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## Abstract

We studied 92 patients with allergic rhinitis in Syodosima, Japan, during the pollen season between April and June to evaluate the cross-reactivity to different antigens, including pollen from the olive tree (*Olea europaea*) and orchard grass (*Dactylis glomerata*). Olive tree pollen was present in the atmosphere for 23 days, from May 19 to June 12, 1994. Specific IgE antibodies for olive tree pollen antigen were present in 21 (26.9%) of the 78 patients with allergic rhinitis. Nine (24.3%) of the 37 patients with allergic rhinitis exhibited positive skin reactivity to an extract of olive tree pollen. Fifteen (88.2 %) of the 17 patients who had IgE reactivity in their sera to olive tree pollen antigen demonstrated allergic reactions to an extract of olive tree pollen. Specific IgE antibodies for orchard grass pollen antigen were present in 43 (48.3%) of the 89 patients with allergic rhinitis and 20 (95.2%) of the 21 patients who had IgE reactivity in their sera to olive tree pollen antigen. The inhibition test using the CAP System revealed that the reactivity of the IgE antibody specific for olive tree pollen antigen was inhibited dose-dependently by an extract of orchard grass pollen. These findings show that there is a reaction in some patients with grass (Gramineae) pollinosis that might be induced by olive tree pollen.

**KEYWORDS:** olive tree pollen, pollinosis, cross-reactivity, grass pollen, orchard grass

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## Cross-Reactivity to Olive Tree Pollen and Orchard Grass Pollen in Patients with Pollinosis

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We studied 92 patients with allergic rhinitis in Syodoshima, Japan, during the pollen season between April and June to evaluate the cross-reactivity to different antigens, including pollen from the olive tree (*Olea europaea*) and orchard grass (*Dactylis glomerata*). Olive tree pollen was present in the atmosphere for 23 days, from May 19 to June 12, 1994. Specific IgE antibodies for olive tree pollen antigen were present in 21 (26.9%) of the 78 patients with allergic rhinitis. Nine (24.3%) of the 37 patients with allergic rhinitis exhibited positive skin reactivity to an extract of olive tree pollen. Fifteen (88.2%) of the 17 patients who had IgE reactivity in their sera to olive tree pollen antigen demonstrated allergic reactions to an extract of olive tree pollen. Specific IgE antibodies for orchard grass pollen antigen were present in 43 (48.3%) of the 89 patients with allergic rhinitis and 20 (95.2%) of the 21 patients who had IgE reactivity in their sera to olive tree pollen antigen. The inhibition test using the CAP System revealed that the reactivity of the IgE antibody specific for olive tree pollen antigen was inhibited dose-dependently by an extract of orchard grass pollen. These findings show that there is a reaction in some patients with grass (*Gramineae*) pollinosis that might be induced by olive tree pollen.

**Key words:** olive tree pollen, pollinosis, cross-reactivity, grass pollen, orchard grass

**T**he olive tree (*Olea europaea*) belongs to the family Oleaceae, class *Dicotyledoneae*, *Angiospermophy-*

*tina*. It releases its pollen during the early summer, and is reported to be one of the most clinically significant allergens in countries with a Mediterranean climate (1). Olive trees have been cultivated in Japan for the past 100 years. Since the 1940s, olives have been an important industry on Syodoshima Island of the Inland Sea District. In an earlier study of olive pollinosis in Japan (2), we reported a high degree of correlation between IgE titers of olive tree pollen and orchard grass pollen. The objective of the present study was to verify the degree of cross-reactivity between them as demonstrated by the CAP System [Pharmacia CAP System FEIA (fluorescence enzyme immunoassay)] inhibition test.

### Subjects and Methods

**Subjects.** A total of 92 Japanese patients from Syodoshima who suffered from allergic rhinitis, including rhinorrhea, nasal obstruction and sneezing, during the pollen season from March to June, 1994, were included in this study. Six patients (Cases 1 to 6; Table 1) whose sera displayed IgE reactivity to olive tree pollen antigen underwent inhibition tests to determine whether they would show cross-reactivity between orchard grass and olive tree pollen.

**Methods.** Skin testing was performed with the scratch method using an extract of olive tree pollen (Holster-Steir Miles Inc. IN, USA). Whealing of more than 5mm and erythema of more than 15mm indicated positivity. IgE antibody specific for olive tree pollen antigen was measured by the CAP System. Patients who exhibited a reaction of 0.7 UA/ml or higher were deemed positive for this antibody in this study. The eosinophil

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Table 1 Results of examinations

Case No.	Eo in nasal fluid	Serum IgE (IU/ml)	Specific IgE antibody (UA/ml)				Number of leucocytes		Results of provocation test		
			Mite <sup>a</sup>	C. jab <sup>b</sup>	Orch. g <sup>c</sup>	Olive	( /mm <sup>3</sup> )	Eo (%) <sup>d</sup>	Serous r. <sup>e</sup>	Swell <sup>f</sup>	Sneezing <sup>g</sup>
1	- <sup>h</sup>	163	0.35 >	0.7	36.35	1.68	6400	2	+ <sup>i</sup>	+	7
2	+	N. D. <sup>j</sup>	0.35 >	34.72	0.9	1.51	N. D	N. D	+	+	1
3	+	2590	N. D	2.3	81.5	15.54	7400	9	+	+	3
4	+	389	0.35 >	3.33	68	15.54	10700	20	+	+	2
5	+	114	0.35 >	0.67	33.42	6.44	11000	10	+	-	13
6	+	105	0.35 >	3.3	0.35 >	1.01	7900	0	+	+	3
7	+	1400	67	2.88	1.96	0.7	4500	17	-	-	0
8	+	1440	100	38.3	7	0.7	5500	10	N. D	N. D	N. D
9	+	118	2.86	10.78	2.97	0.76	7700	4	-	-	0
10	+	188	N. D	0.53	0.84	0.84	N. D	N. D	N. D	N. D	N. D
11	+	97	0.35 >	0.35 >	10.22	0.84	6800	1	+	+	2
12	+	200	0.84	3.64	1.2	0.84	N. D	N. D	+	+	0
13	+	44	8.54	0.35 >	6.86	0.87	7300	10	+	+	1
14	+	815	77.5	2.74	3.78	1.2	6300	17	+	+	5
15	-	287	0.57	0.54	39.6	1.51	8000	1	+	+	14
16	-	104	5.6	0.36	1.85	1.57	9200	N. D	+	+	26
17	-	160	0.35 >	0.39	62	2.27	10300	8	+	+	0
18	+	653	3.11	50	74.5	3.02	7000	10	+	+	3
19	+	357	1.48	18.8	26.27	9.66	7200	9	N. D	N. D	N. D
20	+	567	4.48	42.2	76	10.22	5500	6	N. D	N. D	N. D
21	+	1400	0.76	4.9	100	11.76	9400	4	+	-	7

<sup>a</sup>: Dermatophagoides pteronyssinus; <sup>b</sup>: Cryptomeria japonica; <sup>c</sup>: Orchard grass; <sup>d</sup>: Eosinophil; <sup>e</sup>: Serous rhinorrhea; <sup>f</sup>: Swelling of mucous membrane; <sup>g</sup>: Frequency of sneezing; <sup>h</sup>: Negative; <sup>i</sup>: Positive; <sup>j</sup>: Not done

counts in the nasal secretions and peripheral blood were also observed.

Nasal provocation testing was performed by placing two circular paper filters (each approximately 2 mm in diameter) containing the Holster-Steir extract of olive tree pollen into each inferior turbinate bilaterally. Allergic reactions in the nasal cavity were observed by rhinoscopy within 5 min. The reactivity was evaluated as positive if two or more of the following symptoms developed: sneezing, itching in the nose, swelling of the membrane of inferior turbinate or rhinorrhea. Physiological saline was used as a control substance for the extract of olive pollen. Patients were diagnosed as having allergic rhinitis due to olive tree pollen if they exhibited positive results on the skin test or the CAP System, and if they developed symptoms of allergic rhinitis when administered the olive tree pollen extract.

Olive tree pollen in the atmosphere was collected by means of a Dahrum pollen collector and stained with carbella solution. The number of pollen grains were counted under a microscope and expressed as the num-

ber/cm<sup>2</sup>/day.

**Inhibition test.** Six patients (Cases 1 to 6; Table 1) underwent the CAP System inhibition test. Samples of orchard grass pollen and of olive tree pollen (0.2 g each) (Kabi Pharmacia Allergen, Angelholm, Sweden) were compounded with 4 ml of 0.1 M phosphate buffer (pH 7.4) and agitated for two hours at 4 °C. After the extracts were centrifuged for 30 min at 10,000 rpm, the supernatants were filtered under suction through paper filters to serve as the original solutions for each extract. The protein concentration was measured for each original solution, and was found to be 7.20 mg/ml in the orchard grass extract and 4.54 mg/ml in the olive tree pollen extract. The extracts were diluted 1/10, 1/100, 1/1,000, 1/10,000 using 0.1 M phosphate buffer (pH 7.4) before being added to the patient's serum. The concentrations added to the sera of each patient varied according to the specific IgE antibody titer against orchard grass and olive tree pollen. After the solutions were incubated for two hours at room temperature, the specific IgE in these reacted solutions was measured by the CAP System

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method using antigens of olive tree pollen and orchard grass pollen. For the control solution, the pollen extracts were replaced by the same amounts of phosphate buffer. Nonspecific inhibition of each extract was confirmed using the serum containing specific IgE for *Dermatophagoides pteronyssinus* positive and *Dermatophagoides pteronyssinus* antigen. Results were expressed as the inhibition percentage of the initial reactivity (non-absorbed).

## Results

Levels of olive tree pollen and grass (Gramineae) pollen are shown in Fig. 1. Nine (24.3%) of the 37 patients with allergic rhinitis showed positivity on skin testing with pollen extract. IgE antibodies specific for olive tree pollen antigen were detected in 21 (26.9%) of the 78 patients studied. Fifteen (88.2%) of the 17 patients who were positive on the CAP System test exhibited symptoms of allergic rhinitis in response to exposure to the olive tree pollen extract. IgE antibodies specific for orchard grass pollen antigen were present in 43 (48.3%) of the 89 patients with allergic rhinitis and 20 (95.2%) of the 21 patients who had IgE reactivity to olive tree pollen antigen in their sera. The results of the CAP System inhibition test are presented in Figs 2 and 3. There was IgE reactivity to olive tree pollen antigen and orchard grass pollen antigen in five sera, Cases 1 to 5, and only to olive tree pollen antigen in one serum, Case 6. The olive tree pollen extract inhibited the uptake of IgE

antibodies specific to olive tree pollen antigen in all five patients (Fig. 2a) and inhibited the uptake of IgE antibodies specific to orchard grass in one patient (Case 2; Fig. 2b). The extract of orchard grass pollen inhibited the uptake of IgE antibodies specific to olive tree pollen antigen and orchard grass pollen antigen in all five patients (Fig. 2c/d). The IgE reactivity to olive tree pollen antigen in one serum, Case 6, was not inhibited by orchard grass pollen extract (Fig. 3).

## Discussion

Allergic reactions to olive tree pollen have been observed in residents of Syodosshima, Japan, where there is a high concentration of this allergen (2). Based on published reports, including our previous study which demonstrated a significant correlation between IgE titers for olive tree and orchard grass pollen, we hypothesized that patients with allergic rhinitis due to olive tree pollen would also exhibit allergic reactions to orchard grass. The results of our present immunological study show that orchard grass pollen extract, as well as olive tree pollen extract, was able to inhibit the serum IgE reactivity to olive tree pollen antigen in five sera while olive tree pollen extract inhibited the serum IgE reactivity to orchard grass pollen antigen in one serum. It demonstrated a common antigenicity between pollen from the olive and pollen from orchard grass. Thus we suppose that there is a difference in the numbers of the epitopes between both pollens.

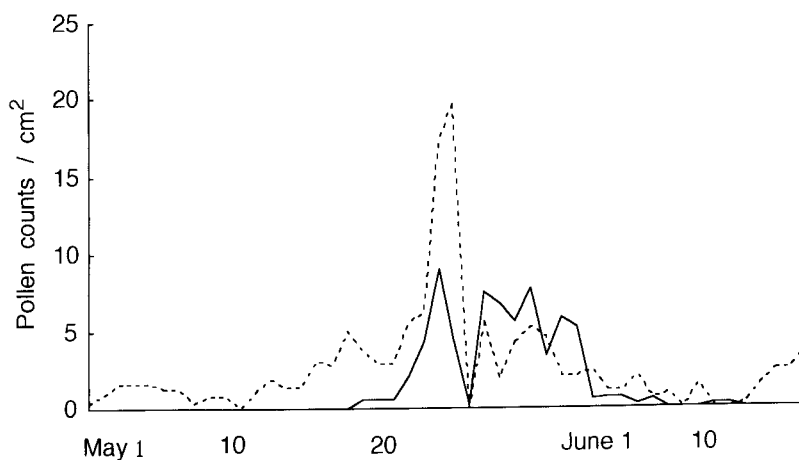
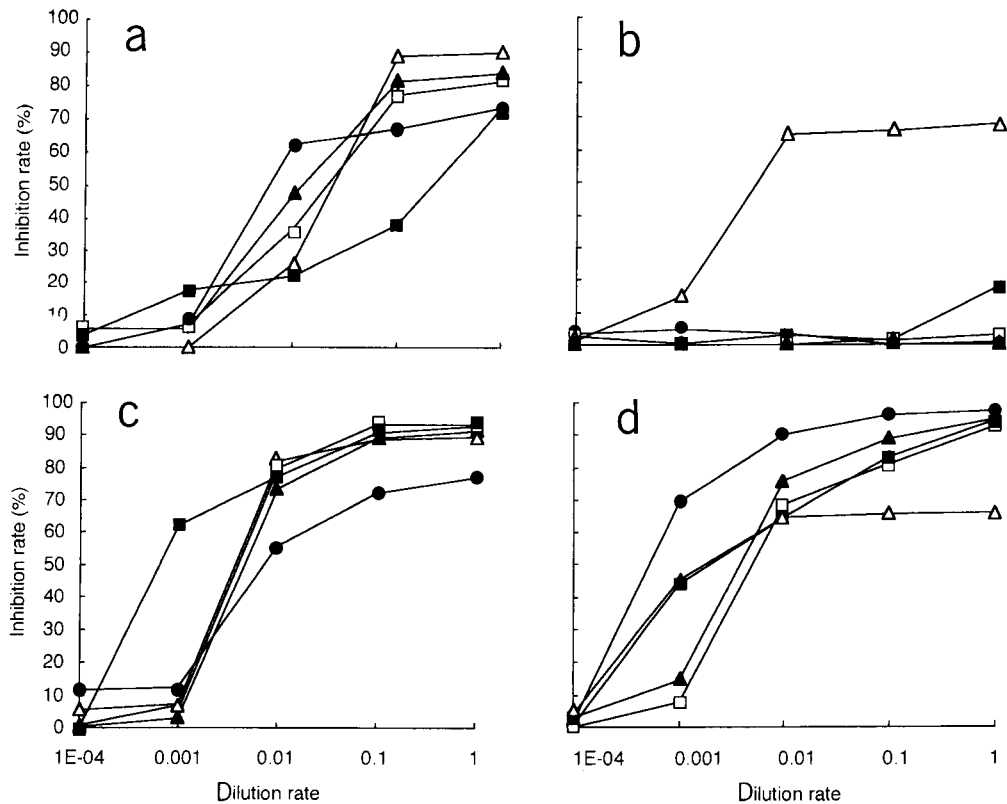
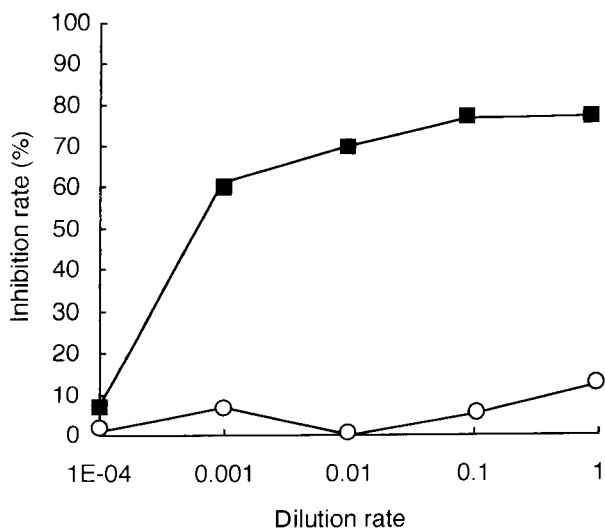


Fig. 1 Level of olive tree pollen and Gramineae pollen in Syodosshima. (—): Olive tree pollen; (---): Gramineae pollen.



**Fig. 2** CAP System inhibition test of olive tree pollen and orchard pollen. The uptake of IgE antibodies specific for the pollen antigen was inhibited by preincubation with pollen extract. Results are those of sera from case 1 (●), case 2 (△), case 3 (▲), case 4 (□) and case 5 (■). **a, c:** Inhibition rate of olive specific IgE antibody preincubated with pollen extracts of olive (**a**) and orchard grass (**c**). **b, d:** Inhibition rate of orchard grass specific IgE antibody preincubated with pollen extracts of olive (**b**) and orchard grass (**d**).



**Fig. 3** Inhibition rate of olive specific IgE antibody in the serum of Case 6. Preincubated with pollen extracts from olive tree (■) and orchard grass (○).

Olive pollinosis patients whose allergic reaction was not induced by the orchard grass pollen were present in our study, but patients having cross-reactivity between olive and orchard grass pollen were also present. Common antigenicity between the olive pollen and the pollen of Gramineae or other Oleaceae plants has also been reported. Baldo *et al.* (3) demonstrated common antigenicity between the pollen of the olive and that of rye grass (*Lolium perenne*, Gramineae). Bousquet *et al.* (1), using the RAST inhibition test, demonstrated a common antigenicity between olive tree pollen and the pollen of other Oleaceae plants such as the ash tree (*Fraxinus excelsior*) and privet (*Ligustrum vulgare*). Obispo *et al.* (4) reported that pollen extracts resembling the main antigen of the olive tree pollen, *Ole e I* (5, 6), were present in Oleaceae plants including the ash, privet, and lilac (*Syringa vulgaris*), and that the molecular weight and sequence of the N-terminal, consisting of 20 amino acids, were the same among them. They also reported that the titers of specific

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IgE antibodies against these pollen extracts were equal to those against olive tree pollen in the sera of patients who were sensitive to the pollen. Gramineae pollen is among the most common irritant in patients with pollinosis. The allergic reactions in such patients seem to be induced by the pollen of olive trees and other Oleaceae plants. Cross-reactivity to these different antigens raises important issues in the diagnosis and treatment of allergic rhinitis. Additional immunologic studies are needed to clarify these issues.

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