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Common antigenicity between Japanese cedar (*Cryptomeria japonica*) pollen and Japanese cypress (*Chamaecyparis obtusa*) pollen, I. H-2 complex affects cross responsiveness to Cry j 1 and Cha o 1 at the T- and B-cell level in mice

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SUMMARY

Common antigenicity among two purified Japanese cedar pollen allergens (Cry j 1 and Cry j 2) and one Japanese cypress pollen allergen (Cha o 1) was explored at the T-cell and B-cell level in mice of different H-2 haplotypes. Cry j 2 did not show any common antigenicity with Cry j 1 or Cha o 1. B10.S (H-2^S) mice immunized with Cry j 1 or Cha o 1 generated T cells and antibodies reactive to both antigens, indicating the common antigenicity of these antigens. C57BL/6 (H-2^b) mice were non-responders to Cry j 1. BALB/c (H-2^d) mice immunized with Cry j 1 or Cha o 1 and C57BL/6 mice immunized with Cha o 1 generated T cells that were only reactive with the respective immunogen, but produced antibody reactive to both Cry j 1 and Cha o 1, indicating that Cry j 1 and Cha o 1 share their B-cell epitope but not their T-cell epitope. This finding may provide a clue for the clarification of the T-cell and B-cell epitopes of Cry j 1 and Cha o 1, even though the data are influenced by H-2 complex restriction in mice. Considering that H-2 complex restriction affects cross responsiveness to Cry j 1 and Cha o 1 at the T- and B-cell level in mice, we assessed the possible situation in humans exposed sequentially to Japanese cedar pollen and Japanese cypress pollen.

INTRODUCTION

Pollinosis caused by Japanese cedar pollen (*Cryptomeria japonica*; 'sugi' in Japanese) is one of the commonest allergic respiratory diseases in Japan.[1–3] In most patients with Japanese cedar pollinosis, it has been noted that symptoms persist long after the end of the pollen season for Japanese cedars (February to March).[4,5] The Japanese cypress (*Chamaecyparis obtusa*; 'hinoki' in Japanese) pollen season runs from March to April and this pollen also causes pollinosis in Japan.[4,6,7] Therefore, antigenicity or common antigenicity between Japanese cedar pollen and cypress pollen has been studied to seek the cause of the prolonged symptoms in cedar pollinosis patients. These studies indicated cross allergenicity between the two types of pollen based on serum levels of antibodies to the pollen or clinical scores.[4,6–11] However, it is difficult to evaluate accurately the existence of common antigenicity as the cause of prolonged symptoms of Japanese cedar pollinosis by such studies, because patients could be sensitized to the two pollens independently.

Two allergenic molecules of *C. japonica* have been purified; Sugi basic protein (SBP; Cry j 1)[12] and Cry j 2.[13] Recently, the complete amino acid sequences of Cry j 1 and Cry j 2 were reported, with no homology being detected between the two antigens.[14,15] In addition, purification and molecular cloning of a major allergenic molecule of *C. obtusa*, Cha o 1, which shows 79–80% homology with the amino acid sequence of Cry j 1, were reported.[16]

Using these purified antigens, we investigated the possibility of common antigenicity between Japanese cedar pollen and Japanese cypress pollen, at the T-cell and B-cell level in mice, taking genetic influences on their responsiveness into account.

The present paper describes the results of experiments in which mice with different major histocompatibility complex (MHC) H-2 haplotypes were immunized with either Cry j 1, Cry j 2, or Cha o 1, after which the proliferative responses of their T cells and binding specificity of their serum antibodies to these antigens were determined.

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Abbreviations: CE, crude extract; LNC, lymph node cells; MHC, major histocompatibility complex; SBP, Sugi basic protein; SDS–PAGE, sodium dodecyl sulphate–polyacrylamide gel electrophoresis.

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MATERIALS AND METHODS

Mice

Male B10.S (H-2^S), BALB/c (H-2^d) and C57BL/6 (H-2^k) mice were purchased from Sankyo Laboratories (Tokyo, Japan) and were housed in our facilities under specific pathogen-free conditions. They were used for experiments at the age of 6–10 weeks. The care and handling of the mice followed the Animal Experimentation Guidelines of Jikei University School of Medicine.

Antigens

Cry j 1 and Cry j 2, major allergens of Japanese cedar pollen, were kindly provided by Hayashibara Biochemical Laboratories (Okayama, Japan). Cha o 1, a major allergen of Japanese cypress pollen, was a kind gift from Dr Takesi Ide (Nara Medical University, Kashihara, Japan).

Immunization

Mice were injected subcutaneously into a hind footpad with 10 μg of either Cry j 1, Cry j 2, or Cha o 1 emulsified in 50% incomplete Freund's adjuvant (Difco, Detroit, MI) plus 2 mg of alum. Ten days after immunization, the popliteal and inguinal lymph node cells (LNC) were collected.

Determination of T-cell proliferative responses to antigen

T-cell proliferative responses were determined by the *in vitro* [^{3}H]thymidine incorporation assay. RPMI-1640 medium (Immuno-Biology Laboratories, Tokyo, Japan) supplemented with 1% normal mouse serum was used to suspend LNC and 8×10^5 cells were seeded into each well of 96-well plates (Nunc Maxisorp, Kamnstrup, Denmark) and cultured with 5 μg of each antigen for 72 hr. The cultures were pulsed with 1 μCi of [^{3}H]thymidine (ICN, Costa Mesa, CA) for the final 16 hr. Cells were harvested with a Labo Mash cell harvester (Laboratory Science Co., Ltd, Tokyo, Japan) and [^{3}H]thymidine incorporated by the cells was determined by measurement of the radioactivity with a Beta-plate reader (Beckman Instruments Inc., Fullerton, CA).

Determination of antigen-binding specificity of serum antibody by Western blotting

Serum collected from mice 10 days after immunization was used. The crude extract antigens (CEs) of Japanese cedar pollen and Japanese cypress pollen were prepared as described previously.[12] Briefly, 10 μl of 10 μg/ml of Cry j 1, Cry j 2, Cha o 1, or CE of Japanese cedar pollen and CE of Japanese cypress pollen were electrophoresed in 10% polyacrylamide gel in the presence of sodium dodecyl sulphate (SDS–PAGE). The proteins separated by SDS–PAGE were transferred to a polyvinylidenedifluoride membrane (Bio-Rad Laboratories, Hercules, CA). The membrane was blocked with 1% milk (Block Ace™, Dainippon Pharmaceutical Co., Osaka, Japan) for 1 hr at room temperature and then incubated with a 1 : 200 dilution of test mouse serum overnight at 4°. After washing, to detect immunoglobulin G (IgG) antibody bound to the antigenic protein, the membrane was treated with a Vectastain Elite ABC Kit™ (Vector Laboratories Inc., Burlingame, CA) containing biotin-labeled anti-mouse IgG antibodies and streptavidin–peroxidase conjugate according to the manufacturer's protocol. Finally, staining with 3′-diaminobenzidine tetrahydrochloride (Nakarai Chemicals) was performed for 1 min at room temperature. Kaleidoscope Prestained Standard (Bio-Rad Laboratories) was used as the molecular markers.

RESULTS

Common antigenicity determined from T-cell proliferative responses

The proliferative responses of LNC from Cry j 1-immunized BALB/c, C57BL/6 and B10.S mice to Cry j 1, Cry j 2 and Cha o 1 are shown in Fig. 1. In Cry j 1-immunized BALB/c mice, LNC specifically responded to Cry j 1, and not to Cry j 2 or Cha o 1. LNC from Cry j 1-immunized C57BL/6 mice

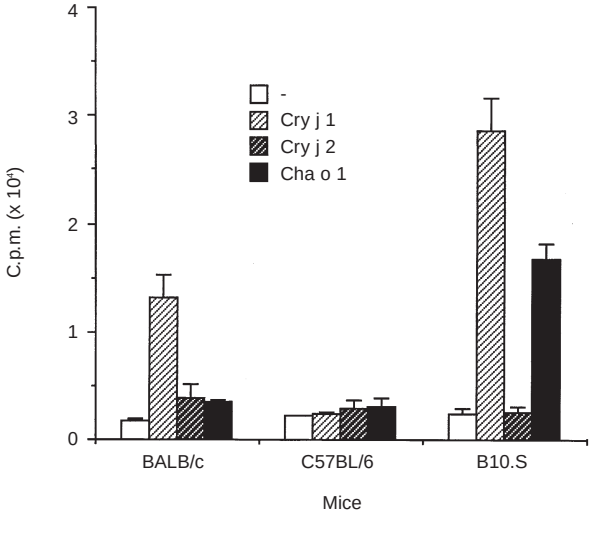

Figure 1. Proliferative responses of T cells from Cry j 1-immunized BALB/c, C57BL/6 and B10.S mice to Cry j 1, Cry j 2 and Cha o 1. LNC obtained from three mice of each strain 10 days after immunization were pooled and used for [^{3}H]thymidine incorporation assay as described in the Materials and Methods. The data are shown as the mean ± SD of triplicate cultures.

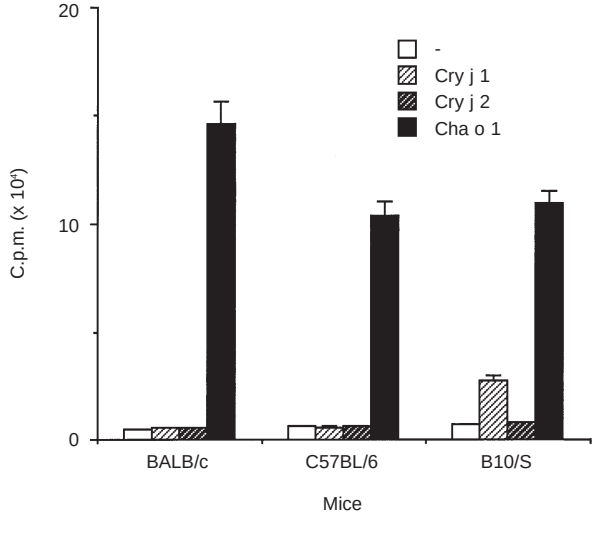

Figure 2. Proliferative responses of T cells from Cha o 1-immunized BALB/c, C57BL/6 and B10.S mice to Cha o 1, Cry j 1 and Cry j 2. Experimental details were the same as described in Fig. 1.

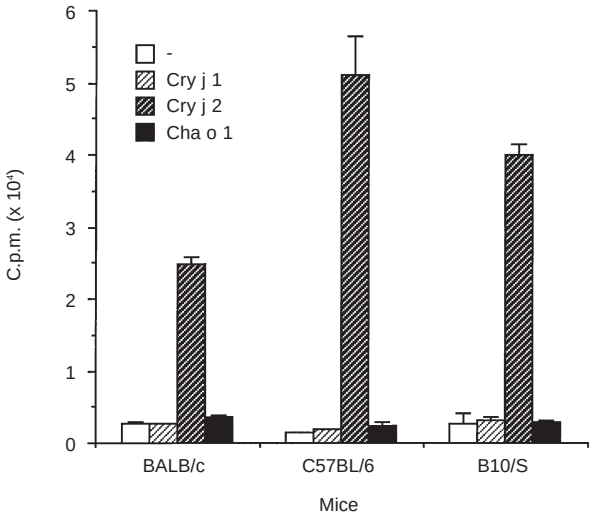

Figure 3. Proliferative responses of T cells from Cry j 2-immunized BALB/c, C57BL/6 and B10.S mice to Cry j 2, Cry j 1 and Cha o 1. Experimental details were the same as described in Fig. 1.

showed no response to any of the antigens, even to Cry j 1, indicating that C57BL/6 mice are non-responders to Cry j 1. In Cry j 1-immunized B10.S mice, LNC responded to Cha o 1, though the response was weaker than to Cry j 1, indicating common antigenicity between Cry j 1(purified from Japanese cedar pollen) and Cha o 1 (purified from Japanese cypress pollen). The LNC showed no response to Cry j 2.

The results of similar experiments performed in Cha o 1-immunized mice are shown in Fig. 2. LNC from Cha o 1-immunized BALB/c and C57BL/6 mice specifically responded to Cha o 1, and not to Cry j 1 or Cry j 2. On the other hand, LNC from Cha o 1-immunized B10.S mice responded to Cry j 1, though the response was weaker than to Cha o 1, indicating common antigenicity between Cha o 1 and Cry j 1. The LNC did not respond to Cry j 2.

LNC from Cry j 2-immunized BALB/c, C57BL/6 and B10.S mice specifically responded to Cry j 2, and not to Cry j 1 or Cha o 1 (Fig. 3).

Common antigenicity determined at the antibody level by Western blotting

Serum samples obtained from BALB/c, C57BL/6 and B10.S mice 10 days after immunization with either Cry j 1, Cry j 2 or Cha o 1 were assessed for their binding specificity to electrophoresed Cry j 1, Cry j 2, Cha o 1 and crude extracts of Japanese cedar pollen (Sugi CE) and Japanese cypress pollen (Hinoki CE).

As shown in Fig. 4, serum IgG from Cry j 1-immunized BALB/c and B10.S mice bound to protein bands of purified Cry j 1, Cha o 1, and the protein bands equivalent to those in Sugi and Hinoki CE, respectively, but did not bind to Cry j 2. The data indicated that common antigenicity at the B-cell level existed between Cry j 1 and Cha o 1. Serum from Cry j 1-immunized C57BL/6 mice did not bind to any of the antigens, indicating that C57BL/6 mice were non-responders to Cry j 1.

The results obtained with serum IgG from Cha o 1-immunized mice are shown in Fig. 5. Serum IgG from Cha o 1-immunized BALB/c, C57BL/6 and B10.S mice bound to protein bands of Cha o 1, Cry j 1 and the equivalent protein bands of Sugi and Hinoki CE, respectively, but did not bind to Cry j 2. This result also indicated that common antigenicity at the B-cell level existed between Cha o 1 and Cry j 1. Serum IgG from Cry j 2-immunized BALB/c, C57BL/6 and B10.S mice specifically bound to protein bands of Cry j 2 and not to those of the other antigens (Fig. 6). Serum from non-immunized mice of all three strains did not bind to any of the antigens (data not shown).

DISCUSSION

In order to explore whether common antigenicity between Japanese cedar pollen and cypress pollen allergens is involved in pollinosis during spring in Japan, we tried to detect common antigenicity in mice at the T- and B-cell level. This study

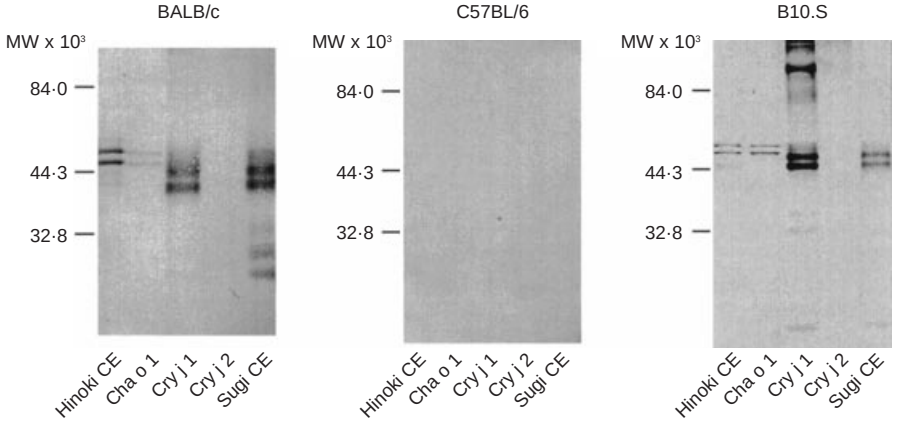

Figure 4. Binding specificity of IgG antibody of Cry j 1-immunized BALB/c and B10.S mice to Cry j 1, Cry j 2 and Cha o 1 in Western blotting. Sera obtained from three mice of each strain 10 days after immunization with Cry j 1 were pooled, diluted to 1 : 200 and used for Western blotting. Ten microlitres of 10 μg/ml Cry j 1, Cry j 2, Cha o 1, Japanese cedar (sugi) pollen CE and Japanese cypress (hinoki) pollen CE were subjected to SDS–PAGE and then transferred to polyvinylidinedifluoride membrane. As molecular markers, Kaleidoscope Prestained Standard (Bio-Rad) was used.

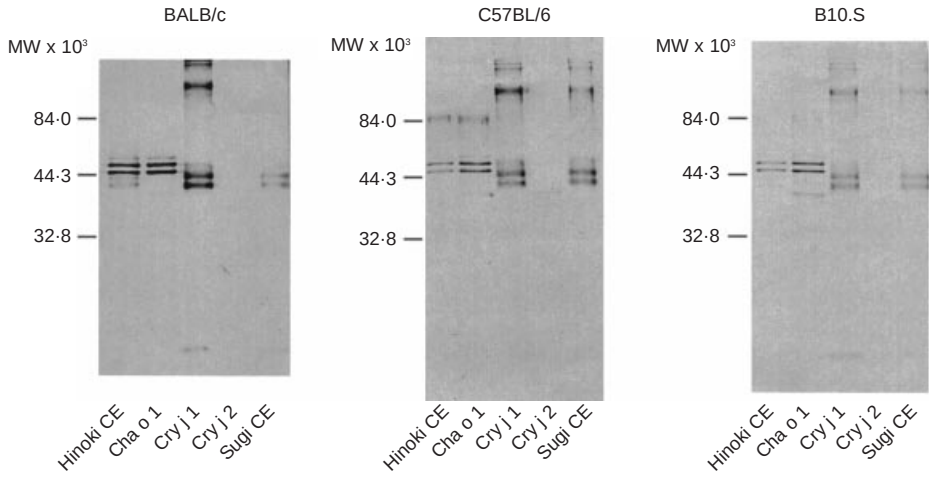

Figure 5. Binding specificity of IgG antibody of Cha o 1-immunized BALB/c, C57BL/6 and B10.S mice to Cha o 1, Cry j 1 and Cry j 2 in Western blotting. Western blotting on the sera from Cha o 1-immunized mice was done in the same way as described in Fig. 4.

assessed the reactivity of T cells and serum IgG antibody generated by mice immunized with Cry j 1 or Cry j 2 (purified Japanese cedar pollen allergens) or with Cha o 1 (purified Japanese cypress pollen allergen). Although IgE antibody reactivity should actually be determined to assess allergy, we investigated IgG antibody as a preliminary measure, because the peak of IgG antibody produced by immunization made it easier to assess cross reactivity.

When B10.S (H-2^S) mice were immunized with either Cry j 1 or Cha o 1, the animals generated T cells and IgG antibody reactive to both antigens, indicating the presence of common antigenicity between Cry j 1 and Cha o 1 at the T-cell and B-cell levels. On the other hand, LNC from BALB/c (H-2^d) mice immunized with Cry j 1 or Cha o 1 specifically responded to the immunized antigen and not to the other antigens, whereas IgG generated in these mice reacted with both Cry j 1 and Cha o 1. These results clearly indicate that the T-cell epitope differs from the B-cell epitope for Cry j 1 and Cha o 1, with these antigens sharing their B-cell epitope but not their T-cell epitope.

The results obtained with Cry j 1-immunized C57BL/6 (H-2^b) mice revealed that this strain was a non-responder to Cry j 1 and did not generate any antigen-specific T cells and antibodies. On the other hand, Cha o 1-immunized C57BL/6 mice generated LNC that reacted specifically with Cha o 1, while they produced IgG that reacted with both Cha o 1 and Cry j 1, again indicating that these antigens share their B-cell epitope but not their T-cell epitope. These findings and the similar results obtained in BALB/c mice (described above) provide an experimental system for exploration of the T-cell and B-cell epitopes of Cry j 1 and Cha o 1. Detailed investigation of the differences between their epitopes may help to improve immunotherapy for pollinosis caused by Japanese cedar pollen and/or Japanese cypress pollen, even though data obtained in mice are subjected to H-2 complex restriction.

Cry j 2, the second major allergen of Japanese cedar pollen, shared no common antigenicity with Cry j 1 or Cha o 1 at the T- and B-cell levels in any of the three strains of mice. These results are in concordance with the finding that the

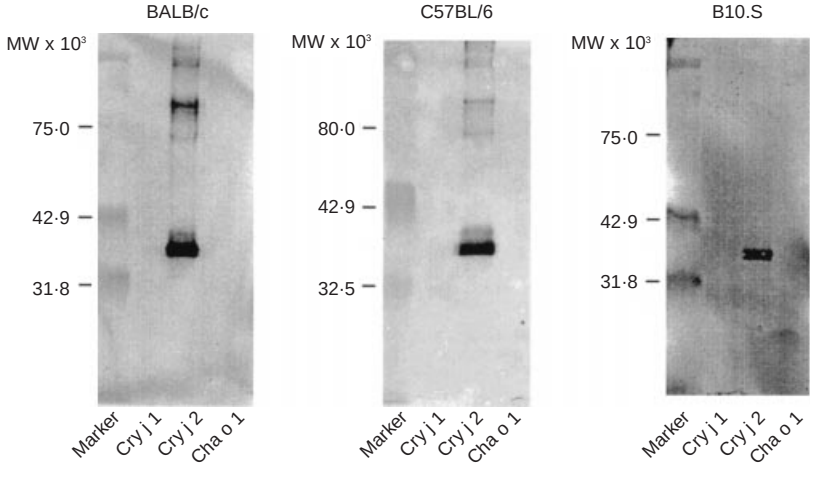

Figure 6. Binding specificity of IgG antibody of Cry j 2-immunized BALB/c, C57BL/6 and B10.S mice to Cry j 2, Cry j 1 and Cha o 1 in Western blotting. Western blotting on the sera from Cry j 2-immunized mice was done in the same way as described in Fig. 4.

amino acid sequence shares no homology between Cry j 2 and Cry j 1,[16] while there is 79–80% homology between Cry j 1 and Cha o 1.[17]

These data showed that the MHC (H-2 complex) of mice affects cross responsiveness to Cry j 1 (Japanese cedar pollen allergen) and Cha o 1 (Japanese cypress allergen) at the T- and B-cell levels. Accordingly, the situation in humans exposed to Japanese cedar pollen and cypress pollen would also depend on their MHC status. After exposure to Japanese cedar pollen, certain individuals may become sensitized to Cry j 1 and Cha o 1 at the T- and B-cell levels, as occurs in B10.S mice, while certain individuals may only show T-cell sensitization to Cry j 1 as well as B-cell sensitization to Cry j 1 and Cha o 1, as in BALB/c mice. In addition, certain individuals may be non-responders to Cry j 1, like the C57BL/6 mice. Since Cry j 2 did not show any common antigenicity with Cry j 1 and Cha o 1 in any of the three strains of mice, humans who are only sensitized to Cry j 2 of Japanese cedar pollen should not respond to Cha o 1 of Japanese cypress pollen.

Panzani et al.[8] reported that cross-reactivity was detected by skin testing, radioallergosorbent test (RAST) and RAST inhibition between the pollens of *Cupressus sempervirens* (common cypress) and *Cryptomeria japonica* (Japanese cedar), when comparing a group of French patients allergic to the pollens of cypress and a group of Japanese patients allergic to Japanese cedar pollen. These data suggest that our findings would need to be considered in future studies of common antigenicity at the T- and B-cell levels between the *Cryptomeria* genus and *Chaecyparis* genus around the world.

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REFERENCES

1. Ishizaki T., Koizumi K., Ikemori R., Ishiyama Y. & Kushibiki E. (1987) Studies of prevalence of Japanese cedar pollinosis among the residents in a densely cultivated area. *Ann Allergy* **58,** 265.
2. Mori A. (1995) Sensitization and onset of Japanese cedar pollinosis in children. *Jpn J Allergol* **44,** 7.
3. Nakamura S. (1996) Seven year study on the incidence of Japanese cedar pollinosis among University students and its transition while in college. *Jpn J Allergol* **45,** 378.
4. Ito H., Nishimura J., Suzuki M., *et al.*, (1995) Specific IgE to Japanese cypress (*Chamaecyparis obtusa*) in patients with nasal allergy. *Ann Allergy Asthma Immunol* **74,** 299.
5. Ito Y., Takahashi Y., Matsuura T. & Kobayashi M. (1996) The period of termination of nasal symptoms in Japanese cedar pollinosis patients and factors participating in the termination of symptoms. *Jpn J Allergol* **45,** 1277.
6. Okano M., Nishioka K., Nagano T., Ohta N. & Masuda Y. (1994) Clinical characterization of allergic patients sensitized to *Chamaecyparis obtusa* using AlaSTAT system. *Jpn J Allergol* **43,** 1179.
7. Ganbo T., Hisamatsu K., Inoue H., *et al.*, (1995) Detection of specific IgE antibodies to Japanese cypress pollen in patients with nasal allergy: a comparative study with Japanese cedar. *Auris Nasus Larynx* **22,** 158.
8. Panzani R., Yasueda H., Shimizu T. & Shida T. (1986) Cross-reactivity between the pollens of *Cupressus sempervirens* (common cypress) and of *Cryptomeria japonica* (Japanese cedar). *Ann Allergy* **57,** 26.
9. Taniai M., Kayano T., Takakura R., *et al.*, (1993) Epitopes on Cry j 1 and Cry j 2 for the human IgE antibodies cross reactive between *Cupressus sempervirens* and *Cryptomeria japonica* pollen. *Mol Immunol* **30,** 183.
10. Enomoto T. & Ohnishi S. (1994) Measurement of specific IgE antibody to false cypress (*Chamaecyparis obtusa*, Hinoki) pollens using *Shionoria* specific IgE. *Jpn J Allergol* **43,** 106.
11. Ito T., Takahashi Y., Fujita T. & Fukuyama S. (1997) Clinical effects of immunotherapy on Japanese cedar pollinosis in the season of cedar and cypress pollination. *Auris Nasus Larynx* **24,** 163.
12. Yasueda H., Yui Y., Shimizu T. & Shida T. (1983) Isolation and partial characterization of the major allergen from Japanese cedar (*Cryptomeria japonica*) pollen. *J Allergy Clin Immunol* **71,** 77.
13. Sakaguchi M., Inoue S., Taniai M., Ando S., Usui M. & Matsuhasi T. (1990) Identification of the second major allergen of Japanese cedar pollen. *Allergy* **45,** 309.
14. Sone T., Komiyama N., Shimizu K., Kusakabe T., Morikubo K. & Kino K. (1994) Cloning and sequencing of cDNA coding for Cry j 1, a major allergen of Japanese cedar pollen. *Biochem Biophys Res Commun* **199,** 619.
15. Komiyama N., Sone T., Shimizu K., Morikubo K. & Kino K. (1994) cDNA cloning and expression of Cry j 2, the second major allergen of Japanese cedar pollen. *Biochem Biophys Res Commun* **201,** 1021.
16. Namba M., Kurose M., Torigoe K., *et al.*, (1994) Molecular cloning of the second major allergen, Cry j 2, from Japanese cedar pollen. *FEBS Lett* **353,** 124.
17. Suzuki M., Komiyama N., Itoh M., *et al.*, (1996) Purification, characterization and molecular cloning of Cha o 1, a major allergen of *Chamaecyparis obtusa* (Japanese cypress) pollen. *Mol Immunol* **33,** 451.