

Peptide-induced nonresponsiveness of HLA-DP restricted human T cells reactive with *Dermatophagoides* spp. (house dust mite)

Julie A. Higgins, PhD, Jonathan R. Lamb, PhD, Steven G. E. Marsh, BSc,^a Susan Tonks, BSc,^a John D. Hayball, BSc, Sandra Rosen-Bronson, PhD,^b Julia G. Bodmer, DSc,^a and Robyn E. O'Hehir, MD, PhD^c London, England, and Washington, D.C.

The activation of CD4+ T lymphocytes, which play a central role in allergic inflammation, depends on the recognition of allergen-derived peptides in association with major histocompatibility complex class II gene products. In this report we demonstrate, at a clonal level, that a component of the T-cell repertoire reactive with Dermatophagoides spp. (house dust mite) in atopic individuals, is restricted by HLA-DP class II molecules. This supports the recent results emerging from genetic epidemiologic studies that indicate positive associations between the HLA-DP phenotype and immune responsiveness to a variety of common allergens. Our findings also reveal that the T cells restricted by HLA-DP recognize a species-specific epitope located in the group I allergen of Dermatophagoides pteronyssinus (residues 101-119). Furthermore, we report that the pretreatment of the T cells restricted by HLA-DP with the Der p I peptide renders them nonresponsive to an immunogenic challenge with house dust mite allergen, and the loss of antigen-dependent proliferation is associated with downregulation of membrane expression of the T-cell antigen receptor. The ability to functionally inactivate T cells restricted by HLA-DP, as well as those that recognize allergen in association with HLA-DR class II molecules, suggests that desensitization with allergen-derived peptides may have therapeutic potential in the management of allergic diseases irrespective of their HLA class II association. (J ALLERGY CLIN IMMUNOL 1992;90:749-56.)

Key words: Peptide, T cells, MHC, HLA-DP, Dermatophagoides spp., house dust mite, Der p I, desensitization

It is now well documented that CD4+ T lymphocytes are critical for the induction of both the specific (IgE) and nonspecific (polymorphonuclear granulocytes) effector mechanisms of allergic inflammation.¹⁻³ The activation of CD4+ T cells requires

Abbreviations used

MHC:	Major histocompatibility complex
IL-2:	Interleukin-2
HDM:	House dust mite
PBMC:	Peripheral blood mononuclear cell
[³ H]TdR:	Tritiated methyl thymidine
EBV:	Epstein-Barr virus
cpm:	Counts per minute
Der p I:	Group I allergen of <i>Dermatophagoides pteronyssinus</i>
APC:	Antigen presenting cell

From the Department of Immunology, St. Mary's Hospital Medical School, Imperial College of Science, Technology and Medicine, Norfolk Place, London, Tissue Antigen Laboratory, Imperial Cancer Research Fund, Lincoln's Inn Fields, London,^a and Immunogenetics Group, Med-Dent Building, Georgetown University, Washington, D.C.^b

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Reprint requests: J. A. Higgins, PhD, Department of Immunology, St. Mary's Hospital Medical School, Norfolk Place, London W2 1PG, England.

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the formation of molecular interactions between T-cell receptor, peptide fragments of antigen, and major histocompatibility complex (MHC) class II gene products.^{4,6} Therefore the importance of MHC molecules in the regulation of immune responses prompted genetic epidemiologic studies in an attempt to establish an association between human leucocyte antigen (HLA) phenotype and allergic immune responses to

environmental allergens. The initial analyses concentrated on pollen antigens, and consequently it was demonstrated that the production of IgE after exposure to the ragweed pollen antigens *Amb a* V, *Amb t* V, *Amb p* V, and *Amb a* VI occurred in individuals expressing DRB1*1501 and DRB5*0101 (DR2 Dw2, DR51*) gene products.⁷⁻¹⁰ With the combined approaches of sequence-specific oligonucleotide typing and antigen-specific T-cell clones,¹⁰⁻¹² these HLA associations for ragweed pollen have been confirmed. The extension of these studies on the linkage between HLA and atopic disease established that specific immune responses to the rye grass antigens of *Lolium perenne* (*Lol p*) I, II, and III, are associated with DR3 and DR5 alleles.^{13, 14} As regards allergic immune responses to house dust mite (HDM), from the analysis of antigen-specific T-cell clones it has also been established that HLA-DRB1, HLA-DRB3, and HLA-DRB5 gene products (principally DR2, DR5, DR52b, DR51) are all able to restrict the recognition of HDM determinants.^{15, 16}

The combined results of these studies, in agreement with many others, indicate that HLA-DR molecules restrict T-cell recognition of the majority of nominal antigens. However, in humans there are two additional families of MHC class II molecules (HLA-DP and HLA-DQ) encoded by the HLA gene complex, both of which may function as restriction elements. As regards HLA-DP, documentation exists that T-cell recognition of viral and alloantigens may be restricted by these class II molecules but may contribute to a minor component of the T-cell repertoire.^{17, 18} However, more recently a dominant HLA-DP restricted response to a mycobacterial heat shock protein 65 epitope has been reported in a patient with rheumatoid and arthritis.¹⁹

The ability to induce nonresponsiveness in allergen-specific T cells offers an approach to the modulation of allergic immune responses, and recently it has been demonstrated that peptide-mediated desensitization of T-cell clones reactive to HDM is accompanied by changes in the cytokine pattern from predominantly interleukin (IL)-4 secretion to that of interferon (IFN)- γ , which may contribute to the downregulation of IgE production.²⁰ Evidence exists to support that, similar to activation, MHC class II molecules of the appropriate specificity are required for peptide-mediated T-cell anergy,²¹ although this analysis is limited to T cells restricted by HLA-DR. Here we extend our findings to report the first isolation of cloned CD4+ T cells that are reactive with a peptide determinant of the group I allergen of *Dermatophagoides pteronyssinus* (*D. pteronyssinus* [Der p I]) in the context of HLA-DPB1*0401 class II molecules. This supports the recent population analyses that demonstrate associations between responsiveness to allergens, including HDM, and expression of the HLA-DP phenotype. Furthermore, the results of experiments described here indicate that it is possible to desensitize T cells reactive with HDM and restricted by HLA-DP with specific peptide.

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MATERIAL AND METHODS

Antigens

Lyophilized extracts of *D. pteronyssinus* and *D. farinae* were the generous gift of Bencard (Brentford, Middlesex, England) and Pharmacia (Uppsala, Sweden). Residues 101-119 (Der p I 101-119) of Der p I were synthesized by use of standard solid-phase methods, purified by high-performance liquid chromatography (HPLC), and identified by amino acid analysis.²²

Antibodies

The monoclonal antibodies L243 (anti-HLA-DR)²³ and B7/21 (anti-HLA-DP)²⁴ were isolated from the culture supernatants of the respective hybridoma cell lines and purified by use of protein A sepharose.

Isolation of human HDM reactive clones

Cloned T cells reactive with Der p I 101-119 were isolated from a subject allergic to HDM by limiting dilution cloning, as published elsewhere.²⁵ In brief, peripheral blood mononuclear cells (PBMCs; 10^6 /ml) were stimulated with an optimal concentration of *D. pteronyssinus* (20 μ g/ml) for 7 days in RPMI-1640 medium supplemented with 2 μ mol/L L-glutamine, 100 IU/ml penicillin/streptomycin (Gibco, Life Technologies, Paisley, Scotland), and 5% screened, inactivated human A+ serum (Blood Transfusion Service, Edgeware England). Lymphoblasts enriched on Ficoll-Paque (Pharmacia) were established as a long-term line in the presence of irradiated autologous PBMCs (5×10^5 /ml; 2500 rad), *D. pteronyssinus* (20 μ g/ml), and IL-2 (10% vol/vol; Lymphocult T, Biotest Folex, Frankfurt, Germany) and subsequently cloned by limiting dilution. For cloning, viable cells (0.3 cells/well) were resuspended in supplemented medium and plated in Microtest II (Nunc; Nunc, Roskilde, Denmark) plates together with irradiated autologous PBMCs, *D. pteronyssinus*, and IL-2. After 7 days, growing clones were transferred to flat-bottom 96-well microtiter plates and subsequently transferred to 24-well plates. The clones were maintained with IL-2 every 3 to 4 days and with irradiated autologous PBMCs and *D. pteronyssinus* every 7 days. Before use in assay systems, clones were rested for 7 to 8 days after the last addition of antigen presenting cells (APCs) and antigen.

Proliferation assays

Cloned T cells (10^5 cells/ml) were stimulated with Der p I 101-119 (1 μ g/ml) or *D. pteronyssinus* (20 μ g/ml) in the presence of an equal number of either irradiated autol-

*Nomenclature used is from the WHO Nomenclature Committee, XI International Histocompatibility Workshop, 1991.

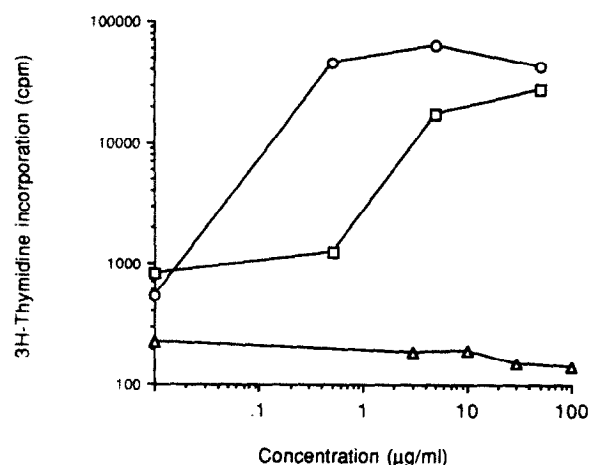


FIG. 1. Antigen specificity of cloned T cells (KS2.12) induced with *D. pteronyssinus*. T cells (10^5 /ml) were stimulated with antigen in the presence of irradiated autologous PBMCs as APCs. Proliferation as correlated with [^{3} H]TdR incorporation was measured at 72 hours. Marked dose-dependent stimulation was observed with the peptide epitope Der p I 101-119 (○) and with unfractionated *D. pteronyssinus* extract (□). No response was observed to the related HDM species, *D. farinae* (△).

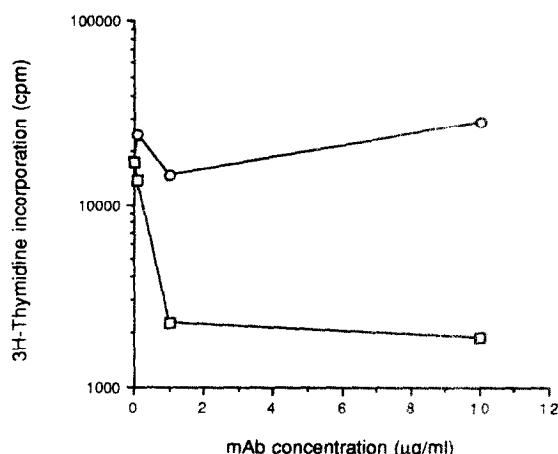


FIG. 2. Anti-HLA-DP class II antibody inhibits the antigen-dependent proliferative response of T-cell clone KS2.12. The blocking effects of murine monoclonal antibodies against human MHC class II molecules were determined in proliferation assays with clone KS2.12 and irradiated autologous PBMCs as accessory cells with *D. pteronyssinus* (20 µg/ml). Marked inhibition was observed with the anti-HLA-DP antibody, B7/21 (□) in contrast to the anti-HLA-DR antibody, L243 (○).

ogenous PBMC (2500 rad) Epstein-Barr virus (EBV)-transformed B cells (5000 rad), or mitomycin C-treated murine fibroblasts expressing HLA-D region gene products, as APCs. The murine fibroblasts (DAP3), transfected with DRB1*0101 or DPB1*0401 genes were kindly provided by Dr. J. Trowsdale, (Imperial Cancer Research Fund), Dr. S. Rosen-Bronson, Georgetown University, Washington D.C., and Dr. H. Inoko, Tokai University School of Medicine, Bosheida Isehara Kanagawa, Japan, respectively. In serologic inhibition assays, antibodies were added over a concentration range at the initiation of cultures. After 60 hours of incubation the cultures were pulsed with tritiated methyl thymidine (1 µCi/well, [^{3} H]TdR; Amersham International Inc., Amersham, U.K.) and the cultures harvested 8 to 16 hours later. Proliferation as correlated with [^{3} H]TdR incorporation was measured by liquid scintillation spectroscopy. The results are expressed as mean counts per minute (cpm) for triplicate cultures. The standard error of the mean for all experiments was <20%. The data plotted in each of the figures and tables are from one experiment; however, each experiment was repeated three times or more with consistent results.

Induction of T-cell nonresponsiveness

The induction of peptide-mediated nonresponsiveness was performed as previously reported for T cells restricted by HLA-DR.²⁶ In brief, cloned T cells (10^6 /ml) were incubated with Der p I 101-119 (100 µg/ml) for 16 hours in the absence of APCs. Control cultures of cells in medium alone were performed in parallel. The cells were washed extensively after the pretreatment before determining their ability to respond to either an immunogenic challenge of

antigen (*D. pteronyssinus* or Der p I 101-119) and APCs, or IL-2 or APC alone.

RESULTS

Antigen specificity of T-cell clone

T cells of clone, KS 2.12, in the presence of accessory cells, proliferated in a dose-dependent manner to the inducing antigen, *D. pteronyssinus* whole extract but not a closely related member of the same genus, *D. farinae* (Fig. 1). With use of overlapping synthetic peptides, the reactivity of the T cells was mapped to residues 101-119 of Der p I.

Serologic inhibition of the antigen-dependent response of T-cell clone KS2.12

To define initially the subsets of MHC class II molecules acting as restriction elements, monoclonal antibodies directed against framework antigens of HLA-D region molecules were added over a concentration range at the initiation of the proliferation assays containing the cloned T cells (Fig. 2). Maximal inhibition of antigen-dependent proliferation was obtained with anti-HLA-DP antibody (B7/21), whereas the anti-HLA-DR antibody (L243), which recognizes a determinant on DR α chains, failed to modulate the proliferative response. The activity of L243 was confirmed by its ability to block antigen-dependent proliferation of other cloned T cells. The anti-HLA-DP

TABLE I. HLA-D region specificities of the panel of EBV-transformed B cells used as APCs

Cell line	HLA-D specificities	HLA alleles							
		DRB1	DRB3	DRB4	DRB5	DQA1	DQB1	DPA1	DPB1
PGF	DR15 Dw2	1501	—	—	O101	O102	O602	O1	O401
HOM2	DR1 Dw1	O101	—	—	—	O101	O501	ND	O401
YAR	Dr4 Dw10	O402	—	O101	—	O301	O302	O1	O401
SWEIG	DR11(5) Dw5	DR52 Dw25	1101	O202	—	O501	O301	ND	O402
AMAI	DR15(2) Dw2	1501	—	—	O101	O102	O602	O1	O402
TOKONAGA	DR15(2) Dw12	1502	—	—	O102	O103	O601	O2	O901
WT24	DR16(2) Dw21	1601	—	—	O201	O102	O502	O1	O301
RML	DR16(2) Dw22	1602	—	—	O202	O501	O301	O1	O402
JVM	DR11(5) Dw'JVM'	DR52 Dw25	1102	O202	—	O501	O301	O1	O201
TISI	DR11	DR52 Dw25	1103	O202	—	O501	O301	O1	O402
FPF	DR11(5) Dw'FS'	DR52 Dw25	1104	O202	—	O103	O6	O1	O201
BM16	DR12(5) Dw'DB6'	DR52 Dw25	1201	O202	—	O501	O301	O1	O201
TER-ND	DR103 Dw'BON'	O103	—	—	—	O101	O501	O1	O201, O401
PMG075	DR1 Dw20	O102	—	—	—	O101	O501	O1,O2	O301,O401

ND, Not determined.

TABLE II. The T-cell clone KS2.12 (10^6 /ml) was cultured with and without *D. pteronyssinus* in the presence of HLA-D region typed EBV-transformed B cells (10^6 /ml) described in Table I, including the autologous EBV-B cells

Cell line	HLA-D specificities	3H-thymidine incorporation (cpm)		
		APC	Dpter (50 µg/ml)	
KSE	DR1 DR2	902	35232	
PGF	DR15 Dw2	5430	23904	
HOM2	DR1 Dw1	2949	46887	
YAR	Dr4 Dw10	2911	45938	
SWEIG	DR11(5) Dw5	DR52 Dw25	2901	27255
AMAI	DR15(2) Dw2		2352	2880
TOKONAGA	Dr15(2) Dw12	1580	1458	
WT24	DR16(2) Dw21	1054	2204	
RML	DR16(2) Dw22	2381	23051	
JVM	DR11(5) Dw'JVM'	DR52 Dw25	7772	8546
TISI	DR11		DR52 Dw25	1285
FPF	DR11(5) Dw'FS'	DR52 Dw25	1840	1870
BM16	DR12(5) Dw'DB6'	DR52 Dw25	3027	3517
TER-ND	DR103 Dw'BON'	4470	25904	
PMG075	DR1 Dw20	854	26182	

Dpter: *D. pteronyssinus*.Proliferation as correlated with [^{3} H]TdR incorporation measured at 72 hours. Results expressed as mean cpm for triplicate cultures. In both test and control groups SE of the mean <20%.

antibody also gave marked inhibition of a *D. pteronyssinus*-specific T-cell line derived from KS showing that a major component of the T-cell repertoire for *D. pteronyssinus* was restricted by HLA-DP (data not shown).

Restriction pattern of cloned T cells with use of panels of B-cell lines transformed by EBV

On the basis of the serologic inhibition data, the cloned T cells were further examined for responsive-

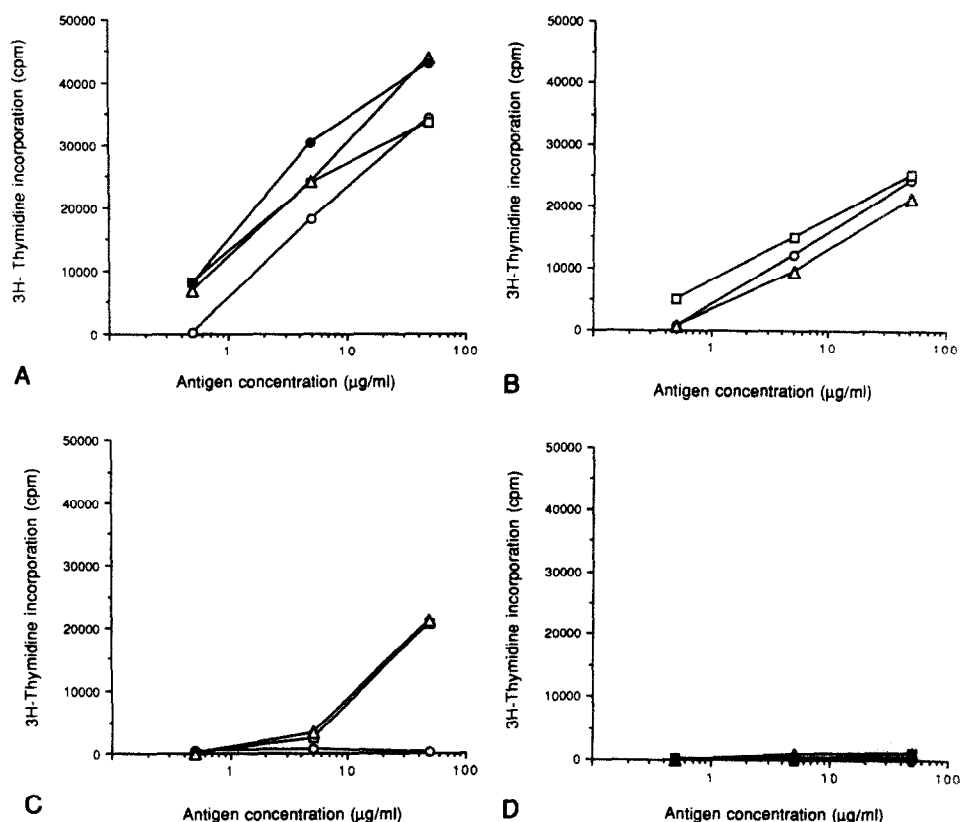


FIG. 3. EBV-transformed B-cell lines expressing HLA-DP class II molecules restrict the recognition of *Der p* I by the T-cell clone KS2.12. Cloned T lymphocytes (KS2.12; 10^5 /ml) were cultured with unfractionated *D. pteronyssinus* extract over a concentration range in the presence of an equal number of HLA typed, irradiated EBV-transformed B cells as a source APCs. Strong proliferative responses (A) were observed with KSE, the autologous control EBV line (○), PGF (□), YAR (●), and HOM2 (△). Moderate proliferation (B) was also induced in the presence of SWEIG (○), PMG075 (□), and TER-ND (△). Weak proliferation (C) was observed with RML (□) and TISI (△) at high antigen concentration (50 μg/ml), but AMAI (○) failed to present HDM. No stimulation (D) was induced with the cells TOKUNAGA (○), WT24 (□), JVM (△), FPF (●), or BM16 (□).

ness by use of an APC panel consisting of HLA-typed, EBV-transformed, B-cell lines (Table I), including the autologous EBV B-cell line, KSE (DR1, DR2) as a positive control (Table II). A dose-dependent proliferative response was induced to *D. pteronyssinus* when presented by those EBV-B cells homozygous for the DPB1 gene product *0401 (PGF, HOM2, and YAR; Fig. 3, A). The B-cell lines PMG075 and TER-ND, which express the DPB1 gene products *0301, *0401, and *0201, *0401, respectively, also presented antigen to the T-cell clone in a dose-dependent manner (Fig. 3, B). The level of proliferation was lower in these cultures than that induced by either the HLA-DPB1*0401 homozygous or autologous B-cell lines. SWEIG, which expresses DPB1*0402, also induced a lower dose-dependent proliferation than that observed with the autologous B-cell line. At supraop-

timal antigen concentrations (50 μg/ml), RML and TISI (DPB1*0402) induced marked proliferation. However, the other EBV-B-cell line expressing *0402 (AMAI) failed to present antigen effectively to the cloned T cells (Fig. 3, C). Those EBV-B-cell lines expressing the DPB1 products *0201 (JVM, FPF, BM16), *0301 (WT24), and *0901 (TOKUNAGA) failed to induce proliferation (Fig. 3, D).

Antigen presentation by murine fibroblasts expressing HLA-DPB1*0401 class II molecules

To confirm the functional role of DPB1*0401 in antigen presentation to T cells of clone KS2.12, murine fibroblasts transfected with the DPB1*0401 gene were used as APCs in proliferation assays with whole *D. pteronyssinus* extract and peptide (*Der p* I 101-

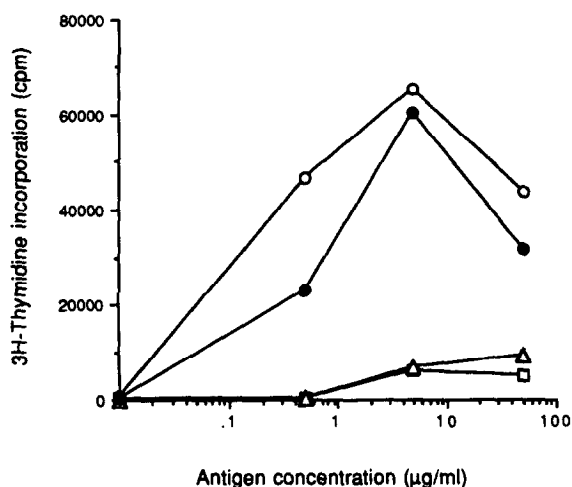


FIG. 4. Recognition of *Der p* I 101-119 by T-cell clone KS2.12 is restricted by the HLA-DPB* 0401 specificity. Cloned T cells (10^5 /ml) were cultured with the peptide *Der p* I 101-119 (0.5 to 50 μ g/ml) in the presence of different APCs (10^5 /ml); autologous EBV-B cells ($\circ$) or mitomycin-C-treated murine fibroblasts expressing DPB1*0401 ($\bullet$), DRB1*0101 (Δ), and DAP3 (untransfected control murine fibroblast $\square$).

119). The transfected fibroblasts induced strong, dose-dependent proliferative responses in the presence of the peptide in a manner analogous to those observed with autologous EBV-transformed B cells as APCs (Fig. 4). In contrast, the nontransfected fibroblast line, DAP3, and a DRB1*0101 transfectant, when used as APCs failed to induce marked antigen-dependent proliferation. Unfractionated HDM extract was only presented to T cells of clone KS2.12 by the DPB1*0401 transfectant at high concentrations (50 μ g/ml), and proliferation was decreased compared with presentation by the autologous EBV-B cells (data not shown).

Induction of antigen-specific nonresponsiveness

Preincubation of the HDM-reactive T cells (KS2.12) with specific peptide (*Der p* I 101-119) at 100 μ g/ml for 16 hours, in the absence of APCs, induced nonresponsiveness, such that the T cells failed to proliferate when rechallenged with an immunogenic challenge of *D. pteronyssinus* extract or with peptide in the presence of autologous PBMCs (Fig. 5). Responsiveness to exogenous IL-2, however, was enhanced. The presence of anergy was associated with downmodulation of CD3 expression and upregulation of CD25 expression (data not shown), as has been described previously in antigen-induced nonresponsiveness in T-cell clones restricted by HLA-DR.²⁰

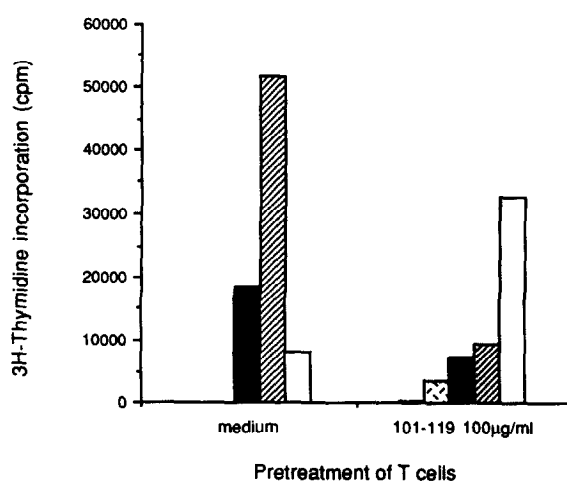


FIG. 5. Peptide-induced inhibition of T-cell proliferation. T cells of clone KS2.12 were cultured for 16 hours in the presence of *Der p* I 101-119 (100 μ g/ml) in the absence of accessory cells or in medium alone. The T cells were then washed thoroughly and rechallenged with an immunogenic concentration of *Der p* I 101-119 (1 μ g/ml; *hatched histogram*) or crude HDM extract, *D. pteronyssinus* (20 μ g/ml; *stippled histogram*) in the presence of irradiated autologous PBMCs as accessory cells, or with medium alone (*solid histogram*), accessory cells alone (*feathered histogram*), or with IL-2 (*open histogram*). Proliferation as correlated with [^{3} H]TdR incorporation was determined at 72 hours. Results are expressed as mean cpm for triplicate cultures. In both test and control groups the SE of the mean was <20%.

DISCUSSION

In this report we demonstrate, in the periphery of an atopic individual, the presence of T cells that respond to the group I allergen of *D. pteronyssinus* (residues 101-119) in association with HLA-DP molecules. Furthermore, the results of the experiments reported here indicate that the administration of a supraoptimal concentration of *Der p* I 101-119 is able to functionally inactivate the T cells such that they are unresponsive to an immunogenic challenge of antigen.

The inhibition of antigen-dependent proliferation with anti-HLA-DP, but not anti-HLA-DR framework antibodies, provided the initial evidence that T-cell recognition was restricted by HLA-DP molecules. This contrasts with previous anti-MHC class II antibody blocking studies examining HDM-specific T-cell lines and clones,^{15, 27} which suggested that HLA-DR molecules were the major restriction elements. The subsequent detailed analysis of the restriction specificity of HDM-reactive T-cell clones with use of murine fibroblasts expressing the appropriate HLA-D region molecules confirmed that HLA-DRB1, HLA-DRB3, and HLA-DRB5 MHC class II molecules are

functional in the presentation of HDM.¹⁶ The multi-determinant nature of HDM may account for this heterogeneity in the HLA class II restriction specificity of the T-cell repertoire reactive with dust mite allergens and, in part, may explain the failure of many genetic epidemiologic studies to establish an HLA linkage. To explore further the HLA-DP restriction of the *Der p* I 101-119 specific T cells, a panel of HLA-typed, EBV-transformed B cells was used as APCs. Antigen-dependent proliferation was observed in the presence of EBV-B cells known to express HLA-DPB1*0401 (PGF, HOM2, YAR, PMG075, and TER-ND). The ability of the DPB1*0402 expressing EBV-B-cell lines SWEIG, RML, and TISI, but not AMAI, to induce proliferation, suggests that different allelic forms of DPB1*0402 may exist with varying structural homology to DPB1*0401. The HLA-DPB1 restriction was confirmed with murine fibroblasts expressing DPB1*0401, which were able to present *Der p* I 101-119 with comparable efficiency to autologous EBV-B cells. In agreement with previous reports examining T-cell recognition of other antigens,¹⁷ our observations demonstrate that HLA-DP molecules are able to restrict T-cell responses to dust mite allergen. It is interesting to note that it has been reported that HLA-DP restricted T-cell clones specific for heat shock protein 65 expressed CD4⁺, CD8⁻, TCR δ phenotype,¹⁹ whereas the *Der p* I 101-119 reactive T cells described here show positive membrane staining with antibodies specific for TCR- $\alpha\beta$ heterodimers. Furthermore, their lymphokine profile was that of the "allergen phenotype,"²⁸ displaying enhanced mRNA levels for IL-4 and IL-5, but not IL-2 and IFN- γ , which is consistent with the ability to support IgE synthesis (data not shown).

The importance of our findings with respect to the role of HLA-DP molecules in atopy is highlighted by recent epidemiologic studies that suggest positive correlations between specific IgE against common allergens and phenotypic expression of HLA-DP. Using the polymerase chain reaction, Eura et al.²⁹ observe increases in DPB1*0401 and *0201 frequencies in the response to the group II allergen of *D. farinae*. The findings in this report, based on the class II restriction specificity of cloned T cells reactive with HDM, support these epidemiologic studies. In parallel studies, Young et al.³⁰ using population analyses report an increase in DPB1*0401 frequencies in atopic individuals, as compared with controls, for a number of important allergens (*Fel d* I; *Can f* I; *Alt a* I; *Phl p* V; *Der p* I, *Der p* II). The expression of DPB1*0401 is 55% in normal individuals, and therefore extension of the population studies combined with the use of cell culture systems is required to dissect the MHC class II

restriction of HDM responsiveness. Nevertheless, together these reports suggest a possible important role for HLA-DP as well as HLA-DR gene products in the regulation of allergic immune responses.

The tissue distribution of HLA-DP class II molecules may be as important as their specificity in allergic immune responses. In 5% of adults and 10% of children atopic disease is manifest as extrinsic asthma. Thus increased expression of HLA-D region molecules, including HLA-DP, on lung dendritic cells, the primary APCs in the airway, may have both qualitative and quantitative effects on local immune responses, as has been suggested for dendritic cells isolated from the synovial fluid of patients with inflammatory arthritis.³¹ Similarities in the inflammatory components of asthma and inflammatory arthritis imply that studies on the expression of class II molecules on airway APCs may be rewarding.

The ability to render peripheral T-cells nonresponsive to specific antigen forms the theoretic basis of allergen-mediated desensitization. It has been reported that peptides and superantigens of the appropriate specificity are able to induce tolerance and modulate the lymphokine production of T cells reactive to HDM toward that of Th1 type cells.²⁰ The in vivo potential of peptide-mediated anergy is suggested by the observation that high doses of peptide are able to induce T-cell nonresponsiveness in the presence of APCs.²⁰ Nevertheless, this analysis has been limited to only those T cells that are restricted by HLA-DR and prompted us to investigate whether or not it was possible to induce tolerance in T cells restricted by HLA-DP molecules. The findings reported here establish that exposure to specific peptide may functionally inactivate T cells restricted by HLA-DP, and that the loss of antigen-dependent proliferation is associated with the transient modulation of the T-cell receptor from the cell surface.²⁰

Our findings demonstrate at a clonal level that a component of the T-cell repertoire reactive with HDM, in atopic individuals, may be restricted by HLA-DP class II molecules and support the recent experimental data on HLA associations in allergy that are emerging from the population studies.^{29, 30} The ability to functionally inactivate the T cells with use of allergen-derived peptides suggests that such an approach may have therapeutic potential in the management of allergic diseases where T-cell allergen recognition is in the control of HLA-DR and/or HLA-DP molecules.

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