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***Dermatophagoides farinae*-1- Derived Peptides and HLA Molecules Recognized by T Cells from Atopic Individuals**

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Abstract

Der f1, the group I allergen in *Dermatophagoides farinae* extracts, is a major source of inhalation allergens in Japan. Using the mixture of a panel of overlapping synthetic peptides that spread over the entire Der f1 molecule, we found that polyclonal Der f1-specific short-term T cell lines prepared from peripheral blood of 6 individuals allergic to Der f who carry most of the common HLA haplotypes seen in the Japanese population can respond to 16 different peptides. Eight of 16 peptides stimulated T cells of more than 2 donors, regardless of the HLA types. Proliferative responses of four T cell lines were markedly inhibited by mAb HU4 (anti-HLA-DRB1+B5), one was inhibited partially by HU11 (anti-HLA-DQ4+5+6), and one was inhibited fully by a combination of HU4, L243 (anti-HLA-DRB1+B4) and PLM16 (anti-HLA-DRB3) but only partially by each of these mAbs. One of these T cell lines, DT, of which the proliferative response was partially inhibited by HU11, was cloned. Indeed, the T cell clones were restricted by DQ6 molecules on an *HLA-DRB1*1501-DRB5*0101-DQA1*0102-DQB1*0602* haplotype. These results indicate that patients' T cells recognize Der f1 in association mainly with HLA-DRA/DRB1, but that DQA1/DQB1, DRA/DRB3 and possibly DRA/DRB4 gene products also function as antigen-presenting molecules. Thus, although some peptides have a more potent T cell-stimulatory activity than others, the T cell receptor ligands formed with the Der f1 molecule are highly heterogeneous.

Key Words

Der f1
T cell epitope
HLA
Peptide

Introduction

Exogenous antigens are engulfed and processed by endosomal proteases to peptides in antigen-presenting cells (APC) and are presented to T cells by appropriate MHC class II molecules present on the cell surface. The CD4+ helper T cells are activated upon recognition of these peptide-MHC complexes by T cell receptor molecules and exert effector functions through various biological activities of secreted lymphokines.

T cell epitopes of several major allergens have been reported [1–7]. House dust mite is the most potent allergen in Japan with more than 70% of patients affected by bronchial asthma during childhood. Two mite species of the genus *Dermatophagoides*, *Dermatophagoides farinae* (Der f) and *Dermatophagoides pteronyssinus* (Der p), are frequently responsible for atopic diseases such as bronchial asthma, allergic rhinitis, and atopic dermatitis. Der f1 and Der p1, major allergens of Der f and Der p, respectively, showed a significant homology, mutually, and with a group of cys-

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teine proteases, homology in sequence of cDNA between these two mite allergens being approximately 80%. Epitopes of Der p 1 but not Der f 1 recognized by human T cells were reported [2]. We established short-term Der f 1-specific polyclonal T cell lines, using HLA-typed panels derived from Japanese patients allergic to Der f, and we identified T cell epitopes on Der f 1 and antigen-presenting molecules.

Materials and Methods

Peripheral Blood Mononuclear Cell Donors

Diagnosis was based on clinical symptoms, positive skin test, and positive radioallergosorbent test results. The donors selected cover frequently observed HLA-DR haplotypes in the Japanese [8]. Thus, individuals allergic to Der f with HLA-DRB1*0901 (30.3% of the general Japanese population), DRB1*0405 (27.8%), DRB1*1502 (23.7%), DRB1*0803 (17.4%), DRB1*1501 (12.6%), DRB1*1302 (10.7%), DRB1*0101 (10.4%), DRB1*1401 (3.2%) haplotypes were selected as peripheral blood mononuclear cell (PBMC) donors. Subjects negative for these haplotypes account for about 20% in the Japanese population.

Synthesis of Der f 1-Derived Peptides

Peptides were synthesized according to amino acid sequence of Der f 1 reported by Dilworth et al. [9], using a solid-phase simultaneous multiple peptide synthesizer PSSM-8 (Shimadzu, Kyoto, Japan), based on the Fmoc strategy. All peptides were purified by C18 reverse-phase HPLC (Millipore, Waters, Milford, Mass.).

Generation of Antigen-Specific T Cell Lines and Clones

Der f 1-specific T cell lines were established from PBMC from 6 patients with type I allergy to Der f. T cell lines were generated by stimulating PBMC (1.5×10^5 /well) with $0.1 \mu\text{M}$ each of Der f 1 peptides in RPMI 1640 medium (Gibco, Grand Island, N.Y.) being supplemented with 2 mM L-glutamine, 100 units/ml of penicillin, 100 $\mu\text{g}/\text{ml}$ of streptomycin, and 10% pooled, heat-inactivated normal human male plasma in 96-well flat-bottomed culture plates (Falcon, Becton Dickinson, Lincoln Park, N.J.). After 7–9 days, irradiated (3,000 cGy) autologous PBMC (1.5×10^5 /well) pulsed with Der f 1 peptide mixture (3.5 μM each), human rIL-2 (50 U/ml), and human rIL-4 (10 U/ml) were added to the culture wells carrying T cell blasts and were thus maintained for another 7 days. Aliquots of growing cultures were combined as a bulk T cell line for an individual patient. The specificity was determined by culturing T cells with either medium, the soluble Der f 1 peptide mixture (0.1 μM each), individual peptide solutions (0.5 μM) or crude Der f protein (10 $\mu\text{g}/\text{ml}$), in the presence of 3,000 cGy-irradiated autologous PBMC (1.5×10^5 /well). Cloning was done in Terasaki plates (Nunc, Roskilde, Denmark), by limiting dilution at 0.3 cells per well in the presence of irradiated (3,000 cGy) autologous PBMC (3×10^4 /well) pulsed with Der f 1 peptide mixture (3.5 μM), human rIL-2 (50 U/ml), and human rIL-4 (10 U/ml), in the same medium described above. Growing microcultures (approximately 5% of all culture wells) were then expanded at weekly intervals first in a 96-well plate, and then in a 24-well plate by feeding irradiated feeder cells (1.5×10^6 /well) pulsed with Der f 1 peptide mixture (3.5 μM) in the presence of rIL-2 and rIL-4.

Antigen-Induced Proliferative Responses of T Cell Clones

Antigen-induced proliferation of the T cell clones was assayed by culturing the T cells (3×10^4 /well) with 3,000 cGy-irradiated autologous PBMC (1.5×10^5 /well), in 96-well flat-bottomed culture plates in the presence of either medium, the soluble Der f 1 peptide mixture (0.1 μM each), individual peptide solutions (0.5 μM) or crude Der f protein (10 $\mu\text{g}/\text{ml}$). Cells were cultured for 72 h, in the presence of 1 $\mu\text{Ci}/\text{well}$ of [^3H]TdR during the final 16-hour period, and the incorporated radioactivity was measured by liquid scintillation counting. To determine restriction molecules for antigen presentation, the T cell clones were cultured with irradiated autologous PBMC, with or without saturating amounts of anti-HLA class II monoclonal antibodies (mAbs) HU-4 (anti-HLA-DRB1+DRB5 IgG2a, monomorphic) [10, 11], L243 (anti-HLA-DRB1+DRB4 IgG2a, monomorphic, reactivity to DRB3 and DRB5 yet to be determined) [12], PLM16 (anti-HLA-DRB3) [13], HU-11 (anti-HLA-DQ4+DQ5+DQ6 IgG2a) [14], HU-18 (anti-HLA-DQ7+DQ8+DQ9 IgG2a) [15], HU-46 (anti-HLA-DQ4 IgG2a) [16], and B7/21 (anti-HLA-DP IgG1, monomorphic) [10]. At the same time, experiments were done in which PBMC of two donors with one shared haplotype and one donor with irrelevant HLA haplotypes were used as APC. In this experiment, allogeneic PBMC (3×10^5 /well) were cultured in plates with peptides (1 μM each) in the culture medium, for 2 h at 37°C, then excess peptides and nonadherent cells were removed by gently washing the plates 3 times with RPMI 1640 media containing 3% human serum, to minimize T-T interaction mediated by soluble peptides. The remaining adherent cells were irradiated at 3,000 cGy and used as APC. Crude extract of Der f which contains 14.3 μg of Der f 1/mg total protein was kindly provided by Torii Pharmaceuticals (Tokyo, Japan).

HLA Typing

HLA class II (DR, DQ) alleles were determined by hybridization of HLA-DR, DQ genes amplified by polymerase chain reaction with sequence-specific oligonucleotide probes distributed in the 11th International Histocompatibility Workshop, as described elsewhere [1]. The nomenclature of the HLA-DR, DQ alleles was according to the WHO Nomenclature Committee for factors of the HLA system [17].

Results and Discussion

Der f 1 Epitopes Recognized by Polyclonal

Der f 1-Reactive T Cell Lines

Short-term Der f 1-specific T cell lines were prepared from PBMC of 6 atopic donors with type I allergy to Der f. These donors carry common HLA haplotypes seen in the Japanese population. These cell lines were tested for proliferative responses to a panel of overlapping synthetic peptides corresponding to the amino acid sequence of Der f 1. As shown in figure 1, sixteen T cell epitopes were identified in these 6 donors (Der f 1 p13–31, 18–38, 27–47, 36–56, 44–64, 49–65, 75–94, 83–102, 110–130, 119–139, 128–148, 137–157, 146–166, 155–175, 200–217, and 206–223). All donors reacted in different patterns; T cell lines established from donors DT, HK and SK exhibited limited antigen specificity, whereas those established from donors KY, HY and KN

³H-thymidine incorporation

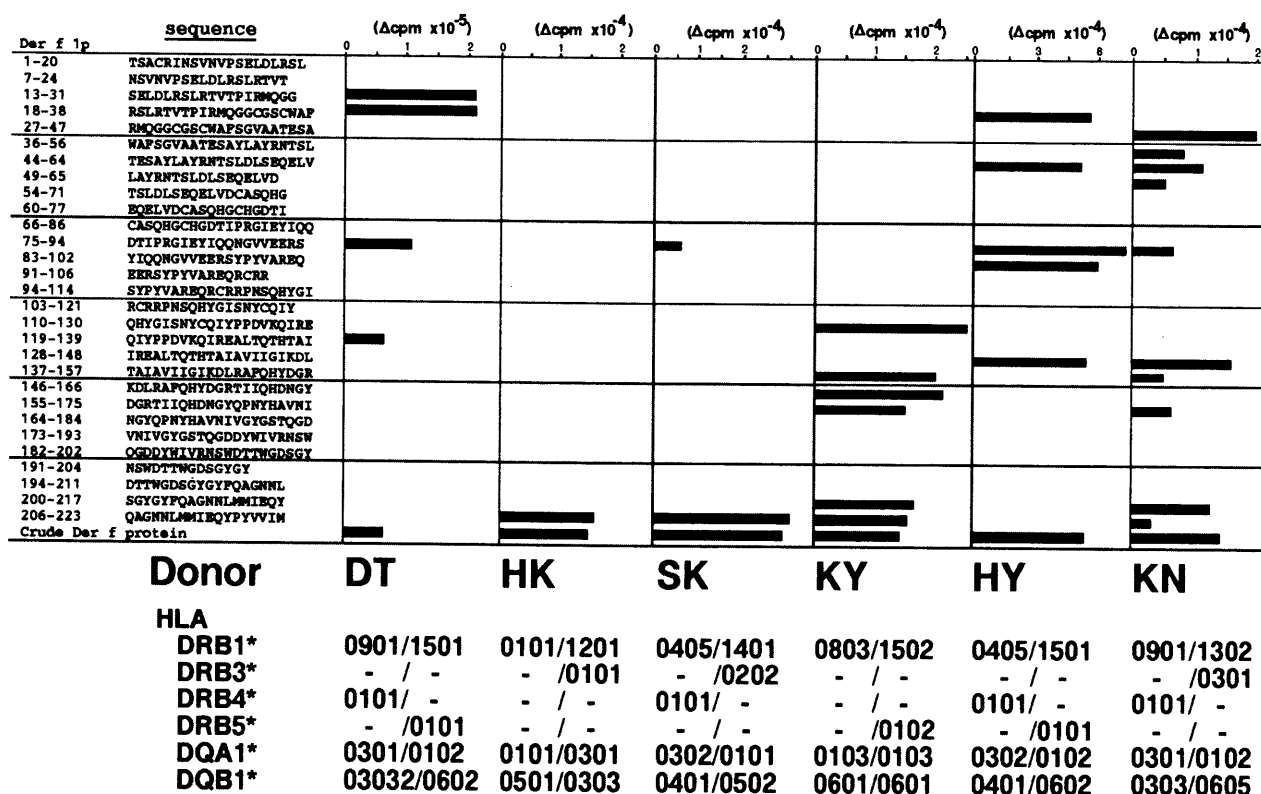


Fig. 1. Proliferative responses of short-term T cell lines to 11- to 16-residue-overlapping synthetic peptides derived from Der f1. Peptides are designated by indicating amino acid residue numbers of the amino- and carboxy-termini. Values shown are the mean cpm of duplicate cultures after subtraction of mean cpm obtained from culture without peptides. The standard error of duplicate culture was <20%. Cultures without peptides showed 87–1,409 cpm (HK, SK, HY, KY and KN) and 17,512 cpm for DT.

showed a heterogeneous specificity. However, regardless of the HLA phenotypes, 8 peptides (Der f1 p18–38, 44–64, 75–94, 128–148, 137–157, 155–175, 200–217, 206–223) were commonly recognized by the different T cell lines. All these lines, when tested for reactivity with crude Der f extract, proliferated significantly. Thus, these T cell lines recognize not only synthetic peptides, but also naturally processed Der f1 peptide fragments.

Antigen-Presenting HLA Molecules

Proliferation inhibition experiments using anti-HLA class II mAbs HU4 (anti-HLA-DRB1+DRB5, monomorphic), HU11 (anti-HLA-DQ4+DQ5+DQ6), HU18 (anti-HLA-DQ7+DQ8+DQ9), HU46 (anti-HLA-DQ4) and B7/21 (anti-HLA-DP, monomorphic) were carried out to determine antigen-presenting molecules. As shown in figure 2A, the addition of HU4 inhibited the proliferative responses of four T

cell lines (HY, HK, KY and KN) by over 50%. However, the T cell line SK was partially inhibited by HU4 (25%), and another T cell line DT was only marginally inhibited by HU11 alone. Since the donor SK carries HLA-DRB3 and DRB4 gene products, a proliferation inhibition experiment was done using L243 (anti-HLA-DRB1+DRB4) and PLM16 (anti-HLA-DRB3). As shown in figure 2B, SK cells were inhibited fully (83%) by the addition of HU4 + L243 + PLM16, despite the partial inhibition by each of these mAbs. Moreover, as shown in figure 2C, both DRB1*0405 haplotype-shared and DRB1*1401 haplotype-shared allogeneic PBMC presented Der f1 p206–223 to the SK line, whereas only DRB1*1401 haplotype-shared PBMC presented Der f1 p75–94. These results suggest that (1) the DRB1, DRB3 and possibly DRB4 gene products are involved in presenting peptide antigens to the SK line, and (2) at least two distinct HLA molecules are involved in presenting Der f1 p206–223. It is also

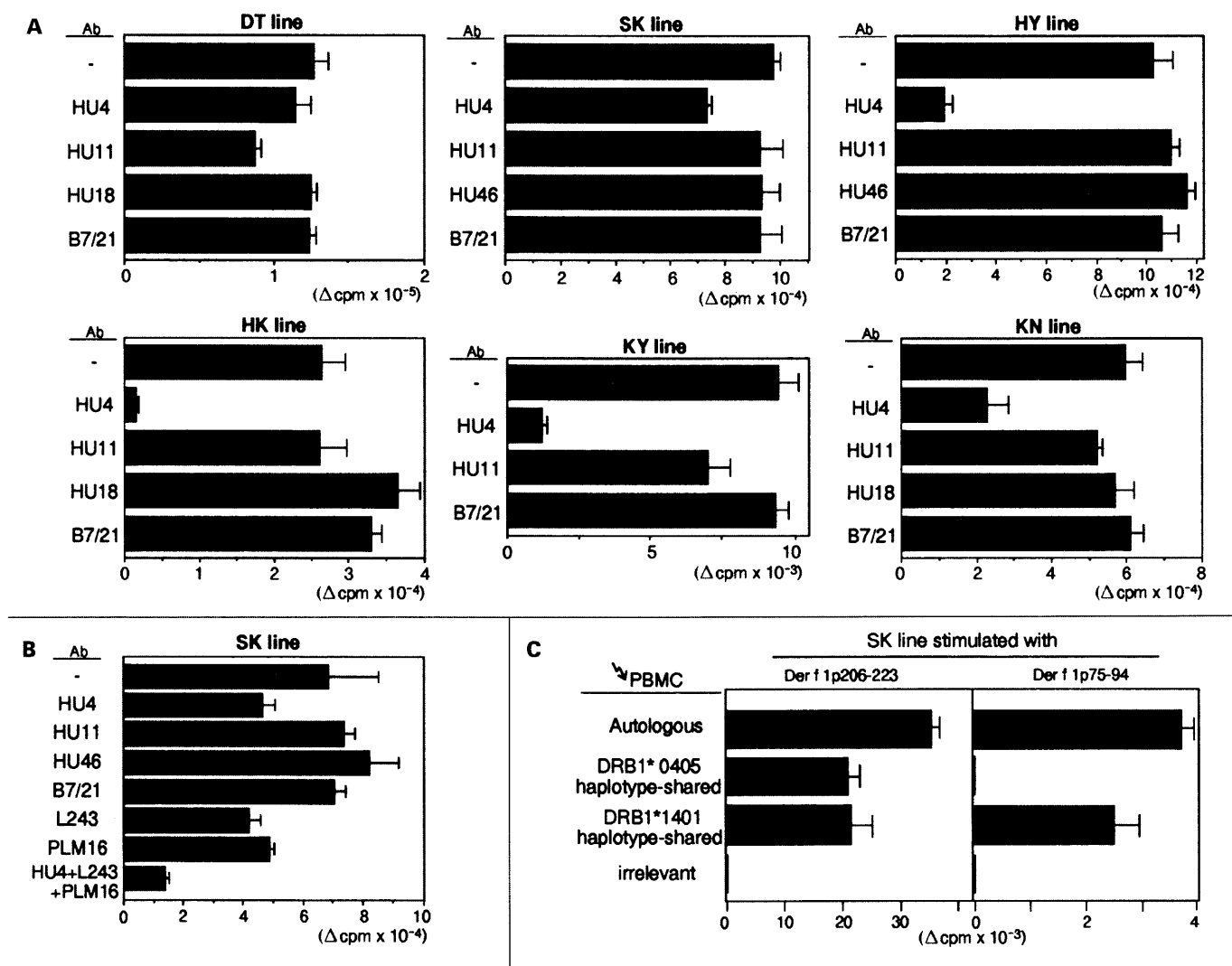


Fig. 2. Proliferation inhibition of six bulk T cell lines using anti-HLA class II mAbs. **A** HU4 (anti-HLA-DRB1+DRB5, monomorphic), HU11 (anti-HLA-DQ4+DQ5+DQ6), HU18 (anti-HLA-DQ7+DQ8+DQ9), HU46 (anti-HLA-DQ4) and B7/21 (anti-HLA-DP, monomorphic) were cocultured with T cells (3×10^4 /well) and irradiated autologous PBMC (1.5×10^5 /well), with or without Der f 1 peptides. Results are expressed as the geometric means of triplicate determinations $\pm$ standard error. **B** Proliferation inhibition of SK cells was tested by using HU11, HU46, B7/21, HU4, L243 (anti-HLA-DRB1+DRB4, monomorphic), PLM16 (anti-HLA-DRB3) and their combination. Results are expressed as the geometric means of triplicate determinations $\pm$ standard error after subtraction of mean cpm obtained from cultures without added peptides. All mAbs in ascites forms were tested at a final dilution of 1:200 that corresponds to 20–25 μ g specific Ab/ml at a final concentration. **C** Proliferation of SK line to Der f 1 p206–223 and Der f 1 p75–94 presented by allogeneic PBMC. T cells were cultured with autologous, DRB1*0405 haplotype-shared, DRB1*1401 haplotype-shared or irrelevant PBMC. PBMC were prepulsed with peptides and irradiated. HLA types of PBMC donors were DRB1*0405-DRB4*0101-DQA1*0302-DQB1*0401 / DRB1*1501-DRB5*0101-DQA1*0102-DQB1*0602, DRB1*1401-DRB3*0202-DQA1*0101-DQB1*0502 / DRB1*0803-DQA1*0103-DQB1*0601, and DRB1*0803-DQA1*0103-DQB1*0601/DRB1*1502-DRB5*0102-DQA1*0103-DQB1*0601, respectively.

important to note that DRA/DRB1 molecules are likely to be major antigen-presenting molecules for donors HY, HK, KY and KN, although HY, KY and KN did show a heterogeneous antigen specificity.

One of these T cell lines, DT, the proliferative response of which was only marginally inhibited by HU11, was cloned by limiting dilution, and was investigated regarding the restriction molecules. Clone DT13.2 which was generated from DT, was cultured in the presence of Der f1 peptides (Der f1 p13–31 and Der f1 p18–38) and autologous PBMC with anti-HLA class II mAbs. As shown in figure 3A, only HU11 inhibited the peptide-driven proliferation of DT13.2. Furthermore, as shown in figure 3B, DT13.2 proliferated in response to Der f1 peptides presented by allogeneic PBMC (MH) which share the *HLA-DRB1*1501-DRB5*0101-DQA1*0102-DQB1*0602* haplotype alone with the donor D.T. In contrast, allogeneic PBMC with another shared haplotype (MM), and with irrelevant haplotypes (KK) did not present Der f1 peptides to DT13.2. All four Der f1-specific clones (DT13.1, DT13.2, DT13.3 and DT13.7) established from the DT line showed the same restriction pattern [data not shown]. These observations indicate that the T cell clone DT13.2 recognized Der f1 peptides (Der f1 p13–31 and Der f1 p18–38) in the context of DQA1*0102/DQB1*0602, which is likely to be the major antigen-presenting molecule for the DT line.

Why the inhibition of a peptide-driven response of clone DT13.2 by HU11 was complete, whereas that of the DT line was only partial is not clear. In this respect, foregoing studies suggested that HU11 is stimulatory for certain T cell clones [18], thereby raising the possibility that HU11 stimulated proliferative responses of nonspecific autoreactivity present in the DT line (17,512 cpm in the absence of peptides, as shown in legend of fig. 1).

Der f1 p206–223 was presented to the SK line by both DRB1*0405 haplotype-shared and DRB1*1401 haplotype-shared allogeneic PBMC, indicating that the reactivity of the SK line of Der f1 p206–223 is composed of at least two clones. It may be that Der f1 p206–223 binds to distinct allelic products, because in our previous studies, certain peptides [19–21] and even peptides carrying a single binding motif [19, 20], were shown to interact with HLA in an allele-nonspecific manner.

Studies on Der p1 by other workers [2, 3] showed that T cells recognize Der p1 p45–67 (DR7), p94–104 (DR2, DR11, DR8), p107–119 (DPB1*0401, DPB1*0402, DPB1*0501), p110–119 (DPB1*0402), p110–131 (DRB1*0101) and p117–143 (DR7) in the context of the HLA molecules shown in the parentheses. However, none of the corresponding sequence pairs are identical between Der p1 and Der f1. Al-

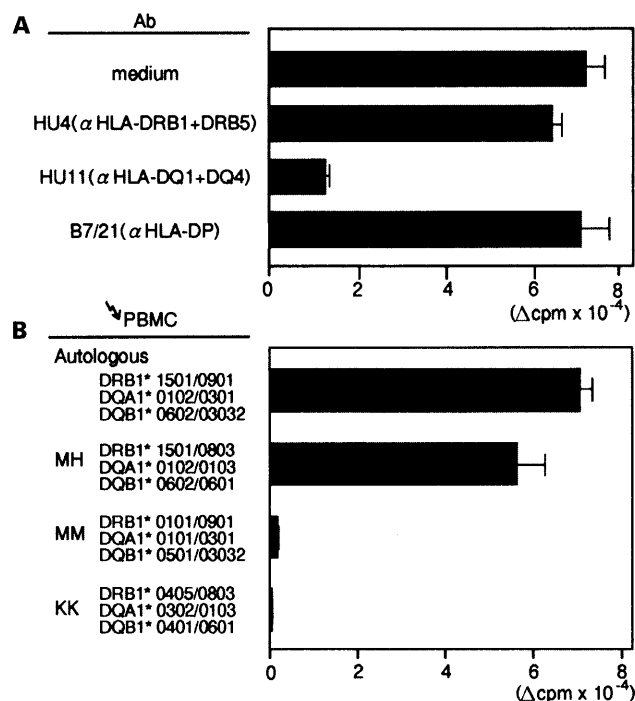


Fig. 3. Antigen-presenting molecules for a clone DT13.2. **A** A clone DT13.2, one of the clones generated from the DT line, was cultured in the presence of Der f1 peptides (Der f1 p13–31 and Der f1 p18–38) and irradiated autologous PBMC with or without anti-HLA class II mAbs. Results are expressed as the geometric means of duplicate determinations $\pm$ standard error. All mAbs were tested at a final dilution of 1:200 that corresponds to 20–25 μg specific Ab/ml at a final concentration. **B** Autologous or allogeneic PBMC (3×10^5 /well) were cultured in a 96-well plate with peptides (1 μM each) in the culture medium for 2 h at 37°C, then excess peptides and nonadherent cells were removed by gently washing the plate 3 times with RPMI 1640 media containing 3% human serum. The remaining adherent cells were irradiated at 3,000 cGy and used as APC. Results are expressed as the geometric means of duplicate determinations $\pm$ standard error.

though possible differences of the HLA phenotypes between the Japanese and Caucasians would need to be considered, these results do suggest that desensitization therapy with Der f1 and Der p1 peptides needs to be designed independently.

Desensitization therapy with Fel d1 peptides [22] was reportedly successful, using two peptides that stimulate T cells of a majority of patients allergic to Fel d1. From a similar standpoint, the combination of Der f1 p18–31 + Der f1 p206–223 or Der f1 p75–94 + Der f1 p206–223 stimulated T cells of all 6 subjects listed in figure 1. While the precise mechanisms for the in vivo tolerance induction with peptide antigens remain to be determined, human T-T inter-

actions with peptide antigens are prone to induce T cell anergy, because human activated T cells express class II HLA molecules [23, 24]. Moreover, it may not be necessary to target all the antigen-specific T cells, if bystander suppression in the microenvironment and/or active suppression by anergic T cells [25] take place. Thus, Der f I-derived peptides that commonly stimulate patients' T cells, as demonstrated in the present study, should contribute to an effective immunotherapy.

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