

LETTER TO THE EDITORS

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Laryngeal symptoms in patients exposed to Japanese cedar pollen: allergic reactions and environmental pollution

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Sirs: In Japan, the number of Japanese cedar (*Cryptomeria japonica*) pollinosis patients has been increasing, with more than 10% of the population now suffering from this seasonal nasal allergy [4]. Many of these patients complain about itching of the throat, persistent cough and feeling like there is a foreign body in the larynx. Additionally, ambient air pollutants probably promote sensitization of airway mucosa and exacerbate allergic respiratory disorders and their symptoms [1, 3, 6, 7]. In an attempt to determine both the existence of laryngeal allergy to cedar pollen and the effect of air pollutants, we undertook a clinical study and a series of animal experiments.

To demonstrate laryngeal manifestations in Japanese cedar pollinosis patients, two hospitals in the Aichi prefecture were chosen. One hospital was situated in a planted cedar woodland region without heavy industry (Shinshiro) and the other in an urban, crowded, heavily industrial area (Nagoya). Between 1991 and 1995, clinical records of patients with Japanese cedar pollinosis attending the two hospitals were examined. The patients were diagnosed as suffering from Japanese cedar pollinosis by the presence of eosinophilia in nasal secretions and serum IgE antibody specific to Japanese cedar pollen. In Shinshiro (woodland region) a total of 276 patients were available for review, while 165 were seen in Nagoya (urban industrial area). No significant differences in age (unpaired *t*-test), sex (χ^2 -test) and serum level of specific IgE antibody (unpaired *t*-test) were found between the two groups in each year of the study. All patients were questioned as to symptoms of nasal stuffiness, itching of the eyes, soreness of the throat, cough, sputum, hoarseness and foreign body sensation in the larynx. U-tests were employed for statistical significance.

Pollen counts in the woodland were 2–5 times higher than those in the urban industrial area (Fig. 1). NO₂ concentrations in the urban industrial area during the same periods were twice as high as those in the woodland (Fig. 2). Also, SO₂ and suspended particulate matter (SPM) levels of the ambient air in the urban area were 2–3 times higher.

The severities of laryngeal symptoms varied directly with the cedar pollen counts, as did nasal and ophthalmic symptoms (Table 1). The large number of patients with Japanese cedar pollinosis who complained of laryngeal symptoms was beyond our expectation, with a foreign body sensation in the larynx being the most frequent complaint in the urban industrial area, and

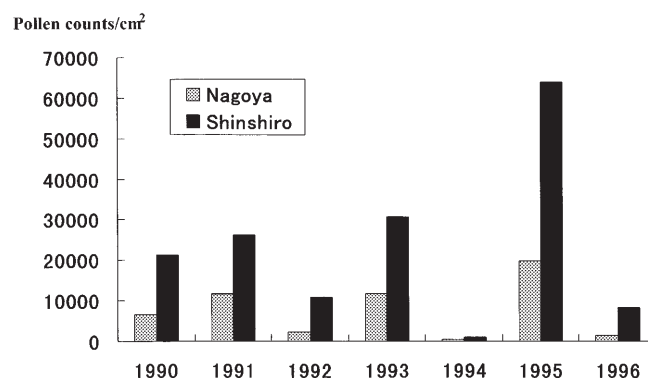


Fig. 1 Counts of airborne Japanese cedar (*Cryptomeria japonica*) and Japanese cypress (*Chamaecyparia obtusa*) pollen in Nagoya and Shinshiro districts

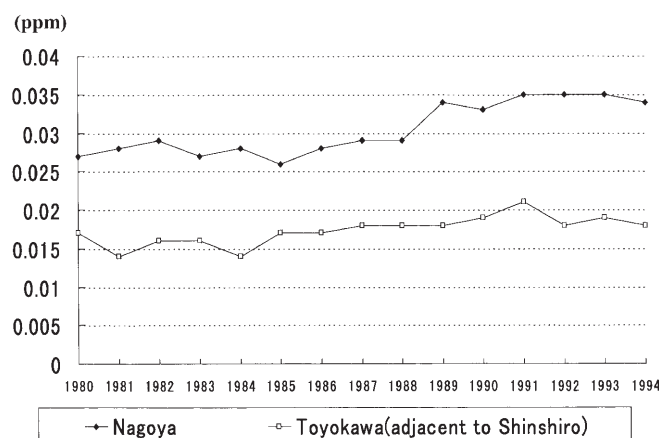


Fig. 2 Variations of environmental NO₂ concentrations in Nagoya and Shinshiro districts

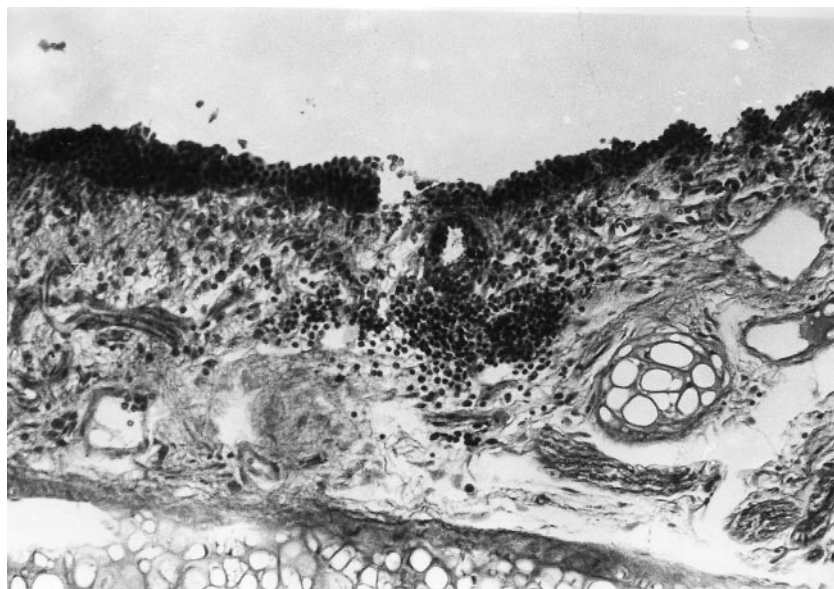
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Table 1 Comparison of upper respiratory symptoms in patients with Japanese cedar pollinosis

Symptoms	Patients	
	Shinshiro area (woodlands, <i>n</i> = 275)	Nagoya (industrial, <i>n</i> = 165)
Itching eyes	236	136
Nasal obstruction	245	138
Sore throat	158	84
Sputum	150	97
Cough	153	97
Hoarseness	98	63
Foreign body sensation in larynx	172	104

Table 2 Tissue eosinophilia in rats sensitized with Japanese cedar pollen in comparison with non-sensitized rats (*NS* not significant)

Nasal mucosa	Grade of eosinophilia			χ^2 -test
	–	+	++	
Control (<i>n</i> = 5)	4	1	0	<i>P</i> < 0.01
Sensitized (<i>n</i> = 8)	1	2	5	
Laryngeal mucosa	Grade of eosinophilia			χ^2 -test
	–	+	++	
Control (<i>n</i> = 5)	4	0	1	<i>P</i> < 0.05
Sensitized (<i>n</i> = 8)	0	5	3	
Tracheal mucosa	Grade of eosinophilia			χ^2 -test
	–	+	++	
Control (<i>n</i> = 5)	5	0	0	<i>NS</i>
Sensitized (<i>n</i> = 8)	8	0	0	

Fig. 3 Histology of typical laryngeal mucosal specimen in rats sensitized to Japanese cedar pollen ($\times 40$)

more severe than those in the woodland region, although the cedar pollen counts in the urban industrial area were 2–5 times lower than those in the woodland region during the period of study.

In order to determine whether an allergic reaction of the larynx was actually due to Japanese cedar pollen, we then investigated rats immunized with Cry j I, which is one of the major allergens of Japanese cedar pollinosis [8]. Six-week-old male Brown Norway rats were immunized twice on day 0 and day 13 with intraperitoneal injections of 10 μ g Cry j I with 4.5 mg aluminum hydroxide gel. Seven days after the last immunization, serum levels of anti-Cry j I IgE antibody were determined by the method of reverse IgE-capture enzyme-linked immunosorbent assay (ELISA). After determining the serum levels of IgE, hematoxylin and eosin-stained laryngeal, nasal and tracheal mucosa were examined microscopically. Each specimen was obtained 6 h after pernasal exposure to 10 μ g Cry j I, and the extent of any eosinophilia present was assessed. Five non-sensitized rats were employed as controls. Additionally, two groups of four rats each were immunized with nasal drips of Cry j I (10 μ g) solution 5 days per week for 6 weeks. During this period four animals were exposed to 0.5 ppm NO₂ as a common air pollutant, while a second group of four immunized rats was not exposed. Another five rats not exposed to either NO₂ or sensitized with Cry j I were employed as controls. Serum levels of specific IgE antibody to Cry j I in the rats were measured at weeks 2, 4 and 7. Laryngeal mucosa was sampled for microscopic detection of eosinophilia 6 h after pernasal exposure to 10 μ g Cry j I.

The mean value of serum levels of specific IgE in eight rats immunized with Cry j I was 307.1 $\pm$ 1.85.3FU (free units)/ml. No significant IgE levels were found in the controls. The grades of eosinophilia in the nasal mucosa of the sensitized rats were higher than those in the non-sensitized rats (Table 2). In the laryngeal mucosa, the grades of eosinophilia in the sensitized rats were higher than those in the controls (Fig. 3). Only small eosinophil accumulations were observed in the tracheal mucosa of both groups.

In the rats exposed to NO₂, the mean serum values of specific IgE to Cry j I were significantly higher than in unexposed rats at week 4 (Table 3). The grades of eosinophilia in the laryngeal mucosa of the rats immunized with Cry j I were greater than

Table 3 Mean levels (free units/ml) of serum-specific IgE antibody to Japanese cedar pollen in rats sensitized with cedar pollen and exposed to NO₂, rats sensitized with cedar pollen but not exposed to NO₂, and untreated controls

	Week 2	Week 4	Week 7
Control (n = 5)	0 ± 0	0 ± 0	0 ± 0
Sensitized without NO ₂ Exposure (n = 4)	1.7 ± 3.3	17.0 ± 20.9	22.9 ± 36.5
Sensitized with NO ₂ Exposure (n = 4)	3.9 ± 4.5	70.2 ± 36.1	105.2 ± 100.8

*P < 0.05

Table 4 Local eosinophilia of the larynx in rats sensitized with Japanese cedar pollen and exposed to NO₂, rats sensitized with cedar pollen but not exposed to NO₂, and untreated controls (NS not significant)

	Grade of eosinophilia		χ^2 -test
	–	≥+	
Control (n = 5)	4	1	* NS
Sensitized without NO ₂ Exposure (n = 4)	0	4	
Sensitized with NO ₂ Exposure (n = 4)	0	4	

*P < 0.05

those of the controls, but the differences between sensitized rats exposed to NO₂ and the unexposed rats were not significant (Table 4).

The number of cedar pollinosis patients has been increasing in Japan because of the increase in ambient cedar pollen counts [5]. Many clinicians have noted associated laryngeal symptoms in these patients, including foreign body sensation in the larynx and persistent cough. In our present study, approximately 40–70% of cedar pollinosis patients were found to suffer from several kinds and severities of laryngeal symptoms that occurred in parallel with seasonal changes of cedar pollen counts. In animal studies we demonstrated the existence of an allergic reaction of the larynx using rats sensitized to Cry j I, which is one of the major allergens in Japanese cedar pollinosis. This finding supports the view that laryngeal manifestations in cedar pollinosis patients might be generated by an allergic reaction of the larynx.

In recent studies, it has been shown that ambient air pollutants such as NO₂, SO₂, diesel exhaust particulate (DEP) and ozone probably promote sensitization of airway mucosa and exacerbate symptoms [1, 3, 6, 7]. In Japanese cedar pollinosis, both the increase in pollen count and environmental pollution have been considered to be causes for the increased patient populations [2]. We found that patients with Japanese cedar pollinosis who were exposed to twice the concentration of NO₂, SO₂ and SPM in the ambient air complained of more severe laryngeal symptoms with nasal and ophthalmic manifestations, even though they were exposed to 2–5 times less airborne antigen. NO₂ was employed as an air pollutant in our animal study because of its ease of use and stability of concentration. We found that exposure to a high concentration of NO₂ (0.5 ppm) enhanced the abil-

ity of the rats to produce specific IgE antibody to Japanese cedar pollen, but the influence on allergic response of the larynx was not clear.

Allergic reactions of the larynx might not occur as frequently or severely as nasal allergy or bronchial asthma, but we have clearly demonstrated its existence in our study. We suggest that the increase in antigen exposure and environmental pollution increases the incidence of allergic disorders and makes laryngeal allergy conspicuous.

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