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Human T Cell Response to Antigen Peptides of Japanese Cedar Pollen (Cry j 1)

Key Words

Pollinosis · Cry j 1 · Peptide · IL-4 · IFN- γ · T cell line · HLA · Immunotherapy

More than 10% of the Japanese population are suffering from Japanese cedar pollinosis from late February to April of every year. Therefore, there has been a strong social demand to develop a more reliable and effective way of controlling the pollinosis. The aim of this study was to determine the peptide epitope reactivity of T cells from patients allergic to Japanese cedar pollen (*Cryptomeria japonica*), and to discuss whether immunotherapy could control T cell response to the allergen, which is the fundamental and essential way of controlling IgE antibody production.

Twelve patients allergic to *C. japonica* with high serum IgE antibodies participated in this study. None of the patients had received oral corticosteroids or pollen immunotherapy before the study, and blood samples were obtained off season. One $\times 10^5$ /well of peripheral blood mononuclear cells (PBMC) were cultured with 2.5 μ g/ml of purified fraction (Cry j 1) of the pollen extract for 7 days and an additional 7 days with human rIL-2 and rIL-4, and pulsed simultaneously with autologous PBMC (APC) irradiated with 3,000 cGy and antigen to obtain T cell lines specific to Cry j 1. The reactivity of the lines were tested by ³HTdR uptake and release of cytokines, IL-4 and IFN- γ , in the cultured supernatant, when the lines were stimulated again with the APC and Cry j 1 (2.5 μ g/ml) or peptides (2.5 μ M), which were synthesized according to amino acid sequence of Cry j 1. HLA class II alleles were determined by hybridization of HLA DR, DQ, DP genes amplified by PCR.

Seventeen of 37 overlapping peptides were recognized by at least one line from every donor of the 12 cases. Of

them, 4 peptides activated the lines from plural cases, such as p61–80 (EY, TH, OT), p115–132 (SK, SPT), p206–225 (MK, YA, ya, KT) and p337–353 (YK, SE, MK, ya, KT) which could be considered as major peptides of Cry j 1. However, the question remains whether these epitopes are dominant in vivo. Cultured supernatant from 9 lines out of the 12 cases were assayed for cytokine release resulting in that most of the activated lines produced either IL-4, IFN- γ or both. IFN- γ dominant secretion was seen in TH (p61–80), SK (p115–132), NT (p271–190) and SE (p337–353). IL-4 dominant cases were ya (p108–125, p201–220, p206–225), SPT (p115–132) and MK (p127–143). Almost equal secretions of IL-4 and IFN- γ were seen in SPT (p13–42), YA (p206–225), MK (p301–321), MK (p337–352) and YK (p337–353) (fig. 1).

Taken together, it seemed to be difficult to determine which particular peptide stimulates specifically Th1, Th2 or Th0 [1]. We have previously found that an established Cry-j 1-specific T cell clone which recognized peptide p335–346 and secreted dominant amount of IL-4 increased the production of IFN- γ when a single amino acid substituted analog peptide (³³⁹threonine to valine) was used as a stimulator [2]. This interesting result suggested that even a single T cell clone may function as either Th1- or Th2-dominant depending on the character of the outcome of the stimulation.

The results of HLA class II typing of the 11 cases indicated that the higher phenotype frequency of DPB1*0501 and DQB1*03 [3] seemed to be associated with the recognition

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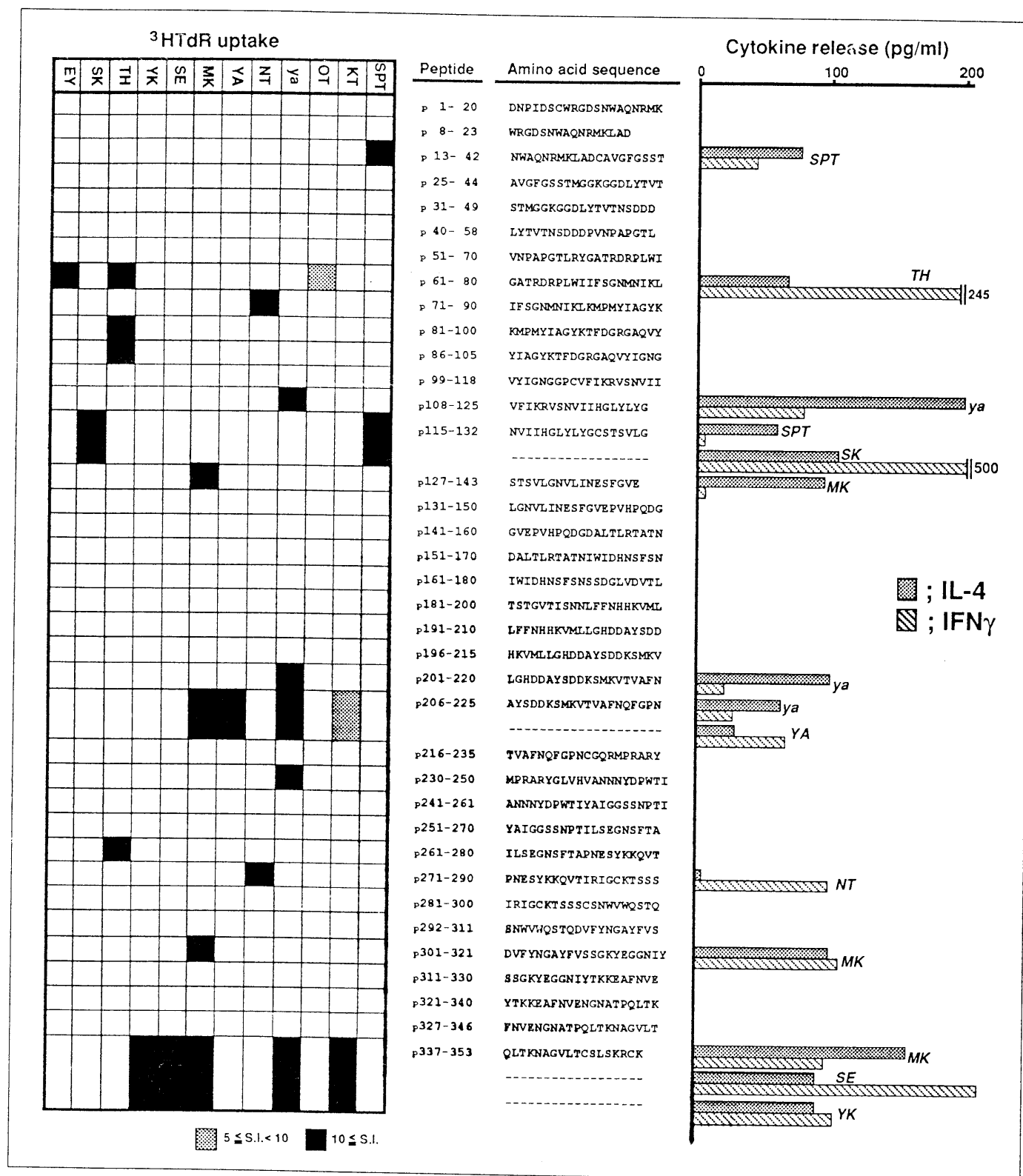


Fig. 1. ³HTdR uptake and cytokine release of T cell lines from patients allergic to Japanese cedar pollen by antigenic peptide stimulation. Responses of T cell lines from 12 Japanese cedar pollinosis were assayed by ³HTdR incorporation when the lines were stimulated by synthesized overlapping peptides of Cry j 1. The cytokine amount in the cultured supernatant after the stimulation was measured by ELISA in the samples from 9 of the above 12 cases.

of p337–353 and p206–225, respectively, and the higher haplotype frequency of DRB1*0405-DPB1*0501 and DRB1*1502-DQA1*0103-DQB1*0601 with the recognition of p337–353 and p61–80, respectively, when compared to the reference table of HLA 1991 (Proceedings of the 11th International HLA workshop).

Theoretically, the induction of T cell anergy [4, 5] or dominant IFN- γ production are the most feasible ways for immunotherapy with peptides to downregulate IgE antibody response to the allergen. In light of this, future progress should aim at investigating which peptides should be used for therapy. Additionally the various routes, such as dermal, nasal or intestinal, for application of the peptides may also be important.

References

- 1 Spiegelberg HL, Beck L, Stevenson DD, Ishio-ka GY: Recognition of T cell epitopes and lymphokine secretion by rye grass allergen *Lolium perenne* I-specific human T cell clones. *J Immunol* 1994;152:4706–4711.
- 2 Ikagawa S, Matsushita S, Chen YZ, Ishikawa T, Nishimura Y: Single amino acid substitutions on Japanese cedar pollen allergen (Cry j 1)-derived peptide induced alterations in human T cell responses and T cell receptor antagonism. *J Allergy Clin Immunol* 1996;97:53–64.
- 3 Shenk S, Breiteneder H, Susani M, Najafian N, Lagger S, Duchene M, Valenta R, Fischer G, Scheiner O, Kraft D, Ebner C: T-cell epitopes of Phl p 1, major pollen allergen of timothy grass (*Phleum pratense*): Evidence for cross-reacting and non-crossreacting T-cell epitopes within grass group I allergens. *J Allergy Clin Immunol* 1995;96:986–996.
- 4 Sloan-Lancaster J, Evavold BD, Allen PM: Induction of T-cell anergy by altered T-cell-receptor ligand on live antigen-presenting cells. *Nature* 1993;363:156–159.
- 5 Briner TJ, Kluo MC, Keating KM, Rogers BL, Greenstein JL: Peripheral T-cell tolerance induced in naive and primed mice by subcutaneous injection of peptides from the major cat allergen Fel d 1. *Proc Natl Acad Sci USA* 1993;90:7608–7612.