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Segregation analysis of IgE responses to *Cryptomeria japonica* pollen antigen in vivo

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Abstract The IgE response to *Cryptomeria japonica* pollen antigen (CPAg) in vivo was determined by radioimmunoassay of the plasma of 525 members from 98 families with known nasal allergies. Based on responses, patients were classified into a non-responder or low-responder group (non/low) and a high-responder group. Segregation analysis revealed that the IgE non/low responsiveness to CPAg involved a single dominant trait. The gene frequency was calculated to be 0.44–0.60. The IgE non/low response to CPAg was found to be mediated by CPAg-specific suppressor T cells. These findings demonstrated that the phenotypic variation of IgE responsiveness to CPAg is not due the immune response gene, but rather is mediated by the immune suppression gene for CPAg, via CPAg-specific suppressor T cells.

Key words *Cryptomeria* pollinosis · *Cryptomeria japonica* pollen antigen · Immune suppression gene
Radioimmunoassay

Introduction

The immune response of mice to natural and synthetic antigens is controlled by immune response (Ir) or immune suppression (Is) genes [1, 5, 15]. The possible existence of human genes that similarly control the immune response is now receiving attention because there is an as-

sociation between a gene marker such as HLA and the immune responsiveness to several natural antigens [7, 12, 20, 22]. We previously reported the existence of an Is gene that controls low responsiveness to the streptococcal cell wall antigen in vitro, via the generation of antigen-specific suppressor T cells [17, 19].

It is known that allergic diseases can be controlled by genetic factors [8, 11, 13]. A genetic analysis of patients with *Cryptomeria* pollinosis (CP) based on clinical manifestations showed that resistance to developing CP was formally a single dominant trait [19]. We designated this gene as “Is-CPAg”, because the non-responders had *Cryptomeria japonica* pollen antigen (CPAg)-specific suppressor T cells [14]. We conducted a segregation analysis of the IgE response to CPAg by measuring the titer of anti-CPAg IgE antibody (Ab) in the plasma of 525 members of 98 families with known nasal allergies, using antigen- and isotype-specific solid-phase radioimmunoassay (RIA).

Materials and methods

Preparation of antigens

The major allergens of *C. japonica* pollen were prepared as previously described [14, 27], with some modifications. In brief, dry pollen was defatted and stirred in 0.125 M ammonium bicarbonate. Solid ammonium sulfate was added to the extract (fraction A), with stirring to 100% saturation. The precipitate was dissolved in 0.05 M TRIS-HCl pH 7.8 and dialyzed against the same buffer. The sample (fraction B) was applied to a DEAE-cellulose column (Whatman, England) and eluted with the same buffer. The eluate (fraction C) was dialyzed against 0.01 M acetate buffer at pH 5.0 and applied to a CM-cellulose column (Whatman). The column was first eluted with the same buffer and then with 0.1 M phosphate buffer at pH 7.0. The second eluate (fraction D) was concentrated, dialyzed against phosphate buffered saline (PBS) and subjected to RIA.

Polyacrylamide gel electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out in 4% to 20% gradient acrylamide gel, as described by Weber and Osborn [25], and was followed by silver staining.

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Rabbit anti-human IgE Ab

Anti-human IgE Ab was prepared by immunizing rabbits with IgE myeloma protein (PS). The anti-serum was purified by different IgE protein (ND)-bound sepharose to exclude possible cross-reactions with the idiotype of PS [6, 23]. This Ab was specific for the epsilon chain, as previously described [14].

RIA for anti-CPAg IgE Ab

Anti-CPAg IgE Ab in plasma was measured by solid-phase RIA [14]. Briefly, 96-well polyvinyl plates were coated with CPAg (50 µg of fraction D/ml). After blocking with 10% fetal calf serum (FCS)/PBS, a sample diluted 2-fold with 10% FCS/PBS was added to each well in triplicate, followed by 4-h incubation at 37°C. Next, ¹²⁵I-labelled anti-human IgE in 10% FCS/PBS was added to each well and incubated for 3 h at room temperature. After washing in tap water, the wells were dried, cut, and counted in an automatic gamma counter. Using this system, the IgE Ab could be measured both in an antigen- and isotype-specific manner [14]. Serially diluted pooled plasma of patients with CP was used as the standard for specific IgE Ab determination. As such, a thousand-fold dilution was determined as one local unit, corresponding to 70 pg/ml of anti-CPAg IgE Ab.

Panels

Heparinized plasma was collected from 525 members of 98 Japanese families during the pollination season of *C. japonica*. The range of age was 15–62 years old (average, 34.7 years old). Numbers of males and females studied were 249 and 274, respectively. Also obtained for study were 38 plasma samples from umbilical cord blood, 37 plasma samples from adults with a negative skin test to CPAg, and 14 plasma samples for patients with positive skin tests to CPAg.

Familial analysis

Nuclear families were selected through the parents (complete ascertainment). Family members were categorized as either "non/low" responders (L) or "high" responders (H) to CPAg, based on the anti-CPAg IgE Ab titer in their plasma. Segregation analysis was performed in 98 families that were classed into three groups, based on the parent's phenotypes: families in which both parents were high responders (H × H), those in which both parents were non/low responders (L × L), and those in which parents differed in their immune responses to CPAg (H × L). [16].

Results

Preparation of CPAg

Figure 1 shows the SDS-PAGE profile of CPAg in both reducing and non-reducing conditions. Fraction D used for RIA included two major glycoproteins (mol. wt. = 41 Da and 46 kDa : *Cry j I*), which showed major allergenic reactivity by the RAST inhibition test [27] and Western blot analysis [14].

Anti-CPAg IgE Ab titer in RIA

Anti-CPAg IgE Ab titers were determined by RIA in the plasma of each family member. As shown in the cumula-

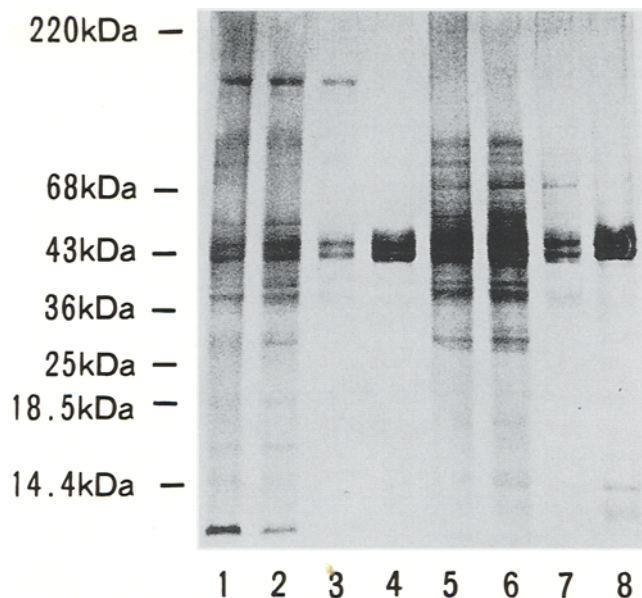


Fig. 1 SDS-PAGE profile of purified CPAg. Lanes 1 and 5: crude extract (fraction A); lanes 2 and 6: ammonium sulfate precipitate (fraction B); lanes 3 and 7: DEAE fraction C; lanes 4 and 8: CM fraction D (1–4 not reduced; 5–8 reduced)

tive frequency distribution (Fig. 2d), panels were classified into three groups: group A (< 11 units/ml), group B (12–23 units/ml) and group C (> 24 units/ml). The anti-CPAg IgE Ab of plasma from umbilical cord blood was classified in group A (Fig. 2a). Thirty-five people with negative skin tests to CPAg belonged to groups A and B, while 2 were in group C (24 units/ml) (Fig. 2b). In addition, the IgE Ab titer of plasma of patients with positive skin tests to CPAg was categorized as group C (36–1700 units/ml) (Fig. 2c).

From the results of skin testing, group A was designated as the non-responders, group B as the low responders and group C as the high responders. For statistical analysis, the panels were separated into two groups with a cut-off point of 23 units/ml; namely, the non/low responders (groups A and B) and the high responders (group C). The 31 patients with IgE Ab titers of 23 ± 2 units/ml were excluded from analysis, because the standard deviation of triplicate assays of anti-CPAg IgE Ab titer was 1.2 units/ml. When either or both of the parents were included in the border range (23 ± 2 units/ml), all members of that family were excluded from study. The segregation pattern in 92 families of the non/low responders or the high responders to CPAg, as assigned by this criterion, is summarized in Table 1.

Mode of inheritance for IgE responsiveness to CPAg

The mode of inheritance was analyzed for IgE responsiveness to CPAg. The hypothesis that a non/low responsiveness to CPAg is a single recessive trait was disregarded here because (a) many of the children who were high re-

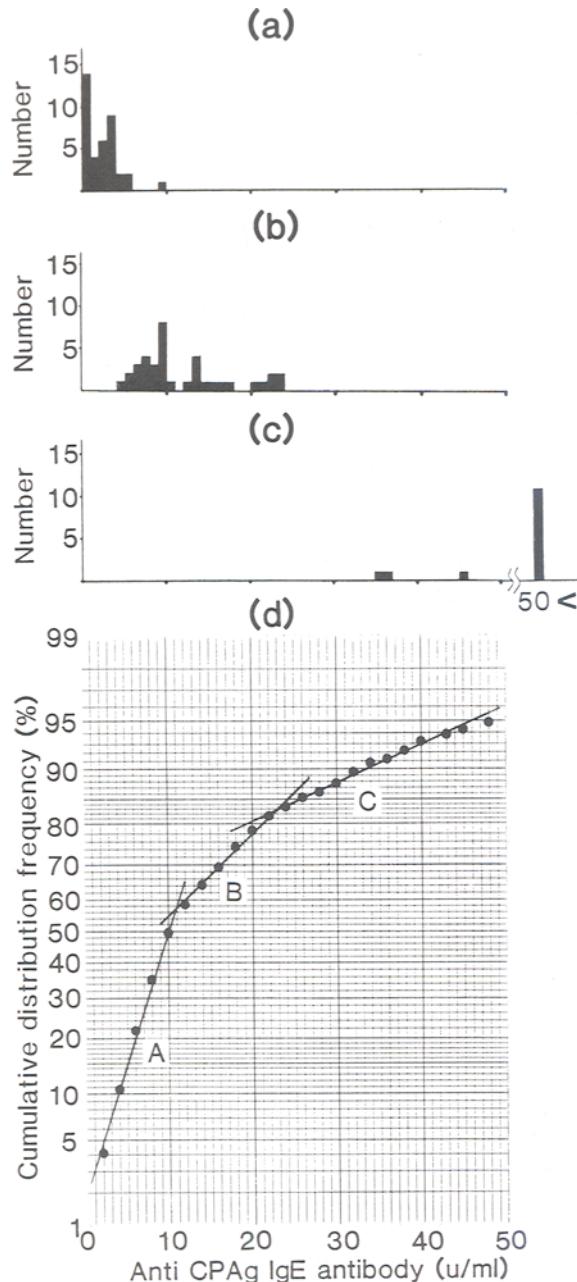


Fig. 2 Distribution of anti-CPAg-IgE antibody titer. *Abscissa* indicates titer of anti-CPAg IgE antibody in units/ml (**a–d**). *Ordinate* indicates the number (**a–c**) and the cumulative distribution frequency (%) (**d**). **a** Titer of plasma obtained from umbilical cord blood; **b** titer of plasma from healthy adults with negative skin tests; **c** titer of plasma from adults with positive skin tests; **d** titer of 525 family members

sponders were segregated from the 27 matings of the non/low responders, and (b) there was a significant difference between the observed versus expected findings ($P < 0.02$) in 15 back-cross families (Table 2A). As shown in Table 2B, when the hypothesis of there being a single recessive trait for high responders was evaluated, no significant differences were found between the observed versus expected findings in 76 inter-cross families ($0.05 < P <$

Table 1 Summary of observed phenotypes of IgE responsiveness to *Cryptomeria japonica* pollen antigen (CPAg) in vivo

Phenotype of parents	Phenotype of children	Number of families
$H \times H^a$	(H, H, H)	1
$H \times L^b$	(H, L)	1
	(L, L)	2
	(H, H, H)	1
	(H, H, L)	2
	(H, L, L)	4
$L \times L$	(L, L, L)	5
	(H)	1
	(L)	1
	(H, L)	4
	(L, L)	8
	(H, H, H)	2
	(H, H, L)	2
	(H, L, L)	16
	(L, L, L)	33
	(H, H, H, L)	1
	(H, H, L, L)	1
	(H, L, L, L)	4
	(L, L, L, L)	2
	(L, L, L, L, L)	1
Total		92

^a High responder

^b Non/low responder

0.1). As shown in Table 2C, this hypothesis cannot be discarded considering the results of the 15 back-cross families ($0.8 < P < 0.9$). In addition, all offspring of the high responder parents were also high responders. These findings support the idea of a single recessive inheritance for high responsiveness to CPAg; that is, there is a single dominant inheritance for non/low responsiveness to CPAg.

Gene frequency of major gene for IgE response to CPAg

The incidence of the major gene for the IgE response to CPAg was calculated as 44–60%, as deduced from the incidence of high responders in the 525 family members, and from the proportion of non-segregating families.

Discussion

Genetic analysis of type I allergy provides certain clues to demonstrating Ir genes in humans. Some investigators have reported a single recessive model for allergy-related diseases [4, 21, 24, 26], while others have described a single dominant model [3]. Black et al. [2] suggested the existence of the Ir gene to ragweed pollen antigens, while Marsh et al. [13], in a population study, suggested the Ir gene (Ir-Rye I and Ir-Ra 3) mediated response to those allergens.

Table 2 Segregation analysis of responders to CPAg in vivo (Morton's method). Tests for goodness of fit of the inheritance of IgE responsiveness to CPAg are based on the hypothesis of (A) an autosomal recessive inheritance for L, or (B) and (C) and an autosomal recessive inheritance for H

(A) Back-cross ($L^b \times H^a$)

Siblings size	Number of L	Observed	Expected	χ^2
2	0	0	0.85	0.85
2	1	1	1.43	0.13
2	2	2	0.72	2.30
3	0	1	1.97	0.40
3	1	2	4.30	1.23
3	2	4	4.30	0.02
3	3	5	1.43	8.88
Total		15	15.00	13.89 (df = 5) $P < 0.02$

(B) Inter-cross ($L \times L$)

Siblings size	Number of H	Observed	Expected	χ^2
1	0	1	1.72	0.30
1	1	1	0.28	1.84
2	0	8	9.05	0.12
2	1	4	2.53	0.85
2	2	0	0.42	0.42
3	0	33	35.76	0.21
3	1	16	12.58	0.93
3	2	2	4.19	1.15
3	3	2	0.75	5.05
4	0	2	4.92	1.74
4	1	4	1.92	2.33
4	2	1	0.95	0.00
4	3	1	0.21	2.95
4	4	0	0.02	0.02
5	0	1	0.57	0.32
5	Others	0	0.43	0.43
Total		76	76.00	18.66 (df = 11) $P > 0.05$

(C) Back-cross ($H \times L$)

Siblings size	Number of H	Observed	Expected	χ^2
2	0	2	1.31	0.36
2	1	1	1.13	0.01
2	2	0	0.56	0.56
3	0	5	4.12	0.19
3	1	4	3.37	0.12
3	2	2	3.37	0.56
3	3	1	1.12	0.01
Total		15	15.00	1.81 (df = 5) $P > 0.8$

^a High responder

^b Non/low responder

CP caused by *C. japonica* (Japanese cedar) pollen prevails every year in Japan from February to April, except in the northern- and southern-most areas of the archipelago. The counts of *C. japonica* pollen do not fall below 40–200/cm² per day during the season, even in urban areas, indicating intense exposure to a large quantity of this antigen [19]. The prevalence of such pollinosis ranges from 7% to 20% of the population. Multiple families with CP are frequently observed. In a previous clinical study, the Is-CPAg was in negative linkage disequilibrium with HLA-DQ3 [19]. These observations led to the idea that susceptibility to CP was genetically based.

If the non/low responsiveness to CPAg was determined by defective function of Ir gene to CPAg, such non/low responsiveness to CPAg would be a single recessive trait. However, previous genetic analysis based on clinical manifestations to CP demonstrated the opposite: that resistance to CP is a single dominant trait [19]. Even non/low responders respond to CPAg, if their suppressor T-cell fraction is depleted from peripheral blood lymphocytes [14]. From these observations, we designated the gene that governs resistance to CP through suppressor T cells as Is-CPAg [14].

In this study, we demonstrated that a non/low IgE responsiveness to CPAg in vivo is a single dominant trait. The observations reconfirmed our view of immune responsiveness to CPAg: i.e., a non/low responsiveness to CPAg is not attributable to the deletion of Ir gene but is due to Is-CPAg via antigen-specific suppressor T cells. Namely, non/low responders are homozygotic or heterozygotic in terms of Is-CPAg, so that the IgE response to CPAg is suppressed by the suppressor T cells. In contrast, high responders to CPAg are homozygous for Is-CPAg(–).

If Is-CPAg controls non-responsiveness to CP and if its absence in the homozygous state produces a responsiveness to CP, one can question the possibility of a gene-dose effect of the Is-CPAg(+) haplotype. The rate of Is-CPAg(+)/Is-CPAg(–) heterozygotes was calculated to be 0.48–0.50, based on the gene frequency of Is-CPAg: 0.44–0.60. This does not correspond to the ratio of low responders (0.32), and indicates that the gene-dose effect cannot fully explain the existence of such low responders. Other minor loci may be involved in the IgE response to CPAg.

The precise mapping of Is-CPAg is not yet feasible. However, our recent work on Is genes for the streptococcal cell wall antigen and *Schistosoma japonicum* antigen suggests that these genes can be mapped within the HLA-DQ subregion [9, 10, 17, 18]. Studies on the identification of the product of Is-CPAg and the precise mapping of Is-CPAg are now under way.

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