the air through a tandem membrane filter system (the first filter, Nuclepore filter with 5 μ m-pores; and the second, Millipore filter with 0.3 μ m-pores). None of the pollen grains was detectable on the 0.3 μ m-pore filter with light microscopy. However, *Cry j* I was detectable in the aqueous extract from the second filter. From these results, we concluded that pollen-free *Cry j* I existed in the air of Yamagata City during the pollen season.

Key words: airborne allergen; *Cryptomeria japonica*; exine-free allergen; Japanese cedar; pollen.

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Japanese cedar (*Cryptomeria japonica*, Cj) pollinosis is one of the most common allergic diseases in Japan. The incidence of Cj pollinosis has increased in recent years (5); Ishizaki et al. (6) reported that, in a densely Cj-cultivated area, 9.6 % of the population were suffering from that disease.

In addition to pollinosis, some researchers have reported that bronchial asthma is also caused by pollen allergens of various species, e.g. Cj (4, 11), birch (8), ragweed (1, 3), rye-grass (12). It is suggested that the bronchial asthma is caused by submicronic and/or paucimicronic form of pollen allergens in ragweed and rye-grass. In this paper, we investigated whether there are airborne Cj pollen allergens of smaller size than that of intact pollen grains.

MATERIAL AND METHODS

Air sampling

Air samples were collected by an Andersen multi-stage air sampler (Model AV-100, Tokyo Dylec Co., Tokyo), and by a sampler with a tandem filter system which was composed of a 5 μ m-pore capillary filter (Nuclepore filter; Nuclepore Corporation, Pleasanton CA, USA) and a 0.3 μ m-pore Millipore filter with diameters of 47 mm. These samplers were placed 20 m above ground level in the central part of Yamagata City, (located in northern Japan and situated at 38° 14' 50" latitude north during the Cj pollen season from early March to mid-April, 1990.

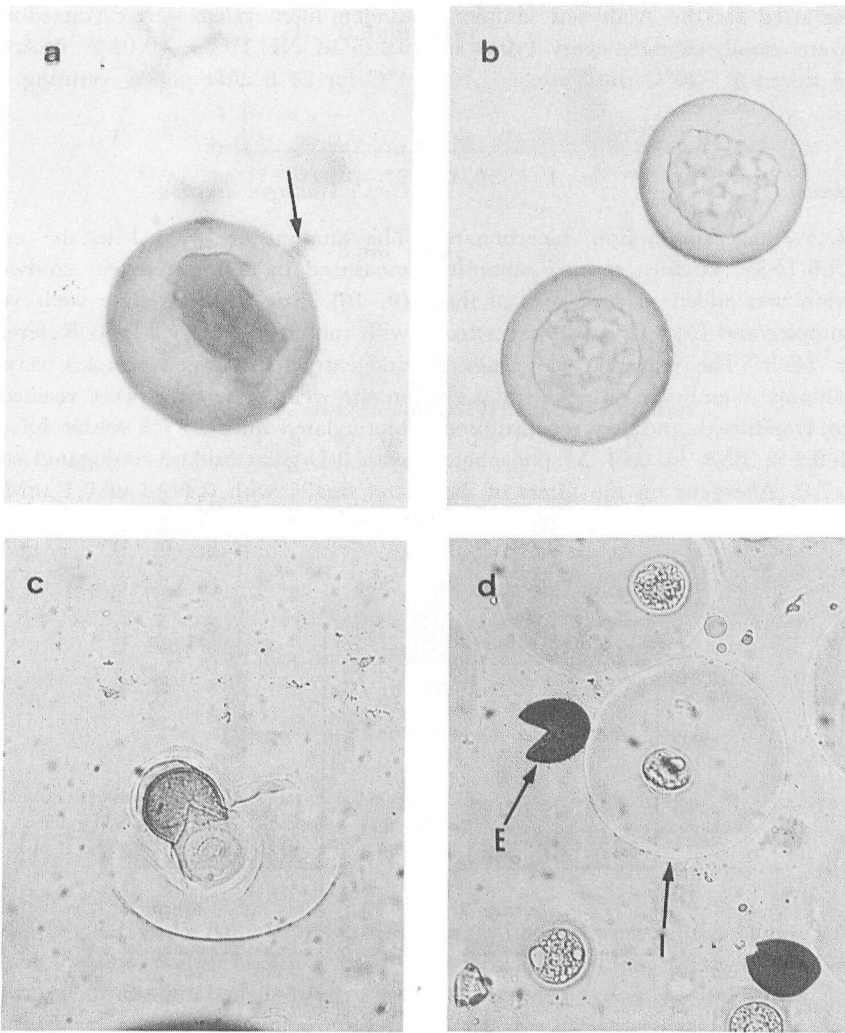


Fig. 1. Morphology of Japanese cedar and False-cypress pollens.

a) *Cj* pollen stained with Carberla solution. Papilla, indicated with arrow, is the typical feature of this pollen. b) *Chamaecyparis obtusa* (False-cypress) pollen. Papilla does not exist in this pollen. c) When *Cj* pollen was extracted with NH_4HCO_3 solution, exine and intine were separated. Figure shows a pollen at the moment of separation. d) Broken exines (E) were stained pink with Carberla solution, but swollen intine (I) particles were not stained.

The Andersen sampler was composed of six stages: according to the manufacturer, airborne particles are separated into $> 7 \mu\text{m}$, $7\text{--}4.7 \mu\text{m}$, $4.7\text{--}3.3 \mu\text{m}$, $3.3\text{--}2.1 \mu\text{m}$, $2.1\text{--}1.1 \mu\text{m}$ and $1.1\text{--}0.65 \mu\text{m}$ size groups. Suction was provided by a low volume air pump (Model L-30, Shibata Scientific Technology Ltd, Tokyo), calibrated to draw 27 ml/min, and the airborne particles

were collected in 27 ml of distilled water in a sampling dish placed at each of the stages. The water volume in the dish was adjusted every 24 h to 27 ml with distilled water to compensate evaporation, and the dishes were changed every 48 to 72 h.

Suction of the tandem filter system was provided by the same pump and under the same

conditions as used for the Andersen sampler. The filters were usually changed every 2 days at 9 a.m., and stored at -20°C until use.

Allergen extraction

Two-ml of 1.7 M ammonium bicarbonate (NH_4HCO_3)-0.14 % bovine serum albumin (BSA) solution was added to each dish of the Andersen sampler and *Cry j* I (7) was extracted at 4°C for 24 h. The extracts were passed through Millipore membrane filters with 0.45 μm -pore size, lyophilised, and then reconstituted in 2 ml of 0.1 % BSA in 0.01 M phosphate buffer, pH 7.0. Allergens on the filters of the

tandem filter system were extracted with 1 ml of 0.125 M NH_4HCO_3 -0.01 % BSA solution at 4°C for 24 h after pollen counting.

Cry j I allergen detection

The amount of *Cry j* I in the extracts was measured by a fluorometric sandwich ELISA (9, 10). Briefly, microplate wells were coated with rabbit anti-*Cry j* I IgG. Reference *Cry j* I and extracted allergen samples were incubated in the wells. The wells were reacted first with biotinylated anti-*Cry j* I rabbit IgG, and then with β -D-galactosidase-conjugated streptavidin, and finally with 0.1 ml of 0.1 mM 4-methyl-

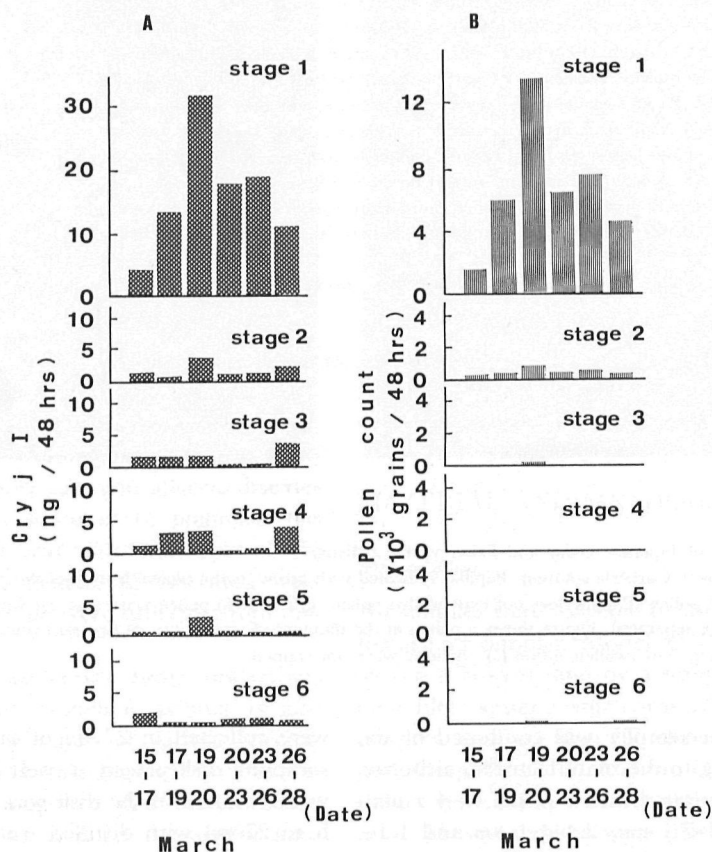


Fig. 2. Pollen count and the amount of *Cry j* I in each stage of an Andersen air sampler during the *Cj* pollen season in Yamagata City, 1990. *Cry j* I in each stage of an Andersen air sampler was immunochemically quantified (A), and the number of *Cj* pollen were counted by $100\times$ light microscopy (B). Each value of *Cry j* I and *Cj* pollen was adjusted to 48 h-value.

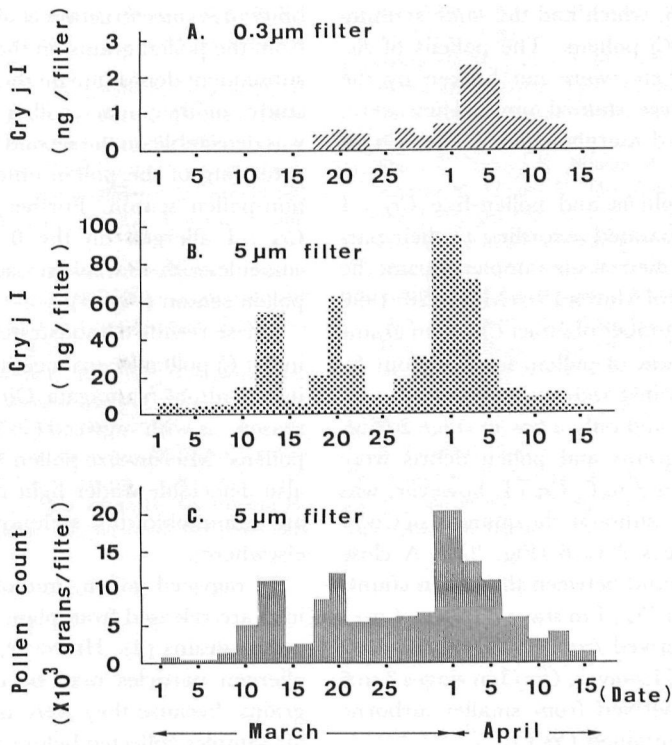


Fig. 3. Relationship between airborne *Cry j* I and pollen count. *Cry j* I on 0.3 µm-Millipore filter connected in line with 5 µm-Nuclepore capillary filter was immunochemically quantified (A). *Cry j* I amounts and *Cj* pollen counts on 5 µm-Nuclepore filter are shown in B and C, respectively.

umbelliferyl-β-D-galactoside. After incubation at 37°C for 2 h, the fluorescence intensity of the enzyme reaction product, 4-methylumbelliferone, was read on a microplate fluorometric reader (Fluoroskan; Flow-Laboratories, McLean, USA). The sensitivity of this ELISA was 0.1 ng of *Cry j* I per ml.

Cross-reactivity of anti-*Cry j* I to the antigens of pollens from the other plants dispersed at the same time was also examined. Weak cross-reactivity was found against *Cypressaceae*, but not against *Betulaceae*, *Taxaceae*, etc.

Pollen counting

Pollen grains collected by Millipore filtration of Andersen sampler specimens were stained with Carberla solution and counted under 100 × light microscopy. Pollen grains on the Nucle-

pore filter were counted under 100 × light microscopy without staining to avoid the *Cry j* I allergen extraction.

In this study, *Cypressaceae* pollens were also found together with *Cj* pollens. However, their number was very small, < 5 % of *Cj* pollen counts.

RESULTS

Cj pollen was the most commonly dispersed pollen during the survey period, although small numbers of pollens of *Cypressaceae*, *Betulaceae*, and *Taxaceae* were observed during the same period. During the allergen extraction procedure, most of *Cj* pollen grains were ruptured by swelling of the intine, and separated into exines and intines (13). The former were stained pink with Carberla solution, but not the latter (Fig. 1). The pollens of *Cypressaceae* were also separated into

exines and intines, which had the same staining character as the *Cj* pollens. The pollens of *Betulaceae*, *Taxaceae*, etc. were not broken by the extraction and were stained pink. They were, however, identified morphologically under light microscopy.

Airborne *Cj* pollens and pollen-free *Cry j* I allergens were separated according to their particle size in an Andersen air sampler during the pollination period of March 15 to March 28, 1990 (Fig. 2). A large number of intact *Cj* pollen grains and a small amount of pollen debris (about 5–30 % of intact grains) were microscopically observed in stage 1, and only a few in stage 2 (Fig. 2B). No pollen grains and pollen debris were detectable in stages 3 to 6. *Cry j* I, however, was found in all stages, although the quantity of *Cry j* I was lower in stages 2 to 6 (Fig. 2A). A close correlation was found between the pollen counts and the amount of *Cry j* I in stage 1. Thus, *Cry j* I in stage 1 was derived from pollen grains after water extraction. However, *Cry j* I in stages 3 to 6 may have been derived from smaller airborne particles which contained *Cry j* I.

Next, we attempted to separate airborne micron-size allergen particles from pollen grains by the tandem filter system described in Material and Methods. First, we checked the reliability of this system. When *Cj* pollens were deliberately spread on the 5 µm-pore Nuclepore filter for 72 h during a non-pollination season, pollen grains and *Cry j* I were detected on the 5 µm-Nuclepore filter, but not on the 0.3 µm filter. This indicated that *Cry j* I did not go from pollen grains on the 5 µm-filter to be deposited on the 0.3 µm-filter during air suction.

Fig. 3A shows the amounts of *Cry j* I on the 0.3 µm-filter during the pollen season. Intact pollen grains and pollen debris were undetectable on the 0.3 µm-filter during the investigation period. However, *Cry j* I was visible on the 0.3 µm-filter after the pollen appeared, and the peak of *Cry j* I on the 0.3 µm-filter seemed to be delayed a few days after the pollen count peak.

DISCUSSION

When airborne pollen allergen particles are analysed by tandem filters, there should be no possi-

bility of *in situ* extraction of allergens by humidity from the pollen grains on the first filter and their subsequent deposition on the second filter. In this study, neither intact pollen nor *Cry j* I allergen was detectable on the second filter after deliberate spreading of the pollen onto the first filter in a non-pollen season. Further, the appearance of *Cry j* I allergen on the 0.3 µm-filter did not coincide with the pollen count peak during the pollen season (Fig. 3).

These results demonstrated the co-existence of intact *Cj* pollen grains together with smaller parts in the air of Yamagata City during the pollen season, as with ragweed (1, 3) and rye-grass (12) pollens. Micron-size pollen allergen particles are also detectable under light microscopy by using an immunoblotting technique (to be published elsewhere).

In ragweed pollen, micron-size allergen particles are released from plant parts other than the pollen grains (2). However, micron-size *Cry j* I allergen particles may be derived from pollen grains, because they were not detectable in any air samples collected before and at the beginning of the pollen season, and the smaller allergen peak was always delayed a few days after the pollen count peak in the pollen season (Fig. 3). We are now interested in investigating whether micron-size allergens exist in the air of big cities, such as Tokyo, which are much further from the mountains where *Cj* trees grow.

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