

Common antigenicity between Japanese cedar (*Cryptomeria japonica*) pollen and Japanese cypress (*Chamaecyparis obtusa*) pollen, II. Determination of the cross-reacting T-cell epitope of Cry j 1 and Cha o 1 in mice

N. OHNO,*† T. IDE,‡ M. SAKAGUCHI,§ S. INOUE¶ & S. SAITO†
**Department of Pediatrics, †Department of Molecular Immunology, Institute of DNA Medicine, The Jikei University School of Medicine, Tokyo, ‡Department of Chemistry, Nara Medical University, Kashihara, §Department of Immunology, and ¶Infectious Disease Surveillance Center, National Institute of Infectious Diseases, Tokyo, Japan*

SUMMARY

We have previously detected common antigenicity between Cry j 1 and Cha o 1 in B10.S mice. B10.S mice immunized with Cry j 1- or Cha o 1-generated T cells and antibodies reactive to both allergens. In the present study, we investigated the cross-reacting and Cry j 1-specific T-cell epitopes in B10.S mice. Lymph node cells from B10.S mice immunized with Cry j 1 recognized Cry j 1 p111–130, p211–230, and p310–330 as well as Cha o 1 p209–228. The existence of the cross-reacting T-cell epitope in Cry j 1 and Cha o 1 was confirmed by the response of newly established p211–230-specific and Cha o 1 p209–228-specific T-cell lines. The minimum peptide sequence (p213–224) of the cross-reacting T-cell epitope was identical in Cry j 1 and Cha o 1. These findings clearly demonstrate that common antigenicity at the T-cell level between Japanese cedar and cypress pollen allergens was caused by the existence of an identical-cell epitope in Cry j 1 and Cha o 1.

INTRODUCTION

In our previous study, we investigated whether there was common antigenicity between Japanese cedar pollen and Japanese cypress pollen at the T- and B-cell levels in mice of different H-2 haplotypes.¹ We showed that B10.S (H-2^S) mice, but not BALB/c or C57BL/6 mice, immunized with Cry j 1 or Cha o 1 generated T cells reactive to both antigens, indicating that there was common antigenicity and that major histocompatibility complex (MHC; murine H-2 complex) influence the cross responsiveness to Cry j 1 and Cha o 1 at the T-cell level. In addition, specific antibodies to each allergen generated in these mice showed reciprocal cross-reactivity. Furthermore, cross-reactivity at the B-cell level between Cry j 1 and Cha o 1 has been reported in humans.^{2,3}

On the basis of the known sequence homology and previous data indicating the existence of common antigenicity at the T-cell level,^{1,4,5} we would expect that a cross-reacting T-cell epitope exists for Cry j 1 and Cha o 1, but there have been no reports of such an epitope in humans or mice. Detection of a cross-reacting epitope recognized by T cells specific for each

allergen could provide useful information to improve our understanding of the common or specific antigenicity of the two pollens. However, in pollinosis patients who are exposed to both pollens, it is difficult to assess the presence of cross-reactive epitopes at the T-cell level.

In the present study, we demonstrated a cross-reacting T-cell epitope of Cry j 1 and Cha o 1, using lymph node cells from B10.S mice immunized with each antigen and each antigen-specific T-cell line.

MATERIALS AND METHODS

Mice

Male B10.S (H-2^S) mice were purchased from Sankyo Laboratories (Tokyo, Japan) and were maintained in our own facilities under specific pathogen-free conditions. The animals were used for the 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 and synthetic peptides

Cry j 1, one of the major allergens of Japanese cedar pollen, was kindly provided by Hayashibara Biochemical Laboratories (Okayama, Japan). Cha o 1, a major allergen of Japanese cypress pollen, was purified as reported previously.^{4,6} A complete series of overlapping peptides spanning the entire

Received 13 September 1999; accepted 5 January 2000.

Correspondence: Dr S. Saito, Department of Molecular Immunology, Institute of DNA Medicine, The Jikei University School of Medicine, 3-25-8 Nishi-Shinbashi, Minato-ku, Tokyo, 105-8461, Japan.

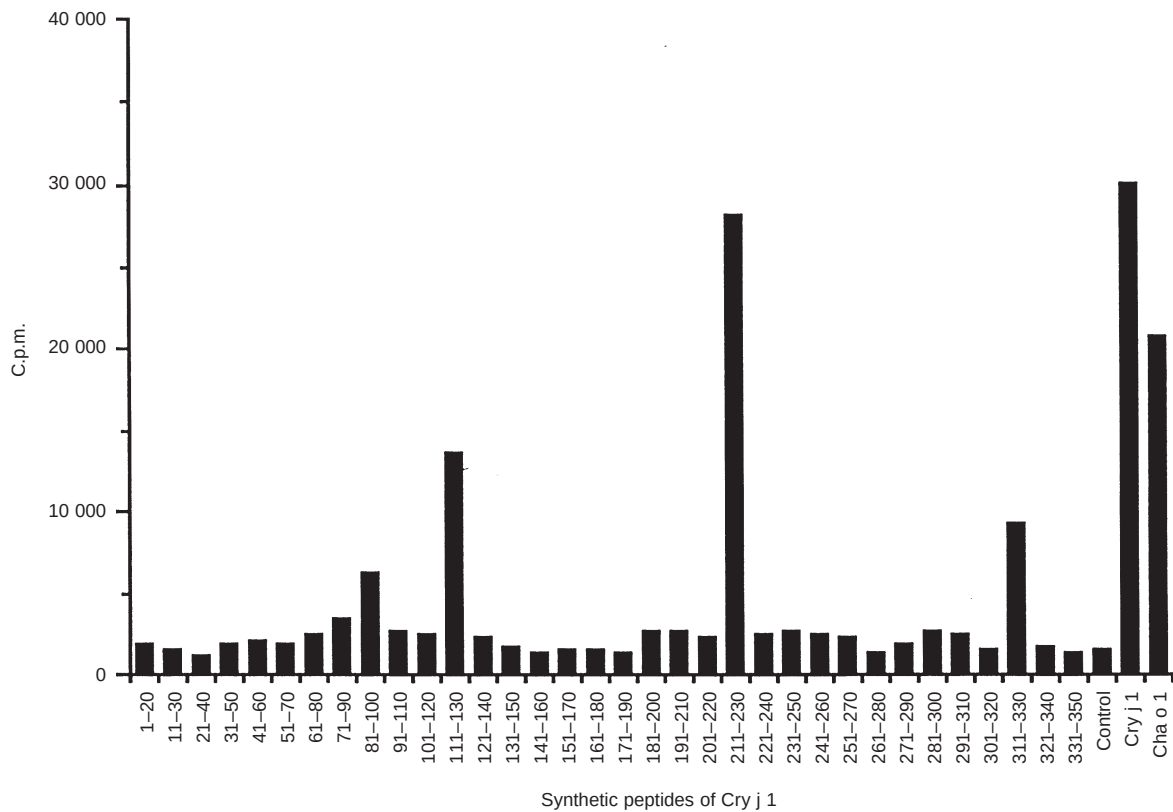


Figure 1. T-cell epitopes on Cry j 1 recognized by LNCs from B10.S mice. The peptides are designated by indicating N- and C-terminal amino acid residue numbers. LNCs were stimulated for 72 hr with 34 overlapping peptides spanning the entire sequence of Cry j 1 and were pulsed for the last 16 hr with 1 μ Ci of [^{3} H]thymidine. Representative data are shown from several comparable experiments.

sequences of Cry j 1 (34 synthesized peptides, 20-mer, advancing by 10 residues) and Cha o 1 (three synthesized peptides, shown in Table 1) were kind gifts from Sankyo Co., Ltd.

Immunization

Mice were injected subcutaneously into a hind footpad with 10 μ g of either Cry j 1 or Cha o 1 emulsified in 50% incomplete Freund's adjuvant (Difco, Detroit, MI) plus 1 mg of alum. Ten days after immunization, popliteal and inguinal lymph nodes were collected and a lymph node cell (LNC) suspension was prepared in culture medium.

Establishment of Cry j 1 and Cha o 1 T-cell lines

Cry j 1- and Cha o 1-specific T-cell lines were established from the LNCs of B10.S mice immunized with Cry j 1 or Cha o 1. Briefly, LNCs from immunized mice were suspended at a density of 4×10^6 /ml in RPMI-1640 medium (Immuno-Biology Laboratories, Tokyo, Japan) containing 1% normal mouse

serum. Then the cells were seeded into the wells of 24-well plates (Nunc Maxisorp, Kamstrup, Denmark) and cultured with 500 ng/ml of Cry j 1 p211–230 or Cha o 1 p209–228 at 37° in a 5% CO₂ incubator. After 7 days, the cells were harvested and then cultured with each antigen in the presence of irradiated (35 Gy) syngeneic spleen cells as antigen-presenting cells (APC). After repeated cycles (more than seven) of stimulation in the presence of APC, antigen-specific T-cell lines were established.

T-cell proliferation assay

LNCs from immunized mice were stimulated with Cry j 1 (2.5 μ g/ml), Cha o 1 (2.5 μ g/ml), or each synthetic peptide (500 ng/ml) in 96-well plates at a concentration of 8×10^5 cells per well. When the T-cell lines were tested, 1×10^4 cells were stimulated with each antigen or synthetic peptide in the presence of APC. The proliferative response of LNCs was measured by [^{3} H]thymidine (ICN, Costa Mesa, CA) incorporation during the last 16 hr of the 72-hr culture period. Cells were

Table 1. Synthetic peptides derived from Cry j 1 and Cha o 1 sequences used in this study

Cry j 1		Cha o 1	
Peptide	Amino acid sequence	Peptide	Amino acid sequence
p111–130	KRVSNVIIHGLYLYGCSTSV	p110–124	MRTVSHVILHGLNIH
p211–230	KSMKVTVAFNQFGPNCGQRM	p209–228	DDKSMKVTVAFNQFGPNAGQ
p311–330	SSGKYEGGNIYTKKEATNVE	p311–325	SSGKNEGTTKKNNNE

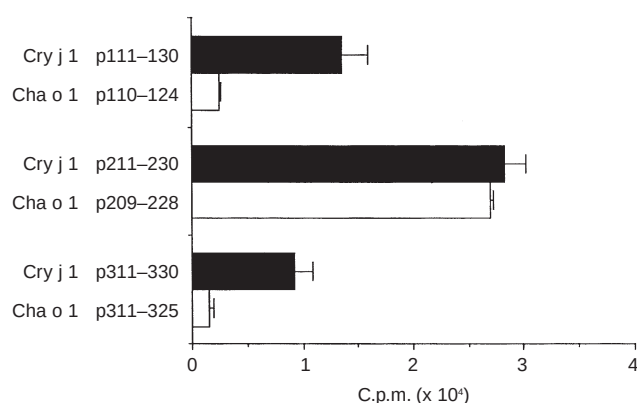


Figure 2. Cross-reacting T-cell epitope of Cry j 1 and Cha o 1. LNCs from B10.S mice primed with Cry j 1 were examined for proliferation in response to the synthetic peptides shown in Table 1. LNCs were stimulated with peptides for 72 hr and were pulsed for the last 16 hr with 1 μ Ci of [³H]thymidine. Representative data are shown from several comparable experiments. Values are the mean $\pm$ SD for triplicate cultures.

harvested with a Labo Mash harvester (Laboratory Science Co., Ltd, Tokyo, Japan) and [³H]thymidine incorporation was determined by measurement of the radioactivity with a Beta-plate reader (Beckman Instruments Inc., Fullerton, CA). The results were expressed as counts per minute (c.p.m.).

RESULTS

Epitope mapping in B10.S mice using of synthetic Cry j 1 peptides

The proliferative response of LNCs from Cry j 1-immunized B10.S mice to stimulation with Cry j 1, overlapping synthetic Cry j 1 peptides, or Cha o 1 was examined. As shown in Fig. 1, LNCs from Cry j 1-immunized mice proliferated in response to

both Cry j 1 and Cha o 1. The strongest proliferative response was observed to p211–230, with weaker responses to p111–130 and p310–330. The results indicated that at least three different Cry j 1 epitopes were recognized by T cells from B10.S mice immunized with Cry j 1.

Analysis of the cross-reacting T-cell epitope in Cry j 1 and Cha o 1

In order to examine the cross-reacting T-cell epitope in Cry j 1 and Cha o 1, we used three synthetic Cha o 1 peptides corresponding to the three major epitopes of Cry j 1 (Table 1). As shown in Fig. 2, LNCs from Cry j 1-immunized B10.S mice responded to Cha o 1 p209–228, the counterpart of Cry j 1 p211–230, but not to the other synthetic Cha o 1 peptides. To further confirm whether Cry j 1 p211–230-specific T cells recognize Cha o 1 p209–228, we established a Cry j 1 p211–230-specific T-cell line (designated as SCR1). As shown in Fig. 3, SCR1 (which specifically recognized Cry j 1 p211–230) showed a strong proliferative response to Cha o 1 p209–228. These data clearly demonstrated that this cross-reactive T-cell epitope was responsible for common antigenicity between Cry j 1 and Cha o 1.

Detailed specificity of the SCR1 T-cell line

We further analysed the minimum size of the epitope within Cry j 1 p211–230 that was recognized by SCR1 in order to determine the core peptide of the cross-reacting T-cell epitope. To localize the minimum epitope, we synthesized the six peptides shown in Fig. 4 and examined the proliferative response of SCR1 to the peptides. SCR1 proliferated most strongly in response to Cry j 1 p211–225, and also recognized Cry j 1 p212–224 and Cry j 1 p213–224. However, SCR1 did not respond to Cry j 1 p212–223.

These results indicated that p213–224 (MKVTV-AFNQFGP) was the minimum epitope for SCR1 and was the

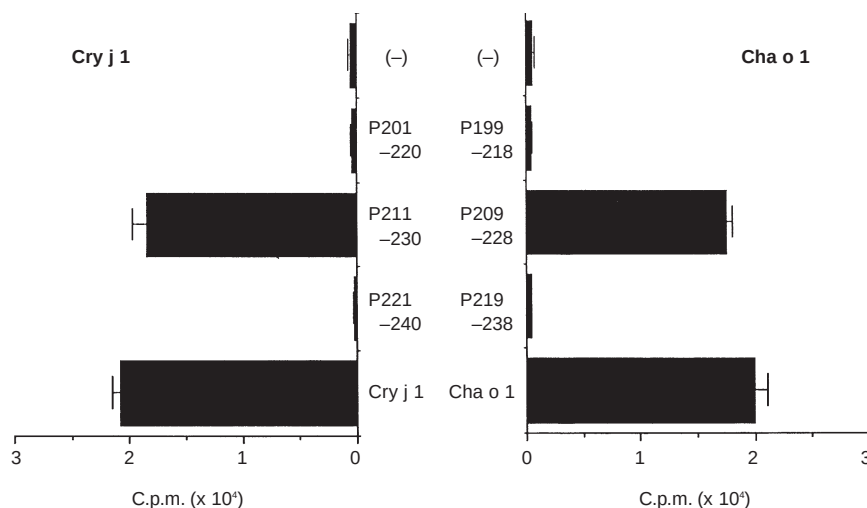


Figure 3. Cross-recognition by a Cry j 1 p211–230-specific T-cell line (SCR1). The SCR1 T-cell line was established as described in the Materials and Methods. T cells were examined for proliferation in response to each synthetic peptide. Peptides are designated by indicating the N- and C-terminal amino acid residues. The experiment was performed as described in the legend to Fig. 2. Values are the mean $\pm$ SD for triplicate cultures.

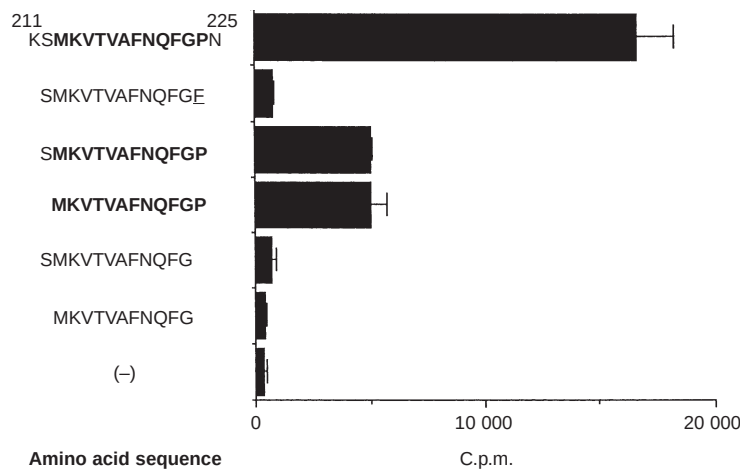


Figure 4. Detailed specificity of the SCR1 T-cell line. SCR1 T cells were examined for the proliferative response to six truncated peptides of Cry j 1 p211–225. The core peptide sequence is shown in bold. Amino acid substitution at position 224 (P → F) is underlined. The experiment was performed as described in the legend to Fig. 2. Values are the mean $\pm$ SD for triplicate cultures.

minimum peptide of the cross-reacting T-cell epitope for Cry j 1 and Cha o 1. In addition, no response was observed to the peptide (p212–224) of the amino acid substitution at position 224 (P → F). These data demonstrated that the proline residue at position 224 of cross-reacting T-cell epitopes p213–224 is essential for T-cell activation in the context of I-A^S molecules.

Recognition of a cross reacting T-cell epitope by a Cha o 1 p209–228-specific T-cell line

To further confirm the existence of a cross-reacting T-cell epitope, we established a Cha o 1 p209–228-specific T-cell line and examined the recognition of Cry j 1 p211–230 by this cell line. A proliferative response of similar magnitude was observed in response to the Cha o 1 p209–228 and Cry j 1 p211–230 peptides (Fig. 5). Taken together, the results clearly

indicated the existence of a cross-reacting T-cell epitope in Cry j 1 and Cha o 1.

DISCUSSION

Previous studies have indicated that there is cross-antigenicity between Cry j 1 and Cha o 1 at the B-cell level,^{2,3} but there has been no report about this at the T-cell level. It is difficult to define cross-antigenicity at the T-cell level by using peripheral blood mononuclear cells from Japanese cedar pollinosis patients who have been exposed to both antigens. In this study, we found a cross-reacting T-cell epitope for Cry j 1 and Cha o 1 using T cells from mice immunized with each antigen separately.

In the preceding study, we showed that B10.S (H-2^S) mice immunized with either Cry j 1 or Cha o 1 generated T cells which responded to a common antigenic site in Cry j 1 and

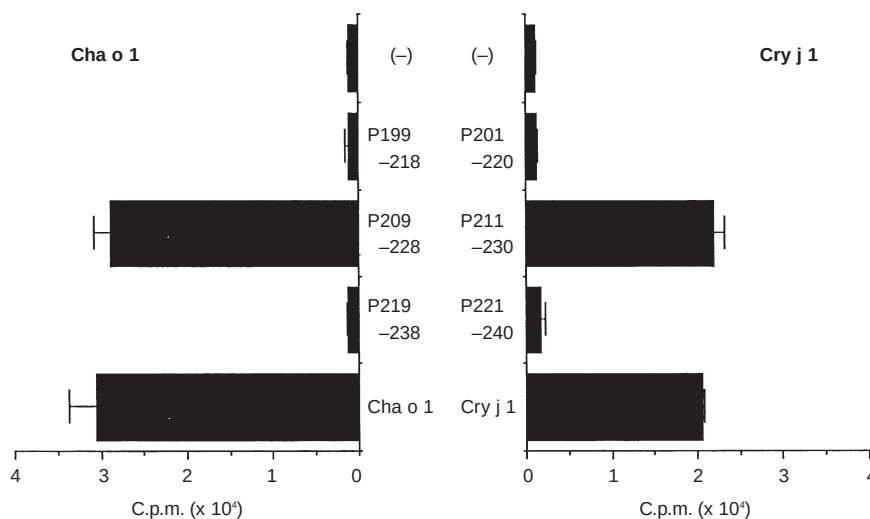


Figure 5. Cross-recognition by a Cha o 1 p209–228-specific T-cell line (Sch1-1-40). The Sch1-1-40 T-cell line was established as described in the Materials and Methods, and T cells were examined for proliferation in response to each synthetic peptide. Peptides are designated by indicating the N- and C-terminal amino acid residue numbers. The experiment was performed as described in the legend to Fig. 2. Values are the mean $\pm$ SD for triplicate cultures.

Cha o 1.¹ Therefore, the present study investigated the cross-reacting T-cell epitope for Cry j 1 and Cha o 1 in B10.S (H-2^S) mice. The T cells of B10.S mice immunized with Cry j 1 recognized Cry j 1 p111–130, p211–230, and p310–330 as well as Cha o 1 p209–228, but not the other Cha o 1 peptides as counterparts of Cry j 1 p111–130 or p310–330. These results indicated that the cross-reacting T-cell epitope exists at p211–230, while the Cry j 1-specific epitopes are at p111–130 and p310–330. Furthermore, existence of a cross-reacting T-cell epitope was confirmed by the finding that a Cry j 1 p211–230-specific T-cell line showed a significant response to Cha o 1 p209–228 and a Cha o 1 p209–228-specific T-cell line also strongly proliferated in response to Cry j 1 p211–230. These findings clearly indicate that there is a cross-reacting T-cell epitope for Cry j 1 and Cha o 1.

To localize the epitope of the Cry j 1 p211–230-specific T-cell line (SCR1), we examined the response of SCR1 to truncated peptides of Cry j 1 p211–230. As a result, the common site of the cross-reacting T-cell epitope (Cry j 1 p211–230) was found to be MKVTVAFNQFGP (Cry j 1 p213–224). The amino acid sequences of the common site were identical between Cry j 1 and Cha o 1. Taken together, these findings indicate that common antigenicity between Cry j 1 and Cha o 1 at the T-cell level in B10.S mice is explained by the existence of an identical T-cell epitope in Cry j 1 and Cha o 1. On the other hand, the amino acid sequences of the Cry j 1-specific epitopes (p111–130 and p310–330) are very different from those of Cha o 1 corresponding to the epitope of Cry j 1 (40% discrepancy for Cha o 1 p111–130 and 30% discrepancy for Cha o 1 p310–330, respectively). Therefore, these regions could not be the cross-reacting T-cell epitope for Cry j 1 and Cha o 1.

In the last few years, our understanding of the mechanisms involved in the interaction between MHC class II molecules and antigenic peptides has greatly increased.^{7,8} Previous studies have shown that a proline residue is important for binding to I-A^S molecules.⁹ In this study, deletion of the proline residue at position 224 or amino acid substitution at position 224 (P → F) in the peptide sequences (p212–224) led to the lack of a T-cell response. These findings are in agreement with the previous reports.⁹ In addition, our data showed that the amino acid sequence of the Cry j 1-specific T-cell epitope did not contain a proline residue. This result suggests that other amino acid residues besides proline are involved in binding to I-A^S molecules, although we did not examine amino acid binding to I-A^S molecules.

The present study demonstrated a cross-reacting T-cell epitope of Cry j 1 and Cha o 1 in B10.S mice. In humans, several T-cell epitopes of Cry j 1 have been reported.^{10,11} Interestingly, one of the major human T-cell epitopes in Cry j 1 contains the core peptide of the cross-reacting T-cell epitope we found in B10.S mice. On the basis of our data, it is suggested that T cells from Japanese cedar pollinosis patients recognize Cry j 1 p211–230 and also respond to Cha o 1. Therefore, cedar pollinosis patients could continue to have allergic symptoms even after the end of the cedar pollen season, which is followed by the cypress pollen season.^{12–14}

In summary, we demonstrated a cross-reacting T-cell epitope of Cry j 1 and Cha o 1, which are major pollen allergens in Japan, using T cells from B10.S (H-2^S) mice immunized with either Cry j 1 or Cha o 1. The existence of an

identical T-cell epitope in Cry j 1 and Cha o 1 may be an important factor in the prolongation of symptoms in cedar pollinosis patients.

ACKNOWLEDGMENT

We thank Professor Yoshikatu Eto (Jikei University School of Medicine) and Nobufusa Serizawa (Medical Research Laboratories, Sankyo Co. Ltd) for helpful advice and critical reading of the manuscript. We sincerely appreciate the technical assistance of Mrs Yoko Nataka and Mrs Junko Kawaguchi. This work was supported by a grant from the Agency for Science and Technology.

REFERENCES

1. KINGETU I., OHNO N., HAYASHI N., SAKAGUCHI M., INOUE S. & SAITO S. (2000) Common antigenicity between Japanese cedar (*Cryptomeria japonica*) pollen and Japanese cypress (*Chamaecyparis obtusa*) pollen: I. H-2 complex affects cross responsiveness to Cry j1 and Cha O 1 at T and B cell levels in mice. *Immunology* **99**, 625.
2. PANZANI R., YASUEDA H., SHIMIZU T. & SHIDA T. (1986) Cross-reactivity between the pollen of *Cupressus sempervirens* (common cypress) and of *Cryptomeria japonica* (Japanese cedar). *Ann Allergy* **57**, 26.
3. TANAI M., KAYANO T., TAKAKURA R., *et al.*, (1993) Epitope 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.
4. 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.
5. KRIHARA M. (1997) Physicochemical and immunological characterization of major allergens of Japanese cedar pollen and false cypress pollen. *Allergology* **3**, 203.
6. IDE T., YAMAMOTO K., TABATA S. & ASHIDA T. (1995) N-terminal amino acid sequence of a major allergen, Cha o 1, of Japanese Cypress pollen. *Jpn J Palynol* **41**, 69.
7. ZUGEL U., WANG R., SHIH G., SETTE A., ALEXANDER J. & GREY H.M. (1998) Termination of peripheral tolerance to a T cell epitope by heteroclitic antigen analogues. *J Immunol* **161**, 1705.
8. ROTHBARD J.B. & TAYLOR W.R. (1988) A. sequence pattern common to T cell epitopes. *EMBO J* **7**, 93.
9. REIZIS B., EISENSTEIN M., MOR F. & COHEN I.R. (1998) The peptide-binding strategy of the MHC class II I-A molecules. *Immunol Today* **19**, 212.
10. SONE T., MORIKUBO K., MIYAHARA M., *et al.*, (1998) T cell epitope in Japanese Cedar (*Cryptomeria japonica*) pollen allergens: choice of major T cell epitopes in Cry j 1 and Cry j 2 toward design of the peptide-based immunotherapeutics for the management of Japanese Cedar pollinosis. *J Immunol* **161**, 448.
11. ISHIKAWA T., IKAGAWA S., MASUYAMA K., MATSUSHITA S. & NISHIMURA Y. (1997) Human T. cell response to antigen peptides of Japanese cedar pollen (Cry j 1). *Int Arch Allergy Immunol* **113**, 255.
12. 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 (Tokyo)* **22**, 158.
13. ITO Y., TAKAHASHI Y., FUJITA T. & FUKUYAMA S. (1997) Clinical effects of immunotherapy on Japanese cedar pollinosis in the cedar and cypress pollination. *Auris-Nasus-Larynx (Tokyo)* **24**, 163.
14. ITO H., NISHIMURA J., SUZUKI M., *et al.*, (1995) Specific IgE. to Japanese cypress (*Chamaecyparis obtusa*) in patients with nasal allergy. *Ann Allerg Asthma Imm* **74**, 299.