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Key Words

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Identification of the Disease-Related T Cell Epitope of Ovalbumin and Epitope-Targeted T Cell Inactivation in Egg Allergy

Abstract

An ovalbumin (OVA)-specific T cell line (TCL) was established from a patient with hen egg allergy. The TCL was CD4+, expressed $\alpha\beta$ T cell receptor, and recognized OVA presented by HLA-DR10. Based on the response of the TCL to synthetic OVA peptides, it was found that the TCL recognized OVA 323-339, which is a major T cell epitope presented by murine I-A^d. The TCL secreted high levels of IL-5, but undetectable amounts of IL-2, interferon- γ , and IL-4 when stimulated with OVA or the OVA 323-339 peptide. Since IL-5 is an important growth and chemotactic factor for eosinophils, it is possible that these OVA 323-339-specific T cells can contribute to human egg allergy. To our knowledge, this is the first demonstration of food allergen-specific TCL establishment and identification of a T cell epitope possibly related to the allergic reaction to food antigens. An analog peptide of the OVA 323-339 which is known to strongly bind to I-A^d partially inhibited the response of the TCL to OVA 323-339 presented by HLA-DR10, raising the possibility of peptide-based immunotherapy of food allergy.

Introduction

Hen egg allergy is a frequent food allergy and ovalbumin (OVA) is one of the major allergens in patients with hen egg allergy [1]. While there have been several reports on IgE antibody responses to OVA [2, 3], no studies to date have dealt with the analysis of epitopes on the OVA molecule that can induce cytokine production and proliferative responses by OVA-reactive T cells in patients allergic to OVA. Since T cell recognition of antigen is pivotal for development of allergy, the analysis of T cell epitopes on OVA is important for our understanding of OVA-induced allergic reactions. Furthermore, protein sequences that elicit T cell responses have potential applications in future therapies [4].

Recent establishment of T cell lines (TCLs) and clones which recognize several allergen [5-7] has facilitated delineation of epitopes of allergens recognized by and cytokine production from allergen-specific T cells. While all the allergens so far investigated were mainly aeroallergens, no detailed T cell response to food allergens has been reported. This might be partly due to a relative difficulty in obtaining enough volume of blood samples from patients with food allergy to establish antigen-specific TCL since the patients are usually infants or young children.

In this report, we established an OVA-specific TCL from a patient with hen egg allergy by use of a recently reported method to generate antigen-specific TCLs, and characterized the TCL for HLA restriction, epitope specificity, and

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antigen-induced cytokine production. Furthermore, we tested whether TCL proliferation to the relevant antigenic peptide could be inhibited by an analog OVA peptide.

Materials and Methods

Patient

The patient studied (HLA-DRB1* 0405, 1001; DRB4* 0101; DQA1* 0101, 0301; DQB1* 0401, 0501; DPA1* 0101; DPB1* 0201) is a 4-year-old atopic patient suffering from urticaria, atopic dermatitis, and bronchial asthma who is clinically allergic to egg white (radioallergosorbent test to egg white is class 4; >17.5 PRU/ml). The patient has high titer of IgE antibodies to OVA detected by ELISA.

Antigens and Reagents

OVA (type VII), ovomucoid and β -lactoglobulin were purchased from Sigma (St. Louis, Mo., USA). Purified protein derivative of *Mycobacterium tuberculosis* (PPD) was obtained from Japan BCG Products (Tokyo, Japan). Mitomycin C was obtained from Kyowa Hakko (Tokyo, Japan). Monoclonal antibodies, W6/32 (antihuman class I Ab) [8], L243 (anti-HLA-DR framework) [9], HU-4 (anti-HLA-DR framework) [10], HU-11 (anti-HLA-DQw1+4) [10], HU-18 (anti-HLA-DQw3) [11], and B7/21 (anti-HLA-DP framework) [12], were used for blocking antigen-induced T cell proliferation. Fluorescein isothiocyanate-labeled WT31 antibody (anti- $\alpha\beta$ T cell receptor) and phycoerythrin-labeled Leu3a antibody (anti-CD4), were purchased from Becton Dickinson (Mountain View, Calif., USA).

Synthetic OVA Fragments

Synthetic peptides that correspond to the sequences of amino acid positions 111–120, 258–281, 323–339 (ISQAVHAAHAEINEAGR), and 370–381 of OVA, the analog peptide of the OVA 323–339 (AKAKVHAAHAAKAK) [13], and an α S1 casein peptide (residues 130–147, QQKEPMIGVNQELAYPYP) were synthesized by automatic solid phase synthesis on a peptide synthesizer (model 430A, Applied Biosystems, Foster City, Calif., USA). Peptides were purified by HPLC and analyzed for purity by amino acid composition and analytical HPLC and amino acid sequence analyses.

Epstein-Barr Virus-Transformed Lymphoblastoid Cell Line and Transfectants

Epstein-Barr virus (EBV) transformation of peripheral blood mononuclear cells (PBMC) was done exactly as described [7]. L cells transfected with DR4 or DR53 were described previously [14], and 3T3 cells transfected with DR10 [15] were kindly provided by Dr. Lang (University of Freiburg, Germany).

Generation of OVA-Specific TCL

An OVA-specific TCL was generated by stimulating PBMC of the patient with OVA by the method described by Pette et al. [16] with minor modifications. Briefly, 2×10^5 PBMC were incubated in 96-well round bottom plates with 30 μ g/ml OVA in 100 μ l of RPMI 1640 medium containing 10% heat-inactivated human serum, 2×10^{-5} M 2-mercaptoethanol, and antibiotics. Fresh medium containing 30 IU/ml recombinant interleukin-2 (rIL-2; generous gift of Takeda Chemical Industries, Osaka, Japan) was added on the 3rd day of the culture and rIL-2 was fed every 4 days for a total period of 18 days. On day 18, cells

in the plate were washed to remove IL-2 and resuspended in 100 μ l of culture medium. Cells in a well were divided into two adjacent wells. A hundred thousand autologous EBV-lymphoblastoid cell line (LCL) that had been treated with mitomycin C (as antigen presenting cells, APC) were added to both wells and OVA was added to only one of them. Proliferating cells in OVA-pulsed wells were transferred to 24-well plates and stimulated with APC and OVA.

Proliferation Assays

Cellular proliferation was measured using a [^{3} H]TdR assay, as has been described [7]. In brief, 2×10^4 T cells were mixed with 5×10^4 mitomycin C-treated EBV-LCL or HLA-DR transfected murine fibroblasts and antigen in flat-bottomed 96-well plates in a total volume of 200 μ l. After 72 h of incubation at 37°C, 0.5 μ Ci [^{3} H]TdR (New England Nuclear, Boston, Mass., USA) was added to each well. Sixteen hours later, the cells were harvested onto glass fiber strips, and the amount of incorporated [^{3} H]TdR was measured by liquid scintillation counting. The results were expressed as the stimulation index or cpm (mean of the triplicate cultures $\pm$ SD).

Determination of Cytokines in the Supernatant

Twenty thousand TCL were stimulated with antigen in the presence of 5×10^4 irradiated PBMC in 96-well plates in a final volume of 0.2 ml for 48 h. Supernatants were harvested and cytokines secreted in the culture supernatant were measured using a sandwich ELISA. Commercial ELISA kits were purchased from Ohtsuka Assay Laboratories (Tokushima, Japan) for IL-2, or from Genzyme (Cambridge, Mass., USA) for IL-4 and interferon- γ (IFN- γ). IL-5 was also measured by a commercial kit (Suntory, Kyoto, Japan) [17]. The sensitivity of the assays was 15 pg/ml for IL-2, 45 pg/ml for IL-4, 100 pg/ml for IFN- γ , and 10 pg/ml for IL-5, respectively.

Results

Establishment of the OVA-Specific TCL

PBMC of a donor allergic to egg white were stimulated with OVA plus rIL-2 and an OVA-specific TCL, designated 2E7, was established. As shown in figure 1, 2E7 specifically proliferated in response to OVA in the presence of autologous EBV-LCL but did not proliferate in response to control antigens (ovomucoid, PPD, and β -lactoglobulin). FACS analysis showed that 2E7 was a CD4+, $\alpha\beta$ T cell receptor+ TCL (fig. 2).

Restriction Element of 2E7

In order to define the subsets of major histocompatibility complex molecules that might be acting as restriction elements in 2E7 recognition of OVA, monoclonal anti-HLA class I and class II antibodies were used to block antigen-specific proliferation of 2E7. Although a small degree of inhibition was observed with W6/32, Hu-11, HU-18 and B7/21 anti-DR antibodies, L243 and HU-4, completely blocked 2E7 proliferation (fig. 3). Since the patient expresses three DR molecules (DR4, DR10, and DR53), the restriction ele-

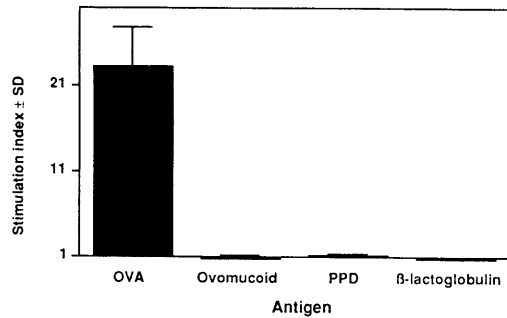


Fig. 1. Antigen-specific proliferation of TCL, 2E7. 2×10^4 2E7 cells were cultured with OVA, ovomucoid, PPD, β -lactoglobulin at the concentration of 50 μ g/ml in the presence of 5×10^4 autologous mitomycin C-treated EBV-LCL for 72 h. [^{3} H]TdR was pulsed for the final 18 h of the culture and radioactivity incorporated by the cells was counted, expressed as stimulation index $\pm$ SD of triplicate cultures.

ment was further investigated using murine fibroblast lines expressing each of these HLA-DR molecules as APC. As in figure 4, only the 3T3-DR10 transfectant could present OVA to 2E7, indicating that DR10 is the restriction element in this system.

OVA Epitope Recognized by 2E7

To determine the epitope of OVA recognized by 2E7, four OVA peptides were synthesized and examined for recognition by 2E7. Among these OVA peptides, only OVA 323–339 was found to stimulate 2E7 efficiently (fig. 5a). This peptide could also be presented to 2E7 by the 3T3-DR10 transfectant (fig. 5b).

Cytokine Production of 2E7

Cytokines secreted from 2E7 on stimulation with the OVA 323–339 peptide were measured by sandwich ELISA. Among four cytokines measured, IL-2, IFN- γ , IL-4 were undetectable in the supernatant [data not shown]. In contrast, very high levels of IL-5 were detected in the culture supernatant when stimulated with OVA and the OVA 323–339 peptide but not by control antigen, β -lactoglobulin (fig. 6).

Inhibition of 2E7 Proliferation by an OVA Analog Peptide

We next tried to inhibit 2E7 activation by an analog peptide of OVA 323–339. Since the binding motif of peptides presented by HLA-DR10 was not clear, we chose the analog peptide of OVA 323–339 which has been shown to be a very

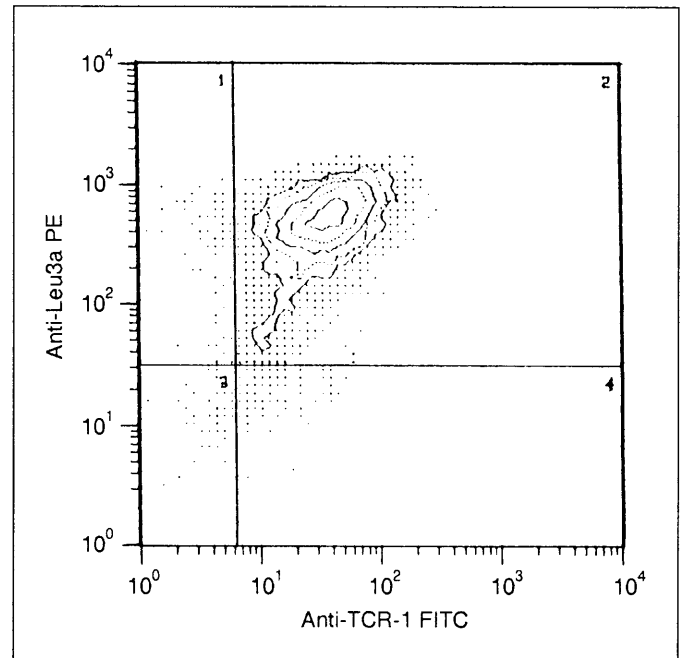


Fig. 2. Two-color flow cytometry analysis of 2E7. Cells were stained with both PE-labeled anti-CD4 and fluorescein isothiocyanate (FITC)-labeled anti-TCR $\alpha\beta$ chain antibody.

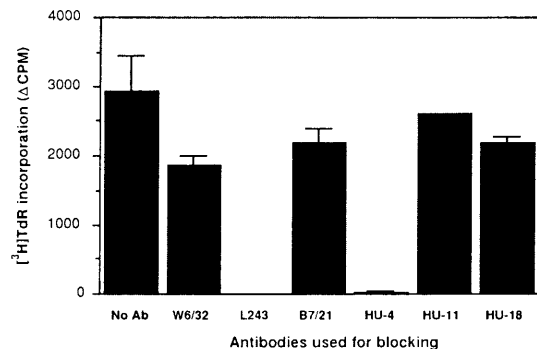


Fig. 3. Inhibition of 2E7 proliferation in response to OVA by monoclonal anti-HLA antibodies. Ascites fluid of each antibody diluted 1:250 was added to the culture and [^{3} H]TdR incorporation was measured after 72 h. The appropriate dilution of the ascites was determined based on FACS analysis in which PBMC were stained by serial dilutions of ascites from each monoclonal antibodies. W6/32: anti-class I; B7/21: anti-DP; L243 and HU-4: anti-DR; HU-11: anti-DQw1+4; HU-18: anti-DQw3. Proliferative response of 2E7 was expressed as Δ cpm (cpm cultured with antigen–background cpm). Ab = Antibodies.

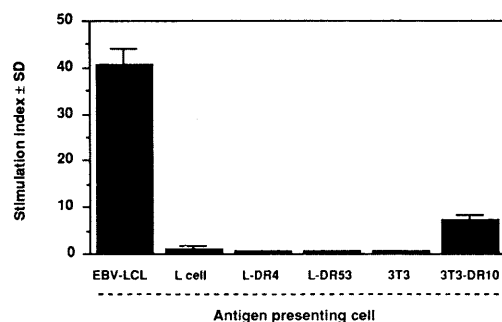


Fig. 4. OVA presentation to 2E7 by HLA-DR molecule-transfected murine fibroblast lines. Proliferative response of 2E7 was measured exactly as described in the legend of figure 1.

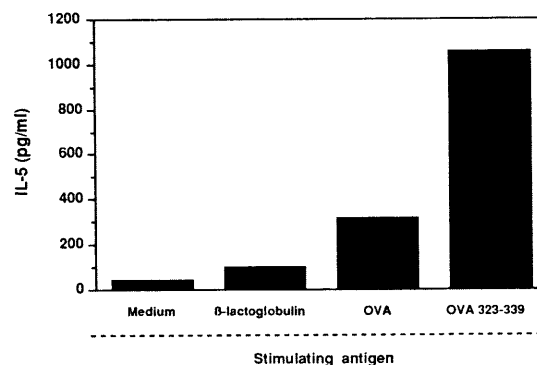


Fig. 6. IL-5 production by 2E7 following Ag-specific activation. 2E7 was cultured with 50 µg/ml β-lactoglobulin, OVA, or 10 µg/ml OVA 323–339 peptide. Supernatant after 48 h culture was harvested and IL-5 in the supernatant was measured by sandwich ELISA as described in Materials and Methods. The results shown are the mean of the duplicate determinations.

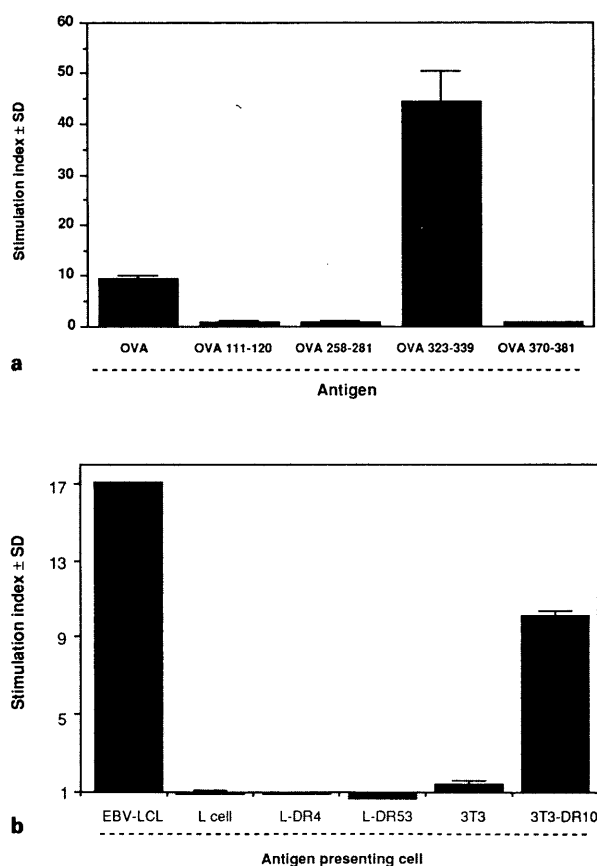


Fig. 5. Proliferative responses of 2E7 to synthetic OVA peptides. **a** 2E7 was cultured with 50 µg/ml OVA or 10 µg/ml of each of OVA peptides in the presence of autologous EBV-LCL. **b** 2E7 cultured with 10 µg/ml of OVA 323–339 peptides in the presence of autologous EBV-LCL or HLA-DR-transfected murine fibroblast. [³H]TdR incorporation by 2E7 was measured after 72 h culture and expressed as the stimulation index ± SD.

strong binder to I-A^d molecule [13] as a candidate to inhibit T cell proliferation to OVA 323–339 presented by HLA-DR10. In the first experiment, 2E7 was stimulated with increasing concentrations of the OVA 323–339 peptide in the presence of mitomycin C-treated EBV-LCL as a source of APC. To these cultures, the OVA analog peptide was added at a fixed concentration (150 µM), and T cell proliferation was determined (fig. 7a). The analog peptide was able to inhibit OVA 323–339 presentation to 2E7 by about 45%. In contrast, an unrelated peptide αS1 casein 130–147 failed to inhibit the proliferation of 2E7. To further confirm that the analog peptide competes with the relevant antigenic peptide at the level of binding to the HLA-DR10 molecule on the APC, APC were incubated with OVA 323–339 in the presence of an increasing amount of the analog peptide. APC were thoroughly washed to remove free peptides, and 2E7 was cultured with APC. Again, 2E7 proliferation was inhibited by the analog peptide in a dose-dependent manner (fig. 7b).

Discussion

Although T cell epitopes of OVA were studied as a model of immune responses to protein antigens [18, 19], no studies have been reported on OVA epitope recognized by human T cells, especially in patients with hen egg allergy. As far as we know, this is the first report on the establishment and char-

acterization of an OVA-reactive TCL in a human patient and identification of one T cell epitope possibly related to hen egg allergy.

The restriction element which presents OVA to the OVA-specific TCL 2E7 was analyzed first by antibody blocking experiments and finally determined by use of murine fibroblast lines transfected with defined HLA-DR molecules. These experiments revealed that 2E7 recognizes OVA in the context of HLA-DR10. The lower magnitude of 2E7 proliferation to OVA presented by 3T3-DR10 transfectant compared to that by autologous EBV-LCL might be due to lower levels of the expression of surface DR10 molecule on the transfectant [data not shown] or to lack of cell adhesion molecules and/or their ligands.

We next tried to determine the epitope which 2E7 recognizes in the context of HLA-DR10. Since OVA is composed of 385 amino acids, it is rather difficult to define an epitope 2E7 recognizes by peptides spanning the whole OVA molecule. Instead, we tested four OVA peptides for recognition by 2E7. Two OVA peptides, OVA 323–339 [18] and OVA 258–281 [19], were chosen since it is known that these peptides contain the T cell epitope in the murine system and that some murine T cell epitopes also can be human T cell epitopes [20, 21]. Another two OVA peptides were created because they were highly amphipathic and predicted to be immunogenic [22, 23]. OVA 323–339 has been known as a T cell epitope restricted by I-A^d molecule in mice [18]. Our results clearly showed that OVA 323–339 is not only a murine but also a human T cell epitope presented by HLA-DR10 molecule.

It is now widely accepted that CD4⁺ Th cells are divided by their cytokine production phenotype into two subpopulations in human as well as in mouse [24, 25]. Paronchi et al. [25] showed that all PPD-specific T cell clones established from normal individuals produce IL-2 and IFN- γ but not IL-4 and IL-5 (Th1), whereas *Toxocara*-specific T cell clones established from the same donors produce IL-4 and IL-5 but no or limited amounts of IL-2 and IFN- γ (Th2). All T cell lines and clones to aeroallergens such as house dust mite established from atopic patients, so far reported, belong to the Th2 subpopulation [7, 26, 27]. 2E7, the OVA-specific TCL, produced very high levels of IL-5, but no or little amounts of IL-2, IFN- γ on stimulation with OVA. Although 2E7 did not produce detectable amounts of IL-4, several subclones derived from 2E7 secreted measurable levels of IL-4 [data not shown]. These results suggest that 2E7 is a Th2-type TCL. Since IL-5 is a potent activator and chemotactic factor for eosinophils [28], it seems likely that OVA 323–339 is one of the epitopes related to development of hen egg allergy. OVA 323–339 recently has been shown

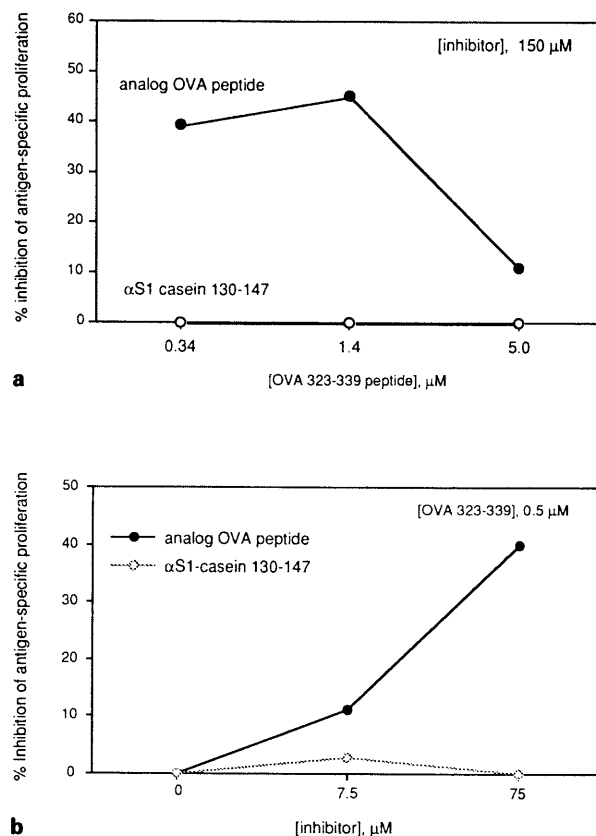


Fig. 7. Inhibition of OVA 323–339 presentation to 2E7 with the OVA analog peptide. **a** 2E7 was stimulated with increasing concentrations of the OVA 323–339 peptide in the presence of autologous mitomycin C-treated EBV-LCL. To these cultures, the OVA analog peptide was added at a fixed concentration (150 μM), and T cell proliferation was determined as described in Materials and Methods. **b** Autologous EBV-LCL were incubated with 0.5 μM OVA 323–339 in the presence of two concentrations of the analog OVA peptide at 37°C. After 2 h incubation, cells were washed thoroughly to remove free peptides and recounted. 2E7 was cultured with these LCL and the proliferation assay was done as shown in Materials and Methods. Inhibition of proliferative response was shown by % inhibition (cpm in antigen culture–cpm with inhibitor/cpm in antigen culture). Standard deviation was less than 20%.

to represent a T and B cell epitope, which is important in the generation and development of immediate hyperresponses in BALB/c mice [29]. In addition, Johnson and Elsayd reported that OVA 323–339 binds human specific IgE from a serum pool of egg-allergic patients [30]. Taken together, it is concluded that OVA 323–339 is the important T and B cell epitope related to an allergic reaction to OVA both in human and mouse.

Identification of OVA epitopes recognized by disease-inducible T cells in patients allergic to OVA is pivotal in terms of therapeutic strategy to control or modify the T cell response to OVA in the patients with hen egg allergy. Epitope-targeted inactivation of T cells in murine experimental autoimmune diseases has been reported by use of the analog peptides [4]. At present, no data has been published for binding motif of the antigenic peptides presented by the HLA-DR10 molecule. Thus, in our study, we took advantage of the binding motif of the OVA 323–339 peptide to the I-A^d molecule that consists of 6 consecutive amino acid residues (VHAAHA). The OVA analog peptide, AKAKV-HAAHAAKAK, which was shown to be a strong I-A^d binder [13], could inhibit OVA 323–339 presentation by HLA-DR10 to 2E7 in vitro by about 50% by competing for binding to HLA-DR10 molecule.

In summary, in this study we first demonstrated a food allergy-related T cell epitope of OVA presented by HLA-DR10. Once epitopes recognized by T cells are defined, blocking by such analog peptides of activation of T cells involved in food-related allergic reactions will be a new approach for the treatment of food allergy.

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References

- Yunginger JW: Food antigens; in Metcalfe DD, Sampson HA, Simon RA (eds): Food Allergy Adverse Reaction to Foods and Food Additives. Oxford, Blackwell Scientific Publications, 1991, pp 36–51.
- Elsayd S, Apold J, Holen E, Vik H, Florvaag E, Dybendal T: The structural requirements of epitopes with IgE binding capacity demonstrated by three major allergens from fish, egg and tree pollen. *Scand J Clin Lab Invest* 1991;51:17–31.
- Kahlert H, Petersen A, Becker WM, Schlaak M: Epitope analysis of the allergen ovalbumin (*Gald II*) with monoclonal antibodies and patients' IgE. *Mol Immunol* 1992;29:1191–1201.
- Urbain JL, Horvath SJ, Hood L: Autoimmune T cells: Immune recognition of normal and variant peptide epitopes and peptide-based therapy. *Cell* 1989;59:257–271.
- Dhillon M, Roberts C, Nunn T, Kuo M: Mapping human T cell epitopes on phospholipase A2: The major bee-venom allergen, *J Allergy Clin Immunol* 1992;90:42–51.
- van Neerven RJJ, van 't Hof W, Ringrose JH, Jansen HM, Aalberse RC, Wierenga EA, Kapsenberg ML: T cell epitopes of house dust mite major allergen *Der p II*. *J Immunol* 1993;151:2326–2335.
- Yssel H, Johnson KE, Schneider PV, Wideman J, Terr A, Kastelein R, de Vries JE: T cell activation-inducing epitopes of the house dust mite allergen *Der p I*. Proliferation and lymphokine production patterns by *Der p I*-specific CD4⁺ T cell clones. *J Immunol* 1992;148:738–745.
- Brodsky FM, Parham P: Monomorphic anti-HLA-A, B, C monoclonal antibodies detecting molecular subunits and combination determinants. *J Immunol* 1982;128:129–135.
- Lampson LA, Levy R: Two populations of Ia-like molecules on a human B cell line. *J Immunol* 1980;125:293–299.
- Sone T, Tsukamoto K, Hirayama K, Nishimura Y, Takenouchi T, Aizawa M, Sasazuki T: Two distinct class II molecules encoded by the genes within the HLA-DR10 subregion of HLA-Dw2 and Dw12 can act as stimulating and restriction molecules. *J Immunol* 1985;135:1288–1298.
- Hirayama K, Nishimura Y, Tsukamoto T, Sasazuki T: Functional and molecular analysis of three distinct HLA-DR4 β -chains responsible for the MLR between HLA-Dw4, Dw15, and DKT2. *J Immunol* 1987;137:924–933.
- Watson AJ, DeMars R, Trowbridge IS, Bach FH: Detection of novel human class II HLA antigen. *Nature* 1983;304:358–361.
- Sette A, Sidney J, Albertson M, Miles C, Colon SM, Pedrazzini T, Lamont AG, Grey HM: A novel approach to the generation of high affinity class II-binding peptides. *J Immunol* 1990;145:1809–1813.
- Kamikawaji N, Fujisawa K, Yoshizumi H, Fukunaga M, Yasunami M, Kimura A, Nishimura Y, Sasazuki T: HLA-DQ-restricted CD4⁺ T cells specific to streptococcal antigen present in low but not in high responders. *J Immunol* 1991;146:2560–2567.
- Marryman P, Gregersen P, Lee S, Silver J, Nunez-Roldan A, Crapper R, Winchester R: Nucleotide sequence of a DRw10 β chain cDNA clone; identity of the third D region with that of DRw53 allele of the β 2 locus and as the probable site encoding a polymorphic MHC class II epitope. *J Immunol* 1988;140:2447–2452.
- Pette M, Fujita K, Wilkinson D, Altman DM, Trowsdale J, Giegerich G, Hinkkanen A, Eppelen JT, Kappos L, Wekerle H: Myelin autoreactivity in multiple sclerosis: Recognition of myelin basic protein in the context of HLA-DR2 products by T lymphocytes of multiple-sclerosis patients and healthy donors. *Proc Natl Acad Sci USA* 1990;87:7968–7972.

- 17 Fukuda Y, Hashino J, Haruyama M, Tsuruoka N, Nakazato H, Nakanishi T: A sandwich enzyme-linked immunosorbent assay for human interleukin-5. *J Immunol Methods* 1991;143: 89–94.
- 18 Shimonkevitz R, Colon S, Kappler JH, Marrack P, Grey H: Antigen recognition by H-2-restricted T cells. II. A tryptic ovalbumin peptide that substitutes for processed antigen. *J Immunol* 1984;133:2067–2074.
- 19 Lipford GB, Hoffman M, Wagner H, Heeg K: Primary in vivo responses to ovalbumin. Probing the predictive value of the K^b binding motif. *J Immunol* 1993;150:1212–1222.
- 20 Martin R, Jaraquemada D, Flerlage M, Richert J, Whitaker J, Long EO, McFarlin DE, McFarland HF: Fine specificity and HLA restriction of myelin basic protein-specific cytotoxic T cell lines from multiple sclerosis patients and healthy individuals. *J Immunol* 1990;145:540–548.
- 21 Hosmalin A, Clerici M, Houghten R, Pendleton CD, Flexner C, Lucy DR, Moss B, Germain RN, Shearer GM, Berzofsky JA: An epitope in human immunodeficiency virus 1 reverse transcriptase recognized by both mouse and human cytotoxic T lymphocytes. *Proc Natl Acad Sci USA* 1990;87:2344–2348.
- 22 Rothbard JB, Taylor WR: A sequence pattern common to T cell epitopes. *EMBO J* 1988;7: 93–100.
- 23 Margalit HM, Spouge JL, Comette JL, Cease KB, DeLisi C, Berzofsky JA: Prediction of immunodominant helper T cell antigenic sites from the primary sequence. *J Immunol* 1987;138: 2213–2229.
- 24 Mossman TR, Coffman RL: Heterogeneity of cytokine secretion patterns and functions of helper T cells. *Adv Immunol* 1989;46:111–147.
- 25 Paronchi P, Macchia D, Piccini MP, Biswas P, Simonelli C, Maggi E, Ricci M, Ansari AA, Romagnani S: Allergen and bacterial antigen-specific T-cell clones established from atopic donors show a different profile of cytokine production. *Proc Natl Acad Sci USA* 1991;88: 4538–4542.
- 26 Wierenga EA, Snoek M, deGroot C, Chretien I, Bos JD, Jansen HM, Kapsenberg ML: Evidence for compartmentalization of functional subsets of CD4⁺ T lymphocytes in atopic patients. *J Immunol* 1990;144:4651–4656.
- 27 Lin K-L, Hsieh K-H, Huang J-H, Wang S-Y: Major histocompatibility complex (MHC) class II restriction, lymphokine production, and IgE regulation of house dust mite-specific T-cell clones. *J Clin Immunol* 1992;12:271–280.
- 28 Yamaguchi Y, Suda T, Suda J, Eguchi M, Miura Y, Harada N, Tominaga A, Takatsu K: Purified interleukin-5 supports the terminal differentiation and proliferation of murine eosinophilic precursors. *J Exp Med* 1988;167:43–56.
- 29 Renz H, Bradley K, Larsen GL, McCall C, Gelfand EW: Comparison of the allergenicity of ovalbumin and ovalbumin peptide 323–339. *J Immunol* 1993;151:7206–7213.
- 30 Johnsen G, Elsayd S: Antigenic and allergenic determinants of ovalbumin. III. MHC Ia-binding peptide (OA 323–339) interacts with human and rabbit specific antibodies. *Mol Immunol* 1990;27:821–827.