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IgE Antibody Responses against Japanese Cedar Pollen in the Mouse

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Abstract IgE antibody responses against Japanese cedar pollen in the mouse were investigated to develop a mouse model of human allergy for combinations of factors including pollen administration routes, elicitation antigens and inbred mouse strains. Daily short term inhalation of native pollen or intratracheal administration of pollen suspended in saline induced IgE antibody responses in DBA/2, BDF₁ and Balb/c mice, but failed to induce any detectable responses in C57BL/6 and C57BL/10 mice. Intraperitoneal injection of pollen suspension also induced IgE antibody responses in DBA/2, BDF₁ and Balb/c mice but not in C57BL/6 mice. IgE antibody responses against pollen described above were detected by passive cutaneous anaphylaxis (PCA) reactions using crude extract of pollen as an elicitation antigen. On the other hand, IgE antibodies specific for antigen Sugi basic protein (AgSBP), which is a major allergen of pollen in humans (Yasueda, H., Yui, Y., Shimizu, T., and Shida, T., 1983. Isolation and partial characterization of the major allergen from Japanese cedar (*Cryptomeria japonica*) pollen. J. Allergy Clin. Immunol. **71**: 77-86), were also detected by PCA reactions using AgSBP in the sera from mice which received secondary or the tertiary stimulation by pollen. These results suggest that IgE antibody responses against Japanese cedar pollen in the mouse can be induced by airway sensitization and that the responses are genetically controlled by H-2-linked immune response genes. The results also suggest that not only IgE antibody responses specific for components other than AgSBP but also responses specific for AgSBP can be induced in the mouse by repeating appropriate sensitization by pollen.

Human allergy to the Japanese cedar (*Cryptomeria japonica*, Sugi in Japanese) pollen, as well as human allergies to the various other weed, grass or tree pollen, is known to be caused by airway sensitization, and controlled genetically by HLA genes (1, 7, 11). For both clinical diagnosis and medical treatment of the allergy, studies on purified allergen of the pollen are important. So far, attempts have been made to purify and characterize the major allergen of pollen (14). On the other hand, the development of a potential animal model for human atopic allergy is essential for establishing the basis of medical treatment of allergies. Few attempts

have been made to develop a mouse model for the allergies caused by airway sensitization by pollen.

The present study was performed to develop a mouse model for allergy to Japanese cedar pollen. IgE antibody responses against the pollen in the mouse were described for combinations of materials and methods including pollen administration routes, elicitation antigens for passive cutaneous anaphylaxis (PCA) and inbred mouse strains.

MATERIALS AND METHODS

Mice. Six-week-old female mice of the following inbred strains were obtained from the Shizuoka Agricultural Cooperative Association for Laboratory Animals, Hamamatsu, Japan: DBA/2, (C57BL/6 × DBA/2)_{F1} (BDF₁), C57BL/6, C57BL/10, and Balb/c mice. Male Sprague Dawley rats weighting 250–350 g were also obtained from the Shizuoka Agricultural Cooperative Association, as recipients for the PCA reaction.

Antigens. Pollen was obtained from the 1984 crop of the Japanese cedar (*Cryptomeria japonica*). Preparation of antigens from the pollen was performed according to the method of Yasueda et al (14) with slight modifications. Briefly, the pollen was mixed with 20 volumes of 0.125 M sodium bicarbonate (NaHCO₃) and incubated at 20 °C for 30 min. After extraction, the supernatant (about 10 ml) was separated from the remainder of the pollen by centrifugation. It was designated as crude extract and used as an elicitation antigen for PCA. Solid ammonium sulfate was added to the supernatant to 80% saturation. After incubation at 4 °C overnight, the precipitate was obtained by centrifugation, dialyzed in 0.05 M Tris-HCl (pH 7.6) and passed through a DEAE-cellulose column. The fraction was dialysed against 0.01 M acetate buffer (pH 5.0) and applied to a CM-cellulose column. Antigen Sugi basic protein (AgSBP) fractions were eluted with 0.1 M phosphate buffer containing 0.3 M sodium chloride (pH 7.0) and used as immunizing antigen and elicitation antigen for the PCA reaction.

Sensitization. Airway sensitization of mice against native pollen was performed by applying the dry pollen daily to the animal at the tip of the nose for one or three 5-day periods with a 2-day interval in between. The treatment took about one minute per day per mouse until the mouse had difficulty in breathing due to ingestion of the pollen in the nasal cavity. Mice were also sensitized by intratracheal administration of 20 µl of pollen (50 mg/ml) suspended in borate buffered saline (BBS). The administration was accomplished by inserting a cannula into the trachea via the mouth. Mice were also sensitized by intraperitoneal (ip) injection of 200 µl of pollen (5 mg/ml) suspended in BBS, or 200 µl of AgSBP (5 mg/ml) adsorbed in alum adjuvant (5 mg/ml) (12).

Serum. Samples of 0.1 ml of blood were collected weekly after sensitization from each animal by orbital puncture with calibrated micropipettes and diluted in 0.2 ml of saline. The resulting dilution of serum was taken as a 1:5 dilution. Serum samples from five mice in each experimental group were pooled and the pooled sera

were stored at -20°C until used for PCA assays.

Measurement of IgE antibodies. IgE antibodies were titrated by the PCA reaction in Sprague Dawley rats (10). Two-fold dilutions of antisera were injected intracutaneously for passive sensitization. The PCA reaction was elicited with a 2-hr sensitization period by an iv injection of allergen, *i.e.*, crude extract (about 2 mg/ml of crude extract which contained about 10 μg of AgSBP) or AgSBP (500 μg) in 1 ml of 1% Evans blue. The test was set up in duplicate and the titer was expressed as the geometric mean. Variation of the titer for the same sample was less than 2-fold.

RESULTS

Sensitization by Inhalation of Native Pollen

Susceptibility of the mice to an allergy to Japanese cedar pollen by inhalation of pollen was examined in different mouse strains. DBA/2, BDF₁, and C57BL/10 mice were exposed to native pollen by repeated inhalation, and IgE antibody responses were measured by PCA reactions using either crude extract or AgSBP. As shown in Table 1, IgE antibody responses, detected by PCA reactions using crude extract, were induced in DBA/2 and BDF₁ mice with either one or three 5-day periods of inhalation. On the other hand, C57BL/10 mice did not show any detectable responses. Repetitive inhalation of native pollen seemed to be required for the induction of IgE antibody responses in these mice because a single inhalation did not induce detectable responses (data not shown). IgE antibody responses specific for AgSBP were not induced under these conditions (data not shown).

Table 1. IgE antibody responses to native cedar pollen in different strains of mice sensitized by inhalation

Strains	Inhalation ^{a)} (Period of treatment)	PCA titers ^{b)}			
		Days after sensitization			
		7	14	21	28
DBA/2	5-day period	10	10	20	40
	5-day period ($\times 3$)	10	5	5	< 5
BDF ₁	5-day period	< 5	< 5	< 5	< 5
	5-day period ($\times 3$)	10	10	10	40
C57BL/10	5-day period	< 5	< 5	< 5	< 5
	5-day period ($\times 3$)	< 5	< 5	< 5	< 5

^{a)} Mice were sensitized by applying dry pollen for about one minute per day to the animal at the tip of the nose for one or three 5-day periods with a 2-day interval in between.

^{b)} The IgE titers of serum pools from each group of mice on various days after sensitization were determined by PCA reactions using crude extract of the pollen as an elicitation antigen. They are shown as the reciprocals of the highest dilutions of serum pools provoking threshold PCA reactions.

Sensitization by Intratracheal Administration of Pollen Suspension

Pollen or their fragments might reach not only the nasal cavity but also the bronchi by inhalation of the pollen (3). To simplify airway sensitization, IgE antibody responses against pollen were induced by intratracheal administration of the pollen in different mouse strains. DBA/2, BDF₁, C57BL/6, and Balb/c mice were given 1 mg of the pollen suspended in saline intratracheally. On days 21 and 42 after the primary sensitization, they were given secondary and tertiary intratracheal administrations of the same dose of antigen. On various days after antigen stimulation, IgE antibody responses were measured by PCA reactions using either crude extract or AgSBP. Table 2 shows the responses detected by PCA reactions using crude extract. IgE antibody responses were induced in DBA/2, BDF₁, and Balb/c mice, whereas no detectable responses were induced in C57BL/6 mice even after the second or tertiary antigen injection. On the other hand, Table 3 shows the IgE antibody responses detected by PCA reactions using AgSBP. DBA/2, BDF₁, and Balb/c mice responded to the pollen and showed IgE antibody responses specific

Table 2. IgE antibody responses elicited by crude extract after intratracheal administration of pollen to different mouse strains

Strains	PCA titers ^{a)}							
	Primary (days)			Secondary (days)			Tertiary (days)	
	7	14	21	7	14	21	7	14
DBA/2	<5	20	5	320	20	20	160	80
BDF ₁	<5	<5	<5	<5	20	<5	<5	40
C57BL/6	<5	<5	<5	<5	<5	<5	<5	<5
Balb/c	<5	5	10	320	40	20	320	160

^{a)} Mice were sensitized by intratracheal administration of 20 μ l of the pollen (50 mg) suspended in 1 ml of BBS. On days 21 and 42 after the primary sensitization, they received secondary and tertiary intratracheal administrations of the same dose of antigen. The IgE titers were determined by PCA reactions using crude extract.

Table 3. IgE antibody responses elicited by AgSBP after intratracheal administration of pollen to different mouse strains

Strains	PCA titers ^{a)}							
	Primary (days)			Secondary (days)			Tertiary (days)	
	7	14	21	7	14	21	7	14
DBA/2	<5	<5	<5	<5	<5	<5	20	<5
BDF ₁	<5	<5	<5	<5	<5	<5	40	40
C57BL/6	<5	<5	<5	<5	<5	<5	<5	<5
Balb/c	<5	<5	<5	<5	<5	<5	20	<5

^{a)} Mice were sensitized by intratracheal administration of 20 μ l of the pollen (50 mg) suspended in 1 ml of BBS on days 0, 21, and 42. The IgE titers were determined by PCA reactions using AgSBP.

for AgSBP after tertiary antigen stimulation. In C57BL/6 mice, responses specific for AgSBP were not induced even after tertiary stimulation. These results indicate that allergy to cedar pollen can be caused by airway sensitization in the mouse, as well as in humans. Moreover, the results suggest that the IgE antibody responsiveness of the mouse strains to cedar pollen is related to their H-2 types; H-2^d mice are responders and H-2^b mice are non-responders. The results also suggest that IgE antibody responses to pollen are first induced against allergens other than AgSBP and then induced against AgSBP after appropriately frequent stimulations.

Sensitization by ip Injection of Pollen Suspension

IgE antibody responses induced by airway sensitization with the pollen were compared with those induced by ip injection of the antigen in different mouse strains. Table 4 shows IgE antibody titers assayed by PCA reactions using either crude extract or AgSBP on various days after primary and secondary antigen stimulation. DBA/2, BDF₁, and Balb/c mice responded to the pollen for IgE antibodies detected by PCA reactions using crude extract, while C57BL/6 mice did not. On the other hand, IgE antibodies detected by AgSBP were also induced in Balb/c and BDF₁ mice after secondary pollen injection. These results correspond to those of responses induced by airway sensitization.

Sensitization by Alum-Adsorbed AgSBP

Experiments were performed to determine further the involvement of AgSBP-specific IgE antibodies in IgE antibody responses to the pollen. Mice were sensitized by ip injection of 1 µg of AgSBP adsorbed in 1 mg of alum. On day 28, they were sensitized again by the same ip injection. Table 5 shows IgE antibody titers against

Table 4. IgE antibody responses elicited by either crude extract or AgSBP after ip injection of pollen into different mouse strains

Strains (H-2 type)	Elicitation antigens	PCA titers ^{a)}					
		Primary (days)				Secondary (days)	
		7	14	21	28	7	14
DBZ/2	Crude extract	20	20	20	80	N.D.	N.D. ^{b)}
(H-2 ^d)	AgSBP	<5	<5	<5	<5	N.D.	N.D.
BDF ₁	Crude extract	<5	<5	<5	<5	10	5
(H-2 ^{b/d})	AgSBP	<5	<5	<5	<5	<5	10
C57BL/6	Crude extract	<5	<5	<5	<5	<5	<5
(H-2 ^b)	AgSBP	<5	<5	<5	<5	<5	<5
Balb/c	Crude extract	<5	40	20	<5	160	80
(H-2 ^d)	AgSBP	<5	<5	<5	<5	20	40

^{a)} Mice were sensitized by intraperitoneal (ip) injection of 200 µl of the pollen (5 mg) suspended in 1 ml of BBS. On day 28 after the primary sensitization, they received a second ip injection of the same dose of antigen. The IgE titers were determined by PCA reactions using either crude extract or AgSBP.

^{b)} N.D., not determined.

Table 5. Comparison of IgE antibody responses elicited by AgSBP with those elicited by crude extract of pollen in AgSBP-sensitized mice

Strains	PCA titers ^{a)}			
	Primary—21 days		Secondary—7 days	
	AgSBP	Crude extract	AgSBP	Crude extract
DBA/2	80	<5	640	<5
BDF ₁	<5	<5	320	<5
C57BL/6	<5	<5	<5	<5
C57BL/10	<5	<5	<5	<5

^{a)} Mice were sensitized by ip injection of 200 μ l of AgSBP (5 μ g) adsorbed in alum (5 mg). Twenty-eight days later, they were sensitized again intraperitoneally with the same antigen. The IgE titers of the serum pools from each group of mice on various days after sensitization were determined by PCA reactions using either crude extract or AgSBP.

AgSBP assayed by PCA reactions using either AgSBP or crude extract for sera from mice on day 21 after the primary injection and on day 7 after the secondary injection. IgE antibody responses against AgSBP were detected by AgSBP, but not by crude extract. Thus, the crude extract of the pollen did not include amounts of AgSBP sufficient for the elicitation of PCA reactions. Table 5 also shows that DBA/2 and BDF₁ mice are responders to AgSBP, whereas C57BL/6 and C57BL/10 mice are non-responders.

DISCUSSION

Human allergy to the Japanese cedar pollen can be characterized as follows. 1) It is caused by inhalation of native pollens and by sensitization *via* the respiratory tract (1, 3). 2) It is under the control of genes linked to alleles of the HLA locus (13). 3) The major allergen of cedar pollen is AgSBP (14). 4) IgE antibody responses in patients allergic to the pollen, as well as in the patients allergic to other environmental allergens (5, 11), are persistent and boosterable by repeated doses of allergens.

The present study was performed to develop a mouse model for allergy to Japanese cedar pollen. Mice responded to the pollen given either by inhalation or by intratracheal administration and showed IgE antibody responses which were determined by PCA reactions using either crude extract of the pollen or AgSBP (Tables 1–3). Moreover, strains with the d allele at the H-2 locus (Balb/c, DBA/2, and BDF₁ mice) were responders to the pollen, whereas strains with the b allele at the H-2 locus were non-responders. These differences were also seen in mice sensitized by ip injection of the pollen (Table 4). Thus, IgE antibody responses against pollen could be induced in the mouse by airway sensitization and seemed to be under the control of genes linked to H-2.

IgE antibodies specific for AgSBP were not induced by a single injection of

pollen in the mouse and could not be detected until the mouse received a tertiary intratracheal or secondary ip injection of pollen (Tables 3 and 4). In addition, **the fact that IgE antibodies in AgSBP-sensitized mice were not detected by PCA reactions using crude extract (Table 5)** supported the concept that the pollen contained only a minute amount of AgSBP. Yasueda et al (14) reported that native pollen contains about 4% crude extract and only about 0.03% AgSBP. These data suggest that persistent IgE antibody responses specific for AgSBP could be induced by appropriate repetitive stimulations of pollen containing a minute amount of AgSBP.

In pollen-sensitized mice, IgE antibodies specific for either AgSBP or the other components in the crude extract of pollen were produced (Tables 1-4). According to Yasueda et al (14), the crude extract of pollen includes more than 90% polysaccharide, a small amount of proteins and low molecular weight materials. It has so far been reported that all of these materials can be allergens in some human allergies (2, 6, 8, 9, 15). Thus, it was suggested that the major components of the crude extract, the polysaccharides, and others are involved in IgE antibody responses of the pollen-sensitized mice as allergens other than AgSBP. Recently, Hagisawa et al (3), who studied childhood asthma from Japanese cedar pollen, reported that skin test-positive but radioallergosorbent test (RAST)-negative children were replaced by skin test-positive and RAST-positive children with increasing age. Skin tests using crude extract detected IgE antibodies specific for all allergens in crude extract. However, RAST cannot detect IgE antibodies specific for non-protein allergens, such as polysaccharides, because cyanogen bromide-activated paper disks are used to prepare solid-phase antigens in RAST. Thus, the observations by Hagisawa et al imply that in early stages of sensitization to pollen, IgE antibodies specific for non-protein allergens are produced in sensitized humans although we cannot exclude the possibility that RAST cannot detect a low level of AgSBP-specific IgE antibodies. We also investigated the specificity of IgE antibodies in sera from 52 patients allergic to Japanese cedar pollen and found that 50 of them had IgE antibodies specific for AgSBP and two of them had only IgE antibodies specific for components other than AgSBP in crude extract (unpublished data). The small number of such patients (two of 52) could be explained by the presence of a minor population of patients who had not been exposed frequently to the pollen. On the other hand, we cannot exclude another possibility that a minority of patients **are genetically non-responders to AgSBP but responders to the other components.** These data suggest that in humans, as well as in the mouse, components other than AgSBP may also be involved in IgE antibody responses to cedar pollen. Studies to characterize allergens other than AgSBP remain to be performed.

IgE antibody responses to pollen seemed to be under the control of H-2 genes; H-2^d mice are responders and H-2^b mice are non-responders (Tables 1-4). Similar variations were found in the response of strains to AgSBP (Table 5). H-2 haplotypes associated with high or low IgE responsiveness to Japanese cedar pollen or to AgSBP appeared to be specific because they were different from that to ovalbumin (OA) or to ovomucoid (OM). Vaz et al (16) demonstrated that H-2^b, H-2^d

and H-2^a are linked to high IgE responsiveness to OA and to low responsiveness to OM in different strains of mice given low-dose antigen intraperitoneally with alum adjuvant. Holt et al (4) showed data different from those of Vaz et al in mice given low-dose antigen intraperitoneally without adjuvant. Further work will be required to confirm the specificity of genetically controlled responsiveness to Japanese cedar pollen.

In the human system, Sasazuki et al (13) showed that an HLA-linked dominant gene controls resistance to cedar pollinosis or non-response to cedar pollen antigen. They also demonstrated that non-responder peripheral lymphocytes contain an active suppressor T cell which completely suppresses the IgE response of HLA-DR haplo-identical responder (allergic patient) lymphocytes to cedar pollen antigen. On the other hand, the results obtained in the present experiments did not indicate that active suppressor T cells were involved in nonresponse to cedar pollen in the mouse. Genetically much better models for human allergy to Japanese cedar pollen remain to be developed.

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