

Original Article

Relationship between pollen counts of *Cryptomeria japonica* and Cupressaceae and the severity of allergic symptoms

Hiroyuki Namba,¹ Katsuyoshi Saitou,² Norio Sahashi,³ Masahiko Yamamoto,⁴ Tomoe Yoshida,⁴ Hiroshi Ogasawara,⁵ Masaaki Fujimoto⁶ and Nobuhiko Asada⁷

¹Pharmacy and ²Department of Internal Medicine, Kurashiki Municipal Kojima Shimin Hospital, Okayama, ³Department of Biology, School of Pharmaceutical Sciences, Toho University, ⁴Department of Otorhinolaryngology, Toho University School of Medicine Sakura Hospital, Chiba, ⁵Department of Otorhinolaryngology, Hyogo College of Medicine, Hyogo, ⁶Fujimoto Clinic of Otorhinolaryngology and ⁷Biological Laboratory, Faculty of Science, Okayama University of Science, Okayama, Japan

ABSTRACT

Background: A study throughout 1999 of the airborne pollens of both *Cryptomeria japonica* and Cupressaceae (*Chamaecyparis obtusa* and *Juniperus rigida* in the present study) demonstrated unique patterns of pollen dispersal at three observation sites, namely Okayama and Nishinomiya cities in the Chugoku region and Sakura city in the Kanto region.

Methods/Results: In contrast with the Sakura site, where there was a low pollen count for Cupressaceae, high pollen counts were found at the two sites in Okayama and Hyogo Prefectures. There was a good correlation between results of pollen prick tests and the capsulated hydrophilic carrier polymer–radioallergosorbent test (CAP-RAST) for *C. japonica*, *C. obtusa* and *J. rigida*. The fact that a positivity rate of 32.6% to the *J. rigida* pollen skin test was obtained in Sakura city in Chiba Prefecture, where no *J. rigida* pollen dispersal was observed, seems to demonstrate the existence of a common antigen epidemiologically. Daily pollen counts, symptom scores, medication scores and symptom–medication scores of 25 patients with vernal pollinosis in Chiba Prefecture in whom the dose and use of internal and external medication was

consistent with the severity of their symptoms, were all significantly related. The efficacy of pharmacotherapy seems to be inadequate when patients are exposed to pollen during a heavy pollen dispersal period without having taken any protection against it. Moreover, there seemed to be a certain critical point beyond which the symptoms rapidly worsened.

Conclusions: These results suggest that it is important to avoid exposure to pollen, based on the regional pollen information, as well as to administer appropriate medication.

Key words: allergic symptoms, *Chamaecyparis obtusa*, *Cryptomeria japonica*, Cupressaceae, *Juniperus rigida*, medication, pollinosis.

INTRODUCTION

The allergy caused by the pollen of Japanese cedar (*Cryptomeria japonica*) is a typical example of vernal pollinosis in Japan, yet symptoms persist after the dispersal of cedar pollen is complete. Because Cupressaceae (*Chamaecyparis obtusa* and *Juniperus rigida* in the present study) pollens, which have been reported to have some cross-antigenicity with the pollen of *C. japonica*,^{1,2} have also been detected during this period, it is most important to understand the timing of pollen dispersion of both *C. japonica* and Cupressaceae in each area.

Although there has been one clinical report³ that concluded the variation in the number of patients with

Correspondence: Hiroyuki Namba, 303-3 Nakai, Okayama City, Okayama 703-8205, Japan. Email: na-2857@po.harenet.ne.jp

Received 15 May 2000. Accepted for publication 27 November 2000.

pollinosis was correlated with yearly *C. japonica* pollen counts and an epidemiological report⁴ on the prevalence of allergy to *C. japonica* pollen, there have been few reports on the relationship between pollen counts, the severity of allergic symptoms from exposure to pollen and pharmacotherapy for pollinosis patients. Moreover, there have been no reports on dispersal conditions with regard to *J. rigida* pollen or of epidemiological surveys on *J. rigida* pollinosis in Japan, except for those studies performed by Horiuti⁵ and Miyoshi *et al.*⁶ in Okayama Prefecture.

In the present study, we have investigated the dispersal of pollens at two sites in the Chugoku region (Okayama and Hyogo Prefectures) and at one site in Chiba Prefecture in the Kanto region, which all have almost the same latitude, in Japan. In addition, we performed skin tests after obtaining the consent of patients who came to the clinic during the *C. japonica* and Cupressaceae pollen-dispersal period and assessed the relationship between *C. japonica*- and *Chamaecyparis obtusa*-specific IgE antibodies. We also investigated the relationship between pollen counts, medication and degrees of subjective symptoms of pollinosis reported by the patients in a diary of pollinosis symptoms.

The present study reveals some important relationships between the severity of pollinosis resulting from exposure to pollen, the doses of medication taken by the patients and the efficacy of these treatments against the symptoms.

METHODS

The three observation sites for the determination of airborne pollen counts and the three hospitals where the skin test and *in vitro* test were performed are as described below.

We used Durham standard pollen samplers⁷ for sampling pollen. Using Calberla liquid (glycerin 5 mL; ethanol 10 mL; Fuchsin Basic saturated liquid (Wako Pure Chemicals, Osaka, Japan) 2 drops; purified water 15 mL),⁸ we stained pollen grains that had fallen naturally onto glass slides and had been trapped within a thin layer of white Vaseline (Maruishi Pharmaceutical, Osaka, Japan) over a period of 24 h from 08.30 h. The grains within a 3.24 cm² area (1.8 × 1.8 cm) were counted under a light microscope and the result expressed as pollen grains/cm². The observation period was from the beginning of February until the end of May 1999.

We recorded pollen dispersion from the day it began until the day it ended, as well as the day of maximum pollen count and the total pollen counts for *C. japonica* and Cupressaceae. Our methods followed those of the Agreement Report of Working Group of Standardization for Pollen Information.⁹

Because the clinic in Okayama Prefecture, in which outpatients were examined, has not recorded data on pollens, pollen counts from Maple Pharmacy, the nearest to the clinic, were used. Patients were also examined at two other clinics, one in Hyogo Prefecture and the other in Chiba Prefecture. These observation sites, the local clinic and two hospitals are shown in Fig. 1. In Okayama Prefecture, data were recorded at the Fujimoto Clinic of Otorhinolaryngology, where outpatients were examined using the skin test and *in vitro* test, whereas the Maple Pharmacy (Okayama City) was the observation site for pollen grains. In Hyogo Prefecture, outpatients were examined at Hyogo College of Medicine (Nishinomiya City) using the skin test and *in vitro* test and pollen grains were also observed here. In Chiba Prefecture, outpatients were examined at the Toho University School of Medicine Sakura Hospital (Sakura City) using the skin test and *in vitro* test and pollen grains were observed here also.

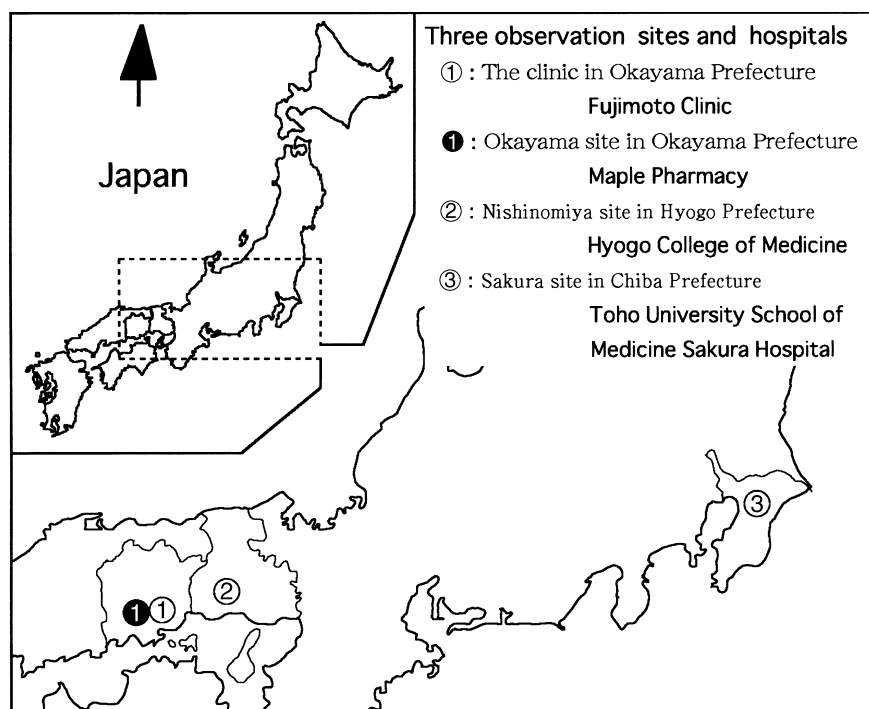
Antigen extraction of *C. japonica* and Cupressaceae, skin test and *in vitro* test

Antigen extractions of pollen grains (*C. japonica*, *C. obtusa* and *J. rigida*) were made according to the method reported by Sheldon *et al.*¹⁰ After being dried and defatted, pollen grains were soaked in an extracting solution (defatted pollen grains 10.0 g; glycerin 25.0 mL; Toluene 1 mL; buffered saline 50 mL; sterile distilled water 415 mL) for 48 h.

Buffered saline was made by adding 5 g sodium chloride, 0.36 g monobasic potassium phosphate, 7.0 g dibasic sodium phosphate and 4.0 g phenol crystals to distilled water and making the solution up to a final volume of 1000.0 mL. The extracted solution was filtered through fluted paper and concentrated to 125 mL in the Seamless Cellophane Tubing 24/32 (Viskase, Chicago, IL, USA). Extracts were clarified by passing them through a millipore filter (pore size 0.22 µm) and 75 mL glycerin was added to produce the skin testing solutions (1 : 20).

The protein content of the pollen antigen preparations was estimated with a spectrometer.¹¹ In order to check the quality of the pollen extracts, sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) in 12.5%

Fig. 1 Three observation sites for *Cryptomeria japonica* and Cupressaceae and the three hospitals that examined patients with pollinosis in Okayama, Hyogo and Chiba Prefectures. (①), the clinic that examined the patients; (●), the site at which pollen grains only were observed; (②), (③), hospitals that observed pollen grains and also examined patients.



gel was performed according to the method of Laemmli¹² and gels were stained with Coomassie Brilliant Blue G250 (Nacalai Tesque, Kyoto, Japan). After placing 15 μ L samples of the pollen extracts (1 : 20) in wells, electrophoresis was allowed to proceed for 150 min at 20 mA. The marker proteins (Pharmacia Biotech, Amersham, UK) were phosphorylase b (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20.1 kDa), and α -lactalbumin (14.4 kDa).

Patients were tested by the prick-puncture skin test, according to the Report of the Working Group on Consultation Guidelines for Allergic Rhinitis.¹³ The skin reaction was detected after 15 min. Wheals greater than 5 mm in diameter or with a flare diameter greater than 10 mm were regarded as positive. If patients reacted to the control solution, we regarded them as positive only if the reaction to the pollen extract was more than twice that of the control. To determine the serum levels of specific IgE antibody to the pollens, the Pharmacia-CAP system RAST (Pharmacia Diagnostics AB, Uppsala, Sweden)¹⁴ was used in accordance with the manufacturer's recommendations. Results are expressed in classes from 0 to 6 and levels above class 1 were regarded as positive. Seventy-three patients (15 from Okayama; 15 from Hyogo; 43 from Chiba) were examined with the skin test

and 56 patients (14 from Okayama; 14 from Hyogo; 28 (*C. japonica*) and 20 (*C. obtusa*) from Chiba) were examined with CAP-RAST. Statistical analysis was performed by using all results from the tests that could be performed in the same patients.

Diary recording pollinosis, symptom score (S score), medication score (M score) and symptom-medication score (SM score)

Patients diagnosed as having pollinosis to *C. japonica* and *C. obtusa* (positive to the prick-puncture skin test or the CAP-RAST examination) were required to keep a diary. Patients not only kept records in their pollinosis diary of the doses of the medication they took each day and the number of topical preparations they used, but they also kept a record of their nasal and eye symptoms and of the degree of interference with their everyday life that they experienced. They were asked to follow a manual^{13,15} that had been prepared in advance with regard to recording these symptoms. We requested patients to record their use of appliances (pollinosis glasses and masks) to protect themselves against pollen. We asked them to either bring the diary to us or to post it in when the pollen dispersal period ended and they no longer experienced any symptoms.

Nasal symptoms of allergic rhinitis and the disturbance to daily life were estimated to fall into one of five grades (ranging from – to +++) and were scored on a scale of 0–4 points according to the guidelines.¹³ Eye symptoms were divided into two types, such as itchy eyes and wiping away of tears. Each symptom was estimated as being in one of the five grades and scored from 0–4 according to the method reported by Okuda.¹⁵ Either eye itchiness or the number of times that tears were wiped was used as the eye symptom score, depending on which was the most severe.¹⁵ The S scores were calculated

according to the total of these three pollinosis symptoms.

The medication prescribed to pollinosis patients followed recommendations and was scored in accordance with the guidelines¹⁵ and the Japanese Society of Allergology.¹⁶ Table 1 shows the pharmacotherapy or M score for patients. The S score combined with the M score was expressed as the SM score. All these scores were averaged according to the number of patients.

The investigation period was from 2 March to 10 April (completed 40 daily diary entries). We have studied the relationships among the daily pollen counts, daily S score,

Table 1 Medication scores for treatments prescribed for pollinosis

Type of medicine	Generic name	Medication score
Oral medication		
Anti-allergic agents		
Suppression of IgE antibody production	Suplatast tosilate	1 point/day
Suppression of chemical mediator release from mast cells	Tranilast	
	Pemirolast potassium	
New-type antihistamines: Suppression of chemical mediator release from mast cells, antihistamine effect etc.	Ebastine	
	Ketotifen fumarate	
	Oxatomide	
	Emedastine difumarate	
	Mequitazine	
	Azelastine hydrochloride	
	Epinastine hydrochloride	
	Astemizole	
	Terfenadine	
	Cetirizine hydrochloride	
	D-Chlorpheniramine maleate	
Old-type antihistamines		
Others		
Combined agents	D-Chlorpheniramine maleate + betamethasone	3 points/day
Chinese herbal medicines	Sho-seiryu-to extract	0.5 points/day
	Other types of herbal medicine	
External medication		
Nasal drops		
Suppression of chemical mediator release from mast cells	Sodium cromoglycate	1 point/day
New-type antihistamines	Ketotifen fumarate	2 points/day
Vasoconstrictors	Tramazoline hydrochloride	
	Naphazoline nitrate	
Steroids	Beclometasone dipropionate	
	Fluticasone propionate	
	Flunisolide	
Eye drops		
Suppression of chemical mediator release from mast cells	Sodium cromoglycate	1 point/day
New-type antihistamines	Tranilast	
Steroids	Ketotifen fumarate	2 points/day
	Fluorometholone 0.02%	
	Dexamethasone sodium Metasulfobenzoate 0.02%	

When pollinosis patients had not taken or used their daily dose of medicine, we divided them by the number of times per day and calculated daily medication scores as the occasion demanded.

daily M score and daily SM score of 25 patients (all patients who recorded the circumstances regarding their use of medication and symptoms without interruption) in Chiba Prefecture.

For statistical evaluation, regression analysis and the χ^2 -test were used. Differences were considered to significant at $P < 0.05$.

RESULTS

State of pollen dispersion for *C. japonica* and Cupressaceae at the three observation sites

Figure 2 and Table 2 show the results for pollen dispersion of both *C. japonica* and Cupressaceae at three observation sites in Okayama, Hyogo and Chiba Prefectures.

Cryptomeria japonica

Release of pollen from *C. japonica* was first observed on 8 February at the Sakura site and lasted for 60 days. Release from the trees at the Okayama site began on 16 February and lasted for 53 days. Pollen dispersion ended at both sites at almost the same time, at the beginning of April. Pollen release began the latest at the Nishinomiya site during the end of February. The expiration day was the earliest at the Nishinomiya site towards the end of March and the pollen dispersal period was the shortest at this site, lasting only 38 days. The day of maximum pollen count, during the early part of March, for *C. japonica* was at almost the same time at all three sites. The maximum pollen count was the highest for the Nishinomiya site (count of 1912 pollen grains), whereas pollen counts at the other two sites were almost the same at approximately 1200 pollen grains.

Cupressaceae

Pollen release from Cupressaceae was first observed at the Okayama site on 12 March. The expiration day at the Okayama site was on 25 May and pollen dispersion continued for the longest period at this site, lasting 74 days. Release began at the Sakura site on 17 March and lasted for 43 days before ending during the final days of April. Release at the Nishinomiya site was the most delayed and did not begin until late March. The pollen dispersion lasted for 32 days at the Nishinomiya site and ended on 24 April after the shortest period of dispersion. The timing of the maximum pollen counts at the three sites was

markedly different. At both the Okayama and Nishinomiya sites, maximum pollen counts were observed from the beginning to the middle of April, whereas at the Sakura site it occurred in late March. The pollen count at the Okayama site was 1163 pollen grains and this was the highest of the three sites, with two peaks being observed. There was a peak pollen count of 566 pollen grains, with only one peak, at the Nishinomiya site. There were only 319 pollen grains counted at the Sakura site, with no clear peak.

Skin test and RAST examination

The protein content of the pollen preparations was 2.47 mg/mL for *C. japonica*, 4.29 mg/mL for *C. obtusa* and 2.16 mg/mL for *J. rigida*. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis was performed in 12.5% gel and the *C. japonica* extract yielded three bands, one with a molecular weight of approximately 94 kDa and two with molecular weights of approximately 43 kDa each. The *C. obtusa* extract yielded five bands, three with molecular weights of approximately 75, 38 and 25 kDa and two with molecular weights of approximately 48 kDa each. The *J. rigida* pollen extract yielded four bands, two with molecular weights of approximately 75 and 62 kDa and two with molecular weights of approximately 40 kDa each.

The testing of patients in Okayama, Hyogo and Chiba who were suspected of suffering from *C. japonica* pollinosis yielded highly positive results in the skin test. In Okayama, the positive rates for the skin test were 73.3, 53.3 and 33.3% for *C. japonica*, *C. obtusa* and *J. rigida*, respectively; in Hyogo, the positive rates were 93.3, 60.6 and 33.3% for *C. japonica*, *C. obtusa* and *J. rigida*, respectively; and in Chiba, the positive rates were 74.4, 58.1 and 32.6 for *C. japonica*, *C. obtusa* and *J. rigida*, respectively. Table 3 shows the results of both the skin test and the RAST examination. Because the RAST assay for *J. rigida* had not been produced in commercial quantities, the diagnosis of pollinosis to *J. rigida* has been examined using only the skin test. All relationships between results for RAST using *C. japonica* and *C. obtusa* antigens and those of skin tests using *C. japonica*, *C. obtusa* and *J. rigida* antigens have correlated well. Two types of relationship between the RAST and skin test results to *C. japonica* and *C. obtusa* antigens were also significant. Moreover, two correspondents of each combination correlated significantly. The relationship of RAST results between *C. japonica* and *C. obtusa* under the same

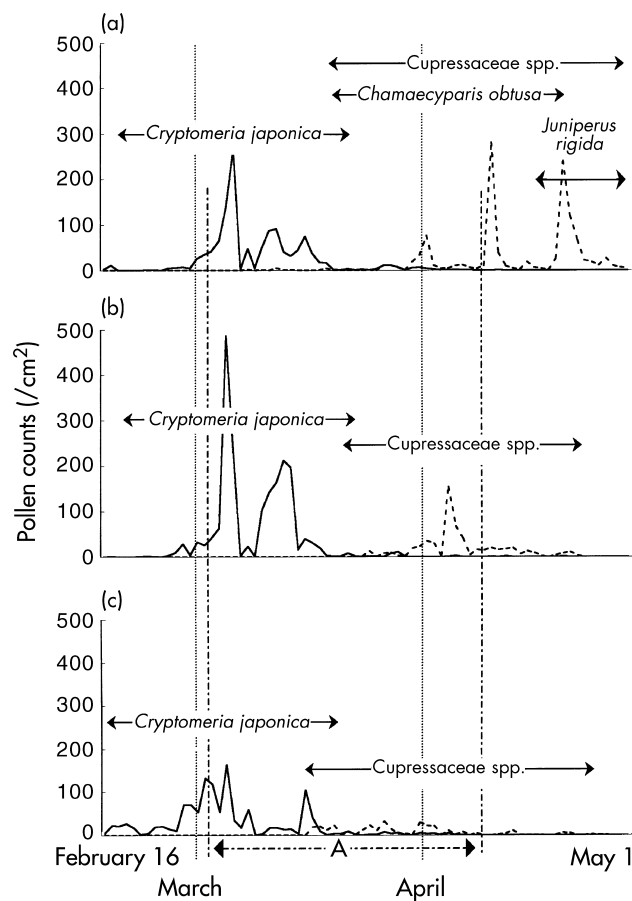


Fig. 2 Annual variation of *Cryptomeria japonica* and Cupressaceae pollen counts at (a) Maple Pharmacy (Okayama), (b) Hyogo College of Medicine (Hyogo) and (c) Toho University School of Medicine, Sakura Hospital (Chiba) in 1999 and the period of investigation for pollinosis patients (A, investigation period was from 2 March to 10 April).

in vitro conditions was also significant. The correspondent was also significant.

There were 12 of 41 patients who were positive to *C. japonica* but negative to *C. obtusa* according to the RAST examination. There was no patient negative to *C. japonica* but positive to *C. obtusa*. Similarly, using the skin test, 16 of 58 patients, who were all positive to *Cryptomeria japonica*, were negative towards *C. obtusa*. There was no patient negative to *C. japonica* but positive to *C. obtusa*. Although two patients were negative for the skin test to *C. japonica* but positive for the RAST to *C. obtusa*, they were positive for *C. japonica* in the RAST examination. These results suggest that all patients positive to *C. japonica* were positive to *C. obtusa*, as shown in Table 4. In contrast, there were no patients negative to *C. japonica* and positive to *C. obtusa*.

Table 2 Dates of pollen surveys at the three observation sites

Observation site	Date at the beginning		Date at the end		Date of maximum		Maximum		Total counts		Total counts
	<i>Cryptomeria japonica</i>	Cupressaceae	<i>Cryptomeria japonica</i>	Cupressaceae	<i>Cryptomeria japonica</i>	Cupressaceae	<i>Cryptomeria japonica</i>	Cupressaceae	<i>Cryptomeria japonica</i>	Cupressaceae	
Okayama site	16 February	12 March	9 April	25 May	6 March	11 April	264	280	1215	1163	2378
Nishinomiya site	21 February	24 March	30 March	24 April	5 March	5 April	486	154	1912	566	2478
Sakura site	8 February	17 March	8 April	28 April	5 March	27 March	168	32	1202	319	1521

Date at the beginning, the first day we observed more than one pollen grain/cm² per day for 2 days without a break after 1 January; date at the end, the day before we were unable to observe pollen grains for 3 days without a break when the season for that pollen was ending.

Table 3 Correlation between each of the RAST results and skin test results for three types of pollen grains

		RAST results				Skin test results							
		CJ		CO		CJ		CO		JR			
RAST results	CJ	+	-	+	-	+	-	+	-	+	-	+	
	CO	+	-	+	-	+	-	+	-	+	-	+	
Skin test results	CJ	+	-	+	-	+	-	+	-	+	-	+	
	CO	+	-	+	-	+	-	+	-	+	-	+	
Skin test results	JR	+	-	+	-	+	-	+	-	+	-	+	
	JR	+	-	+	-	+	-	+	-	+	-	+	

The table is symmetrical; the upper right consists of 2×2 tables, whereas the lower left gives correlation coefficients (χ^2 -test), sensitivity (Se), specificity (Sp) and correspondent (C).

CJ, patients with *Cryptomeria japonica* pollinosis; CO, patients with *Chamaecyparis obtusa* pollinosis; JR, patients with *Juniperus rigida* pollinosis.

Pollen counts for *C. japonica* and Cupressaceae, severity of pollinosis symptoms and medication

The relationship between daily pollen counts for both *C. japonica* and *C. obtusa* and the daily S score of 25 patients is shown in Fig. 3. There were two types of clear correlation between the daily pollen counts and daily S score, the daily pollen counts and daily SM score ($r = 0.690$; $P < 0.0001$; $n = 40$). The relationship between daily S score and daily M score is shown in Fig. 4. The daily S score also correlates well with daily M score. It was quite conspicuous that daily M score followed daily S score significantly until it reached approximately 1.5 points of the M score. However, daily S score then seemed to rise rapidly above the M score.

DISCUSSION

Highly characteristic patterns of pollen dispersion for both *C. japonica* and Cupressaceae were found at the

Table 4 Correlation between *Cryptomeria japonica* (RAST or skin test) results and *Chamaecyparis obtusa* (RAST or skin test) results (χ^2 -test).

Cryptomeria japonica

			$\Phi = 0.506$
Positive	10	33	$P < 0.001$
			Sensitivity = 100%
Negative	5	0	Specificity = 33%
			Correspondent = 79%
	Negative Positive		

Chamaecyparis obtusa

three observation sites in the Chugoku (Okayama site in Okayama Prefecture; Nishinomiya site in Hyogo Prefecture) and Kanto regions (Sakura site in Chiba Prefecture). Although there were differences between the sites with regard to when pollen release from *C. japonica* began, when it ended and the periods of dispersion, the days of maximum pollen counts and the patterns of pollen dispersion were similar. In contrast, the patterns of Cupressaceae pollen dispersion were different. There were two peaks of Cupressaceae pollen release at the Okayama site. It seems most likely that the first peak was from *C. obtusa* and the second from *J. rigida*.

It was difficult to distinguish the pollen grains of *J. rigida* from *C. obtusa* under the light microscope. Miyoshi *et al.*⁶ first reported the pattern of pollen dispersion and the possibility of pollinosis to *J. rigida* distributed mainly around the Seto Inland Sea. Thus, it was considered that this pollen dispersion was important for prefectures facing onto the Seto Inland Sea. The findings of Miyoshi *et al.*⁶ differed from those of the present study in that there was only one peak of Cupressaceae pollen and this was thought to be from *C. obtusa* at the Nishinomiya site in Hyogo Prefecture and the neighboring Okayama Prefecture. In addition, it was apparent that only a small amount of *J. rigida* was contained in the Cupressaceae pollen. When compared with the sites in the Chugoku region, the clear peak with Cupressaceae was not observed and there was a low Cupressaceae pollen count at the Sakura site in Chiba Prefecture. Most notably, there were no *J. rigida* trees found from the field research and their pollen was unlikely to be dispersed in Chiba Prefecture.

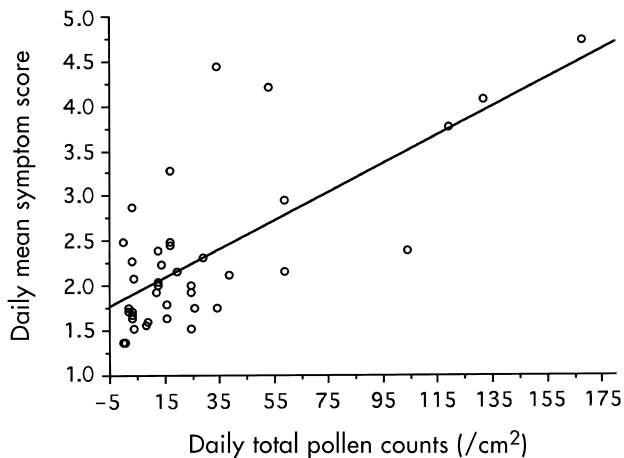


Fig. 3 Relationship between daily total pollen counts for both *Cryptomeria japonica* and *Chamaecyparis obtusa* and daily mean symptom score of 25 patients in Chiba Prefecture (regression analysis, $y = 0.016x + 1.85$, $r = 0.709$, $P < 0.0001$; $n = 40$).

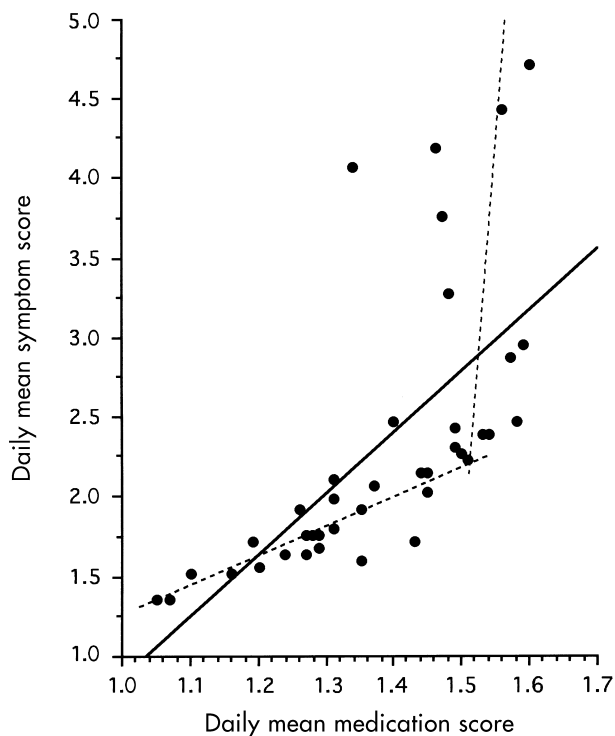


Fig. 4 Relationship between daily mean medication score and daily mean symptom score of 25 patients in Chiba Prefecture (regression analysis, $y = 3.855x - 2.983$, $r = 0.661$, $P < 0.0001$; $n = 40$).

In comparison with the plantation area of *C. japonica* and *C. obtusa* in the Japanese Archipelago as arranged by Sahashi *et al.*,¹⁷ the plantation area of *C. obtusa* was wider than that of *C. japonica* in the Chugoku region, while *C. japonica* was more likely to be cultivated in the Kanto region. The plantation areas¹⁸ of *C. japonica* in both Okayama and Chiba prefectures were almost the same at approximately 45 000 ha, while that of Hyogo Prefecture, which faces both the Seto Inland Sea and the Sea of Japan, was the widest with 111 000 ha. The plantation area¹⁸ of *C. obtusa* in Okayama Prefecture, at approximately 109 000 ha, was the widest of the three Prefectures, the second being 78 000 ha in Hyogo Prefecture and the smallest being in Chiba Prefecture, at 8000 ha. The results of the present study indicate that the airborne pollen count was proportional to the area of tree plantation. However, this cannot be said to be consistent with our results in each Prefecture, because Namba *et al.*¹⁹ have reported a discrepancy between pollen counts of the area that was the source of the airborne pollen and pollen counts at a site some distance away in Okayama Prefecture. Especially at the Okayama site, Cupressaceae pollen counts were most influenced by *J. rigida*.

The existence of cross-antigenicity between *C. japonica* and Cupressaceae, including *J. rigida*, has been reported previously.^{1,2} Takahashi *et al.*¹ have clearly demonstrated, using immunoelectron microscopy, that there is an antigen on the wall of *C. obtusa* able to react to antibodies against *C. japonica*. Ide and Ashida² have suggested that *C. japonica* was predominant over *C. obtusa* when tested for antigenicity with the RAST inhibition test. As a result of the skin test using both *C. japonica* and *C. obtusa* pollen, 53 of 229 patients, who were all positive to *C. japonica*, were negative to *C. obtusa*, while there were no patients positive to *C. obtusa* and negative to *C. japonica*.

We used both the skin test and the RAST assay for IgE levels to determine the extent of pollinosis in the patients. The pollen extracts prepared by us seemed to contain the protein antigens reported by Ide and Ashida² and Horiuti.⁵ All relationships between results for RAST using *C. japonica* and *C. obtusa* antigens and those of skin test using *C. japonica*, *C. obtusa* and *J. rigida* antigens have correlated well. Moreover, a positive rate of 32.6% to *J. rigida* was obtained in Chiba Prefecture where there is little *C. obtusa* pollen and where no *J. rigida* pollen dispersal has been observed. This result seems to prove the existence of cross-antigenicity between *J. rigida*,

C. japonica and *C. obtusa* in our epidemiological study. All patients who were sensitized to *C. obtusa* were positive to *C. japonica*. We have confirmed that *C. japonica* was predominant over *C. obtusa*.

The RAST test is valid for the diagnosis of all types of allergic diseases. However, there are times when it is impossible to use it, as in the case of *J. rigida* pollen, because it has not been marketed commercially. In such instances, it seems important to prepare a scratch solution and perform skin testing in order to identify the antigen responsible. The positive criteria for skin test on adults have not been unified among the guidelines^{13,20} and immediate development of a standardized approach is expected in Japan.

To detect the severity of symptoms in the patients, we used three kinds of symptom score, nasal symptoms, eye symptoms and disturbances to daily life. There have not been any standard point systems for the pharmacotherapy of allergic symptoms among the guidelines^{13,16} and the Japanese Ophthalmological Society. The efficacy of herbal medicine has been reported by the Japanese Society of Allergology;¹⁶ however, these are not mentioned in the Guideline for the Diagnosis of Nasal Allergy.¹³ Therefore, we have referenced these guidelines and estimated the medicine prescribed.

Based on the study of nasal symptoms, Yoshida *et al.*²¹ have reported that the daily cumulative symptom scores were well correlated with the square root of the cumulative pollen counts. In the present study, the daily pollen count significantly correlated with the S score and the SM score. Moreover, the S score significantly correlated with the M score. This indicates that the severity of symptoms was influenced by daily pollen counts. It has been suggested that the dose and use of internal and external applications should coincide with the severity of symptoms. It was found, in a detailed investigation, that patients had sometimes forgotten to take internal medicines when they had no symptoms and that patients had taken excessive doses of externally applied preparations when their symptoms were exacerbated. In addition, hardly any of the patients recorded that they used masks or glasses (there were only four patients who occasionally used only pollen masks).

It was of note that these patients, who were not under strictly controlled pollen exposure, have not been able to control their allergic symptoms well with pharmacotherapy in accordance with the severity of symptoms. In other words, symptoms can be controlled by occasional pharmacotherapy, because the sensitivity of the nasal

mucosa and the conjunctiva is not exacerbated much during the period of low pollen counts. However, when symptoms are improved by occasional pharmacotherapy, their sensitivity gradually increases as a result of continuous exposure to a small amount of pollen. It appears that the critical point of the symptoms is then reached and the symptoms rapidly worsen during a heavy pollen dispersal period without taking any protection against the airborne pollens.

These results suggest the importance of avoiding pollen grains, because it is not sufficient to control the allergic symptoms only with pharmacotherapy. Thus, it is our responsibility, as health-care professionals, to explain to patients the importance of balancing the following aspects of pollinosis control: the side effects of therapy, the importance of taking prescribed medicines according to directions and the necessity to avoid pollen grains.

Predicting yearly pollen counts for an area and daily pollen counts for patients is beneficial. There have been many reports on the state of pollen dispersion and the yearly total pollen counts for both *C. japonica* and Cupressaceae.^{3,8,19,22} Sahashi *et al.*²³ have gathered information together and have drawn up a chart showing the day of pollen release for *C. japonica* in accordance with the latitude. However, the day of maximum pollen count and the expiration day of pollen dispersion have not yet been investigated in all parts of Japan. The state of pollen dispersion for both *C. japonica* and Cupressaceae has been found to be characteristic for each area in the present study. Therefore, it seems necessary to gather and investigate the day of pollen release, the expiration day of pollen dispersion, the day of maximum pollen count and the yearly pollen counts for both *C. japonica* and Cupressaceae in all parts of Japan because of their cross-antigenicity.

ACKNOWLEDGMENTS

We are grateful to Mr T Oka (formerly Head Pharmacist, Tamano City Hospital, Okayama, Japan) who advised us on how to make the skin test solution and the Bisan Air-borne Pollen Society, which provided the pollen information.

REFERENCES

- 1 Takahashi Y, Ide T, Ashida T, Tabata S, Araki T. Major allergens of hinoki- and sugi-pollen exist on their grain wall. *Jpn. J. Palynol.* 1992; **38**: 51–7.

- 2 Ide T, Ashida T. Cross-reactivity between the arboreal pollens of *Cryptomeria japonica* (Japanese cedar) and Cupressaceae. *Allergy Practice* 1991; **11**: 174–8 (in Japanese).
- 3 Kishikawa R, Nagano H, Katsuta M, Sou N. The annual variations in airborne *Cryptomeria japonica* pollen counts and meteorological conditions in Fukuoka city. *Jpn. J. Allergol.* 1988; **37**: 355–63 (in Japanese).
- 4 Nishihata S, Inoue S, Saiga T et al. Prevalence rate of allergy to Japanese cedar pollen in Tokyo. *Jpn. J. Allergol.* 1999; **48**: 597–604 (in Japanese).
- 5 Horiuti T. Evaluation of allergenicity of the extract of Japanese juniper pollen reacting to sera from asthmatic children. *Jpn. J. Allergol.* 1992; **41**: 1459–65 (in Japanese).
- 6 Miyoshi N, Oka T, Horibe T. On pollinosis caused by Japanese juniper. *Jpn. J. Palynol.* 1990; **36**: 137–42 (in Japanese).
- 7 Durham OC. The volumetric incidence of atmospheric allergens. IV. A proposed standard method of gravity sampling, counting, and volumetric interpolation of results. *J. Allergy* 1946; **17**: 79–86.
- 8 Namba H, Tsuboi A, Miyoshi N. Annual variations in counts of airborne *Cryptomeria japonica* and Cupressaceae pollen and possibility of pollen forecasting. *Jpn. J. Palynol.* 1997; **43**: 87–96 (in Japanese).
- 9 Sahashi N, Kishikawa R, Nishima S, Nagano H. Standardization of procedures for atmospheric pollen counts and pollen forecast in Japan. *Jpn. J. Palynol.* 1993; **39**: 129–34 (in Japanese).
- 10 Sheldon JM, Lovell RG, Mathews KPA. *Manual of Clinical Allergy*. Philadelphia: WB Saunders, 1967.
- 11 Gornall AG, Bardawill CJ, David MM. Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.* 1949; **177**: 751–66.
- 12 Laemmli UK. Cleavage of structural proteins during assembly of the head of bacteriophage T₄. *Nature* 1970; **227**: 680–5.
- 13 Bi-allerugi Shinryou Gaidorain Sakusei linkai. *Bi-Allerugi Shinryou Gaidorain*. Tokyo: Life-Science Medica, 1999 (in Japanese).
- 14 Ewan PW, Coote D. Evaluation of a capsulated hydrophilic carrier polymer (the immuno Cap) for measurement of specific IgE antibodies. *Allergy* 1990; **45**: 22–9.
- 15 Okuda M. *Allergic Rhinitis: Basic and Clinical Medicine*, 1st edn Tokyo: Iyaku Drug Journal, 1999 (in Japanese).
- 16 Nippon Allerugi Gakkai Kaihou. Reexamination Committee report on assessment of severity of asthma. *Jpn. J. Allergol.* 1994; **43**: 71–80 (in Japanese).
- 17 Sahashi N, Takahashi Y, Murayama K. *Everything on the Cryptomeria japonica Pollen (Japanese Cedar)*. Tokyo: Medical Journal, 1995 (in Japanese).
- 18 Sakaguchi K, Iwakawa M, Hashimoto Y et al. *The Encyclopedia of Japanese Cedar (Cryptomeria japonica)*. Tokyo: National Forestry Extension Association in Japan, 1983 (in Japanese).
- 19 Namba H, Saitou K, Sahashi N. Assumption of the area supplying Okayama Prefecture with *Cryptomeria japonica* and Cupressaceae airborne pollen, and of their scattering routes. *Jpn. J. Allergol.* 1999; **48**: 1337–47 (in Japanese).
- 20 Sukuratti Kenkyu Han. Sukuratti hannou. *Jpn. J. Allergol.* 1972; **21**: 50–63 (in Japanese).
- 21 Yoshida H, Shimizu H, Fukami S. Relation between symptom and counts of scattering Japanese cedar pollen. *Jpn. J. Allergol.* 1996; **45**: 49–61 (in Japanese).
- 22 Sahashi N, Ikuse M, Ohmoto T, Saito Y, Tanahashi Y, Takeda H. Relationship between seasonal and annual total pollen counts of *Cryptomeria japonica* and Cupressaceae and number of outpatients with sugi pollinosis in central Japan. *Rev. Palaeobot. Palynol.* 1990; **64**: 79–86.
- 23 Sahashi N, Murayama K. Change in the northward movement of pollen front of *Cryptomeria japonica* in Japan, during 1986–91. *Aller. Immunol.* 1993; **25**: 150–3.