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Skin Tests of Pollen Grains of Taxodiaceae and Cupressaceae in Children with Bronchial Asthma

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Atmospheric cedar pollen in the southern region of Okayama Prefecture (situated in south-western Japan) has been counted since 1988. Pollen of different species of the Taxodiaceae family (*Cryptomeria japonica*, *Sequoia sempervirens* and *Metasequoia glyptostroboides*) and Japanese juniper (*Juniperus rigida*) in the Cupressaceae family, which are propagated mainly in the southern region of Okayama Prefecture, were found among the atmospheric pollen. Scratch tests using the pollen extract from Taxodiaceae and Cupressaceae were performed on children with bronchial asthma. Forty (25%) and 30 (18.8%) of the 160 patients reacted positively to an allergen extract from the pollen grains of Japanese cedar (*Cryptomeria japonica*) and Japanese juniper, respectively.

Key Words

Pollinosis, Cupressaceae, *Juniperus rigida*, Scratch test, Bronchial asthma

Introduction

In recent years, an increase in bronchial asthma due to pollen has been detected from inhalation provocation tests. Among the pediatric asthma patients, 55.5% were positive to Japanese cedar pollen, 52.6% to grass pollen, and 50% to mugwort pollen [1]. Azuma [2] reported that 61 (46.2%) of the 132 pediatric allergy patients had complications of bronchial asthma. Morita et al. [3] reported that 47.6% of their Japanese cedar radioallergosorbent test (RAST) positive patients had bronchial asthma, and suggested that nasal symptoms raise oral breathing, and

that the inhalation of dry air increases the hypersensitivity of the respiratory tract, which then causes bronchial asthma. In the skin test, 25% of the asthmatic children were positive to Japanese cedar pollen, and these pollen grains proved to be important antigens causing bronchial asthma.

The atmospheric cedar pollens in the southern region of Okayama Prefecture have been counted since 1988. Pollens of different species in the Taxodiaceae family and *Juniperus rigida* (Japanese juniper) in the Cupressaceae family, which originate mainly in the southern region of Okayama Prefecture, were found among the atmospheric pollen [4].

The present study successively examined the allergic reactions caused by Japanese cedar (*Cryptomeria japonica*), *Sequoia* (*Sequoia sempervirens*), *Metasequoia* (*Metasequoia glyptostroboides*), and Japanese juniper (*Juniperus rigida*) in asthmatic children.

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Materials and methods

Subjects

One hundred and sixty children with bronchial asthma were studied between May and July in 1990 at the pediatric asthma clinic of Tamano City Hospital (which is situated on the southern region of Okayama Prefecture). They consisted of 111 boys and 49 girls from 3 years old to 18 years and 8 months old (mean 10 years and 6 months $\pm$ 3 years and 4 months). The severity of bronchial asthma according to the criteria of the Japanese Society of Pediatric Allergy and Clinical Immunology [5] were mild in 78 patients (48.8%), moderate in 75 (46.9%), and severe in 7 (4.4%). There were 80.0% children with allergic rhinitis and 18.8% with allergic conjunctivitis. The serum IgE level was 13–8,300 IU/ml (mean titer was $1,150 \pm 1,356$ IU/ml). The RAST⁺ rates were 25.56% for Japanese cedar and 94.4% for mites (*Dermatophagoides pteronyssinus*; Table 1).

Antigen solution

Pollen was collected from the trees in forests and parks in the southern region of Okayama Prefecture in May 1990. Collected pollen was defatted and dried with ether. Then 1 ml toluen, 50 ml glycerol and 100 ml phosphate-buffered saline (pH 8) were added to 5 g of pollen, and the volume was made up to 250 ml with distilled

water. Pollen was extracted for 3 days at 4°C. After filtration, pollen was concentrated with a 24/32 inch dialyzing tube until 100 ml at 4°C, and filtered again by a 0.45 μ m filter [6].

Scratch test solution

The antigen solution was used as a scratch test solution. The protein concentration of Japanese cedar in the scratch test solution was 2.2 mg/dl; it was 2.4 mg/dl for Sequoia, 2.6 mg/dl for Metasequoia and 6.4 mg/dl for Japanese juniper.

Scratch test

Scratch tests were performed on the forearm or skin of the back by the linear method [7]. The skin reactions were evaluated after 15 min; if the diameters of the flares were larger than 15 mm or the weal was larger than 5 mm, the test was considered positive.

Results

Forty (25%), 22 (13.8%), 20 (12.5%) and 30 (18.8%) of the 160 patients proved positive in the scratch tests with the allergen extract of Japanese cedar, Sequoia, Metasequoia and Japanese juniper, respectively (Table 2). Ten (25%) of the 40 Japanese cedar-positive patients proved positive with the allergen extract from pollen grains of Sequoia, and 30 (75%) of those proved negative (Table 3). Six (15%) of the 40 Japanese cedar-positive patients proved positive to Metasequoia, and 34 (85%) of those proved negative (Table 4). Ten (25%) of the 40 Japanese cedar-positive patients proved positive to Japanese juniper, and 30 (75%) of these proved negative (Table 5).

Table 1. Clinical data for the subjects

Patients (n = 160)		
Sex	111 Male	49 Female
Age range	3 years–18 years 8 months	
Mean age	10 years 6 months $\pm$ 3 years 4 months	
Severity of asthma		
Mild	78 (48.8%)	
Moderate	75 (46.9%)	
Severe	7 (4.4%)	
Accompanied with		
allergic rhinitis	128 (80.0%)	
allergic conjunctivitis	30 (18.8%)	
IgE titer	13–8,300 IU/ml	
Mean IgE titer	$1,150 \pm 1,356$ IU/ml	
RAST ⁺ scores		
Japanese juniper	41 (25.6%)	
Mite	151 (94.4%)	

Table 2. Positive rate of pollen scratch tests in asthmatic children

Pollen	Cases	Positive cases	Positive rate (%)
Japanese cedar	160	40	25.0
Sequoia	160	22	13.8
Metasequoia	160	20	12.5
Japanese juniper	160	30	18.8
All 4 pollens	160	4	2.5

Table 3. Cross-reactivity between Japanese cedar and Sequoia in the scratch tests

	Reaction to Sequoia		
	+	-	Total
Reaction to Japanese cedar			
+	10 (6.3)*	30 (18.8)*	40 (25.0)
-	12 (7.5)	108 (67.5)	120 (75.0)
Total	22 (13.8)	138 (86.3)	160 (100.0)

* $P < 0.01$

Table 4. Cross-reactivity between Japanese cedar and Metasequoia in the scratch tests

	Reaction to Metasequoia		
	+	-	Total
Reaction to Japanese cedar			
+	6 (3.8)*	34 (21.3)*	40 (25.0)
-	14 (8.8)	106 (66.3)	120 (75.0)
Total	20 (12.5)	140 (87.5)	160 (100.0)

* $P < 0.01$

Table 5. Cross-reactivity between Japanese cedar and Japanese juniper in the scratch tests

	Reaction to Japanese juniper		
	+	-	Total
Reaction to Japanese cedar			
+	10 (6.3)*	30 (18.8)*	40 (25.0)
-	20 (12.5)	100 (62.5)	120 (75.0)
Total	30 (18.8)	130 (81.3)	160 (100.0)

* $P < 0.01$

Four (2.5%) of the 160 patients proved positive for all four pollens in the scratch tests (Table 2).

Discussion

Pollinosis has been gathering interest in the field of pediatric allergology in recent years. In this study, scratch tests were performed using extract from pollen grains of Taxodiaceae (*Cryptomeria japonica*, *Sequoia sempervirens* and *Metasequoia glyptostroboides*) and Cupressaceae (*Juniperus rigida*), and the positive rates of scratch tests using extracts from Sequoia, Metasequoia and

Japanese juniper were compared with those obtained from Japanese cedar.

The results of the scratch test suggested a low cross-allergenicity between the Sequoia, Metasequoia and Japanese juniper pollens and Japanese cedar pollen.

The pollen grains of Japanese juniper gave the highest positive rate in the skin tests next to Japanese cedar. In a previous report, Ueno suggested the possibility of pollinosis due to Japanese juniper [8], although the allergenicity of Japanese juniper pollen extract has not been reported. Pollen of another species of Japanese juniper, *Chamaecyparis obtusa*, was reported to be a cause of pollinosis in 1945 by Ordman [9]. In France, Panzani was the first to publish cases of common cypress (*Cupressus sempervirens*) allergy, which was then very rare. Since 1975, the increase of cypress allergy in southern France has been observed [10]. In Japan, cypress pollen is an important antigen as a cause of pollinosis. Shimizu et al. [11] suggested that, after the pollination period of Japanese cedar, cypress pollinosis follows Japanese cedar pollinosis. Shimizu et al. proved that there is cross-reactivity between the pollen extracts of Japanese cedar and cypress using the Praunitz-Küstner (P-K) reaction, RAST inhibition tests and immune precipitation, and suggested that the pollen extracts of Japanese cedar have a major allergen and those of cypress have a minor allergen of Japanese cedar pollen extract [11]. Panzani et al. reported a clear cross-reactivity between the pollen of common cypress and Japanese cedar by skin testing, RAST and RAST inhibition, and suggested that an antigen in cypress is cross-reactive with Sugi Basic Protein (SBP), which is a major allergen of Japanese cedar [12]. The pollination period of Japanese cedar from mid-February to the end of March; that of Japanese cypress is between the end of March and the end of April, with peak pollination early in April; and that of Japanese juniper is between early and late April [1]. The results of these studies indicate that pollen grains of Japanese juniper cause pollinosis after the pollination of Japanese cedar.

Because of the increase of Metasequoia along the streets of Tamano City, located in the

for 3 days at 4°C. concentrated with a until 100 ml at 4°C, µm filter [6].

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atch tests in asth-

Positive rate (%)

25.0
13.8
12.5
18.8
2.5

southern region of Okayama Prefecture, an increase of atmospheric pollen is expected. The number of positive scratch tests with the pollen extracts of *Metasequoia* should increase with the increase in atmospheric pollen from the 12.5% incidence reported here. Careful administrative management of these trees is needed.

In conclusion, scratch tests using extracts from *Taxodiaceae* and *Cupressaceae* were performed on children with bronchial asthma. Forty (25%) of the 160 patients proved positive in the scratch tests with the allergen extract of Japanese cedar. Thirty (18.8%) of the 160 children proved positive in the scratch tests with the allergen extracts from the pollen grains of Japanese juniper.

The results of these scratch tests indicate that the pollen grains of Japanese juniper may cause pollinosis.

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