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Sequence and expression of MHC-DPB1 molecules of the New World monkey *Aotus nancymaae*, a primate model for *Plasmodium falciparum*

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Abstract *Aotus nancymaae* represents an animal model for the pre-clinical evaluation of blood-stage vaccine candidates against *Plasmodium falciparum* and *Plasmodium vivax*. We present here the nucleotide sequences of exon 2 and 3 of *MHC-DPB1* genes. In a group of seven unrelated animals captured in the wild, three alleles of *MHC-DPB1* exon 2 could be identified. Phylogenetic analysis shows that in contrast to *Aona-DRB* and *-DQB*, the *Aona-DPB1* exon 2 amino acid sequences cluster in a species-specific manner. No evidence could be found for the conservation of allelic lineages pre-dating the divergence of Old and New World monkeys. Additionally, two nucleotide sequences of *MHC-DPB1* exon 3 could be identified differing in one synonymous base exchange. Phylogenetic analysis of *Aona-DPB1* exon 3 amino acid sequence shows that it clusters together with human sequences separately from the New World monkey *Saguinus oedipus*. *Aona*-DP heterodimers are expressed on the surface of *Aotus* cells, as detected by staining with a cross-reactive monoclonal antibody, and can therefore present antigenic peptides to the cellular immune system.

Keywords *Aotus nancymaae* · Malaria · *Platyrrhini* · *Catarrhini* · *MHC-DPB1*

The nucleotide sequence data reported in this paper have been submitted to the EMBL/GenBank nucleotide sequence databases and have been assigned the accession numbers AF486448 to AF486450.

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Introduction

The protective function of T cells depends on their ability to recognize cells that are harboring pathogens or that have internalized pathogens or their products. T cells recognize antigens in form of complexes consisting of peptides and molecules encoded in the major histocompatibility complex (MHC) (Zinkernagel and Doherty 1974). Proteins belonging to MHC class I and class II molecules collect, transport, and present peptides on the cell surface, where the peptide-MHC complexes are continuously surveyed by the cellular immune system (Kappes and Strominger 1988). Three MHC class II loci, named *HLA-DR*, *-DQ*, and *-DP*, produce functional antigen-presenting heterodimers in humans. Each heterodimer is made up of one α and β glycopeptide chain (Kappes and Strominger 1988). MHC class II molecules are highly polymorphic and the polymorphism is largely confined to the second exon of both chains encoding the functional domains that form together the peptide-binding region of the molecule (Jones 1997).

Aotus spp. belonging to the New World monkeys (*Platyrrhini*) have been shown to be susceptible to various infectious diseases affecting mankind, such as bilharziasis, leishmaniasis, and hepatitis A (Lujan et al. 1986; Noya et al. 1998; Polotsky et al. 1994). *Aotus nancymaae* sustains in a predictable way infections with the apicomplexan parasite *Plasmodium falciparum* without prior splenectomy, and the World Health Organization recommended this model to test the efficacy of malaria blood-stage vaccine candidates (Gysin 1998). The evaluation of protection conferred by immunization of *A. nancymaae* with protein sequences derived from the *P. falciparum* asexual blood stages and different vaccine formulations against experimental challenges has been one of the crucial steps in the development of blood-stage vaccine candidates (Chang et al. 1996; Herrera et al. 1992; Jones et al. 2001; Siddiqui et al. 1987; Sim et al. 2001; Stowers and Miller 2001; Stowers et al. 2001). However, it is now debated whether New World monkeys can in fact model critical human immune responses to blood-

stage malaria antigens and whether they should be therefore necessarily included in the developmental pathway of blood-stage vaccine candidates (Heppner et al. 2001; Stowers and Miller 2001). A direct comparison between immune responses of *Aotus* monkeys and humans mounted against identical antigen preparations has not been conducted. Additionally, the knowledge of the immunogenetic background of *Aotus* monkeys and the availability of reagents to study immune responses is highly limited when compared with humans (Heppner et al. 2001). In order to evaluate the suitability of this non-human primate model for the pre-clinical evaluation of potential vaccine candidates, we have recently conducted a series of studies aimed at the systematic characterization of the immunogenetic background of *A. nancymaae* (Daubenberger et al. 2001b; Diaz D et al. 2000; Diaz OL et al. 2000; Favre et al. 1998; Nino-Vasquez et al. 2000; Vecino et al. 1999). The building blocks of the synthetic malaria vaccine SPf66 were defined using systematic protection studies in *Aotus* monkeys (Patarroyo et al. 1987), and this vaccine has been extensively tested in human trials (Graves and Gelband 2000). T-cell clones from two SPf66-immunized volunteers specific for one of the components of SPf66, a peptide derived from the N-terminus of the merozoite surface protein 1 (MSP-1), have been established and characterized (Daubenberger et al. 2001a). The MSP-1-specific responses were restricted by both HLA-DR and HLA-DP molecules (Daubenberger et al., unpublished results). The restriction of MSP-1-specific T-cell responses by HLA-DP molecules prompted us to investigate the presence and polymorphism of *MHC-DPB1* nucleotide sequences in *A. nancymaae*. We demonstrate here that in a group of seven *A. nancymaae* animals limited sequence polymorphism of exon 2 and 3 *MHC-DPB1* gene segments could be found. The phylogenetic analysis conducted with *MHC-DPB1* exon 2 amino acid sequences of Old and New World monkeys indicates that these *MHC-DPB1* genes are located on a separate branch. Hence, *Aona-DPB1* genes might evolve rapidly, leading to a loss of the trans-species conservation of sequence motifs. The expression of *Aona*-DP molecules on the cell surface was confirmed by using cross-reactive anti-HLA-DP monoclonal antibody and fluorescence-activated cell sorting analysis (FACS).

Materials and methods

Nomenclature

Official designations for the *Aona-DPB1* alleles were obtained from R.E. Bontrop and Natasja G. De Groot (Biomedical Primate Research Centre-TNO, Rijswijk, The Netherlands). They are based upon shared sequence motifs, phylogenetic analysis and comparison with sequences that are found in other New World monkeys. In accordance with the proposed nomenclature for MHC in non-human species, *MHC-DPB1* from *A. nancymaae* are designated as *Aona-DPB1* alleles (Klein et al. 1990).

Animals

Animals caught in the Colombian Amazon area close to Leticia were selected at random to ensure the presence of a representative repertoire of different alleles. Leukocytes from seven healthy *A. nancymaae* monkeys were obtained either by density gradient separation of peripheral blood obtained by venous puncture or by splenectomy as described previously (Garraud et al. 1994). The animals were kept in accordance with the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, National Research Council, National Academy of Science Press, Washington D.C., 1996).

PCR, cloning, and sequencing of exon 2 and 3 of *Aona-DPB1* gene segments

Attempts to amplify *Aona-DPB1* exon 2 sequences with a primer pair suitable for the amplification of *HLA-DPB1* exon 2 sequences (5′-GCTGCAGGAGAGTGGCGCCTCCGCTCAT-3′ and 5′-CGGATCCGGCCCAAAGCCCTCACT-3′) (Bugawan et al. 1991) failed. Therefore, a strategy was devised based on the amplification of a fragment encompassing exons 2 and 3 using the primer pair sense (5′-AGGGATCCCCGCAGAGGATTTCGTGTACC-3′) (Bugawan and Erlich 1991) and anti-sense (5′-GTGCTCCACGTGGCAGGTGTAGAC-3′) binding to a conserved region in human exon 2 and 3 sequences. Amplifications were performed with the following profile: 2 min 95°C; 33×(30 s 96°C, 30 s 55°C, 30 s 72°C); 7 min 72°C; soak at 4°C. For amplification cDNA derived from cells of monkeys 11190, 11192, 17999, 18058, 18091, 18094, and 18095 were used. Total RNA was isolated from PBMC using the NucleoSpin RNA kit (Machery-Nagel, Oensingen, Switzerland) according to the manufacturer's protocol. After reverse transcription using Superscript and oligo $(dT)_{16}$ primer (Gibco-BRL Life Technologies, Basle, Switzerland), PCR products were purified using a PCR product purification kit (Roche Molecular Biochemicals, Rotkreuz, Switzerland) according to the manufacturer's protocol and cloned into the pGEM5 T-vector (Promega, Catalys, Wallisellen, Switzerland). After isolation of plasmids using the Nucleo Spin kit (Machery-Nagel, Oensingen, Switzerland), double-stranded plasmid DNA was sequenced and analyzed employing an ABI PRISM 310 genetic analyzer (Perkin Elmer, Foster City, Calif., USA) and the ABI PRISM Sequencing Analysis 3.3 and MT Navigator 1.0.2. software. The reported alleles represent the consensus sequence of at least three identical sequences that were obtained after independent amplifications from the same animal or at least two sequences derived from two or more different animals.

Phylogenetic analyses

Phylogenetic analysis was performed employing the PHYLIP 3.572 software package available under http://bioweb.pasteur.fr. The phylogenetic tree was constructed according to the neighbor-joining method based on Kimura two-parameter distances estimates (Kimura 1980; Saitou and Nei 1987). In accordance with the proposed nomenclature for MHC in non-human species, *DPB1* alleles from *Homo sapiens* are referred to as *HLA-DPB1*, *Pan troglodytes* as *Patr-DPB1*, *Pan paniscus* as *Papa-DPB1*, *Gorilla gorilla* as *Gogo-DPB1*, *Pongo pygmaeus* as *Popy-DPB1*, *Macaca mulatta* as *Mamu-DPB1*, *Saguinus oedipus* as *Saoe-DPB1*, and *A. nancymaae* as *Aona-DPB1* (Klein et al. 1990).

Flow cytometric analysis of activated lymphocytes of *A. nancymaae*

Spleen cells of *A. nancymaae* were diluted to 1×10^6 cells/ml in culture medium and cultivated in culture medium in 48-well plates (Nunc) in the presence of 1 mg/ml phytohemagglutinin (PHA) plus 100 units/ml recombinant human interleukin-2 (rhIL-2) essentially as described (Daubenberger et al. 2001b). Culture medium consist-

ed of RPMI 1640, 10% heat-inactivated human AB serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 100 units/ml penicillin, 100 µg/ml streptomycin, and 1 mM non-essential amino acids. Cells were recovered from the wells 14 days after in vitro cultivation and stained for MHC-DR, -DP, and -DQ expression with a series of cross-reactive monoclonal antibodies (mAbs) specific for defined HLA class II isotypes. Briefly, cells were resuspended in Hanks' balanced salt solution containing 1% bovine serum albumin and 0.01% sodium nitrite (FACS buffer) at a concentration of 5×10^6 cells/ml and 100 µl was dispensed in every FACS tube. After centrifugation, the supernatant was discarded and the cells were mixed with 100 µl of a 1:5 diluted hybridoma supernatant containing antibodies specific for human cell surface antigens. After incubation at 4°C for 30 min, the cells were washed once with FACS buffer, resuspended in 100 µl of appropriately diluted goat-anti IgG mouse fluorescein isothiocyanate-conjugated antibody (Sigma) and incubated for another 30 min on ice. After three washing steps, the cells were resuspended in 100 µl of FACS buffer. Unstained cells and cells incubated with secondary reagent only were included as controls. Fluorescence was measured on a FACScan (Becton Dickinson). Cells were gated using forward and side scatter parameters for dead cell exclusion. In each sample, 10,000 events were measured and data were analyzed using CellQuest (Becton Dickinson) to determine the frequencies and mean fluorescence intensities. The antibodies used included: anti-HLA-DR (L243), anti-HLA-DP (B7/21), anti-HLA-DQ (SPV-L3), and anti-pan HLA-class II (HB145). Hybridoma cell lines secreting these antibodies were obtained from American Type Culture Collection (Manassas, Va., USA).

Results

Activated lymphocytes of *A. nancymaae* express homologues of MHC-DR, -DQ, and -DP molecules

At present, only a highly limited number of nucleotide sequences of *MHC-DP* alleles of non-human primates are available (Bontrop et al. 1999) and several attempts to trace the existence of MHC-DP molecules in the New World monkey *Callitrix jacchus* have failed (Antunes et al. 1998). In order to establish whether *A. nancymaae* expresses MHC-DP molecules, we tested a series of mAbs against human MHC class II antigens for cross-reactivity with the *Aotus* homologues. Spleen cells of three animals (11190, 17999, and 18058) were expanded in vitro for 14 days by stimulation with PHA and rhIL-2, immunostained, and analyzed by flow cytometry (Daubenberger et al. 2001b). For comparison, one human EBV-LCL established and maintained in our laboratory was included in the analysis. Representative results of the FACS analyses are shown in Fig. 1. The HLA class II-specific mAbs L243 (HLA-DR), B7/21 (HLA-DP), SPV-L3 (HLA-DQ), and HB145 (pan class II) seem to be reactive with framework determinants that are conserved between humans and *Aotus* class II isotypes. Both in *Aotus* and humans the surface expression of MHC-DR and -DQ was higher than of -DP.

Analysis of nucleotide sequence polymorphism of *Aona-DPB1* exon 2

As a next step we analyzed the nucleotide sequence polymorphism of *Aona-DPB1*. We failed to amplify *Aona-DPB1* exon 2 gene segments using primer pairs described for the amplification of *HLA-DPB1* and switched to a primer combination that amplifies from cDNA a gene segment encompassing *Aona-DPB1* exons 2 and 3. A total of 22 sequences were generated from seven unrelated animals and three different alleles of *Aona-DPB1* exon 2 were found among these. Each of the alleles was obtained from at least two different animals. The derived exon 2 *Aona-DPB1* nucleotide sequences are aligned with nucleotide sequences of selected *MHC-DPB1* alleles from human and non-human primates (Fig. 2a). The corresponding amino acid sequences are shown in Fig. 2b. None of the sequences derived from *Aotus* display features that would suggest that they are pseudogenes. The three alleles can be divided into two groups, differing from each other in a sequence motif of three amino acids located at position 45–47 (YLA vs FRS). The two alleles belonging to same group (*Aona-DPB1*02* and *Aona-DPB1*03*) differ in a single non-synonymous point mutation at amino acid position 88 (Fig. 2a, b). Interestingly, the amino acids present at positions 10–13 in all *MHC-DPB1* alleles described to date are missing in *Aotus*. The BLAST program was used to compare the new *Aotus* alleles with human *DPB1* exon 2 amino acid sequences deposited in the

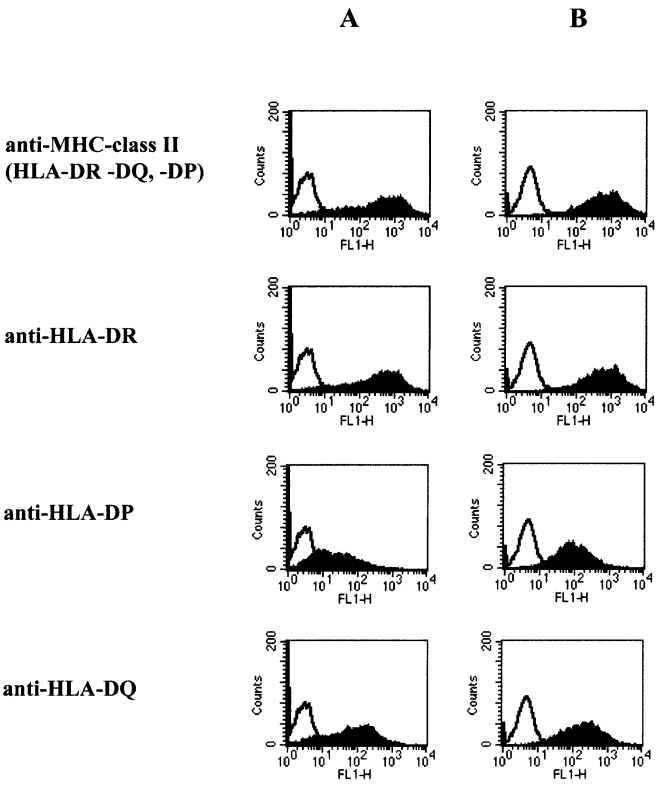

Fig. 1 Flow cytometric analysis of the MHC class II antigen expression by stimulated *Aotus nancymaae* peripheral blood mononuclear cells (animal 11190) (**a**) and human EBV-LCL (**b**). The *filled graphs* represent staining with HLA class II-specific monoclonal antibodies (mAbs), while the *open graphs* depict the background fluorescence with the secondary mAb only. mAbs included in this study are pan anti-class II (HB145), anti-HLA-DR (L243), anti-HLA-DP (B7/21), and anti-HLA-DQ (SPV-L3)

a

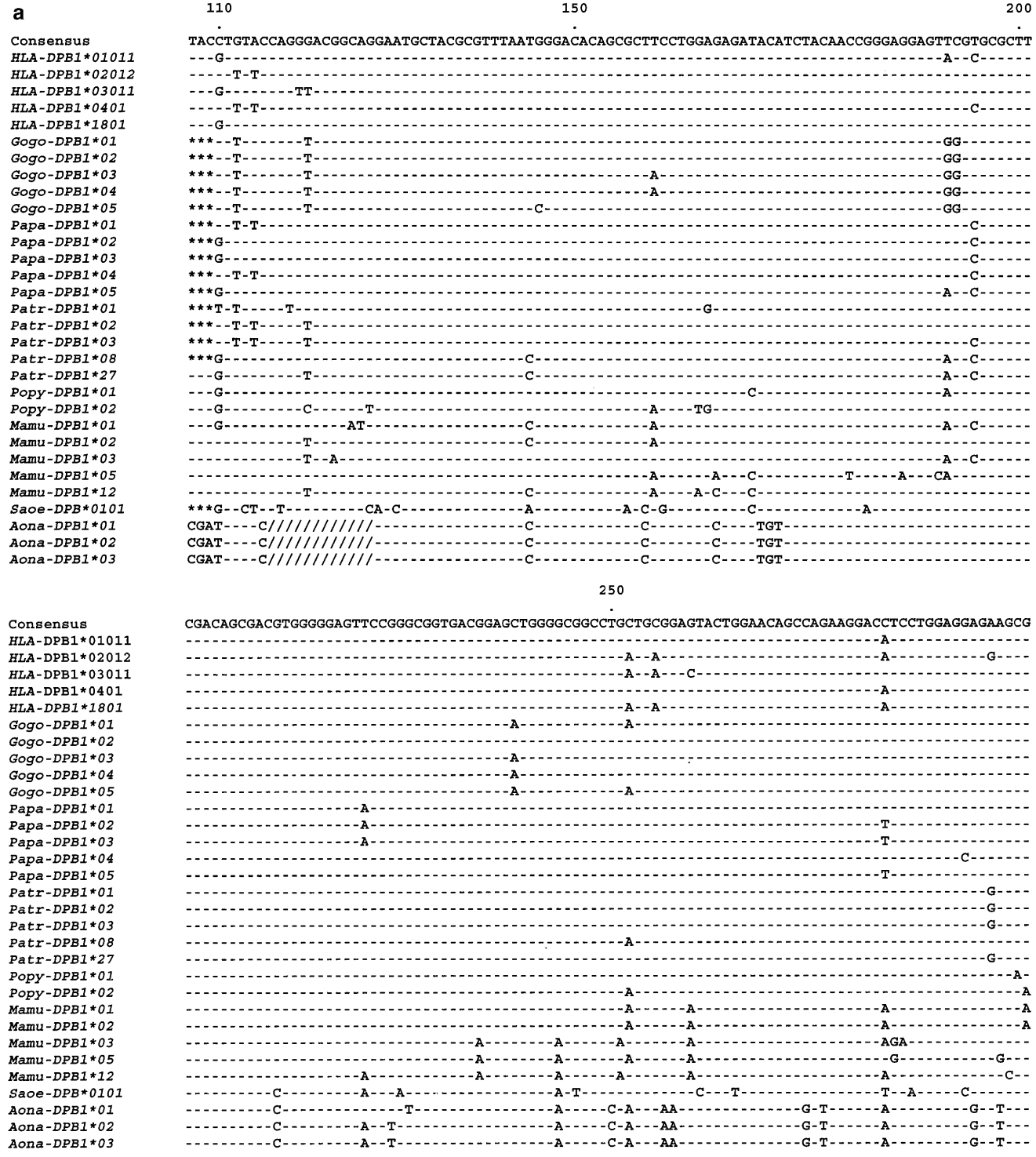

Fig. 2 **a** Alignment of nucleotide sequences of *MHC-DPB1* exon 2 alleles derived from human (accession numbers in ImMunoGeneTics/HLA database at http://www.ebi.ac.uk/hla; HLA00514, HLA00517, HLA00520, HLA00521, HLA00535), common chimpanzee (*P. troglodytes*, *Patr*, Genbank accesion numbers U38865, U38866, U38646, U38871, AF024559), pygmy chimanzee (*P. paniscus*, *Papa*, GenBank accession numbers U38879–U38883), Gorilla (*gorilla gorilla*, *Gogo*, GenBank accession numbers U38885–U38889), orang utan (*P. pygmaeus*, *Popy*, GenBank accession number AF024552, AF024553), rhesus macaque (*M. mulatta*, *Mamu*, GenBank accession numbers Z32402–Z32404, Z32409, Z32413), and cotton-top tamarin (*S. oedipus*, *Saoe*, GenBank accession number AF027966). A simple majority consensus sequence is given at the *top*. The *dash (–) marks* identity with the consensus sequence, *slash (/)* deletion of a nucleotide base, and *asterisk (*)* lack of availability of sequence information. The numbering corresponds to the numbering system used in the ImMunoGeneTics database (http://imgt.cines.fr:8140). **b** Alignment of deduced amino acid sequences of *MHC-DPB1* exon 2 alleles derived from humans. For information on the accession numbers see Fig. 2a. A simple majority consensus sequence is given at the top. *Dash (–)* indicates identity with the consensus sequence, *slash (/)* deletion of nucleotide bases, and *asterisk (*)* lack of availability of sequence information. The numbering corresponds to the numbering system used in the ImMunoGeneTics database

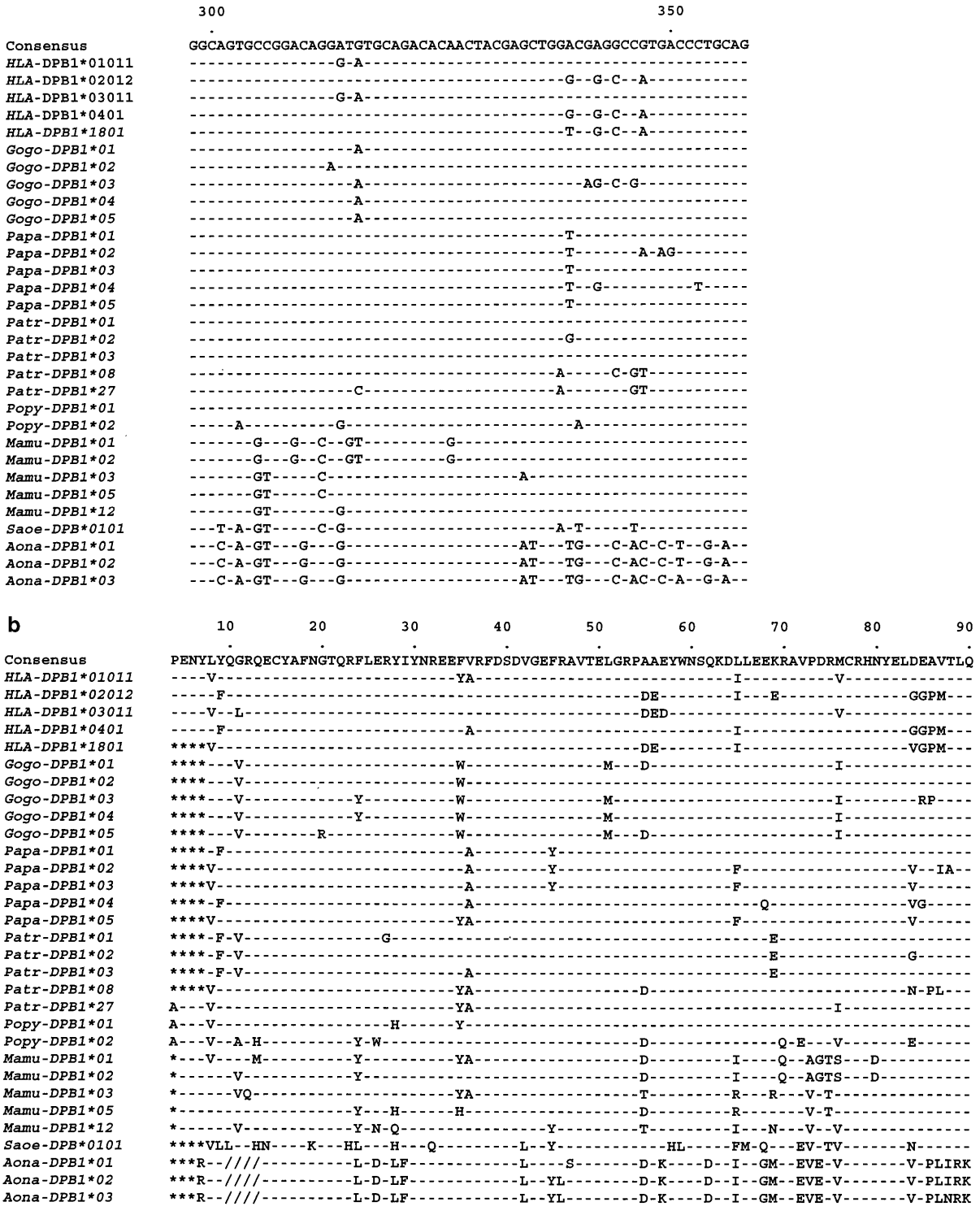

Fig. 2 continued

ImMunoGeneTics/HLA database at http://www.ebi.ac.uk/hla. The *HLA-DPB1* allele displaying the closest amino acid identity with the *Aotus* homologues was *HLA-DPB1*1801* (77%). The sequence motif PLI/NRK at positions 86–90 at the C-terminus of *Aona-DPB1* exon 2 sequences is unique to *A. nancymaae*, explaining the failure to amplify these gene segments with primers suitable for *HLA-DPB1*. A high proportion of the polymorphic sequence motifs are not conserved between *Aotus* and the other species aligned, except for the motif at position 35–36 (FV) that can be found in alleles of human and *Aotus* (Fig. 2b).

The phylogenetic relationship among *MHC-DPB1* exon 2 alleles of human and non-human primates is depicted in Fig. 3. The tree shows that the *DPB1* alleles of New World monkeys cluster together on one branch. The majority of the sequences derived from *M. mulatta*, *G. goril-*

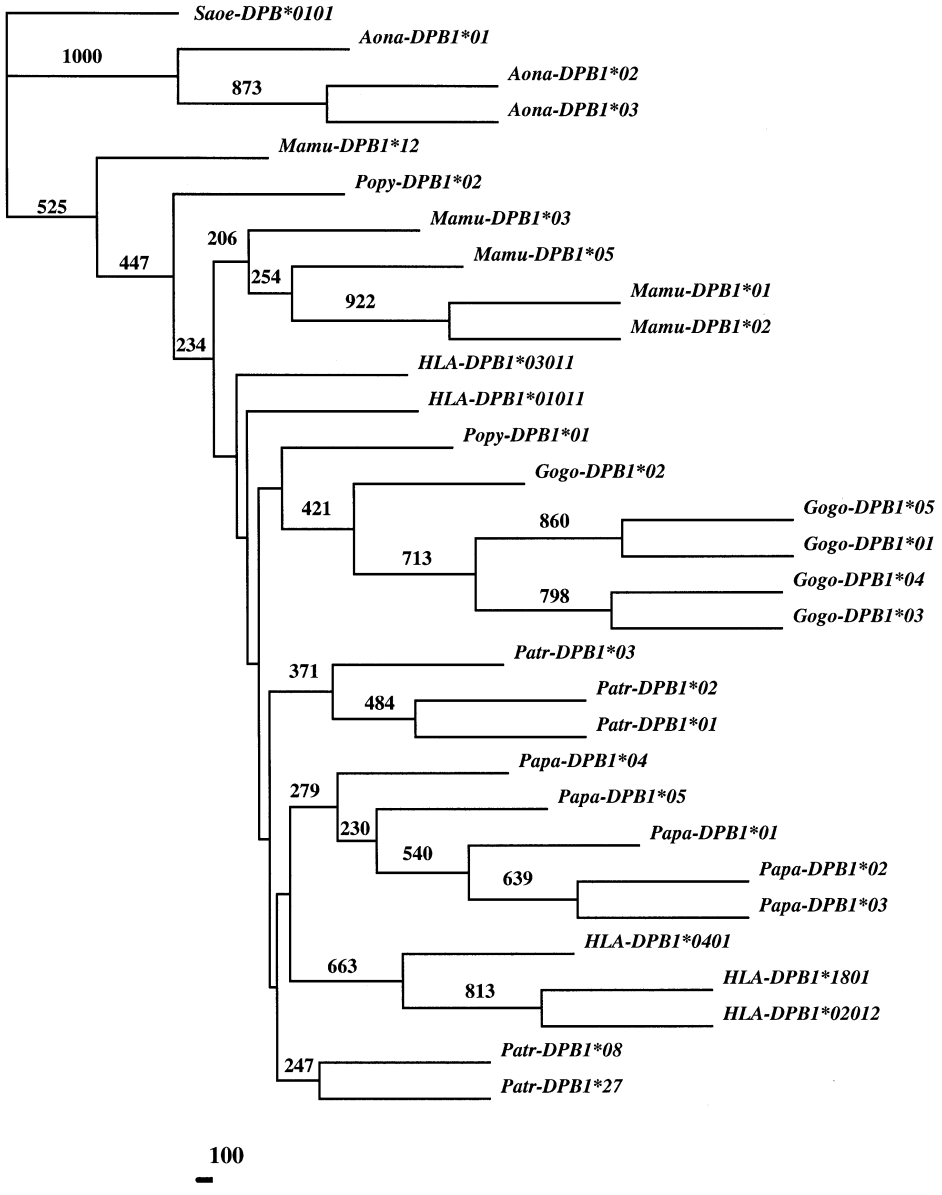

Fig. 3 Phylogenetic tree constructed according to the neighbor-joining method (Saitou and Nei 1987). The tree was constructed from the amino acid sequences given in Fig. 2b using the neighbor-joining algorithm of the PHYLIP 3.572 program package available at http://bioweb.pasteur.fr. The tree was rooted using the *S. oedipus* sequence as the outgroup. The numbers at the nodes indicate the percentage of recovery of that node in 1,000 bootstrap replications

la, orangutan, and chimpanzee species cluster together in a species-specific fashion on separate branches (Fig. 3).

Analysis of nucleotide sequence polymorphism of *Aona-DPB1* exon 3

We have identified two different exon 3 gene segments of *Aona-DPB1* in 22 nucleotide sequences derived from seven animals. *Aona-DPB1*01* and *Aona-DPB1*02* differ in exon 3 in one synonymous point mutation at nucleotide sequence position 531 (Fig. 4a). *Aona-DPB1*02* and *Aona-DPB1*03* share identical exon 3 nucleotide sequences. The corresponding deduced amino acid sequence aligned with representative sequences of human and *S. oedipus* are depicted in Fig. 4b. The *HLA-DPB1* exon 3 sequences displaying the closest amino acid identity with *Aotus* are *HLA-DPB1**01011 and *HLA-DPB1*03011* (97%). The phylogenetic analysis demonstrated that compared with the cotton-top tamarin the *A. nancymaae*-derived sequence is located on a separate branch between human-derived alleles (Fig. 5). The sequences of *S. oedipus* are functional alleles taken from Kriener et al. (2001) and follow a tentative designation in form of roman numerals.

Discussion

Pre-clinical evaluation of malaria vaccine candidates in one of the two New World monkey species susceptible to *P. falciparum* (*Aotus* spp. and *Saimiri* spp.) provides efficacy data prior to the costly production of clinical-grade material, reduces the need for extensive field trials, and provides data to develop and validate in vitro surrogate markers of protection (Stowers and Miller 2001). However, one of the pre-requisites for the inclusion of this

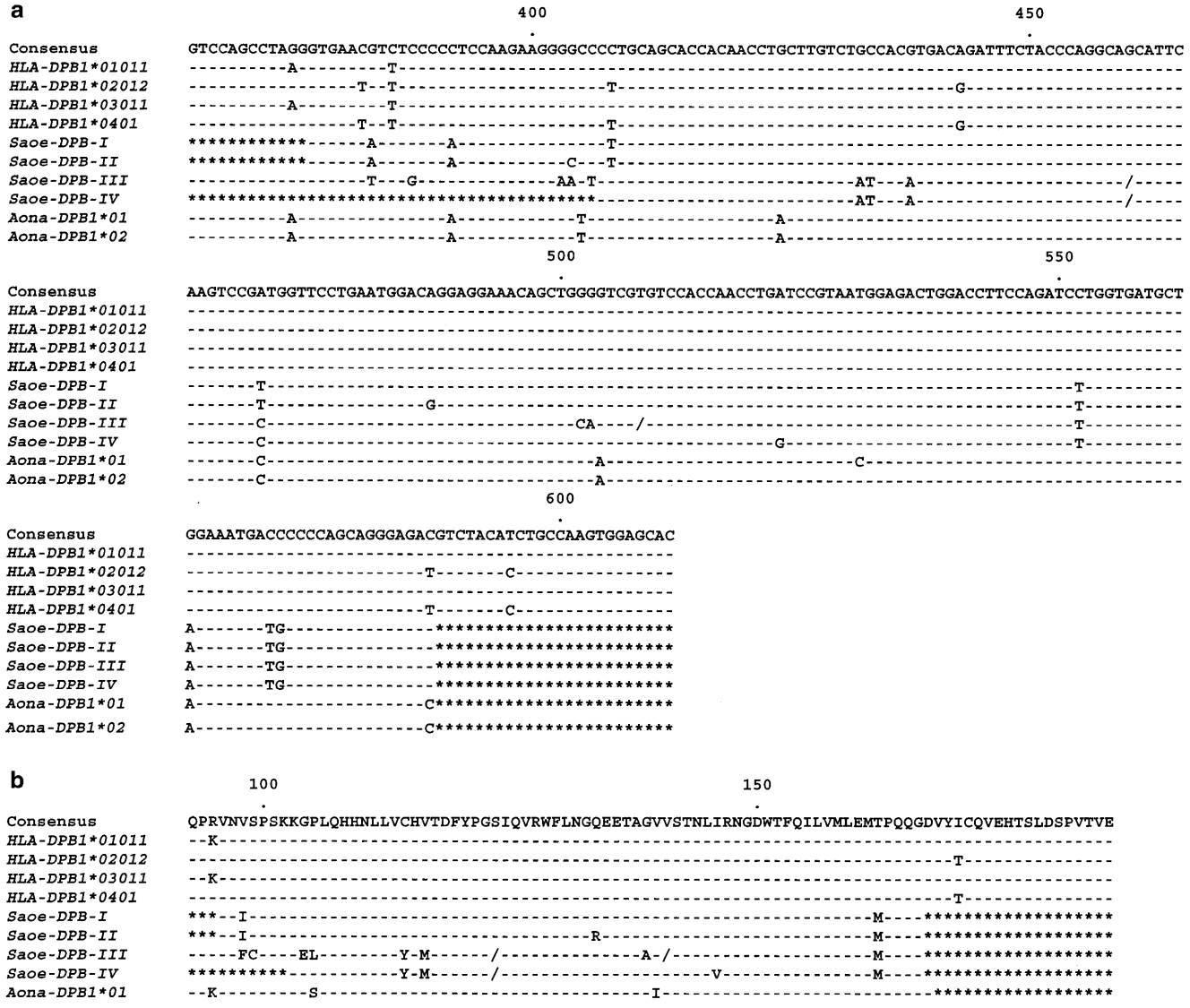

Fig. 4 **a** Alignment of nucleotide sequences of *MHC-DPB1* exon 3 of human, *A. nancymaae*, and cotton-top tamarin. A simple majority consensus sequence is given at the top. *Dash (–)* indicates identity with the consensus sequence, *slash (/)* deletion of nucleotide bases, and *asterisk (*)* lack of availability of sequence information. Numbering starts with first nucleotide of the exon 3 according to *HLA-DPB1* sequences. The sequences were obtained from the GenBank under the accession numbers AY013369, AY013370, AY013371, AY013373, and the ImMunoGeneTics/HLA database at http://www.ebi.ac.uk/hla (HLA00517, HLA00521, HLA00514, HLA00520). **b** Alignment of *MHC-DPB1* exon 3 deduced amino acid sequences of human, *A. nancymaae*, and cotton-top tamarin. A simple majority consensus sequence is given at the top. *Dash (–)* indicates identity with the consensus sequence, *slash (/)* deletion of a nucleotide base, and *asterisk (*)* lack of availability of sequence information. Numbering of amino acid positions is according to *HLA-DPB1* sequences. The sequences were obtained from the GenBank under the accession numbers AY013369, AY013370, AY013371, AY013373, and the ImMunoGeneTics/HLA database (http://www.ebi.ac.uk/hla; HLA00517, HLA00521, HLA00514, HLA00520). The conventional amino acid one-letter code is shown

approach into the development strategy of blood-stage vaccines against malaria is that the immune systems of human and *Aotus* recognize and mount comparable immune responses against the candidates investigated (Stowers and Miller 2001).

In the present study we show that *Aotus* expresses MHC-DPB1 molecules on the surface of activated lymphocytes. Staining with cross-reactive mAbs specific for HLA-DP demonstrates the expression of *Aona*-DP on the cell surface of in vitro stimulated lymphocytes. RT-PCR sequencing analysis reveals a limited polymorphism of *Aona-DPB1* exon 2 gene segments with three alleles identified in a population of seven animals analyzed. As a unique feature, four amino acid residues at positions 10–13 are lacking when the new *Aona-DPB1* alleles were compared with all other *MHC-DPB1* alleles described to date. In humans, the *DPB1* locus is the second most polymorphic class II locus next to *DRB1*. This is clearly in contrast to *Aotus* monkeys where the three alleles differ mainly in a single sequence motif encompassing three amino acids. In humans and rhesus monkeys it has been

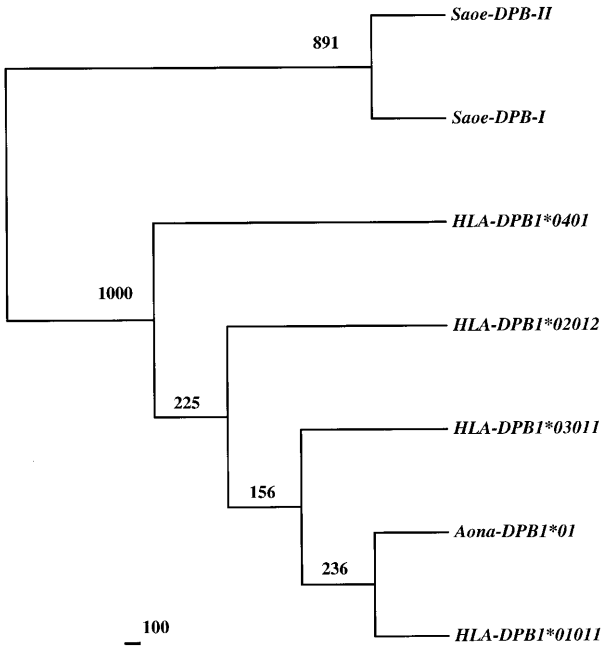

Fig. 5 Phylogenetic tree constructed according to the neighbor-joining method showing the relationship of exon 3 sequences between the identified *Aona-DPB1* allele and selected functional *MHC-DPB1* alleles from *S. oedipus* and human (Saitou and Nei 1987). The tree was constructed from the amino acid sequences given in Fig. 4b using the neighbor-joining algorithm of the PHYLIP 3.572 program package available at http://bioweb.pasteur.fr. The tree was rooted using the *S. oedipus* sequences as the outgroup. The numbers at the nodes indicate the percentage of recovery of that node in 1,000 bootstrap replications

reported that the *MHC-DPB1* alleles are not evenly distributed, but single predominant alleles are usually found in separate populations (Gyllensten et al. 1996; Otting et al. 1998). Therefore, the identification of three alleles in a group of seven monkeys caught in the region around Leticia could be a reflection of a similar phenomenon.

The organization of the *MHC-DP* region has been shown to be remarkably stable and shared between hominoids, Old and New World monkeys (Bontrop et al. 1999). However, the phylogenetic analysis of *MHC-DPB1* exon 2 sequences demonstrates that *Aotus* expresses novel sequences compared with other non-human primate species and humans included in the analysis. The only described *DPB1* exon 2 sequence derived from another New World monkey is located on the same branch as the *Aotus* sequences and bootstrap values indicate that this localization is fairly robust.

The major biological role of the MHC is to protect the host from invaders by contributing to the development of T cells specific for pathogen-derived peptide sequences. Polymorphism at the MHC loci ensures that one pathogen cannot exterminate a complete population by avoiding the recognition through loss of MHC binding. Therefore, lineages that have a beneficial effect on the host might be conserved over long evolutionary distances, as has been described for the *MHC-DR3* cluster, which might be involved in the presentation of conserved bacterial heat shock proteins (Elferink et al. 1993). Other loci might evolve more rapidly in response to changes in the microbial environment. The species-specific clustering of *MHC-DPB1* exon 2 sequences in the phylogenetic tree is in contrast to the clustering observed with *MHC-DRB* and *-DQB* (Diaz D et al. 2000; Nino-Vasquez et al. 2000). Therefore, the *MHC-DPB1* locus might, just like the *HLA-B* locus, evolve more rapidly than the *MHC-DR* and *-DQ loci*. It is conceivable that the polymorphism of *MHC-DPB1* has been generated within the life-span of an individual species and that the trans-species conservation of allelic lineages is difficult to trace (Otting et al. 1998; Slierendregt et al. 1995).

The similarity between the *Aotus DRB* and *-DQB* and Catarrhini exon 2 sequences described previously (Diaz D et al. 2000; Nino-Vasquez et al. 2000) could be explained as a result of convergent evolution driven by positive selection for repeated but independent creation of similar sequence motifs, as also suggested by others (Kriener et al. 2000, 2001). Exon 3 of the MHC class II isotypes is not subject to extensive evolutionary pressure but displays polymorphism suitable to distinguish different genes. To our knowledge, non-human primate *MHC-DPB1* exon 3 sequences have only been reported from the cotton-top tamarin (Kriener et al. 2001). The phylogenetic analysis of *MHC-DPB1* exon 3 sequences shows that the sequences of *S. oedipus* cluster together in a mono-phyletic group while the *A. nancymaae* and human sequences form a separate group. Hence, the results of this phylogenetic study do not support the view that some MHC class II lineages shared between Catarrhini and Platyrrhini might be paralogous rather than orthologous lineages (Kriener et al. 2000, 2001). However, we have not analyzed intron sequences and therefore more investigations are needed to establish the evolutionary relationship between Catarrhini and Platyrrhini MHC loci.

In summary, we present novel sequences of *Aona-DPB1* exon 2 and 3 gene segments. The polymorphism of *Aona-DPB1* exon 2 sequences seems to be limited and in contrast to *Aona-DRB1* and *-DQB* exon 2 sequences the trans-species character of *Aona-DPB1* is obscured. *Aona*-DP heterodimers are expressed on the surface of *Aotus* cells, as detected by staining with cross-reactive mAbs, and are therefore functional in the presentation of peptides to the cellular immune system.

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