

T CELLS SENSITIZED TO SYNTHETIC HLA-DR3 PEPTIDE
GIVE EVIDENCE OF CONTINUOUS PRESENTATION OF
DENATURED HLA-DR3 MOLECULES BY HLA-DP

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It is well established that the activation of various types of T lymphocytes requires recognition by TCRs in association with MHC molecules on the surface of APC or target cells. Class II MHC molecules regulate the recognition of antigens by Th cells. It has been suggested that the antigens presented by class II molecules are preferentially ingested or internalized exogenous proteins (1) and that these proteins are presented in a degraded or a denatured form, rather than in the native form (2, 3). Several studies have shown that short peptides, corresponding to mapped antigenic sites of proteins, indeed have the same stimulatory capacity as the intact protein (4). The proteins used for these studies are derived from viruses or bacteria and, thus, foreign proteins. An interesting question that arises from these findings is whether peptides derived from self proteins like MHC molecules are also presented to Th cells. Kourilsky et al. (5) have postulated that self, as well as nonself, proteins can yield peptides presented by the MHC and that alloreactivity could result from MHC molecules presenting different sets of self peptides, including peptides derived from MHC molecules themselves. This hypothesis has been supported by the work of Maryanski et al. (6), who have shown that synthetic peptides derived from class I molecules can induce class I-specific CTL to lyse mouse cells (6, 7). It has, however, never been proven that class II peptides derived from the MHC are presented to and recognized by Th cells. Here, we show that we have obtained T cell clones that recognize HLA-DR3 peptides as well as endogenous, denatured HLA-DR3 presented by a molecule from the MHC complex, the HLA-DPw3 molecule.

Materials and Methods

Peptide Synthesis. The peptide U6 was synthesized in the amide form, using solid phase peptide synthesis methodology (8-10) and checked using analytical reversed phase HPLC and amino acid analysis (8).

The peptide is homologous to the third hypervariable region of the second domain of the DR3 BI chain (10a) (amino acid 67-85) and has the following amino acid sequence: L L E Q K R G R V D N Y C R H N Y G V.

T Cell Lines and Clones. 10⁵ PBLs were primed in the presence of peptide in a concentration of 10 µg/ml in RPMI 1640 supplemented with 20% pooled human serum and gentamycin

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(50 mg/liter) in V-shaped wells (96-well plates; Cel-Cult, Tissue Culture Products, Feltham, UK). After 7 d the cultures of ~60 wells were collected, spun down, and restimulated with irradiated autologous feeder cells (3,000 Rad) at a ratio of 10:1 and peptide in 24-well plates (Costar, Cambridge, MA). The line was cloned by limiting dilution to 10 or 0.5 cells per well in the presence of T cell growth factor (TCGF; Biotest Serum Institut, Frankfurt, FRG) and autologous irradiated feeder cells. The T cell lines and clones were expanded by serial exposure to TCGF and peptide as presented by autologous feeder cells. T cell lines and clones were frozen in small aliquots and stored in liquid nitrogen before use.

Proliferation Studies. For T cell lines and T cell clones, 20,000 and 5,000 responder cells per well were used, respectively. The T cell lines or clones were cocultured with 10^5 irradiated stimulator cells (2,000 Rad) in a total volume of 200 μ l culture medium in flat-bottomed microtiter plates. After 48 h of incubation (37°C, 5% CO₂) the cultures were pulsed with 0.05 ml (20 μ Ci/ml) of tritiated thymidine and harvested 14–16 h later. [³H]Thymidine incorporation was then measured by liquid scintillation counting. The indicated cpm represent the median value of triplicate cultures. The stimulation index (SI) is calculated by dividing the cpm measured when stimulator and responder are cultured together by the sum of the cpm measured when stimulator and responder are cultured separately.

mAbs. The DR backbone-specific mAb B 8.11.2 (11) was provided by Dr. B. Malissen (the Ludwig Institute for Cancer Research, Lausanne); the DQ antibody SPV.L3 (12) was provided by Dr. H. Spits (the Netherlands Cancer Institute, Amsterdam); and the mAb A3 (anti-DP) was obtained from the Tenth International Histocompatibility Workshop, New York (Becton Dickinson & Co., Mountain View, CA).

Antibody Blocking Assays. Ascitic fluid was added to the culture in a final dilution of 1:300 at the initiation of the T cell proliferation assay.

Results and Discussion

Panel Reactivity of the Anti-U6 T Cell Line ThoU6. PBLs from individual Tho (DR4,5; DQw3; DPw3,w4) were in vitro stimulated with a synthetic DR3 peptide (peptide U6). The obtained T cell line ThoU6 was tested in a proliferation assay for recognition of peptide U6 in the presence of autologous irradiated APC and for recognition of PBLs of different HLA types. A representative experiment is depicted in Table I.

As expected, ThoU6 recognized peptide U6 presented by autologous APC. In this experiment two PBL donors also stimulate ThoU6 in the absence of U6. These PBLs express DR3 and share the expression of DPw3 with Tho. Therefore, stimulation of ThoU6 might, in these cases, be due to presentation of processed DR3 mole-

TABLE I
Recognition of Peptide U6 and PBLs by T Cell Line ThoU6

Stimulator	HLA type			Responder	
				ThoU6	None
				<i>cpm</i>	
SdK	DR3,3	DQw2	DPw2,w3	78,992	335
Mun	DR2,3	DQw1,w2	DPw3,w4	66,779	380
PvtS	DR2,3	DQw1,w2	DPw2,w4	805	425
Bier	DR2,3	DQw1,w2	DPw2,w4	890	390
Tho	DR4,5	DQw3	DPw3,w4	555	425
HeyL	DRw6	DQw1	DPw3	635	525
JB	DR2	DQw1	DPw4	415	290
Gul	DRw6	DQw1,w3	DPw1,w4	480	110
Tho + peptide U6 (1 μ g/ml)				31,470	285
Tho + peptide U6 (10 μ g/ml)				86,108	285

The counts represent the median value of triplicate cultures.

cules by DPw3. To examine this possibility an extended panel of PBLs was tested for its stimulatory capacity in the presence and in the absence of synthetic peptide.

24 PBLs were tested in the presence of peptide U6 in a concentration of 10 $\mu\text{g/ml}$. 11 of 11 DPw3⁺ PBLs, but none of the 13 DPw3⁻ PBLs, were able to stimulate the line in the presence of peptide.

52 PBLs were tested in the absence of peptide. The results are shown in Fig. 1. There is a highly significant difference between the stimulation induced by DR3 DPw3⁺ PBLs and PBLs expressing only one or none of these antigens ($p < 0.0001$).

From the results of the panel studies, it can be concluded that T cell line ThoU6 recognizes not only synthetic peptide U6 presented by HLA-DPw3, but also a naturally occurring peptide that is most likely a product of denaturation of the DR3 molecule. Although we have no formal proof that the presented antigen is a product of DR3, we will refer to it as the natural DR3 peptide. Possibly, the DR3 molecule is internalized and processed by the APC.

Cloning of ThoU6. To exclude the possibility that synthetic peptide and denatured DR3 molecules are recognized by two different sets of T cells, ThoU6 was cloned. Nine clones, five of which originated from 10 cells/well and four from 0.5 cells/well, were tested against a panel of eight PBLs in the presence and absence of peptide U6. The clones all recognized peptide U6 presented by autologous APC or DPw3⁺ PBLs ($n = 2$) as well as DR3 DPw3⁺ PBLs ($n = 2$). None of the DR3⁺ DPw3⁻ ($n = 2$) or DR3 DPw3⁻ ($n = 2$) PBLs were able to stimulate the clones. As there are no differences in the reactivity patterns of the clones, we conclude that peptide U6 and the natural DR3 peptide are recognized by the same set of T cells.

Proliferation Inhibition Studies with mAbs. Because all APC stimulating T cell line ThoU6 express DPw3, it is very likely that this molecule presents the DR3 peptide to the responder cells. To prove that the DP molecule is responsible for the presentation of antigen, we performed blocking studies with monomorphic mAbs against DR, DQ, and DP. Results of the experiment using the original line and two clones are shown in Fig. 2, *a-c*. When the autologous APC (Tho) and DPw3⁺ DR3⁻ PBLs (HeyL) were used as stimulators in the presence of peptide U6, the anti-DP monoclonal could completely block the recognition of the peptide. This was also the case when a DR3 DPw3⁺ stimulator was used in the absence of peptide U6 (Slot). Therefore, it can be concluded that the DP molecule is indeed responsible for the presentation of synthetic peptide as well as for the presentation of natural DR3 peptide.

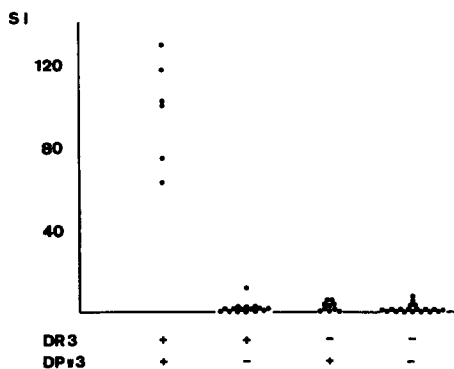


FIGURE 1. Reactivity of T cell line ThoU6 against a panel of 52 PBLs. Specificities DR1 to DRw15 and specificities DPw1 to DPw5 are represented. The panel is divided into DR3⁺ DPw3⁺, DR3⁺ DPw3⁻, DR3⁻ DPw3⁺, and DR3⁻ DPw3⁻ stimulators, as indicated along the *x*-axis. The stimulation index (SI) is indicated along the *y*-axis.

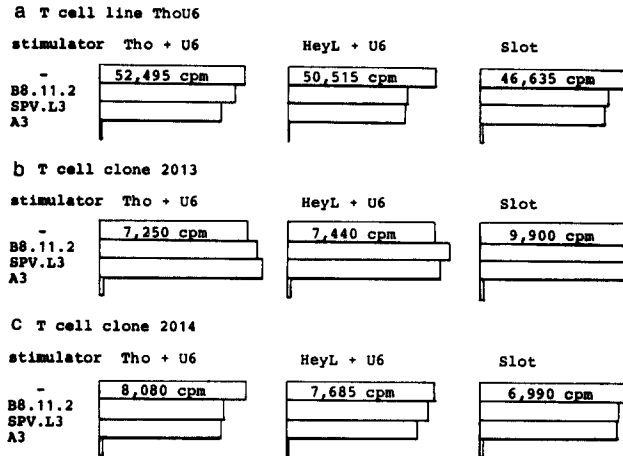


FIGURE 2. Results of the blocking studies with mAbs B8.11.2 (DR), SPV.L3 (DQ), and A3 (DP). Stimulators are autologous APC (Tho) in the presence of U6; DPw3⁺ DR3⁻ APC (HeyL) in the presence of U6; or DR3 DPw3⁺ PBLs (Slot) in the absence of U6. Responders are (a) the original T cell line ThoU6; (b) clone 2013; and (c) clone 2014. Results of the assays without the addition of mAbs represent 100% proliferation.

It has been suggested that allrecognition does not necessarily have to be recognition of the intact HLA molecule, but can be the recognition of a processed HLA molecule presented as nominal antigen (5–7, 13, 14). Kourilsky and Claverie (15) hypothesized that peptides derived from the breakdown of most or all cellular proteins are able to associate with class I or class II antigens and would thus be continuously presented at the surface of cells. Peptides derived from MHC molecules would be no exception to this rule (5, 15). So far, there has only been proof for the class I-restricted presentation of class I molecules (6, 7 13). Here, we report of a DP-restricted response to DR3 after *in vitro* sensitization of PBLs with synthetic DR3 peptide. Our results, furthermore, imply a continuous presentation of natural DR3 peptides by DP molecules.

We have recently been able to raise a second T cell line that is responsive to both synthetic and natural DR3 peptide. This time, the response was restricted through HLA DRw6 (data not shown). This finding, together with the results discussed in the present article, suggests that the DR3-derived peptide can probably be presented by several HLA class II molecules. The presentation of natural, MHC-derived peptides by different self class II molecules might have implications for the induction of self tolerance (16) and, through that, for autoimmunity and organ transplantation (17).

Summary

T cell clones raised against a synthetic peptide, identical to the third hypervariable region of the DR3 BI chain, were tested for secondary proliferative responses against a panel of PBLs. All seven DR3 DPw3⁺ stimulators could induce proliferation. DR3⁻ DPw3⁺ PBLs were recognized when the synthetic peptide was added to the cultures. Inhibition studies with mAbs showed that in both cases the HLA-DP molecule is involved in the recognition of both types of stimulators. We conclude that the clones recognize the DR3 peptide presented by HLA-DPw3. This stimulus can be obtained in two different ways: (a) by addition of synthetic peptide to DPw3⁺ PBLs or (b) by using DR3 DPw3⁺ stimulator cells where DR3 peptides are present

in the culture as a product of denaturation of the DR3 molecule. Because all DR3 DPw3⁺ PBLs tested could stimulate the line and clones, we assume that the presentation of the DR3 peptide by DP is a naturally and continuously occurring phenomenon.

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