Major Histocompatibility Complex Class II–restricted Antigen Presentation across a Species Barrier: Conservation of Restriction Determinants in Evolution

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Summary

The existence of at least three alleles of the HLA-*DRB3* gene within the human population is evident. These alleles express DRw52 determinants and react with monoclonal antibody (mAb) 7.3.19.1. The polymorphic epitope recognized by 7.3.19.1 is not only present on human cells but is also expressed on chimpanzee (*Pan troglodytes*) class II–positive cells. The 7.3.19.1 determinant already existed before speciation of man and chimpanzee, and is at least 5,000,000 yr old. Two-dimensional gel electrophoresis demonstrated that the various HLA- and Patr-DRw52 molecules that are reactive with 7.3.19.1 exhibit isoelectric point differences due to primary amino acid heterogeneity, as was confirmed by sequencing data. Sequence comparison allowed us to map the binding site of mAb 7.3.19.1 to the α helix of the major histocompatibility complex (MHC) class II DRB1 domain surrounding the antigen-binding cleft. Despite MHC sequence variation, chimpanzee antigen-presenting cells can present antigen (purified protein derivative) to human T cell lines and vice versa. Only the HLA- and Patr-DRw52 molecules were shown to function as restriction elements for antigen presentation across this species barrier. It is concluded that these particular restriction determinants probably have been conserved in evolution. The HLA- and Patr-DRw52 molecules represent alleles displaying polymorphism that has been selected for in evolution. Such "biomutants" may thus be more useful to study the biological significance of MHC molecules than MHC variants that have been generated by in vitro mutagenesis experiments.

One of the requirements for activation of Th lymphocytes is the complex formation of TCRs with MHC class II molecules that are loaded with selected antigen fragments (1). Such Th cells recognize denatured or processed antigen only in the context of self MHC class II structures, a phenomenon known as MHC restriction (2, 3). The structure of the antigen-binding site of MHC class II molecules has been predicted according to a model (4) based upon the three-dimensional crystal structure of HLA-A2 class I molecules (5). Binding studies provided evidence that MHC class II molecules have a single antigen-binding site (6) also named the cleft or groove.

In man, three major types of MHC class II molecules have been characterized, namely HLA-DP, -DQ, and -DR (7). Each of them has the capacity of acting as restriction element for antigen recognition by Th cells (8, 9). Class II molecules are transmembrane glycoproteins composed of a H (α) and a L (β) chain, both encoded by the MHC complex (10). The HLA-DP, -DQ, and -DR subregions contain multiple genes (11). The highest degree of polymorphism is observed for the HLA-*DRB1*, *-DQA1*, *-DQB1*, and *-DPB1* genes, whereas the HLA-*DPA1*, *-DRB3*, and *-DRB4* genes are clearly less polymorphic (12–16). The HLA-*DRA1* gene appears to be invariant (17). The majority of the polymorphic MHC class II residues are clustered around the antigen-binding site, indicating that these residues may influence the binding of selected antigen segments and interaction with the TCR complex.

The polymorphism of MHC class II molecules has been studied with a wide variety of techniques and reagents. Especially, mAbs have been shown to be powerful reagents to describe the heterogeneity of MHC class II molecules. For example, mAb 7.3.19.1 has been reported to be reactive with an HLA-DRw52 determinant present on HLA-DR3-, -DR5-, and -DRw6-positive cells (18). Immunoprecipitation studies showed that the 7.3.19.1 epitope is located on the gene products encoded by the HLA-*DRB1* and *-DRB3* genes of HLA-DR3-positive cells, whereas it is only present on HLA-DRB3 molecules expressed by HLA-DR5 and -DRw6 haplotypes (19–22). Three alleles have been defined at the HLA-*DRB3* locus (23). The various types of 7.3.19.1-reactive HLA-DR

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molecules are functional as restriction determinants (RDs)[1] in antigen presentation (22, 24) and may also act as stimulus for alloreactive T cells (25).

We report here that the epitope recognized by mAb 7.3.19.1 is not only present on human cells but is also expressed on chimpanzee (*P. troglodytes*) peripheral blood cells. Thus, this epitope predates speciation of man and chimpanzee and is therefore at least 5,000,000 yr old (26). The MHC of the chimpanzee has been named Mhc-Patr, according to a recent proposal (27). The HLA- and Patr-DRw52 molecules analyzed were found to exhibit isoelectric point (pI) differences suggesting primary amino acid variation. Alignment of human and chimpanzee DR β chain sequences confirmed this sequence heterogeneity but also made it possible to pinpoint the 7.3.19.1-binding site. The antigen presentation capacity of the different HLA- and Patr-DRw52 molecules was tested in MHC restriction assays. Some of the Patr- and HLA-DR molecules reactive with 7.3.19.1 were shown to function as restriction elements in antigen presentation to both human and chimpanzee Th cells, indicating that the particular RDs involved have been conserved in evolution.

Materials and Methods

Cells. PBMC were isolated from heparinized venous blood samples obtained from humans and chimpanzees by lymphocyte separation medium (Organon Teknika Corp., Durham, UK) density centrifugation (specific density, 1.077 g/ml). Cells were washed three times with HBSS (Gibco Laboratories, Grand Island, NY) and resuspended in RPMI 1640 (Gibco Laboratories) supplemented with streptomycin (100 μg/ml) and penicillin (100 U/ml) supplemented with 10% FCS, obtained from Flow Laboratories, Inc., Paisley, Scotland.

Production of Radiolabeled Cell Extracts. Human B lymphoblastoid cells or PHA-stimulated PBMC (10^7) obtained from chimpanzees were labeled for 16 h at 37°C with 0.5 mCi ^{35}S-methionine (1,000 Ci/mmol; New England Nuclear, Boston, MA). Radiolabeled cells were washed twice with 10 ml RPMI 1640 (Gibco Laboratories) supplemented with 0.1% BSA and subsequently lysed in a buffer containing 10 mM Tris-HCl, 5 mM $MgCl_2$, 20 mM NaCl, 0.1 mM PMSF, and 1% NP-40 (BDH Chemicals, Poole, UK), pH 7.6. Cell extracts were spun at 12,000 *g* for 15 min. Supernatants were used for immunoprecipitation purposes.

mAbs. 7.3.19.1 (IgG2b) recognizes a DRw52 determinant on HLA-DR3-, -DR5-, and -DRw6-positive donor cells (18). The determinant detected by 7.3.19.1 is located on HLA-*DRB3*-encoded gene products of HLA-DR3-, -DR5-, and -DRw6-positive cells, whereas also HLA-*DRB1* gene products of HLA-DR3-positive cells express this epitope (18–22, 25).

mAb B8.11.2 (IgG2b) reacts with a DR framework structure present on all types of HLA-DR molecules (21, 22), whereas mAb SPV-L3 (IgG2a) detects a framework structure on HLA-DQ molecules (28, 29). PL15 (anti-DP monomorphic) was a kind gift of Dr. R. Knowles, Sloan-Kettering Memorial Hospital, New York (30).

Immunoprecipitation Assays and Two-dimensional (2D) Gel Electrophoresis. The methods used for immunoprecipitation and neuraminidase treatment of the samples have been described in detail (31). 2D gel electrophoresis was performed as described by Goyert et al. (32).

Antigen Presentation. The methods used to generate antigen-specific bulk T cell lines and to present purified protein derivative (PPD) to such T cells by autologous and allogeneic PBMC or by EBV-transformed B cells have been reported (33, 34). Briefly, antigen-specific T cells (10^4) were cultured together with irradiated PBMC (5×10^4) or with lymphoblastoid B cells (5×10^4) and PPD, (10 μg/ml) in 96-well flat-bottomed microtiter plates in triplicate. Inhibition of proliferative responses was achieved by the addition of mAb to the test system. As a control, nonimmune mouse ascites was tested. All antibodies were used in a final concentration comparable with the ascites solution diluted 1:100 or 1:200, which represents an excess of antibody. Proliferation of lymphoblastoid T cells was measured by [^{3}H]thymidine (6.7 Ci/mmol; Radiochemical Centre, Amersham International, Amersham, UK) incorporation. Means and SEMs of the means were computed from the cpm of triplicate tests.

Results

2D-Gel Electrophoresis of MHC Class II Molecules. Immunoprecipitation studies performed on human donor cells have demonstrated the reactivity of mAb 7.3.19.1 with HLA-DR3, -DR5, and -DRw6 haplotype-encoded HLA-*DRB3* gene products and with HLA-*DRB1* gene products of HLA-DR3-positive cells (18–22, 35). At least three alleles of the HLA-*DRB3* gene are found within the human population, named HLA-*DRB3*0101* (DRw52a), -*DRB3*0201* (DRw52b), and -*DRB3*0301* (DRw52c), respectively (23, 36).

A panel of chimpanzee cells, RFLP typed for their Patr-DR specificities (Bontrop et al., manuscript in preparation), was screened for the presence of the 7.3.19.1-defined DRw52 epitope by conducting immunoprecipitation studies followed by 2D gel electrophoresis. All 65 chimpanzees tested were found to be reactive with 7.3.19.1. Based upon electrophoretic mobility, at least three different types of Patr-DRw52 molecules could be detected. As can be seen (Fig. 1), mAb 7.3.19.1 isolates one type of Patr-DRw52 ($\alpha\beta$c) molecules in the case of chimpanzee Ton, whereas two types of Patr-DRw52 ($\alpha\beta$b + $\alpha\beta$c) molecules are observed for the chimpanzee Loek. Within the chimpanzee colony, at least one other Patr-DRw52 molecule with a distinct isoelectric point (pI) is present, designated Patr-DRw52 ($\alpha\beta$a) (data not shown). For control purposes, 7.3.19.1 was also used to isolate HLA-DRw52 molecules from the human cell line WVE, which is heterozygous for its MHC region and types as HLA-DRw13-Dw19-DQw6, -DRw8-DQw3 positive. Immunoprecipitation with 7.3.19.1 allows the isolation of the HLA-*DRB3*0301* (DRw52c) gene product, which is found in strong linkage disequilibrium with the HLA-DRw13-Dw19 specificity within the caucasoid population. HLA-DRw8-positive cells are known to lack the HLA-*DRB3* gene and express no gene product reactive with 7.3.19.1 (18, 21). 2D gel analysis showed that the pI of the HLA-*DRB3*0301* (DRw52c) gene product is lying intermediate between the two types of Patr-DRw52 β chains (Fig. 1). None of the presently described Patr-DRw52 β chains were found to share pIs with the HLA-*DRB3*0101* (DRw52a), -*DRB3*0201* (DRw52b), and -*DRB3*0301* (DRw52c) gene products. Immunoprecipitation with mAb B8.11.2 proved

[1] *Abbreviations used in this paper*: pI, isoelectric point; PPD, purified protein derivative; RD, restriction determinant; 2D, two dimensional.

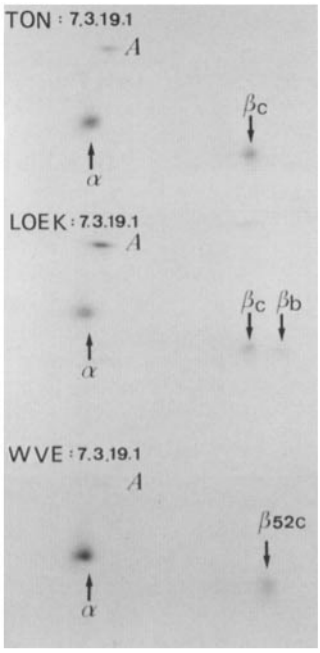

Figure 1. 2D gel electrophoretic analysis of neuraminidase-treated Patr-DRw52 and HLA-DRw52 molecules isolated with mAb 7.3.19.1 from chimpanzee and human cells. Patr-DRw52 molecules were isolated from the chimpanzees Ton and Loek, whereas HLA-DRw52c (HLA-*DRB3*0301*) molecules were isolated from the human B cell line WVE. All samples were run under identical conditions. In addition to the class II α and β chains, the position of actin (A) has been marked.

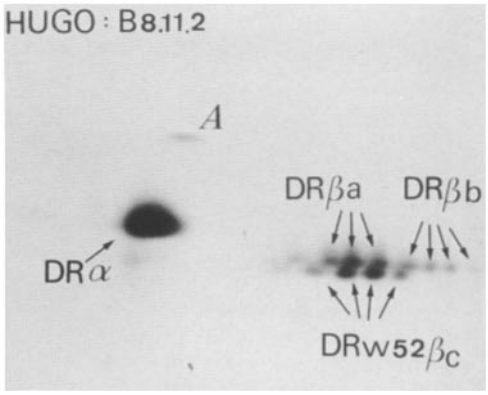

Figure 2. 2D gel analysis of the (non-neuraminidase-treated) Patr class II molecules isolated from the chimpanzee cell line Hugo with the mAb B8.11.2. mAb 7.3.19.1 isolates only the Patr-DRw52 αβc molecules. The position of actin (A) has been marked.

that the Patr-DRw52 molecules reactive with 7.3.19.1 are also bearing a B8.11.2-defined DR framework determinant (Fig. 2). In man too, it is known that the different HLA-DRw52 molecules express both the 7.3.19.1 and B8.11.2 determinant (18–22).

The distribution of the different types of Patr-DRw52 class II molecules among a selected panel of chimpanzees used for the present study has been summarized in the left part of Table 1.

Antigen Presentation Assays. It was investigated whether chimpanzee APC are able to present PPD to human antigen-specific Th cells. Xeno-antigen presentation was not observed using 10 chimpanzee APC representing five RFLP-determined DR specificities in combination with HLA-DR1-, -DR2-, -DR4-, and -DR7-positive Th cells (data not shown). In contrast, successful antigen presentation by chimpanzee APC was seen in combination with Th cells originating from the HLA homozygous typing cell donors CAA (HLA-DR3-Dw24-DQw2), HHK (HLA-DRw13-Dw18-DQw6), and the heterozygous individual WVE (HLA-DRw13-Dw19-DQw6, -DRw8-DQw3) (Table 1). Although chimpanzee APC could present antigen to these human PPD-specific Th cells, differential reactivity patterns were observed. As can be seen in Table 1 and Fig. 3, only four (Carolina, Loek, Ton and Hugo) of

Table 1. *MHC Class II-restricted Antigen Presentation across a Species Barrier*

			Human Th Cell Proliferation			
	Patr-		HHK	CAA	QBL	WVE
Chimpanzee APC	DR RFLP	DRw52 (2D)	DRw52a B3*0101	DRw52a B3*0101	DRw52b B3*0201	DRw52c B3*0301
Carolina	2,2	52c	ND	–	–	+ +
Loek	2,4	52b,c	–	–	–	+ +
Ton	2,2	52c	–	–	–	+ +
Hugo	2,2	52c	–	–	–	+ +
Socrates	4,10	52b	–	–	–	–
Wodka	1,4	52b	–	–	–	–
Plato	4,10	52b	–	–	–	–
Nicoline	4,10	52b	–	–	–	–
Samson	4,5	52a,b	ND	+	–	–
Ulla	5,10	52a,c	+	+	–	+

–, <5,000 cpm; +, a range of 5,000–20,000 cpm; + +, >20,000 cpm.

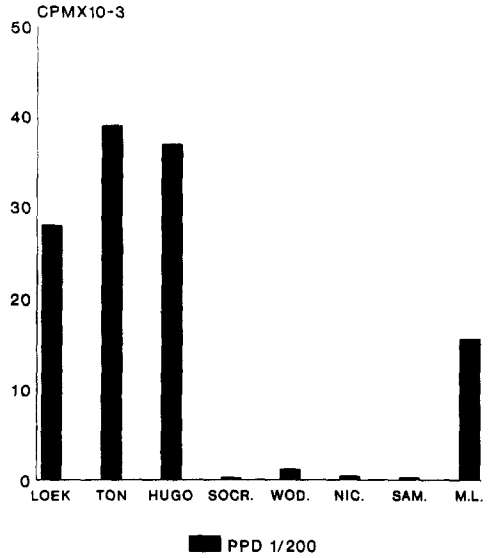

Figure 3. Antigen presentation by chimpanzee APC to human antigen-specific (PPD) Th cell line WVE. Loek, Ton, Hugo, Socrates, Wodka, Nicoline, and Samson are chimpanzees, whereas M. L. is a human control cell (HLA-DRw13-Dw19-DQw6 positive). PHA induced a proliferation of 93,000 ± 5,000 cpm, whereas proliferation without antigen to medium was 1,000 ± 100 cpm.

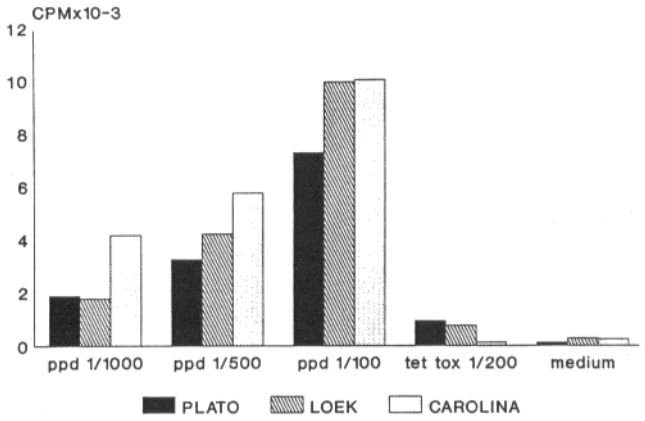

Figure 4. Antigen presentation by human WVE APC to PPD antigen-specific T cell lines obtained from the chimpanzees Plato, Loek, and Carolina.

the 10 tested chimpanzee APCs could present antigen to the human T cell line WVE (HLA-DRw13-Dw19-DQw6, -DRw8-DQw3). A second cluster of reactivity was seen for the Patr-DR5-positive cells that were found to present PPD fragments to HHK and CAA antigen-specific Th cells (Table 1). Both the HHK and CAA donor cells express the HLA-*DRB3*0101* (DRw52a) gene product (Table 1). Patr-DRw52a-induced proliferation was not seen for QBL and WVE Th cells (Table 1).

It was also tested whether human APC could present antigen to chimpanzee T cells. To this purpose, PPD-specific Th cells were generated from the chimpanzees Plato, Carolina, and Loek. APC from the individual WVE were able to present antigen to these chimpanzee Th cell lines. Tetanus toxoid was used as a control antigen and did not induce Th cell proliferation, whereas species-mismatched presentation of PPD was observed for all three chimpanzee T cells lines tested (Fig. 4). The fact that WVE APC can present antigen to Plato PPD antigen-specific T cells, but that the reciprocal situation (Table 1) is not observed, may be due to differences in the T cell repertoire.

Inhibition of antigen-specific T cell proliferation was assayed with mAbs to determine the type of MHC class II molecules involved in antigen presentation across a species barrier. Well-characterized mAbs, the reactivity patterns of which had been defined in immunoprecipitation assays, such as 7.3.19.1 (DRw52), B8.11.2 (DR-backbone), SPV-L3 (DQ-backbone), and PL15 (DP-backbone), were selected for inhibition purposes. These antibodies had been shown to precipitate the corresponding Patr–class II region products in chimpanzees too (Bontrop et al., manuscript in preparation). Proliferation by antigen-specific Th cells of WVE to PPD by APC from chimpanzee Loek was inhibited to the same extent with the antibodies 7.3.19.1 (DRw52) and B8.11.2 (DR), whereas SPV-L3 (DQ) and PL15 (DP) did not inhibit T cell proliferation (Fig. 5). Similar inhibition patterns were observed for the other combinations listed in Table 1, which showed positive Th cell proliferation, emphasizing that the Patr-DRw52 molecules are used as restriction elements.

Discussion

One of the milestones in recent immunology has been the demonstration that T cells recognize degraded antigen segments in the context of self MHC, a phenomenon known as MHC restriction. Consequently, antigen presentation to antigen-specific Th cells is normally observed with autologous or with allogeneic APC sharing identical MHC molecules. The present panel data show that also chimpanzee APC can present antigen to human antigen-specific Th cells and vice versa. Similar observations were made by Lechler et al. (37), who described the species-mismatched presentation of antigen by mouse I-E molecules to an HLA-DR1-positive

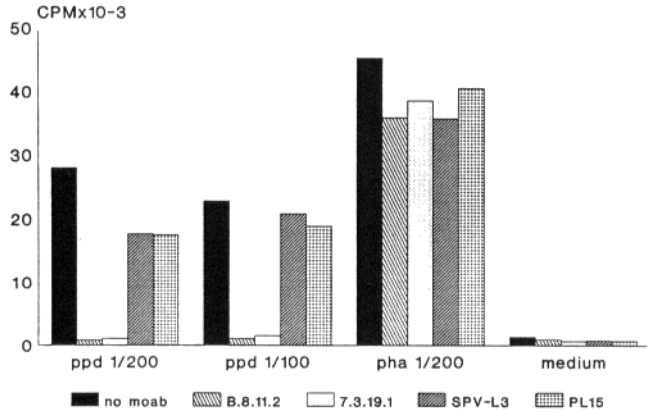

Figure 5. Inhibition of antigen-specific proliferation with MHC class II–specific mAbs of APC derived from chimpanzee and human Th cells.

influenza-specific Th clone, whereas Waters et al. (38) reported antigen presentation by murine and human cells to a murine T cell hybridoma. These data could be explained as degeneracy of MHC restriction (37). On the other hand, species-mismatched restriction determinants that are functional in xeno-antigen presentation assays more likely reflect the existence of evolutionary preserved structures. Alleles containing such preserved segments are probably encoded by ancestral haplotypes or segments of such haplotypes (39).

Immunoprecipitation studies followed by gel electrophoresis analyses showed that man and chimpanzee express identical epitopes on MHC class II molecules reactive with both the mAbs 7.3.19.1 and B8.11.2 (Figs. 1 and 2). The various HLA- and Patr-DRw52-detected molecules display pI differences, even after neuraminidase treatment, suggesting heterogeneity due to primary amino acid variation. According to the transspecies hypothesis of MHC polymorphism (40–42), the evolution of MHC alleles does not start at the inception of a species, but is rather a process in which a major group of alleles is passed on in the phylogeny from one species to another. Thus, the existence of the polymorphic 7.3.19.1 determinant predates speciation of man and chimpanzee (26), and therefore, this epitope is at least 5,000,000 yr old.

Antigen presentation across the species barrier between man and chimpanzee was only observed with HLA- or Patr-DRw52 molecules as antigen presenting structures (Figs. 3 and 5). Antigen-specific Th cell proliferation could be inhibited with the mAbs 7.3.19.1 (DRw52) and B8.11.2 (DR), but not with SPV-L3 (DQ) and PL15 (DP), pinpointing the Patr-DRw52 $\alpha\beta$c molecules as the antigen-presenting structures. Th cells derived from WVE should have TCRs educated to recognize antigen fragments in the context of the HLA-*DRB3*0301* (DRw52c) gene product, which is reactive with 7.3.19.1 (Fig. 1). Particular WVE-TCR are not only able to recognize antigen fragments presented by autologous MHC class II structures, but apparently also recognize PPD fragments in the context of a Patr-DRw52 $\alpha\beta$c molecule. Patr-DR2-positive cells were not able to present antigen to CAA-, HHK-, or QBL-derived antigen-specific T cells that express other alleles at the HLA-*DRB3* locus (Table 1).

Until now, no antigen presentation by chimpanzee APC has been shown to HLA-*DRB3*0201* (DRw52b) and -DRw52 epitope-negative HLA-DRB1-educated TCRs (Table 1). This may be due to the fact that not enough combinations were tested. Another explanation would be that the MHC-DRB1 restriction epitopes of man and chimpanzee have been conserved in evolution to a lesser extent than those of the DRw52 determinant–positive MHC-DRB3 molecules. If the latter explanation is true, this would suggest that MHC-DRw52 molecules have been successful in the regulation of an immune response to the same type of pathogens that are a threat to distinct species.

Three different types of Patr-DR molecules expressed by the chimpanzee Hugo have recently been sequenced (43). From the Hugo cDNA library, a clone designated C4-2 was isolated that shares a high amount of sequence homology with the human *DRB3*0301* (DRw52c) gene but clearly is not completely identical to its human equivalent. The corresponding gene product of the Hugo C4-2 clone is most likely reactive with mAb 7.3.19.1, and thus can be isolated by immunoprecipitation assays (Fig. 2). To detect the putative binding site of mAb 7.3.19.1, the amino acid sequences of various HLA- and Patr-C4-2 DR β first domain sequences were lined up (Fig. 6) according to a recently published method (44). As mentioned earlier, 7.3.19.1 detects a DRw52 determinant present on HLA-DR3 haplotype-encoded DRB1 and

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                    10        20        30        40        50        60        70        80        90
                    |         |         |         |         |         |         |         |         |
HLA-
DRB1*0101 GDTRPRFLWQ LKFECHFFNG TERVRLLERC IYNQEESVRF DSDVGEYRAV TELGRPDAEY WNSQKDLLEQ RRAAVDTYCR HNYGVGESFT VQRR
DR2-Dw2A  --------Q- D-Y------- -----F-H-D ------DL-- ---------- ---------- ------F--D ---------- ---------- ----
DR2-Dw12A --------Q- D-Y------- -----F-H-G ------N--- ---------- ---------- ------F--D ---------- ---------- ----
DRB1*0301 --------EY STS------- -----Y-D-Y FH----N--- ------F--- ---------- ---------- K-GR--N--- -----V---- ----
DRB1*0302 --------EY STS------- -----F---Y FH----N--- ---------- ---------- ---------- K-GR--N--- ---------- ----
DRB1*0401 --------E- V-H------- -----F-D-Y F-H---Y--- ---------- ---------- ---------- K--------- ---------- ----
DRB1*0402 --------E- V-H------- -----F-D-Y F-H---Y--- ---------- ---------- ------I--D E--------- -----V---- ----
DRB1*0403 --------E- V-H------- -----F-D-Y F-H---Y--- ---------- ---------- ---------- ---E------ -----V---- ----
DRB1*1101 --------EY STS------- -----F-D-Y F-----Y--- ------F--- -------E-- ------F--D ---------- ---------- ----
DRB1*1102 --------EY STS------- -----F-D-Y F-----Y--- ------F--- -------E-- ------I--D E--------- -----V---- ----
DRB1*1201 --------EY STG--Y---- ---------H FH----LL-- ------F--- ------V--S ------I--D ---------- ----AV---- ----
DRB1*1301 --------EY STS------- -----F-D-Y FH----N--- ------F--- ---------- ------I--D E--------- -----V---- ----
DRB1*1302 --------EY STS------- -----F-D-Y FH----N--- ------F--- ---------- ------I--D E--------- ---------- ----
DRB1*1401 --------EY STS------- -----F---Y FH----N--- ---------- ---------- ---------- ---------- ---------- ----
DRB1*1402 --------EY STS------- -----F-D-Y FH----F--- ---------- ------A--H ---------R ---E------ -----V---- ----
DRB1*0701 ---Q------ G-YK------ ----QF---L F-----F--- ---------- ------V--S ------I--D --GQ---V-- ---------- ----
DRB1*0801 --------EY STG--Y---- -----F-D-Y F-----Y--- ---------- ------S--- ------F--D ---L------ ------
DRB1*0802 --------EY STG--Y---- -----F-D-Y F-----Y--- ---------- ---------- ------F--D ---L------ ---------- ----
DRB1*0901 ---Q----K- D--------- -----Y-H-G ------N--- ---------- ------V--S ------F--R ---E---V-- ---------- ----
DRB1*1001 --------EE V--------- ---------R VH----YA-Y ---------- ---------- ---------R ---------- ---------- ----
DRB4*0101 ---Q----E- A-C----L-- ----WN-I-Y ------YA-Y N--L---Q-- ---------- ---------R ---E------ Y----V---- ----

DRB3*0101 --------EL R-S------- -----Y-D-Y FH----FL-- ---------- ------V--S ---------- K-GR--N--- ---------- ----
DRB3*0201 --------EL --S------- -----F---H FH----YA-- ---------- R--------- ---------- K-GQ--N--- -----V---- ----
DRB3*0202 --------EL --S------- -----F---H FH----YA-- ---------- R--------- ---------- K-GQ--N--- ---------- ----
DRB3*0301 --------EL --S------- -----F---Y FH----F--- ---------- ------V--S ---------- K-GQ--N--- -----V---- ----

Patr-
C4-2      --------EL V-S------- -----F---Y F-----Y--- ---------- --P---V--S ---------- K-GQ--N--- ----AV---- ----
```

Figure 6. Definition of the 7.3.19.1 epitope by comparison of different HLA- and Patr-DR β first domain sequences. The putative binding site of mAb 7.3.19.1 has been boxed.

DRB3 gene products, whereas this epitope is exclusively located on HLA-DR5 and -DRw6 haplotype-encoded DRB3 gene products (18–25). The unique reactivity pattern of 7.3.19.1 correlates with the boxed motive KRG at amino acid numbers 71–73 (Fig. 6). As would be expected, this motive is also present on the C4-2 clone–derived amino acid sequence from Hugo (Fig. 6). This KRG motive maps to the α helix section in the antigen-binding cleft of MHC class II molecules (44).

APC derived from the chimpanzee Hugo can successfully present antigen to WVE Th cells that have been educated to see PPD in the context of the HLA-*DRB3*0301* (DRw52*c*) gene product. However, the Patr-C4-2 and HLA-*DRB3*0301* gene products display amino acid sequence differences (Fig. 6), as already could be predicted based upon pI differences detected after 2D gel electrophoresis (Fig. 1). Thus, amino acid substitutions at positions 11, 32, 37, 53, and 85 within the Patr-DRw52 $\alpha\beta$c molecules apparently do not interfere with the presentation of PPD to a human WVE-derived TCR (Fig. 6). On the other hand, Patr-DRw52 $\alpha\beta$c gene products are not able to present antigen to the T cell lines CAA and HHK, which have TCRs educated to see antigen fragments in the context of HLA-*DRB3*0101* gene products (Table 1). Therefore, it is concluded that the amino acid substitutions found at position 28, 38, 74, and 85 of the Patr-DRw52βc gene product (Fig. 6) probably do interfere with T cell recognition of HHK and CAA-TCR that have been educated to see antigen in the context of an HLA-*DRB3*0301* gene product.

In conclusion, the availability of chimpanzee APC in combination with information on MHC class II genes and their products provides a set of MHC class II "biomutants" that can be used to study the molecular basis of MHC restriction without performing in vitro mutagenesis studies. Moreover, such biomutants probably have been selected during evolution based on function (45), and thus are biologically more meaningful than artificially generated MHC mutants.

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