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The chimpanzee *Mhc-DRB* region revisited: gene content, polymorphism, pseudogenes, and transcripts

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Abstract

In humans, great apes, and different monkey species, the major histocompatibility complex (MHC) class II *DRB* region is known to display considerable copy number variation. The microsatellite D6S2878 has been shown to be a valuable marker for haplotyping the *DR* region in humans and macaque species. The present report illustrates that chimpanzee haplotypes also can be discriminated with this marker. The analyses resulted in the description of nine different region configurations, of which seven are present within the West African chimpanzee population studied. The region configurations vary in gene content from two up to five *DRB* genes. Subsequent cDNA sequencing increased the number of known full-length *Patr-DRB* sequences from 3 to 32, and shows that one to three *Patr-DRB* genes per haplotype apparently produce functional transcripts. This is more or less comparable to humans and rhesus macaques. Moreover, microsatellite analysis in concert with full-length *DRB* gene sequencing showed that the *Patr-DRB*W9* and *-DRB3*01/02* lineages most likely arose from a common ancestral lineage: hence, the *Patr-DRB*W9* lineage was renamed to *Patr-DRB3*07*. Overall, the data demonstrate that the D6S2878 microsatellite marker allows fast and accurate haplotyping of the *Patr-DRB* region. In addition, the limited amount of allelic variation observed at the various *Patr-DRB* genes is in agreement with the fact that chimpanzees experienced a selective sweep that may have been caused by an ancient retroviral infection.

Keywords

HLA; HIV; Microsatellite; Patr

Introduction

Chimpanzees (*Pan troglodytes*) are the closest living relative of humans, and both species share approximately 98.7% similarity at the nonrepetitive DNA level (Fujiyama et al., 2002). The split between the ancestors of humans and chimpanzees happened about 5–6 million years ago (Fujiyama et al., 2002). Chimpanzees can be divided into four different subspecies, designated as *Pan troglodytes troglodytes* (*P.t.t.*), *P.t. schweinfurthii* (*P.t.s.*), *P.t. verus* (*P.t.v.*), and *P. t. vellerosus* (Gonder et al., 1997; Morin et al., 1994).

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The major histocompatibility complex (MHC) is a multigene family, which is present in most vertebrate species, and two clusters of cell-surface proteins are recognised. MHC class I gene products are expressed on nucleated cells, whereas the MHC class II proteins are expressed on B cells, macrophages, and other antigen-presenting cells. The MHC plays a key role in generating adaptive immunological responses, and the main function is the binding of pathogen-derived peptides and presenting them on the cell surface for T-cell recognition. The MHC class II gene products in humans and chimpanzees are designated *HLA-DP*, *-DQ*, and *-DR*, and *Patr-DP*, *-DQ*, and *-DR*, respectively. All three loci possess one *A* gene and at least one *B* gene. The *DR* region is the most complex, and several *B* genes are characterised in humans: they have been named *HLA-DRB1* to *-DRB9* (Marsh et al., 2005). In chimpanzees, the orthologues of these genes are described, and as in humans, the *-DRB2* and *-DRB6* to *-DRB9* are considered to be pseudogenes (Bontrop et al., 1999). Chimpanzees, however, seem to have lost during their evolution the equivalent of the *HLA-DRB1*04* lineage. In both species, the various types of *DRB* loci display different levels of polymorphism, and most of this variation is confined to exon 2. Additionally, the unique number of *DRB* genes present per region configuration differs and ranges from one to four in humans and from two to five in chimpanzees (de Groot et al., 2008b; Doxiadis et al., 2007). Allelic variation is observed within region configurations, and as a consequence different haplotypes can be discriminated per region configuration.

A complex dinucleotide repeat, D6S2878, located in close proximity of exon 2 of most *HLA-DRB* genes (Andersson et al., 1987; Epplen et al., 1997; Riess et al., 1990), was found to represent an accurate and valuable marker for haplotyping humans and two macaque species (de Groot et al., 2008a; Doxiadis et al., 2007). The genetic profile of this microsatellite showed its usefulness in the description of *Patr-DRB1* alleles (Bak et al., 2006). In humans, the *HLA-DRB1*, *-DRB3*, *-DRB4*, and *-DRB5* genes produce functional transcripts that are translated into products. To date there are limited data available on the actual transcription of *Patr-DRB* alleles (Fan et al., 1989), and even a thorough chimpanzee population study is lacking.

Chimpanzees are known to have very effective cytotoxic T-cell (CTL) responses against human immunodeficiency virus type 1 (HIV-1) infection (Balla-Jhaghoorsingh et al., 2003). Moreover, like particular human individuals, chimpanzees in general are relative resistant to developing acquired immunodeficiency syndrome (AIDS) after natural or experimental infection with HIV-1 or chimpanzee-derived simian immunodeficiency virus (SIV_{cpz}, the closest relative of HIV-1) (Heeney et al., 2006; Keele et al., 2009; Novembre et al., 1997). In humans, resistance to AIDS is strongly associated with the presence of the HLA-B*2705 or -B*5701 molecules (Goulder and Watkins, 2008); also in chimpanzees CTL responses are found that are directed against conserved HIV-1 epitopes in the context of particular *Mhc* class I molecules (Balla-Jhaghoorsingh et al., 1999). Based on these observations, and on the fact that chimpanzees possess a reduced *Mhc* class I repertoire, we hypothesised that the *Mhc* class I gene repertoire reduction may have been caused by SIV_{cpz} or a related ancestral retrovirus. As such, the contemporary chimpanzee populations have modified MHC class I repertoires but are able to cope with their natural environment and with (retro)viral infections such as HIV-1/SIV_{cpz} (de Groot et al., 2002). Evidence for such a selective sweep was further substantiated by the analyses of the MIC locus, which maps near *Patr-B* (de Groot et al., 2005), and by comparative genomics using different microsatellite markers mapping inside and outside the MHC region (de Groot et al., 2008b).

MHC class II molecules play a pivotal role in providing CD4⁺ T-cell-mediated help and antibody production. This effector function is also important for the development and maintenance of CD8⁺ memory T cells (Williams et al., 2006). Although the MHC class I repertoire of chimpanzees is reduced, these animals do mount effective CTL responses against various pathogens such as HCV, HIV-1, and malaria (Balla-Jhaghoorsingh et al., 2003; Bottius

et al., 1996; Erickson et al., 1993). We aimed to characterise the *Mhc-DRB* region of a *P.t.verus* colony thoroughly and to investigate which different *Patr-DRB* alleles may play a role in providing immunological help to CD8⁺ memory T cells.

Material and methods

Source of RNA and DNA

Thirty-five chimpanzees originating from Sierra Leone represent the founder population that is the basis of the West African chimpanzee colony studied. The colony increased to more than 200 animals, covering three generations. The offspring was pedigreed based on serological specificities (*Patr-A* and *-B*) and molecular-defined class I and II polymorphisms (Bontrop et al., 1995; de Groot et al., 2000). Epstein-Barr virus- transformed B-cell lines were used to obtain RNA and genomic DNA (gDNA). Human DNA samples, originating from unrelated Caucasoid individuals, were provided by the department of Immunohaematology and Blood Bank of the Leiden University Medical Centre (Doxiadis et al., 2007) and were included for comparison. In addition, samples of four *P.t.t.* and two *P.t.s.* animals were included in the panel.

Patr-DRB short tandem repeat (STR) genotyping and sequencing

The microsatellite marker D6S2878 was used for *DRB* genotyping. The genotyped cohort comprised of 114 different chimpanzee DNA samples of founder animals and offspring. The relevant DNA segment was amplified with the primers (0.2 µM) described for the human samples and the genotyping techniques were performed as published (de Groot et al., 2008a; Doxiadis et al., 2007).

Forty three (31 *P.t.v* and 12 *P.t.t/P.t.s.*) different *Patr-DRB* alleles were sequenced from exon 2 to intron 2, and this area included the microsatellite. The same primers are used as described for humans (Doxiadis et al., 2007). The PCR products were purified using the QIAquick Gel Extraction Kit (Qiagen) and were subsequently blunt-end ligated into the vector pJET1.2/blunt vector (Fermentas, St. Leon-Rot, Germany). Transformation of the XL1Blue *E. coli* cells was performed with the TransformAid Bacterial Transformation Kit (Fermentas). A minimum of 32 clones were examined per PCR reaction. The sequence samples were prepared using 1 µl of ABI Prism BigDye Terminator v3.1 Cycle Sequencing mixture (Applied Biosystems, Foster City, CA), 0.2 µM of pJET1.2 forward or reverse primer (Fermentas), and 2 µl of 5X sequencing dilution buffer (400mM Tris-HCL, 10mM MgCl₂) in a 10 µl reaction, and sequenced on an ABI 3130xl genetic analyser (Applied Biosystems). The data were analysed using the programmes Lasergene SeqMan Pro version 7.2.1 (Dnastar, Inc Madison, USA) and MacVector version 10.0.2. (MacVector, Inc Cambridge, UK). At least two independent PCR reactions were performed and/or the alleles were confirmed by their presence in different animals.

Patr-DRB cDNA sequencing

For 21 different animals (17 *P.t.v.* and 4 *P.t.t.*), covering most of the genotyped *Mhc-DRB* haplotypes, RNA was extracted using the RNeasy mini kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations. An RT-PCR reaction (Promega, Madison, WI, USA) in accordance with the manufacturer's recommendation was performed using *Mhc-DRB* specific primers described previously (Lekutis and Letvin, 1995). The purification of the PCR products, the ligation, transformation, and sequencing was performed as described above.

Phylogenetic analysis and detection of transposable elements

Neighbor-Joining (NJ) trees were constructed with the PAUP* programme version 4.0b10 for Macintosh (Swofford, 2002). Pairwise distances were calculated using the Kimura-2 parameter method. Bootstrap values were calculated based on 1000 replicates. Alleles of the *Patr-DRB3*01/02* and *-DRB*W9* lineages were analysed for their repetitive elements using the CENSOR software tool from the Genetic Information Research Institute website (<http://www.girinst.org/censor>) (Kohany et al., 2006).

Nomenclature and re-designation of the *Patr-DRB*W9* lineage

Allele names were assigned according to a standardized protocol (Ellis et al., 2006; Klein et al., 1990; Robinson et al., 2003). The full-length *DRB* sequences are deposited at the EMBL database, and received accession numbers **FN424191** to **FN424222**. The newly described exon 2 sequence *Patr-DRB1*0215* received accession number **FN424223**. All sequences are documented at the IPD-MHC-NHP-database (www.ebi.ac.uk/ipd/mhc/nhp).

Based on gDNA and cDNA sequences, the *Patr-DRB*W9* lineage seems to be highly related to the *-DRB3*01/02* lineages. The present data elucidated that the above-mentioned lineages possess a highly similar microsatellite. Furthermore, an analysis of the introns of the *Patr-DRB3*01/02* and *-DRB*W9* alleles in the CENSOR database showed that intron 1 is similar in length and possesses the same Alu inserts (Kohany et al., 2006). The similarity observed between the *Patr-DRB*W9* and *-DRB3*01/02* lineages must reflect the common ancestry. Thus, the alleles that were given the *Patr-DRB*W9* designations in the past are more likely alleles of a lineage of the *Patr-DRB3* locus. For a more consistent nomenclature, we renamed the *Patr-DRB*W9* alleles as follows: *Patr-DRB*W901* became *-DRB3*0701*, *-DRB*W902* became *-DRB3*0702*, and *-DRB*W903* became *-DRB3*0703*. The renaming is documented at the IPD-MHC-NHP-database (www.ebi.ac.uk/ipd/mhc/nhp).

Results and discussion

Patr-DRB haplotype definition by microsatellite analysis

In the past, restriction fragment length polymorphism (RFLP) followed by sequencing of the exon 2 segment of the *DRB* loci and Denaturing Gradient Gel Electrophoresis (DGGE) were used as techniques to describe the *Patr-DRB* profile of the West African chimpanzee colony, and resulted in the description of six different region configurations (Bontrop et al., 1990; de Groot et al., 2008b; Kenter et al., 1992). The region configurations and haplotypes were firmly established based on segregation analyses. The present analyses, using the D6S2878 marker, showed that all exon 2- positive *Patr-DRB* genes possess the *DRB*-STR repeat. In the chimpanzee samples, 6 to 10 *DRB*-STR amplicons could be detected, which have highly variable lengths ranging from 135 to 226 base pair (bp). Subsequent sequencing of exon 2/*DRB*-STR alleles made it possible to link unequivocally a microsatellite to a particular exon 2. This approach resulted in a more accurate definition of *Patr-DRB* haplotypes (Table 1), and for the *P.t.v.* colony seven different region configurations can now be distinguished (Fig. 1A). The analyses of the *P.t.t.* and *P.t.s.* animals resulted in the detection of two additional region configurations (Fig. 1B), each composed of an exclusive combination of *Patr-DRB* genes: the combination of *DRB*-STR alleles seems to be unique for a given haplotype. Additionally, some *DRB*-STR alleles are highly predictive for the presence of a particular *DRB* allele. For example, the 157 fragment is observed in combination with *Patr-DRB1*0701*, the 155 fragment is observed in combination with *Patr-DRB6*0305*, and the 135 fragment segregates in combination with *Patr-DRB7*0101* (Table 1). There are also a few haplotypes in which the microsatellite displays length differences for a particular *Patr-DRB* allele: for instance, the *DRB1*0201*-linked *DRB*-STR of haplotype Ia (Table 1A). These slight length differences originate from different founder haplotypes, as was confirmed by segregation analysis.

In humans, five *DRB* region configurations are recognised and all display an abundant amount of allelic variation for the *DRB1* locus. Another situation is detected in rhesus and cynomolgus macaques. The latter species possess a high number of region configurations that display hardly any allelic variation within a given configuration (de Groot et al., 2008a; Doxiadis et al., 2007). The present data demonstrate that chimpanzees show a moderate amount of allelic variation within certain - but not all nine - described region configurations (Table 1). In general, the situation involving chimpanzees seems to resemble more closely the one found for the *HLA-DRB* region configurations. The human and chimpanzee region configurations contain similar *DRB* genes, illustrating that these genes must have been present in a common ancestor. For instance, region configuration IV is completely identical between humans and chimpanzees, indicating that it was present in a common ancestor and inherited 'untouched' during primate evolution. The stability of this region configuration may be due to an antisense integration of a retroviral insert within the *DRB7* pseudogene (Doxiadis et al., 2008a). Moreover, all *p.t.v.* region configurations are found to contain a *DRB1* gene, which is comparable to humans. In the *p.t.t.* and *p.t.s.* animals analysed region configuration IX was found to lack a *DRB1* gene, but most likely the prominent task of the *HLA-DRB1* gene was taken over by one of the other genes on this region configuration. Chimpanzee region configuration I shows the highest degree of polymorphism and this is mostly due to the allelic variation within the *Patr-DRB5*03* lineage. Haplotype Ii is only observed in one animal, and contains an allele of the *Patr-DRB3*01* lineage, whereas all the other haplotypes of region configuration I possess an allele of the *Patr-DRB3*02* lineage (Table 1A). Nevertheless, a similar haplotype is present in the chimpanzee genome database (contig NM_01236523), thus confirming the existence of haplotype Ii. Another lineage that possesses several alleles is the *Patr-DRB1*03* lineage. It is the most polymorphic lineage, and distinct alleles are present on three different region configurations in the *p.t.v.* population studied (Table 1A). A similar observation has been made with regard to the *Patr-DRB3* locus, which is present on several different region configurations and shows lineage and allelic polymorphism. The different *Patr-DRB* alleles were analysed phylogenetically together with *HLA-DRB* alleles from a Caucasoid population (Fig. 2A). The results equal the earlier published phylogenetic analysis on *DRB* exon 2 sequences (Kenter et al., 1992), and demonstrate that chimpanzee and human alleles of identical lineages/loci cluster tightly together. In addition, the composition of the corresponding microsatellites is shown. The *DRB-STR* is a composite microsatellite, and the comparison of different *DRB* sequences of several primate species indicated that the ancient structure of the microsatellite most likely must have been (GT)x(GA)y (Bergstrom et al., 1999; Doxiadis et al., 2007; Kriener et al., 2000; Riess et al., 1990). Thus far, most *DRB* gene-associated microsatellites encountered are constructed of four sections: namely, a (GT)x, a (GA)z-mix, a (GA)y, and a (GC)n part. The 5' (GT)x repeat represents the longest segment, is mostly uninterrupted by other nucleotides, and is therefore known to evolve rapidly. For example, the *HLA-DRB5*010101* allele can be detected with different *DRB-STR* lengths, (GT) 18–24 (Fig. 2B). This illustrates that the repeat itself evolves faster, due to a higher mutation rate, than the adjacent exon 2 sequence. Interruption of a repeat by other nucleotides usually results in more stability. This is, for instance, evidenced by the *Patr-DRB5* alleles, which all have a highly similar (GT)x part that is interrupted by a GA (Fig. 2B). The (GA)z-mix part is mostly shorter and interrupted by other dinucleotides, and appears to correlate with the different *DRB* lineages (Fig. 2B). Identical/similar color codes highlight that within particular lineages the relevant microsatellite is quite similar between humans and chimpanzees (Fig. 2B). Thus, not only the exon 2 sequences but also the *DRB-STR* alleles appear to reflect the common ancestry of *DRB* lineages. The (GA)y part often correlates with lineages as well, although this is less prominent. The (GC)n part forms the 3' end of the repeat. The *Patr-DRB1*1001* allele was found not to cluster together with its human equivalent, and both the *HLA-* and *Patr-DRB1*1001* alleles appear to possess a different microsatellite (Fig. 2). These observations suggest that the *HLA-* and *Patr-DRB1*1001* alleles belong to different lineages, and therefore the *Patr-DRB1*1001* allele is currently under investigation to unravel its evolutionary history.

The presence of two different *Patr-DRB6* loci on one chromosome is unique for haplotype VII (Table 1A). The compositions of the microsatellites of the loci vary (Fig. 2B); indicating that most likely the haplotype arose via recombination rather than via duplication of one of the *DRB6* genes.

As compared to *Patr-DRB* typing strategies used earlier, the DRB-STR typing protocol appears to be an accurate and speedy method. The present analysis illustrates that next to humans and old world monkeys (de Groot et al., 2008a; Doxiadis et al., 2007), great ape species can also be haplotyped with the D6S2878 microsatellite marker. With the DRB-STR protocol it is possible to haplotype several different kinds of species for the *Mhc-DRB* region; hence, this microsatellite may find its application in pedigree analysis and can be used for conservation biology and breeding management in zoos.

Full-length cDNA analysis of *Patr-DRB* alleles

To determine which *Patr-DRB* alleles are transcribed, a panel of cell lines of different animals was selected that covered most of the known haplotypes (Table 1A/B). All *Patr-DRB1* and -*DRB5* alleles present on the different haplotypes were found to produce *bona fide* transcripts. For the *Patr-DRB3* locus, we detected transcripts for the -*DRB3*0102*, -*DRB3*0103*, -*DRB3*0201*, -*DRB3*0214*, and -*DRB3*07* alleles. For the *Patr-DRB3*0208* and -*DRB3*0209* alleles, no transcripts were observed. The *Patr-DRB3*0309* is observed only on one haplotype and for only one animal a cell-line was available to analyse this allele. Thus, there is a possibility that due to primer inconsistencies the allele was not amplified, and therefore this allele needs further investigation. The *Patr-DRB3*0208* allele appears to be a pseudogene, as sequencing analysis on genomic DNA resulted in the detection of a premature stopcodon in exon 3 (Doxiadis et al., 2008b). No transcripts were detected for the *Patr-DRB4*0104* and -*DRB7*0101* alleles, present on region configuration IVa/b (Table 1A/B). *Patr-DRB4*0104* was deemed to represent a non-functional allele because of the presence of a premature stopcodon at the end of exon 2, whereas the -*DRB7*0101* allele is considered to be a pseudogene because of the presence of deletions and a premature stopcodon (Doxiadis et al., 2008a; Kenter et al., 1992). However, the *Patr-DRB4*0201* allele, representing the other lineage within the *Patr-DRB4* locus, produces a transcript. Previously, it was described that this allele most likely arose from a recombination event between a *Patr-DRB4*01* allele and an unknown donor allele (Kenter et al., 1992). The data indeed illustrate that the composition of the DRB-STR is different between the alleles of the *HLA-* and *Patr-DRB4*01* and *Patr-DRB4*02* lineages (Fig. 2B). The comparison of the full-length sequences of the *HLA-DRB4*0101* and *Patr-DRB4*0201* alleles shows that the sequences diverged, and only a small motif in exon 2 characteristic for the *DRB4* locus is shared between the alleles. These observations suggest a hybrid character for the *Patr-DRB4*0201* allele, and therefore currently the introns of this allele are sequenced to unravel its evolutionary history. Moreover, with the primer set used, no full-length transcripts are observed for the *DRB6* locus. This is expected, as this locus lacks its exon 1 and is therefore considered to be a pseudogene (Kenter et al., 1992; Mayer et al., 1993). Taken together, the present communication increases the number of known full-length *Patr-DRB* sequences from 3 to 32. The full-length sequences of the *Patr-DRB1*0201*, -*DRB3*0201*, and -*DRB5*0301* alleles are identical to the earlier published sequences W1, C4-2, and B3-5, respectively (Fan et al., 1989).

The *Patr-DRB5*0313* allele (present on haplotype Ij) detected in one of the *p.t.t.* animals is very similar to *Patr-DRB5*0306* (present on haplotype Ib and Iic). Although, at position 13 in exon 2 of the *Patr-DRB5*0313* allele, the triplet TGT coding for a cysteine is substituted by CAT, which encodes for a histidine. A histidine is rare at this position and is only observed in alleles of the *HLA-DRB1*04* and *Patr-DRB4*01* lineages (Fig. 3). However, chimpanzees are known to lack the equivalent of the *HLA-DRB1*04* lineage, and *Patr-DRB4*01* seems to

represent a non-functional lineage. As such, the substitution of the cysteine by a histidine in the *Patr-DRB5*0313* allele suggests the rescue of a residue, most probably by recombination, originating from a different functional chimpanzee lineage.

In humans, only haplotypes with one or two transcribed *DRB* alleles are known (Robinson et al., 2003). Most chimpanzee haplotypes of the *P.t.v.* subspecies possess two transcribed *DRB* alleles. Only haplotype IVa contains one, whereas haplotypes Ia, Ie, Ii, and IIa to IIc contain three *DRB* alleles that are potentially translated into a polypeptide (Table 1A). The analysed animals of the *P.t.t.* subspecies possess haplotypes with one (r.c. IVb) or two (r.c. IIIe, VIIla, and IXa) transcribed *DRB* allele(s) (Table 1B). In comparison, rhesus macaques, which possess haplotypes with up to six different *Mamu-DRB* genes, are known to transcribe one to three *DRB* genes/alleles (de Groot et al., 2004). In humans, the presence of only one transcribed *DRB* gene per haplotype (HLA-DR1 and -DR8 serotypes) does not seem to have any disadvantage, as HLA-DR1 and DR8 homozygous individuals are not rare and appear to be healthy (Marsh, 2000; Robinson et al., 2003). Nonetheless, the transcription of three *DRB* alleles per haplotype is most probably the limit, as more *DRB* transcripts per haplotype have not yet been reported. This assumption seems to be plausible if one envisages that the expression of too many MHC class II molecules would result in a high number of T cells that would be deleted during thymic education. As a consequence, such individuals would possess a reduced T-cell repertoire and therefore may become susceptible to infectious diseases.

The complete cDNA sequences of the analysed *Patr-DRB* alleles and particular *HLA-DRB* alleles were subjected to phylogenetic analysis (Fig. 4). The analysis confirms the results of the exon 2 phylogenetic comparison (Fig. 2A and (Kenter et al., 1992)), and illustrates that for most of the full-length *HLA-* and *Patr-DRB* genes a transspecies mode of evolution is observed (von Salome et al., 2007). Even if intron sequences of both species are compared, the footprint of a transspecies mode of evolution is retained. This is, however, different from the situation observed in rhesus macaques. The *Mamu-DRB* exon 2 sequences represent old entities that predate primate speciation, whereas these exons are embedded in genes that appear to represent relatively young entities (Doxiadis et al., 2008b). The full-length sequence of *Patr-DRB3*0103* appears to cluster with *Patr-DRB3*0201* (Fig. 4). This is different from what observed in the exon 2 phylogenetic comparison (Fig. 2A), and indicates that also in chimpanzees exon 2 shuffling in the *Patr-DRB* genes may appear. This seems to be, however, a rare event.

Chimpanzee *DRB* alleles and their potential contribution to resistance to AIDS

Analysis of the MHC region revealed that chimpanzees experienced a selective sweep affecting mainly the *Mhc* class I repertoire. This selective sweep may have been caused by a HIV-1/SIV_{cpz}-like retrovirus (de Groot et al., 2002). In addition to *Mhc* class I, other parts within the *Mhc* region also show signs of a repertoire reduction, such as the major histocompatibility chain-related gene (*MIC*), located centromeric of the *Mhc-B* locus (de Groot et al., 2005; Kulski et al., 2002). Comparative genomics showed that the strongest repertoire reduction maps to the chimpanzee *Mhc* class I region. However, different parts in the *Mhc* class II region show signs of a repertoire reduction as well (de Groot et al., 2008b).

A recent study performed in a cohort of Kenyan sex workers reported that particular *HLA-DRB* alleles are associated with resistance or susceptibility to HIV-1 mediated disease (Lacap et al., 2008). We compared these alleles with the *Patr-DRB* alleles present in our cohort. Equivalents of the *HLA-DRB1*070101*, *-DRB1*1503*, and *-DRB5*010101*, associated with susceptibility, can be found in chimpanzees (Fig. 4). For the *HLA-DRB1*010101* and *010201* alleles, associated with resistance, no apparent equivalents are detected in chimpanzees. The *HLA-DRB1*1102* allele, associated with resistance, seem to cluster phylogenetically together with alleles of the *Patr-DRB1*03* lineage. However, the *HLA-*

*DRB1*030201* allele, associated with susceptibility, seems to cluster as well with the *Patr-DRB1*03* lineage. This suggests that it is difficult to define a contribution of the *Patr-DRB* lineages in HIV-1/SIV_{cpz} infection based on phylogenetic comparisons of sequences. Indeed, in humans it has recently been shown that each of the nine antigens of the HLA-B44 supertype possess unique peptide binding motifs (Hillen et al., 2008). This observation, together with the knowledge that the peptide binding affinity of a MHC class II molecule is less stringent than the binding affinity of a MHC class I molecule, corroborates the notion that caution must be exercised in drawing conclusions based only on the similarity observed in phylogenetic comparisons (Hammer et al., 1993; Rammensee et al., 1993). With the availability of the full-length cDNAs of different *Patr-DRB* alleles, we are now able to construct single antigen-expressing cell lines and study in detail the function and the MHC/peptide interaction of the *Patr-DRB* molecules.

Nevertheless, the human study indicated that the DRB-specific CD4⁺ T-cell responses are an important factor in resistance/susceptibility to HIV-1 infection (Lacap et al., 2008). For chimpanzees, it is known that they maintain CD4⁺ T-cell responses after HIV-1/SIV_{cpz} infection. Comparing the *HLA*- and *Patr-DRB* region, it is obvious that both species have a slightly different number of region configurations, but the region configurations possess identical *DRB* genes. Humans on the one hand seem to have acquired a large amount of allelic variation within the different *DRB* region configurations, whereas chimpanzees on the other hand, although known to be an older species, show only limited allelic variation. Additionally, chimpanzees lack the equivalent of the *HLA-DRB1*04* lineage. These observations support that the chimpanzee MHC class II region may have experienced a reduction in the repertoire as well. The contemporary MHC class II alleles were most likely positively selected due to genetic linkage to the MHC class I alleles that survived the selective sweep. The presently available repertoire of *Patr-DRB* alleles seems to contribute efficiently to the maintenance of the CD4⁺ T-cell responses, and therefore chimpanzees seem to have successful combinations of CD4⁺/CD8⁺ T-cell responses that are able to cope with pathogens of their natural habitat and with HIV-1/SIV_{cpz} infection.

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Figure 1.

A) *DRB* region configurations in the *P.t.v.* subspecies. The haplotypes are arranged based on *DRB1* locus lineages; therefore, haplotype VII is situated between haplotype II and III. **B)** Additional *DRB* region configurations detected in the *P.t.t.* and *P.t.s.* subspecies. The *DRB9* gene is not included but is present on all region configurations. Black boxes represent pseudogenes. For example, in configuration I *DRB3*01/02* means that lineage polymorphism is detected for this locus. Configuration IV is shared between humans and chimpanzees. *The *Patr-DRB3*07* lineage was previously named *Patr-DRB*W9* (details in material and methods).

Figure 2.

A) Rooted phylogenetic comparison of the exon 2 sequences of *DRB* alleles detected in chimpanzees (*Patr*, blue) and humans (*HLA*, red). The common marmoset allele *Caja-DRB*W1601* is taken as an outgroup (Doxiadis et al., 2006). Relevant bootstrap values are indicated. The brackets specify the different *DRB* loci/lineages. *The *Patr-DRB3*07* lineage was previously named *Patr-DRB*W9*. **B)** Composition of the microsatellite sequence (D6S2878) corresponding to the *DRB* allele in Figure 2A. Identical or similar colours indicate identity or similarity of the (GA)z-mix part of the microsatellite.

	1	10	20
<i>HLA-DRB1*010101</i>	GD	TRPRFLWQLKF	EC
<i>HLA-DRB1*040101</i>	---	---	---
<i>Patr-DRB1*0201</i>	---	---	---
<i>Patr-DRB1*0302</i>	---	---	---
<i>Patr-DRB1*0701</i>	---	---	---
<i>Patr-DRB1*1001</i>	---	---	---
<i>Patr-DRB3*0102</i>	---	---	---
<i>Patr-DRB3*0201</i>	---	---	---
<i>Patr-DRB4*0104N</i>	---	---	---
<i>Patr-DRB4*0105N</i>	---	---	---
<i>Patr-DRB4*0106N</i>	---	---	---
<i>Patr-DRB4*0107N</i>	---	---	---
<i>Patr-DRB4*0201</i>	---	---	---
<i>Patr-DRB5*0101</i>	---	---	---
<i>Patr-DRB5*0301</i>	---	---	---
<i>Patr-DRB5*0306</i>	---	---	---
<i>Patr-DRB5*0313</i>	---	---	---
<i>Patr-DRB6*0105N</i>	---	---	---
<i>Patr-DRB6*0301N</i>	---	---	---
<i>Patr-DRB7*0101N</i>	---	---	---

Figure 3.

Deduced protein alignment of the beginning of exon 2 of a representative allele of the *HLA-DRB1*04* lineage and representative alleles of the different *Patr-DRB* lineages. The *HLA-DRB1*010101* is taken as consensus from the IMGT/HLA database (www.ebi.ac.uk/imgt/hla). Identity to the consensus sequence is indicated by *dashes*, whereas amino acid replacements are depicted by the conventional *one letter code*. The *Patr-DRB5*0306* and *-DRB5*0313* alleles are marked in orange. The histidine at position 13 is marked in green. N means null-allele.

Figure 4.

Rooted phylogenetic comparison of the full-length chimpanzee (*Patr*, blue) cDNA sequences. Particular *HLA-DRB* alleles (red) extracted from the IMGT/HLA-database were included (Robinson et al., 2003), the sequences of *HLA-DRB1*110201* and *-DRB1*030201* were not full-length, but this did not alter the phylogenetic comparison. The *Caja-DRB*W1601* allele is taken as an outgroup (Doxiadis et al., 2006). Relevant bootstrap values are indicated. The brackets specify the different *DRB* loci/lineages. A * indicate the *HLA-DRB* alleles associated with resistance and ** indicate the alleles associated with susceptibility to HIV-1 infection found in a cohort of Kenyan sex workers.

Table 1
***Patr-DRB* haplotypes defined by *DRB*-STR genotyping and exon 2 sequencing for the *P.t.v.* subspecies (A) and *P.t.t.* (B) and *P.t.s.* subspecies (C)**

The haplotypes are arranged based on *DRB1* locus lineages; therefore, haplotype VII is situated between haplotype II and III. The last column specifies the number that each haplotype is present in the genotyped cohort. For alleles marked in yellow, a transcript has been found, and the full-length sequences are deposited at the EMBL database. Newly described exon 2 alleles are presented in bold. Data in parentheses indicate STR lengths detected only once or twice.

Haplotype	<i>DRB1</i> locus	STR	<i>DRB3</i> locus	STR	<i>DRB6</i> locus	STR	<i>DRB5</i> locus	STR	<i>DRB7</i> locus	STR	<i>DRB4</i> locus	STR	#
A <i>Pan troglodytes verus</i>													
Ia	<i>DRB1*0201</i>	207, 209, 211, 217	<i>DRB3*0201</i>	186	<i>DRB6*0108</i>	144	<i>DRB5*0301</i>	174					41
Ib	<i>DRB1*0201</i>	209	<i>DRB3*0208</i>	220	<i>DRB6*0109</i>	145	<i>DRB5*0306</i>	174					20
Ic	<i>DRB1*0201</i>	209	<i>DRB3*0208</i>	204	<i>DRB6*0108</i>	145	<i>DRB5*0304</i>	174					6
Id	<i>DRB1*0201</i>	205	<i>DRB3*0208</i>	202	<i>DRB6*0108</i>	145	<i>DRB5*0307</i>	174					7
Ie	<i>DRB1*0204</i>	205	<i>DRB3*0201</i>	188	<i>DRB6*0108</i>	144	<i>DRB5*0301</i>	174					6
If	<i>DRB1*0204</i>	205, 221, 225	<i>DRB3*0208</i>	208, 212	<i>DRB6*0109</i>	145	<i>DRB5*0101</i>	170					35
Ig	<i>DRB1*0204</i>	205	<i>DRB3*0208</i>	208 (200)	<i>DRB6*0109</i>	145	<i>DRB5*0102</i>	170					22
Ih	<i>DRB1*0204</i>	205	<i>DRB3*0208</i>	210	<i>DRB6*0109</i>	145	<i>DRB5*0103</i>	170					1
Ii	<i>DRB1*0214</i>	228	<i>DRB3*0103</i>	186	<i>DRB6*0109</i>	145	<i>DRB5*0101</i>	170					1
Ila	<i>DRB1*0302</i>	182	<i>DRB3*0102</i>	186	<i>DRB6*0305</i>	155	<i>DRB5*0312</i>	174					6
Ilb	<i>DRB1*030701</i>	164, 174	<i>DRB3*0102</i>	186	<i>DRB6*0305</i>	155	<i>DRB5*0301</i>	174					5
Ilc	<i>DRB1*030702</i>	166	<i>DRB3*0102</i>	186	<i>DRB6*0305</i>	155	<i>DRB5*0306</i>	174					2
VII	<i>DRB1*0305</i>	166, 168	<i>DRB3*0208</i>	214, 218, 222 (208)	<i>DRB6*0108</i>	145	<i>DRB6*0305</i>	155	<i>DRB5*0310</i>	174			38
IIla	<i>DRB1*0302</i>	190 (184)	<i>DRB3*0102</i>	186	<i>DRB6*0305</i>	155							3
IIlb	<i>DRB1*0309</i>	176	<i>DRB3*0102</i>	186	<i>DRB6*0305</i>	155							2
IIlc [§]	<i>DRB1*0309</i>	176	<i>DRB3*0703*</i>	190	<i>DRB6*0305</i>	155							3
IIId	<i>DRB1*0311</i>	184	<i>DRB3*0701*</i>	190	<i>DRB6*0305</i>	155							1
IVa	<i>DRB1*0701</i>	157							<i>DRB7*0101</i>	135	<i>DRB4*0104</i>	186	8
V	<i>DRB1*1001</i>	170	<i>DRB3*0208</i>	208	<i>DRB6*0108</i>	145	<i>DRB5*0310</i>	174					2
VI	<i>DRB1*1001</i>	168			<i>DRB6*0108</i>	145	<i>DRB5*0310</i>	174					4
B <i>Pan troglodytes troglodytes</i>													
Ij	<i>DRB1*0202</i>	226	<i>DRB3*0209</i>	206	<i>DRB6*0109</i>	145	<i>DRB5*0313</i>	195					2
Ik [§]	<i>DRB1*0215</i>	211	<i>DRB3*0208</i>	212	<i>DRB6*0109</i>	145	<i>DRB5*0311</i>	172					2
IIle	<i>DRB1*0308</i>	178	<i>DRB3*0102</i>	186	<i>DRB6*0305</i>	155							1
IVb	<i>DRB1*0702</i>	153							<i>DRB7*0101</i>	135	<i>DRB4*0104</i>	186	3
VIIla	<i>DRB1*0205</i>	214	<i>DRB3*0214</i>	182									1
IXa			<i>DRB3*0702*</i>	192, 194							<i>DRB4*0201</i>	138	2
C <i>Pan troglodytes schweinfurthii</i>													
II	<i>DRB1*0202[^]</i>	195	<i>DRB3*0208</i>	212	<i>DRB6*0109</i>	143 [§]	<i>DRB5*0311</i>	172					1
VIIlb	<i>DRB1*0213</i>	213, 217	<i>DRB3*0214</i>	182									2
IXb			<i>DRB3*0702[§]</i>	192							<i>DRB4*0201</i>	138	1

[§] No cell-line available for this haplotype.

* The alleles *Patr-DRB3*0701/0702/0703* were previously named *Patr-DRB*W901/W902/W903*, respectively.

[^] Allele sequenced using *Patr-DRB1*02* specific primers (5'ACGTTTCCTGTTGCAGCC3' and 5'CCCCGTAGTTGTGTCTGC3') and expected to be linked to the STR allele 195.

[§] The STR length represents a sequencing result and therefore differs from the other *DRB6*0109* STR length.