

Evolutionary Conservation of Major Histocompatibility Complex-DR/Peptide/T Cell Interactions in Primates

By Annemieke Geluk,* Diënne G. Elferink,* Bastiaan L. Sliereendregt,†
Krista E. van Meijgaarden,* René R. P. de Vries,*
Tom H. M. Ottenhoff,* and Ronald E. Bontrop†

From the *Department of Immunohematology and Blood Bank, University Hospital, 2300 RC Leiden; and the †Institute of Applied Radiobiology and Immunology TNO, 2280 HV Rijswijk, The Netherlands

Summary

Many major histocompatibility complex (MHC) polymorphisms originate from ancient structures that predate speciation. As a consequence, members of the *Mhc-DRB1*03* allelic lineage are not only present in humans but in chimpanzees and rhesus macaques as well. This emphasizes that *Mhc-DRB1*03* members must have been present in a common ancestor of these primate species that lived about 30 million years ago. Due to the accumulation of genetic variation, however, alleles of the *Mhc-DRB1*03* lineage exhibit species-unique sequences. To investigate the biological importance of such conservation and variation, we have studied both the binding and antigen presentation capacity of various trans-species *Mhc-DRB1*03* lineage members. Here we show that p3-13 of the 65-kD heat-shock protein (hsp65) of *Mycobacterium leprae* and *M. tuberculosis* binds not only to HLA-DR17(3) but also to some chimpanzee and rhesus macaque class II-positive cells. Comparison of the corresponding human, chimpanzee, and rhesus macaque *Mhc-DRB1*03* lineage members revealed the presence of uniquely shared amino acid residues, at positions 9–13 and 26–31, of the antigen-binding site that are critical for p3-13 binding. In addition it is shown that several nonhuman primate antigen-presenting cells that bind p3-13 can activate HLA-DR17-restricted T cells. Certain amino acid replacements, however, in *Mhc-DRB1*03* lineage members did not influence peptide binding or T cell recognition. Therefore, these studies demonstrate that some polymorphic amino acid residues (motifs) within the antigen-binding site of MHC class II molecules that are crucial for peptide binding and recognition by the T cell receptor have been conserved for over 30 million years.

Peptides are able to bind to specificity pockets within the peptide-binding site of MHC molecules (1–3). The proposed model for the structure of MHC class II molecules (4), which has been determined based on the resolution of the structure of HLA class I (5), shows that most polymorphic amino acid residues map within the peptide-binding site. *Mhc* polymorphism thus will result in the differential selection of peptides available for T cell recognition.

Mycobacterial heat-shock proteins (hsp)¹ are dominant T cell antigens in the response of healthy as well as mycobacterial disease-affected individuals (6–8). The antigenicity of, but also the high homology between, several mammalian and bacterial hsp has led to the hypothesis that T cells initially

triggered by an infection may become crossreactive with self-peptides. Eventually this results in, or may even prolong, autoimmune responses at local sites of inflammation (9). The best characterized mycobacterial hsp is hsp65 (10), which is recognized in the context of several different HLA-DR molecules by CD4-positive, antigen-specific T cells (7, 8).

Previously it was reported that hsp65 p3-13 is immunodominant in the mycobacterium-specific T cell response of HLA-DR17(3)-positive individuals and is not recognized in the context of any other HLA-DR molecule (11). This DR17-restricted T cell recognition most probably arises from the fact that p3-13 binds to motifs uniquely present in HLA-DR17 molecules (12).

Comparison of nucleotide sequences showed a high degree of similarity between some nonhuman primate *Mhc* alleles and certain HLA orthologs (13–17). The degree of similarity between related *Mhc* class II alleles of different spe-

¹ Abbreviations used in this paper: B-LCL, B lymphoblastoid cell line; HA, hemagglutinin; hsp, heat-shock protein.

cies is always higher than that found for alleles grouping in distinct lineages of the same species, as is consistent with the trans-species hypothesis (18). This hypothesis states that closely related *Mhc* alleles in different species originate from common ancestral alleles, predating the divergence of these species. Trans-species alleles that group into a lineage are generally unique to a species due to the accumulation of genetic variation, mainly caused by point mutations or crossing over events (19–21).

In this study we have investigated the functional importance of these conserved motifs that are present in the diverged members of the *Mhc-DRB1*03* allelic lineage of humans, chimpanzees (*Pan troglodytes*), and rhesus macaques (*Macaca mulatta*). The capacity of different primate *Mhc-DRB1*03* members to bind and present hsp65 p3-13 was analyzed using non-human primate-derived EBV-B lymphoblastoid cell lines (BLCL) as “biomutant” APC. As the corresponding nucleotide sequences of the *Mhc-DRB* molecules of these cells have been determined (20, 21), it is now possible to pinpoint the essential *Mhc-DRB1*03*-encoded residues involved in the binding of hsp65 p3-13. Moreover, the functionality of the p3-13 binding was analyzed in T cell stimulation assays using HLA-DR17-restricted, hsp65 p3-13-reactive human T cell clones and nonhuman primate-derived EBV-BLCL as APC.

Materials and Methods

Synthetic Peptides. Synthesis of p3-13 (KTIAYDEEARR) and p307-319 (PKYVKQNTLKLAT) has been described (12). In short, peptides were made by standard solid-phase methods on a peptide synthesizer using Fmoc amino acid pentafluorophenylesters (Cambridge Research Biochemicals Ltd., Cambridge, England). The long chain biotinylated analogues of the peptides were made by coupling of 6-(Fmoc amino) hexanoic acid and biotin (Serva, Heidelberg, Germany), respectively, at the end of the synthesis. The peptides were purified by gel filtration chromatography on a Sephadex G-25 (superfine) column (Pharmacia, Uppsala, Sweden) followed by reversed-phase HPLC (C18) purification, and analyzed by fast atom bombardment mass spectrometry.

Binding Assay. In the binding assay (12, 22), EBV-BLCL (3×10^5 /sample) were incubated with the biotinylated peptide (50 μ M) at 37°C for 4 or 20 h. As a control, cells were labeled in each experiment with a biotinylated mAb specific for HLA-DR (5 μ l; Becton Dickinson & Co., Mountain View, CA) at 4°C for 1 h. Peptide or anti-DR preincubation were followed by labeling with FITC-avidin D (10 μ g/ml; 100 μ l; Vector Labs, Burlingame, CA) at 4°C for 30 min. Incubation with FITC-avidin D was followed by incubation of biotinylated anti-avidin D (10 μ g/ml; 100 μ l; Vector Labs) and FITC-avidin D again. After each incubation, excess reagents were washed off at 4°C using PBS containing 0.1% BSA. Stained cells were analyzed by flow cytometry on a FACScan® analyzer (Becton Dickinson & Co.). Dead cells were excluded from the analysis by propidium iodide staining. To measure the relative amount of FITC-avidin D bound, the mean fluorescence of 5,000 stained cells was determined. Background fluorescence, measured in the absence of peptide, was subtracted.

mAbs. The following mAbs were used in this study: anti-DR (B8.11.2); anti-class I (W6/32); anti-class II (1C2); anti-DQ (SPVL3); anti-DP (B7/21); anti-DR52 (7.3.19.1). All antibodies were used in a final concentration comparable with the ascites solution diluted 1:100 (in the binding assays) and 1:200 (in T cell

proliferation assays). As a control, nonimmune mouse ascites were tested.

T Cell Proliferation Assays. Proliferation was assayed by mixing 10^4 T cells, irradiated DR-matched allogeneic PBMC (5×10^4 /well), or EBV-BLCL (3×10^4 /well) and antigen in 96-well flat-bottomed microtiter plates in triplicate. After 66 h of culture, 1 μ Ci [3 H]thymidine was added to each well, and 18 h later cells were collected on glass fiber strips, and the radioactivity incorporated into the DNA was determined by liquid scintillation counting. Mean and SEMs were computed from the cpm of triplicate tests.

Competition Experiments. Inhibition of activation of the HLA-DR17-restricted, p3-13-reactive T cell clone CAAP15 1-1 was studied in T cell proliferation assays by mixing 10^4 T cells, irradiated PBMC (5×10^4 /well), derived from rhesus macaques, stimulator peptide (10 ng/ml) and competitor peptide (final concentration: 0.1-, 1.0-, 10-, 100- and 1,000-fold excess relative to the stimulator peptide p3-13). Toxicity of competitor peptides, for either T cells or APC, was checked by mixing either T cells (10^4 /well) and 10% IL-2 (Lymphocult-T; Biotest, Frankfurt, Germany) or PBMC (5×10^4 /well) and 0.5% PHA (Wellcome Diagnostics, Dartford, UK) with competitor peptide (final concentration, 10 μ g/ml). To exclude the possibility that the nonresponsiveness to the competitor peptides was due to toxicity of those peptides for either the T cells or the APC, we tested the influence of these peptides on both the IL-2-dependent activation of the HLA-DR17-restricted T cells and the PHA-induced proliferation of the APC. The presence of the competitor peptides did not result in reduction of proliferation of the T cells nor did it disturb the activation by PHA.

Nonhuman Primate Mhc Nomenclature and Cells. The chimpanzee and rhesus macaque *Mhc*'s have recently been designated *MhcPatr* and *MhcMamu*, respectively (23). Official locus and names of chimpanzee and rhesus macaque *Mhc* class II alleles were given according to the rules formulated by the nonhuman primate *Mhc* nomenclature committee and were based on similarities between *HLA-DRB* (human), *Patr-DRB* (chimpanzee) (20), and *Mamu-DRB* (rhesus macaque) (21) sequences. The Mamu-DR specificities depicted in Fig. 1 were determined by serologic and RFLP techniques. For example, the DR1 specificity was determined by serology, and the A, B, C, and D designations that may follow represent subtypes defined by RFLP (24). Alloantisera that recognize Patr-DR molecules are at present not available. The Patr-DR types of various chimpanzees that are shown in Fig. 1 have been determined by RFLP (25). There is no obvious relationship between serologic HLA-DR specificities and serologically or RFLP-determined Patr- or Mamu-DR types, meaning that the nonhuman primate DR specificities have been named arbitrarily.

The human homozygous typing EBV-BLCL used in this study are: OOS (HLA-DR1/Dw1), HAR (HLA-DR17, DR52a), and AVL (HLA-DR17, DR52a). The following chimpanzee cells have been used and corresponding relevant sequence information (if present) and typings are depicted: Yvonne (*Patr-DRB1*0204*, *-DRB1*0307/DR4,5*), Gwen (*-DRB1*0305/DR10*), Louise (*DRB*0201/DR4*), Debbie (*-DRB1*0701/DR9,10*), Annette (*-DRB1*0201*, *-DRB1*0309/DR2,8*), Pearl (*-DRB1*0203*, *-DRB1*0306/DR4B,7*), Wodka (*-DRB1*0201*, *-DRB1*0102/DR1,4*), Sherry (*-DRB1*1001/DR3,4*), Dennis (*-DRB1*0204/DR4B,4C*), Brigitte (*-DRBX*0102*, *-DRB1*0702*, *DRB4*0201/DR9,12*), Victoria (*-DRB1*0308/DR13*). EBV-BLCL of the next rhesus macaques, homozygous for Mamu-A, -B, and -DR, were derived from the Institute of Applied Radiobiology and Immunology-TNO colony: 1WM (*Mamu-DRB1*0303/DR1A*), JY (*-DRB1*0405/DR1B*), 1RK (not sequenced/DR2), 3C (*-DRB1*0309/DR3A*), 1KM (*-DRB1*0306/DR3C*), 2Y (not

sequenced/DR4), 1MC (*DRB1*0404/DR5C,5D*), 3081 (not sequenced/DR6), 2849 (*-DRBw8*0101/DR7A*), 2B (*-DRB1*0310/DR8*), 1ZA (*-DRB1*0305/DR9*).

Results

Binding of hsp65 p3-13 and Hemagglutinin (HA) p307-319 to EBV-BLCL Derived from Chimpanzees and Rhesus Macaques. Hsp65 p3-13 of *Mycobacterium leprae* and *M. tuberculosis* functions as an important T cell epitope only in HLA-DR17-positive individuals (11), since it selectively binds to (a pocket in) DR17 molecules (12). It is anticipated that the presence of certain polymorphic amino acid residues (motifs) in the highly polymorphic DR β 1 chain, situated in the MHC class II peptide binding site, is required for binding of p3-13 (13). To measure the binding of p3-13 to different Mamu-DR and Patr-DR molecules, 11 different DR homozygous

rhesus macaque and 11 disparate chimpanzee EBV-BLCL, selected for distinct DR specificities, were pulsed with biotinylated hsp65 p3-13. As a control for binding, the biotinylated analogue of the influenza HA peptide p307-319 (PKYVKQNTLKLAT) was used. This peptide is recognized by a HLA-DR1-restricted T cell clone (26) and is known to bind well to most or all HLA-DR molecules other than HLA-DR17 (22).

The results of the binding experiments with the nonhuman primate BLCL are shown in Fig. 1. For reference purposes the data for HLA-DR17-positive and -DR1-positive BLCL, good binders for p3-13 and HA p307-319, respectively (12), are also shown. For the chimpanzee-derived BLCL, binding of p3-13 is only found for cells with the Patr-DR13 specificity, whereas none of the other cell lines reach significant levels of binding (Fig. 1 A). P3-13 binds to 3 of the 11 rhesus macaque cells tested with the specificities Mamu-DR1, -DR3C,

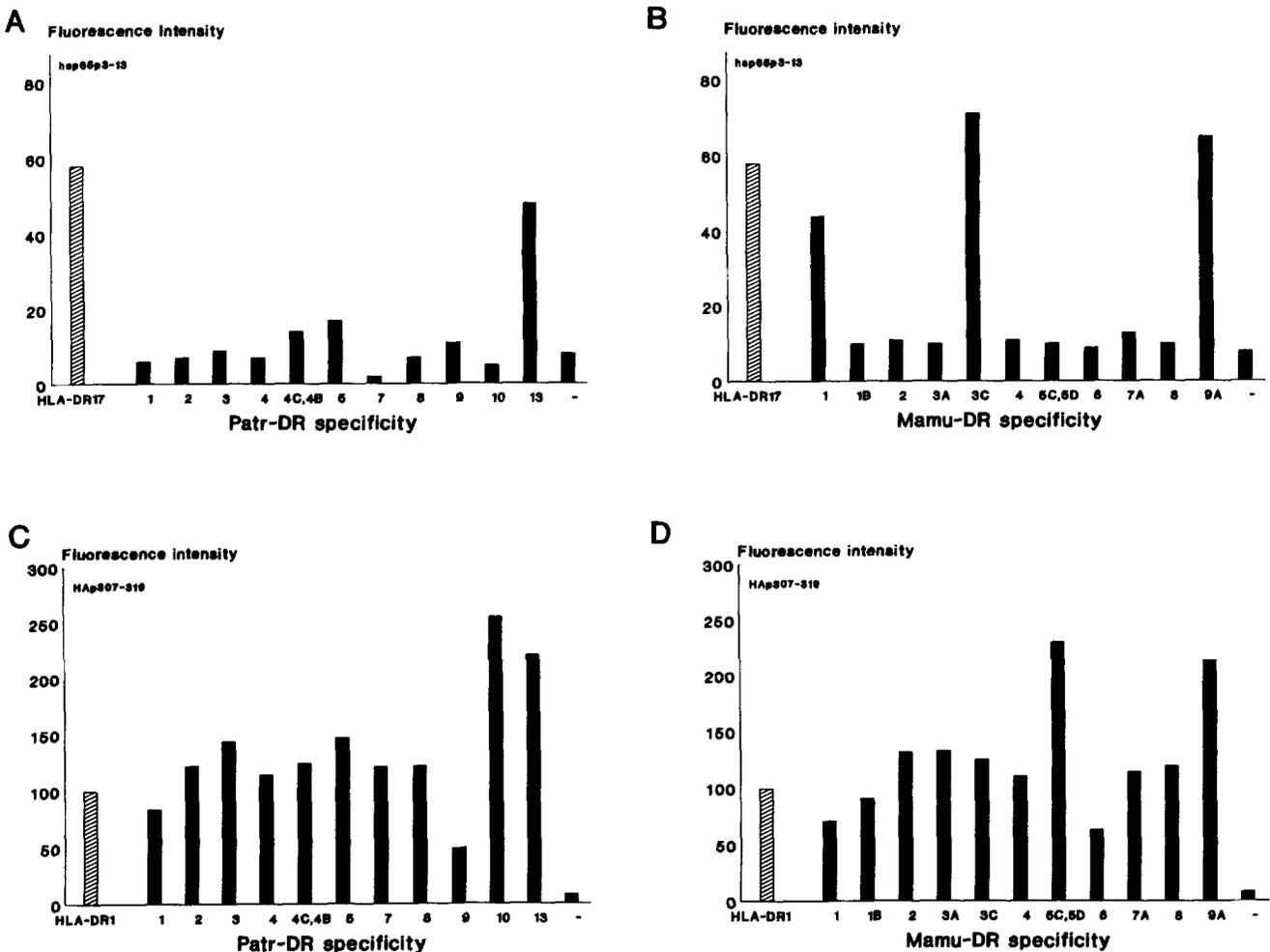


Figure 1. Binding of the biotinylated analogues of hsp65 p3-13 (A and B) and HA p307-319 (C and D) to the surface of living EBV-BLCL. Results of binding for BLCL from chimpanzees (A and C) and rhesus macaques (B and D) given in filled bars, for human BLCL, in striped bars. Values on the y-axis represent the mean fluorescence intensity, corrected for background fluorescence. Background fluorescence, determined in the absence of BLCL by incubation medium with FITC-avidin D, is indicated by a dash on the x-axis. The MHC specificities are given on the x-axis. Sequencing demonstrated that rhesus monkeys with the DR1, DR3C, and DR9A specificities are positive for the *Mamu-DRB1*0303*, *-DRB1*0306*, and *-DRB1*0308* alleles, respectively, whereas the Patr-DR13 specificity is associated with a *Patr-DRB1*0308* allele. More detailed information on the MHC typing of various chimpanzees and rhesus macaques is given in Materials and Methods. The results represent the mean of three independent experiments.

	10	20	30	40	50	60	70	80	p3-13 binding
HLA-									
DRB1*0101	-	WQLKF	ECHFFNGTERVR	LLERCI	YNQEESVRFDSVGEYRAVTELGPRDAEYWNSQKDLLEQRRRAAVDTYCRHNYGVGESF	-	-	-	-
DRB1*0301	-	EYSTS	-----	Y-D-YF	H---N-----F-----	-----	-----	K-GR--N-----V--	+
DRB1*0302	-	EYSTS	-----	F--YF	H---N-----F-----	-----	-----	K-GR--N-----V--	-
DRB1*0401	-	E-V-H	-----	F-D-YF	H---Y-----F-----	-----	-----	K-GR--N-----V--	-
DRB1*1101	-	EYSTS	-----	F-D-YF	H---Y-----F-----	-----	-----	K-GR--N-----V--	-
DRB1*1302	-	EYSTS	-----	F-D-YF	H---N-----F-----	-----	E-----F-D-----	I-DE-----	-
DRB1*0801	-	EYSTG	-Y-----	F-D-YF	H---Y-----F-----	-----	S-----F-D-----L---	F-D-----L---	-
DRB1*0901	-	K-D---	-----	Y-H-G-	H---N-----F-----	-----	V-S-----F-R-----E--V--	F-R-----E--V--	-
DRB3*0101	-	ELR-S	-----	Y-D-YF	H---FL-----V-S-----	-----	V-S-----K-GR--N-----	K-GR--N-----	-
Patr-									
DRB1*0302	-	EYSTS	-----	F-D-YF	H---Y-----V-S-----I--D--GQ--N-----	-----	-----	I--D--GQ--N-----	-
DRB1*0305	-	EYSTS	-----	F-D-YF	H---Y-----F-----D--GQ--N-----AV--	-----	-----	D--GQ--N-----AV--	-
DRB1*0306	-	EYSTS	-----	F-D-YF	H---Y-----F-----D--GQ--N-----R-V--	-----	-----	D--GQ--N-----R-V--	-
DRB1*0307	-	EYSTS	-----	F-D-YF	H---Y-----F-----YV-DE-----A--	-----	-----	YV-DE-----A--	-
DRB1*0308	-	EYSTS	-----	Y-D-YF	H---Y-----F-----V--Y--YV-DE-----	-----	-----	V--Y--YV-DE-----	+
DRB1*0309	-	EYSTS	-----	F-D-YF	H---Y-----F-----YV-DE-----A--	-----	-----	YV-DE-----A--	-
DRB3*0201	-	EYV-S	-----	F--YF	H---Y-----P--V--S-----K-GQ--N-----AV--	-----	-----	P--V--S-----K-GQ--N-----AV--	-
DRB5*0102	-	K-D-Y	-----	Y-H-D-	H---N-----V-----I--R-----A--	-----	-----	V-----I--R-----A--	-
DRB4*0201	-	E---	-----Q--	Y--YF	H---YA-YN-L-----V-----	-----	-----	YA-YN-L-----V-----	-
Mamu-									
DRB1*0303	-	EYSTS	-----	Y-D-YF	H---Y-----H-S-----I--DQ--S--F--R-A--	-----	-----	Y-----H-S-----I--DQ--S--F--R-A--	+
DRB1*0305	-	EYSTS	-----	Y-D-YF	H---N-----I--K--R--N--C--	-----	-----	N-----I--K--R--N--C--	+
DRB1*0306	-	EYSTS	-----	Y-D-YF	H---N-----I--K--R--N--F--	-----	-----	N-----I--K--R--N--F--	+
DRB1*0309	-	EY-TS	-----	F--YF	H---L-----E-S-----I--K--E--N--R--	-----	-----	L-----E-S-----I--K--E--N--R--	-
DRB1*0402	-	E-V-H	R-----	Y--HF	H---FLH--L--F-----L--G--A--	-----	-----	FLH--L--F-----L--G--A--	-
DRB1*0403	-	E-V-H	-----	F-D-YF	H---Y-----RS-----I--D--M--	-----	-----	Y-----RS-----I--D--M--	-
DRB3*0401	-	E-A--	-----	Y-Q-YF	H---F-----V-----F--K--N--Y--V--	-----	-----	F-----V-----F--K--N--Y--V--	-

Figure 2. Alignment of the deduced amino acid sequences of relevant *HLA-*, *Patr-*, and *Mamu-DRB* second exon sequences (20, 21, 51). Sequences were obtained from references 20 and 21. As a consensus sequence the *HLA-DRB1*0101* allele is used. Differences with this consensus are given by letter substitutions, while dashes refer to identical amino acid residues. The regions that are important for p3-13 binding have been boxed, whereas the motifs important for binding are printed in bold.

and -DR9A (Fig. 1 B). In contrast, HA p307-319 binds to all nonhuman EBV-BLCL tested, although the level of fluorescence varies (Fig. 1, C and D).

Comparison of Mhc-DRB1 Sequences of Human, Chimpanzee, and Rhesus Macaque. Since the polymorphic amino acid residues of MHC-DRB molecules involved in binding peptides are likely to be located in the hypervariable regions of the DR β chain (4), the amino acid sequences of the second exons of relevant *HLA-*, *Patr-*, and *Mamu-DRB* gene products were aligned (Fig. 2). One would expect that the various p3-13 binding MHC-DR β chains share specific amino acid residues important for binding. As can be seen, all DR β chains capable of binding p3-13 (*Patr-DRB1*0308*, *Mamu-DRB1*0303*, *-DRB1*0305*, and *-DRB1*0306*) share two motifs namely at positions 9-13 (EYSTS) and 26-31 (YLDRYF), which therefore seem to be important for binding (Fig. 2). These important motifs for p3-13 binding have been indicated in Fig. 3, depicting the hypothetical antigen-binding site of MHC class II molecules.

A single mutation, 26Y→F, as seen in *Patr-DRB1*0302*, *-0305*, *-0306*, *0307*, and *-0309* abrogates binding of p3-13 completely. These data extend the observation on the substitutions of 26Y→F and 28D→E, which are the only differences in the second exon of *HLA-DRB1*0301* (HLA-DR17) vs. *HLA-DRB1*0302* (HLA-DR18) in humans and abrogates binding of p3-13 as well (Fig. 2, and reference 12).

Various differences between the primate MHC-DRB molecules that are able to bind p3-13, located at positions 32 (H→Y), 37 (N→Y), 47 (F→Y), 57 (D→V,H), 60 (Y→S), 61 (W→Y), 67 (L→Y,I), 68 (L→V), 70 (Q→D), 71

(K→E,Q), 73 (G→A), 74 (K→A,S), 77 (N→T), 78 (Y→F), 84 (G→R), and 86 (V→G,A,C,F), do not significantly influence peptide binding. Most of these residues do not map within the two motifs important for binding of this particular peptide (4), as can be seen in Fig. 3.

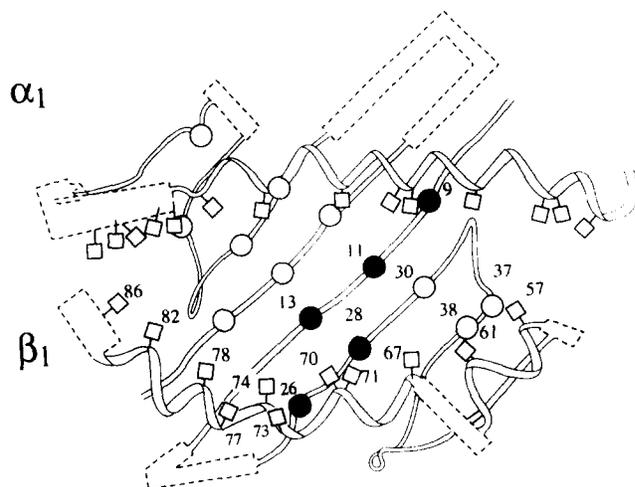


Figure 3. Schematic representation of the hypothetical model of the antigen-binding site of MHC class II molecules, showing the important conserved (black) and variant (white) residues involved in p3-13 binding. Circles represent amino acid residues that lie in the β -pleated sheet; squares represent amino acid residues that are part of the α -helical structure of the molecule.

Specific Activation of HLA-DR17-restricted T Cells by p3-13 Presented by MHC-DR17-like Nonhuman Primate-derived BLCL. The results above indicate that the two motifs for p3-13 binding have been conserved in evolution within at least part of the *Mhc-DRB1*03* lineage members. *Mhc*-peptide complexes are recognized by T cells only in the context of self-MHC, a phenomenon known as MHC restriction (27). In this light it was investigated whether, apart from the critical peptide-binding residues, polymorphic residues for T cell recognition had also been conserved in evolution. In that case, one would expect that T cells that are selected to see p3-13 in the context of HLA-DR17 may recognize this peptide presented by some nonhuman equivalents that bind the peptide. To test this hypothesis the capacity of the BLCLs of 1WM (*Mamu-DRB1*0303*), 1KM (*Mamu-DRB1*0306*), 1ZA (*Mamu-DRB1*0305*), and Victoria (*Patr-DRB1*0308*), which all bind p3-13, to present the peptide to a HLA-DR17-restricted, p3-13-reactive T cell clone, CAAp15 1-1, was analyzed in a T cell proliferation assay (Fig. 4). As a positive control human PBMC (DR2,3) were used as APC. All APC were tested for presentation of hsp65 p418-427 to the hsp65-reactive, DR2-restricted T cell clone R2F10 (28). As a negative control rhesus macaque BLCL that did not bind p3-13 (Fig. 1), such as 1MC (*Mamu-DRB1*0404*) and 2849 (*Mamu-DRBw8*0101*), were used as APC as well. All BLCL derived from rhesus macaques that are able to bind p3-13 are also able to present the peptide to the p3-13-reactive human T cell clone (Fig. 4). Thus, the peptide not just binds to these cells but can also be presented by these nonhuman primate HLA-DR17-like alleles and give rise to T cell recognition and proliferation. BLCL of Victoria (*Patr-DRB1*0308*), however, are not able to present p3-13 to human T cells (Fig. 4). This indicates that although the p3-13 binding residues are conserved in this chimpanzee-derived allele, the TCR contact residues or, alternatively, the conformation of the mole-

cules have not remained the same. As expected, the nonbinding BLCL did not activate the T cells. None of the APC tested, except for the heterozygous human PBMC (DR2,3), were able to present the DR2-specific peptide, hsp65 p418-427, to the HLA-DR2-restricted human T cell clone R2F10.

Inhibition of p3-13-mediated T Cell Activation of a HLA-DR17-restricted T Cell Line by mAbs. To confirm the specificity of the binding of p3-13 to the rhesus macaque DR molecules, we coincubated p3-13 with either α -DR, α -DP, α -DQ, α -DR52, α -class I, or α -class II mAbs in T cell proliferation assays using either HLA-DR17-positive or Mamu-DR-positive EBV-BLCL as APC (Fig. 5). Here, it is evident that binding of p3-13 is inhibited only by α -DR, α -DR52, and α -class II backbone mAbs. Thus, as expected, the presentation of p3-13 is HLA-DRB1*03 restricted. The fact that the binding is also blocked by α -DR52 seems to be unexpected since 7.3.19.1 reacts with residue 73 (Gly) of DRB1, which is present on some *Mhc-DRB1*03* and *-DRB3* molecules (29). Examination of the *Mamu-DRB1*0305* allele, however, shows the absence of the epitope for 7.3.19.1 binding (Fig. 2). Thus, in this particular case, T cell proliferation is probably inhibited by steric hindrance because mAb 7.3.19.1 binds to the MHC class II molecules of the human T cell, which does express the epitope in question.

Competition Experiments. Peptides with single amino acid substitutions, which are able to bind to MHC molecules, may not activate T cells (30–32). Such competitor peptides of hsp65, p4-13, have been demonstrated to inhibit the HLA-DR17-restricted T cell response of p3-13-reactive T cell clones (33). These peptides were now tested for their ability to inhibit the response of T cell clone CAAp15 1-1 to p3-13 in the context of rhesus macaque-derived PBMC. The results of the competition experiment are shown in Fig. 6. P4-13 substituted at position 6 (A→Q) was able to inhibit the response induced by p3-13. A control peptide, p4-13 (8D→P),

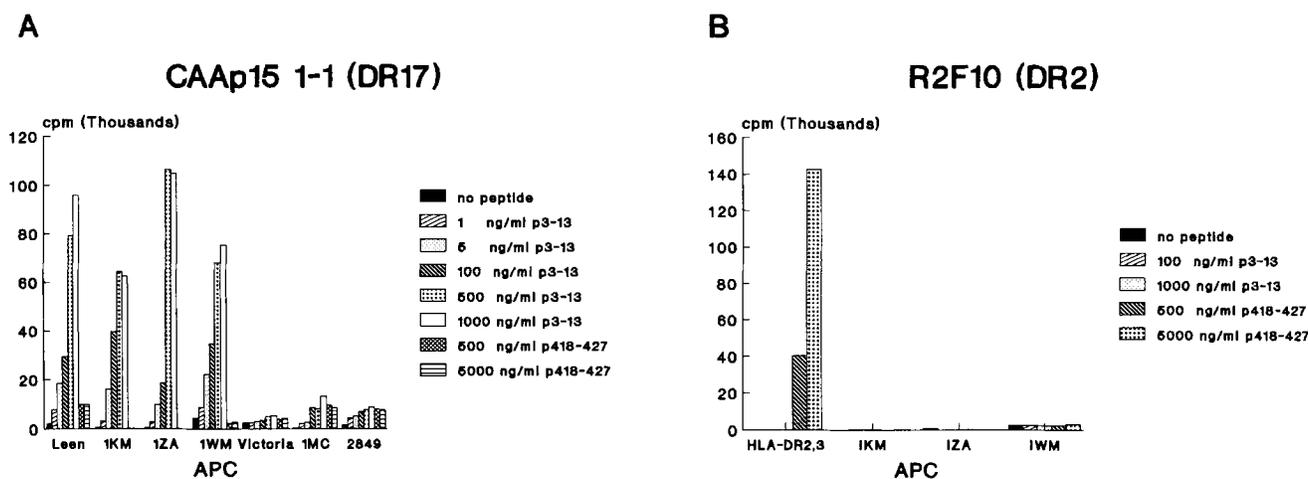


Figure 4. Effect on T cell proliferation of the hsp65 p3-13-reactive, DR17-restricted clone, CAAp15 1-1 (A), and the hsp65 p418-427 (LQAAPALDKL)-reactive, DR2-restricted clone, R2F10 (28) (B). Peptides are presented by four different APC. Proliferation assays were performed as described in Materials and Methods. The APC used, given on the x-axis, are human heterozygous PBMC named Leen (HLA-DR2,3) and rhesus macaque-derived EBV-BLCL 1KM (Mamu-DR3C), 1ZA (-DR9), 1WM (-DR1), 1MC (-DR5) and 2849 (-DR7).

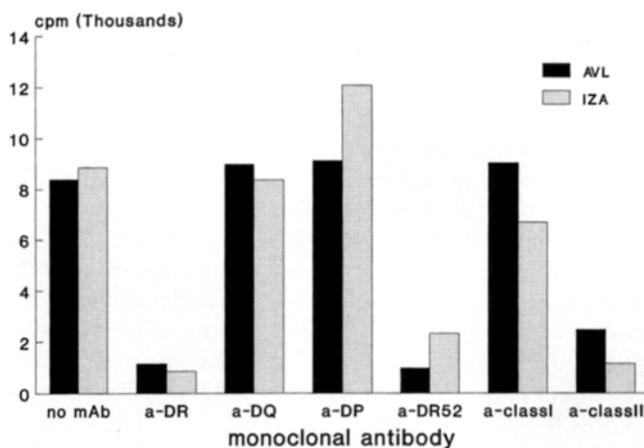


Figure 5. Inhibition of hsp65 p3-13-stimulated activation of the PPD-reactive T cell line v.Es-Menken (HLA-DR2,3) by α -DR, α -DQ, α -DP, α -DR52, α -class I, and α -class II. The mAbs are indicated on the x-axis. EBV-BLCL used as APC are AVL (HLA-DR17) and 1ZA (Mamu-DR9). Proliferation is expressed on the y-axis (cpm).

which does not bind to HLA-DR17, was not able to inhibit the T cell response at all.

Discussion

Humans, chimpanzees, and rhesus macaques are primate species that share a progenitor that lived ~ 30 million yr ago (34). The divergence between humans and chimpanzees took place ~ 5 –7 million yr ago (35). Consequently, these closely related species may share highly similar immune systems. Sequencing studies have demonstrated that many MHC polymorphisms predate speciation, supporting the trans-species hypothesis (18), and indicating that some of the polymor-

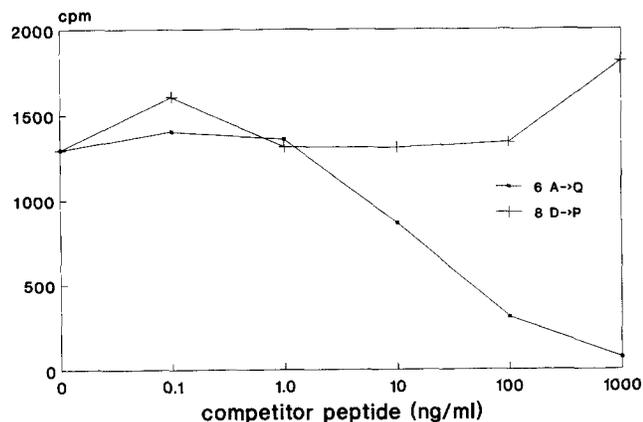


Figure 6. Inhibition of hsp65 p3-13 (10 ng/ml)-stimulated activation of DR17-restricted T cell clone CAAp15 1-1 by a single amino acid substituted analogue of p4-13. Inhibition peptide concentration is indicated on the x-axis. Proliferation is expressed on the y-axis (cpm). Stimulation index for competitor peptides without p4-13 were ≤ 1 . Experience with nonhuman primate cells taught that proliferative responses using PBMC as APC are generally lower than when BLCL are used.

phisms are relatively old and represent stable structures. The finding that MHC class II polymorphism is maintained by strong selective forces (36–39) implies that polymorphism must have some important function. The biologic significance of polymorphism observed within *Mhc* class II lineages was investigated by *Mhc*-peptide binding and T cell proliferation assays.

Here it is demonstrated that humans, chimpanzees, and rhesus macaques have particular *Mhc-DRB1*03* lineage members in common. Only *Mhc-DRB1*03* molecules that contain residues 9–13 (EYSTS) and 26–31 (YLDRYF) are able to bind p3-13. This indicates that at least two motifs are necessary for effective p3-13 binding (Fig. 2). As can be seen, the absence of one of these motifs at 9–13 (EYSTS), as found in *HLA-DRB3*0101*, diminished p3-13 binding significantly. Apart from other residues, a critical residue for p3-13 binding is 26 (Y), as the substitutions at this position (Y→F) abrogates p3-13 binding (Fig. 2). The presence of residue 26 (Y), however, is, on its own, not enough for p3-13 binding as is demonstrated by the lack of p3-13 binding for *HLA-DRB1*0901*, *Patr-DRB1*0102*, *Mamu-DRB1*0402*, and *-DRB3*0401*.

The positively charged residues 71 (K) and 74 (R) are not present in other HLA alleles besides *HLA-DRB1*03*, and are supposed to be located in the peptide-binding site (4). Therefore, it was proposed that these residues could contribute to p3-13 binding by interacting with the negative charge on the peptide (12). However, by using nonhuman primate BLCL, we now demonstrate that p3-13 binding can occur also to *Mhc* alleles *Patr-DRB1*0308* and *Mamu-DRB1*0303*, which lack positively charged residues such as 71 (K) and 74 (R).

MHC molecules bearing the combination of 9–13 (EYSTS) and 26–31 (YLDRYF) motifs described above are present in human, chimpanzee, and rhesus macaque populations. This indicates that they may have been selected as polymorphic peptide contact residues (motifs) in the population in order to preserve binding of certain epitopes from important pathogens. Here one such combination is described. However, we envisage that other preferential MHC-peptide combinations may have been conserved within distinct primate species as well.

The reason for this interspecies conservation of class II motifs may have resulted from the fact that these related species can share the same pathogenic threats like mycobacterial infections (40). Thus, some *Mhc-DRB* lineages, present in different primates, might have been maintained due to a selective advantage with regard to diseases, as described for HLA-B53 and severe malaria in West Africa (41). This may also be the case for mycobacterial diseases, notably tuberculosis cases that have been described in nonhuman primates (42). In addition, it has been reported that both chimpanzees (43) and rhesus macaques (44) can develop leprosy in experimental models, but even cases of naturally acquired leprosy in chimpanzees have been documented (45). These examples emphasize that distinct but closely related species may suffer from shared pathogenic threats and diseases. Mycobacteria are important pathogens, and immunity against these pathogens is strictly T cell dependent both in healthy and in mycobacterial dis-

ease-affected individuals (6–8). P3-13 is the only peptide of hsp65 that is seen as a T cell epitope in HLA-DR17-positive individuals (11). The conservation of a peptide-binding pocket for this immunodominant hsp65 peptide to primates of the *Mhc-DRB1*03* lineage may reflect the importance of the T cell recognition of the peptide in the context of the same MHC molecules and the consequent survival of these species.

Nonhuman primate MHC molecules from rhesus macaques were not only able to bind p3-13 but also to present this peptide to HLA-DR17-restricted, p3-13-reactive human T cell clones (Fig. 4). This indicates that p3-13 probably binds in the same conformation to nonhuman primate-derived BLCL and that besides the *Mhc*-peptide binding residues, the *Mhc*-TCR contact residues have also been conserved. However, the chimpanzee allele that showed positive binding, *Patr-DRB1*0308*, could not present p3-13 to human T cells. This indicates that either p3-13 binding residues have been conserved in this allele and that the TCR contact residues have not, or that this peptide binds in a different conformation to the MHC molecule, which is not recognized by the TCR.

The β -pleated sheet and α helix sections of the second domain of MHC class II molecules appear to have different evolutionary histories (17). Especially, the β -pleated sheet-associated polymorphisms have been conserved between species whereas the α helix accumulated considerably more variation (46). The present data suggest that the β -pleated sheet section-encoded polymorphisms have been maintained in evolution because they encode motifs that are essential for the binding of some peptides needed to initiate an immune response. The α helix section of MHC class II molecules is probably recognized by the TCR. Since one individual encodes a high amount of different T cell receptors, the chance that nonresponsiveness is induced by amino acid replacements in the α helix sections is relatively low. For that reason the α helix is probably allowed to accumulate more variation than the β -pleated sheet.

The conservation of MHC class II molecules for ~ 30 mil-

lion yr stands in contrast to the relatively fast evolution of some MHC class I molecules, as HLA-B antigens (47, 48). This may be due to the difference in origin of the antigens presented by either MHC class I or II molecules being endogenous and exogenous peptides, respectively. Since endogenous viral peptides change rapidly, the MHC class I molecules that present those peptides may have to adapt fast to avoid that, or such pathogens may escape CTL recognition. However, peptides derived from exogenous antigens are selected by MHC class II molecules after binding one of the polymorphic immunoglobulins on B cells and subsequent processing. Therefore, peptides with a more conserved nature can be selected to initiate antibody response, as is exemplified by the conservation of a peptide binding site for >30 million yr for the *Mycobacterium*-derived hsp65 p3-13.

Furthermore, the p3-13 reactive T cell response of the HLA-DR17-restricted T cell clone CAAp15 1-1, which possesses the TCR V β 1 segment, could be inhibited with specific, single amino acid substitution analogues of hsp65 p4-13 (33) using rhesus monkey-derived PBMC that were able to bind to p3-13 as APC. In this context it is relevant to note that not only the MHC may be highly similar in primate species but that equal observations may be done for other systems that are important for immune recognition. Recently, it was documented that rhesus macaques possess TCR V β 1 segments that show 95.6 and 96% nucleotide similarity with their human equivalent (49). The observations described above demonstrate that human and nonhuman primate species share highly similar immune systems. As the HLA-DR3 allele is associated with several autoimmune and allergic diseases, such as SLE, celiac disease, insulin-dependent diabetes mellitus, and Graves' disease (50), the finding of comparable peptide-presenting capacity and specificity for certain nonhuman primate-derived APC could, therefore, open possibilities to study means of preventing HLA-DR3-associated diseases at a preclinical stage in experimental models of autoimmune disease in nonhuman primates.

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Address correspondence to A. Geluk, Department of Immunohematology and Bloodbank, University Hospital, P.O. Box 9600, 2300 RC Leiden, The Netherlands.

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