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Sequence and diversity of MHC *DQA* and *DQB* genes of the owl monkey *Aotus nancymaae*

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Abstract The New World primate Aotus nancymaae has been recommended by the World Health Organization (WHO) as a model for evaluation of malaria vaccine candidates, given its susceptibility to experimental infection with the human malaria parasites Plasmodium falciparum and Plasmodium vivax. We present here the nucleotide sequences of the complete cDNA of MHC-DQA1 and of the polymorphic exon 2 segments of MHC-DQB1/DQB2. In a group of three nonrelated animals captured in the wild, five alleles of MHC-DQA1 could be identified. They all belong to one lineage, namely Aona-DQA1*27. This lineage has not been described in any other New World monkey species studied. In a group of 19 unrelated animals, 14 Aona-DQB1 alleles could be identified which are grouped into the two lineages Aona-DQB1*22 and Aona-DQB1*23. These lineages have been described previously in the common marmoset and cotton-top tamarin. In addition, two Aona-DQB2 sequences could be identified which are highly similar to HLA-DQB2 sequences. Essential amino acid residues contributing to MHC DQ peptide binding pockets number 1 and 4 are conserved or semi-conserved

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 AF201293 – AF201297 and AF213629 – AF213644

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M. Naegeli · G. Pluschke · C.A. Daubenberger (⊠) Swiss Tropical Institute, Socinstrasse 57, 4002 Basel, Switzerland E-mail: Claudia.Daubenberger@unibas.ch Phone: +41-61-2848236 Fax: +41-61-2718654 between HLA-DQ and Aona-DQ molecules, indicating a capacity to bind similar peptide repertoires. These results fully support the use of *Aotus* monkeys as an animal model for evaluation of future subunit vaccine candidates.

Key words Actus nancymaae \cdot MHC class II DQ genes \cdot Allelic lineages \cdot Polymorphism \cdot Peptide binding

Introduction

The function of major histocompatibility complex (MHC) class I and class II molecules is to collect peptide fragments inside the cell and transport them to the cell surface, where the peptide-MHC complex is surveyed by the immune system (Germain and Margulies 1993). In humans, three MHC class II loci, called HLA-DR, -DQ and -DP, produce functional antigen-presenting heterodimers. Each class II heterodimer is made up of the non-covalent association of two glycopeptide chains: the α chain and the β chain, encoded by, for example, for DQ, DQA and DQBgenes. MHC DQA and DQB genes are described in various nonhuman primate species (Bontrop 1994). Some MHC DQB lineages are at least 30 million years old and predate the divergence of hominoid and Old World primate species (Otting et al. 1992). Two sets of closely related gene DQ pairs have been identified in humans: DOA1-DOB1 and DOA2-DOB2. The polymorphic DQA1-DQB1 genes encode the MHC DQ molecules, whereas product of the а DQA2-DQB2 genes has not been identified in humans (Bontrop et al. 1999). Polymorphism of MHC DQ molecules appears to be responsible for variations in the immune responses of individuals to antigens and may contribute to susceptibility or resistance against infectious diseases and autoimmune disorders (Hill 1998; Todd et al. 1987).

The contemporary living primates can be classified into New World monkeys (Platyrrhini) and Old World simians (Catarrhini). The New World monkeys and the Old World simians radiated about 58 million years ago (Ciochon and Chiarelli 1980). One of several species of New World monkeys employed in biomedical research over the past several decades is *Aotus spp*, which can sustain in a predictable way the development of asexual forms of the two major human malaria parasites, *Plasmodium falciparum* and *Plasmodium vivax* from different geographic areas (Gysin 1998). In 1988, the WHO recommended *Aotus spp*. as an experimental model for *P. falciparum* blood-stage infections and for the evaluation of candidate malaria vaccines (Gysin 1998).

The immunogenetic background of *Aotus* monkeys has been investigated to confirm the suitability of this model for the evaluation of potential peptide vaccine candidates (Patarroyo et al. 1987). After the characterization of the *TCRAV* and *TCRBV* repertoire (Favre et al. 1998; Vecino et al. 1999) and the exon 2 of MHC *DRB* genes (Nino-Vasquez et al. 2000), we present here results of an analysis of the *DQA1* (*MhcAona-DQA1*) and *DQB1/2* (*MhcAona-DQB1* and *MhcAona-DQB2*) genes of *A. nancymaae*.

Materials and methods

Animals

The animals analyzed in this study were caught in the Colombian Amazon area close to Leticia and were kept at the monkey colony of the Instituto de Inmunologia. Mononuclear cells from 19 healthy monkeys were obtained by femoral venous puncture and density gradient separation using Ficoll hypaque, or by spleenectomy followed by density gradient separation as described (Garraud et al. 1994). One B lymphoblastoid cell line was established from monkey 3026 by transformation with Epstein-Barr virus.

Amplification of Aona-DQA1 genes by polymerase chain reaction

Total RNA was isolated from spleen cells of monkeys 11190, 11192 and 11145 using a RNeasy Mini Kit (Qiagen, Germany) according to the manufacturer's protocol. After reverse transcription using Superscript (Gibco-BRL, Oensingen, Switzerland) and oligo dT_{12-18} primer (Gibco-BRL), the complete cDNA of MHC-DQAI was amplified by PCR with primers DQA-Amp5 (5'-AAAAAGCTAGCACAGCTCAGARCAG-CAACTG-3') and DQA-Amp3 (5'-GGGGGCTCGAGATTCA-CAAKGGCCCTTGGTG-3') as described (Yasunaga et al. 1996). The following temperature profile was employed: 5 min 94°C; 30 cycles of 1 min 94°C, 1 min 59°C, 1 min 72°C, and the reaction was completed by a final extension step of 7 min at 72°C. The same cDNAs were amplified in a second amplification with primers DQA-Amp5 and DQA-Amp3.2 (5'-GATGGCGATGCACCTTCCCTTCC-3'). This set of primers was employed to amplify the complete cDNA, since they are located outside the coding regions. The PCR reaction was run under the same conditions as described above.

Genomic DNA was extracted either from PBMC, spleen cells or the lymphoblastoid cell line from monkey 3026 using the Nucleospin C+T kit (Macherey-Nagel, Oensingen, Switzerland) according to the manufacturer's protocol. DNA samples were amplified by PCR with primers DQB-DB130 (5'-AGG-GATCCCCGCAGAGGATTTCGTGTACC-3') and DOB-DB131 (5'-TCCTGCAGGGCGACGACGCTCACCTCCCC-3') as described (Bugawan and Ehrlich 1991). PCR cycling conditions were as follows: 5 min 96°C; 35 cycles of 1 min 96°C, 1 min 60°C, 1 min 72°C; 7 min 72°C; soak at 4°C. From monkeys 11190, 11192 and 11145, the second exon of MHC DQB was amplified from cDNA using the same primer pair and conditions as described for genomic DNA. The amplicon was visualized on a 3% agarose gel (Gibco-BRL).

Cloning and DNA sequencing reaction

The PCR products were purified using a High Pure PCR product purification kit (Boehringer Mannheim, Indianapolis, Ind.) according to the manufacturer's protocol and were cloned into pGEM5 T vector (Promega, Madison, Wis.). Plasmid doublestranded DNA was isolated using the Nucleospin kit (Macherey-Nagel). Plasmid inserts were sequenced in both directions using ABI Prism 310 Genetic Analyzer (Perkin Elmer, Foster City, Calif.) and analyzed using the provided software.

Nomenclature

Official designations were obtained from R. E. Bontrop and N. G. de Groot (Biomedical Primate Research Centre, Rijswijk, The Netherlands) based upon shared sequence motifs, phylogenetic analysis and comparison with sequences found in other New World monkeys (Antunes et al. 1998). The reported alleles represent at least three identical clones that were obtained after independent amplifications from the same animal or in different animals.

Phylogenetic analysis

Phylogenetic analysis was performed employing the PHYLIP 3.572 package available under http://bioweb.pasteur.fr. A neighbor-joining phylogenetic tree (Saitou and Nei 1987) was constructed from genetic distance values (Kimura 1980).

Results

Polymorphism of Aona-DQA1 alleles

The cDNA from three randomly chosen *A. nancy-maae* monkeys was used to amplify the full-length MHC *DQA1* gene. The resulting PCR products were cloned and 28 inserts were sequenced. Five different alleles were identified. These *Aona-DQA1* nucleotide sequences are given in Fig. 1A and have been assigned the GenBank accession numbers AF201293 – AF201297. The deduced amino acid sequences are shown in Fig. 1B. None of the sequences display features that would suggest they are pseudogenes. From every monkey, two different alleles could be amplified (Table 1). All five identified *Aona-DQA1* alleles belong to the same lineage, namely *Aona-DQA1*27*.

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Aona-DQA1*2702								A					ATA			T							A			
Aona-DQA1*2703								A					ATA			т							A			
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Aona-DOA1*2702								n										7								-č-
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HLA-DQA1*0101 Aona-DQA1*2701 Aona-DQA1*2702 Aona-DQA1*2703 Aona-DQA1*2704 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2701	CTC /	ATT C C C C	TGT C C C C C	CTT GAG 	GTG ACC	GAC AGC 	AAC 140 TTC 	ATC CTC 	TTT TCC	CCT AAG 	CCT AGT	GTG GAT	120 GTC T T CAT	AAC 	ATC TTC 	ACA TTC 	TGG AAG 	CTG T T T T ATC	AGC AGT 	AAT TAC 	GGG CTC 	CAG C C C C ACC	TCA TTC 	GTC CTC 	ACA 	GAA TCT
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HLA-DQA1*0101 Aona-DQA1*2701 Aona-DQA1*2702 Aona-DQA1*2703 Aona-DQA1*2704 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2701 Aona-DQA1*2702 Aona-DQA1*2703 Aona-DQA1*2704 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2701	GGT GGT 1	ATT C C C C GTT GAT	TGT C C C C TCT C TCT C C 	CTT GAG ATT	GTG ACC TAT	GAC AGC GAC	AAC 140 TTC TGC	ATC CTC AAG	TTTT TCCC GTG GTG	CCT AAG GAG 	CCT AGT CAC	GTG GAT TGG	120 GTC T T CAT T CAT T	AAC 	ATC TTC GAC G	ACA TTC CAG G	TGG AAG CCT	CTG T T T ATC CTT 	AGC AGT CTG 	AAT TAC AAAA	GGG CTC 180 CAC 	CAG C C C C ACC C ACC TGG	TCA TTC G AG -	GTC CTC 	ACA 	GAA TCT
HLA-DQA1*0101 Aona-DQA1*2701 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2704 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2702 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2701 Aona-DQA1*2701 Aona-DQA1*2701 Aona-DQA1*2702	GGT () GGT () 160 GCT ()	ATT C C C GTT GTT GAT	TGT C C C C TCT C TCT C C	CTT GAG ATT 	GTG ACC TAT 	GAC AGC GAC 	AAC 140 TTC TGC 	ATC CTC AAG 	TTT TCC GTG 	CCT AAG GAG 	CCT AGT CAC 	GTG GAT TGG 	120 GTC T T T CAT T CAT 	AAC 	ATC TTC GAC G G	ACA TTC CAG G G	TGG AAAG CCT 	CTG T T T ATC CTT 	AGC AGT CTG 	AAT TAC AAA	GGG CTC 180 CAC 	CAG C C C C ACC C ACC C TGG	TCA TTC G AG G AG - -	GTC CTC SXON G CCT	ACA 	GAA TCT
HLA-DQA1*0101 Aona-DQA1*2701 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2704 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2701 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2701 Aona-DQA1*2701 Aona-DQA1*2702 Aona-DQA1*2702 Aona-DQA1*2703	GGT () GGT () 160 GCT ()	ATT C C C GTT GTT GAT	TGT C C C TCT GAG 	CTT GAG ATT ATT	GTG ACC TAT TAT 	GAC AGC GAC 	AAC 140 TTC TGC 	ATC CTC AAG 	TTT TCCC GTG GTG 	CCT AAG GAG GAG	CCCT AGT CAC 	GTG GAT TGG 	120 GTC T T T CAT T CAT 	AAC 	ATC TTC GAC G G	ACA TTC CAG G G G	TGG AAAG CCCT 	CTG T T T T T T CTT CTT 	AGC AGT CTG CTG	AAT TAC AAA	GGG CTC 180 CAC 1.80	CAG C C C C ACC TGG 	TCA TTC G AG G AG 	GTC CTC SXON G CCT 	ACA 	GAA TCT
HLA-DQA1*0101 Aona-DQA1*2701 Aona-DQA1*2702 Aona-DQA1*2703 Aona-DQA1*2704 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2701 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2704 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2701 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703	GGT 0	ATT C C C GTT C GTT GAT	TGT C C C TCT C TCT GAG GAG 	CTT GAG ATT 	GTG ACC TAT 	GAC AGC GAC GAC 	AAC 140 TTC TGC TGC	ATC CTC AAG AAG 	TTT TCC GTG GTG 	CCT AAG GAG GAG 	CCT AGT CAC 	GTG GAT TGG TGG	120 GTC T T T CAT T CAT 	AAC 	ATC TTC GAC 	ACA TTC CAG G G G G	TGG AAAG CCT CCT 	CTG T T T T T T CTT CTT C	AGC AGT CTG CTG 	AAT TAC AAA 	GGG CTC 180 CAC CAC 1.80	CAG C 	TCA TTC 	GTC CTC 3 CCT 	ACA 	GAA TCT
HLA-DQA1*0101 Aona-DQA1*2701 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2704 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2702 Aona-DQA1*2702 Aona-DQA1*2703 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2701 Aona-DQA1*2703 Aona-DQA1*2704 Aona-DQA1*2704 Aona-DQA1*2704	GGT 0 	ATT C C C GTT C GTT GAT 	TGT C C C C TCT C 	CTT GAG CTT ATT 	GTG ACC TAT 	GAC AGC GAC 	AAC 140 TTC TGC TGC	ATC CTC AAG 	TTT TCC GTG 	CCT AAG GAG 	CCT AGT CAC 	GTG GAT TGG 	120 GTC T T T CAT T CAT T GGC 	AAC 	ATC 	ACA TTC CAG G G G G G	TGG AAG CCT 	CTG T T T T ATC CTT CTT 	AGC AGT CTG 	AAT TAC TAC AAAA AAAA	GGG CTC 180 CAC 180 CAC 	CAG C 	TCA TTC G AG G AG G - 	GTC CTC SXON G CCT 	ACA 	GAA TCT
HLA-DQA1*0101 Aona-DQA1*2701 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2704 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2702 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2701 Aona-DQA1*2702 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2704 Aona-DQA1*2705	GGT () GGT ()) 160 GCT () 	ATT 	TGT C C C TCT GAG GAG 	CTT GAG ATT 	GTG ACC TAT 	GAC AGC GAC 	AAC 140 TTC TGC TGC	ATC CTC AAAG	TTT TCC GTG 	CCT AAG GAG GAG 	CCT AGT CAC CAC	GTG GAT TGG TGG	120 GTC T T T CAT GGC GGC	AAC 	ATC TTC GAC 	ACA TTC CAG G G G G G	TGG AAG CCT 	CTG T T T T ATC CTT 	AGC AGT CTG 	AAT TAC AAAA 	GGG CTC 180 CAC 180 	CAG C C C ACC C C TGG TGG 	TCA TTC G AG - - - - -	GTC CTC SXON G CCT 	ACA 	GAA TCT
HLA-DQA1*0101 Aona-DQA1*2701 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2704 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2701 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2702 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2704 Aona-DQA1*2705	GGT (ATT 	TGT C C C TCT C C C 	CTT GAG ATT 	GTG ACC TAT 	GAC AGC GAC 	AAC 140 TTC TGC TGC	ATC CTC AAG 	TTT TCC GTG GTG 	CCT AAG GAG 	CCT AGT CAC 	GTG GAT TGG TGG	120 GTC T T T CAT T GGC GGC 	AAC 	ATC TTC GAC 	ACA TTC CAG G G G G G	TGG AAAG CCT 	CTG T T T T ATC CTT 	AGC AGT CTG 	AAT TAC AAA 	GGG CTC 180 CAC 	CAG C C C C ACC TGG 	TCA	GTC CTC 	ACA 	GAA TCT
HLA-DQA1*0101 Aona-DQA1*2701 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2704 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2701 Aona-DQA1*2702 Aona-DQA1*2703 Aona-DQA1*2704 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2704 Aona-DQA1*2704 Aona-DQA1*2705 HLA-DQA1*0101	GGT (ATT 	TGT C C C TCT C C TCT GAG GAG C C C	CTT GAG ATT ATT ATT	GTG ACC TAT TAT 	GAC AGC GAC GAC GAG	AAC 140 TTC TGC TGC CTC	ATC CTC AAG AAG	TTT TCC GTG GTG GTG GAG	CCT AAG GAG GAG AAT	CCT AGT CAC CAC GTG	GTG GAT TGG TGG GTC	120 GTC T T T CAT T CAT 	AAC 	ATC 	ACA TTC CAG G G G G G G G G G G	TGG AAAG CCT TTG	CTG T T T T T ATC CTT CTT TCT	AGC AGT CTG GTG GTG	AAT TAC AAA 	GGG CTC 180 CAC CTC	CAG C C C C ACC C GTG	TCA TTC TTC G[]AG 	GTC CTC CTC CTC CTC CTC CTC 	ACA 	GAA TCT
HLA-DQA1*0101 Aona-DQA1*2701 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2704 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2702 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2701 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2705	GGT () GGT () GGT () 160 GCT () 160 CCA ()	ATT 	TGT C C C TCT GAG GAG GAG CCT 	CTT GAG ATT ATG 	GTG ACC TAT TCA	GAC AGC GAC GAC GAG	AAC 140 TTC TGC CTC 	ATC CTC AAG AAG AAG 	TTT TCC GTG GAG 	CCT AAG GAG 	CCT AGT CAC CAC GTG 	GTG GAT TGG TGG GTC 	120 GTC T T T CAT GGC GGC TGC 	AAC 	ATC TTC GAC 	ACA TTC CAG G G G G G G G G	TGG AAAG CCT TTG 	CTG T T T T ATC CTT CTT 	AGC AGT CTG CTG 	AAT TAC AAA AAA GGGC	GGG CTC 180 CAC CTC 	CAG 	TCA TTC TTC G AG - GGC GGC	GTC CTC SXON G CCT 	ACA 	GAA TCT
HLA-DQA1*0101 Aona-DQA1*2701 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2704 Aona-DQA1*2704 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2701 Aona-DQA1*2701 Aona-DQA1*2701 Aona-DQA1*2701 Aona-DQA1*2701 Aona-DQA1*2701	GGT () GGT ()) 160 GCT () 	ATT 	TGT C C C TCT C TCT GAG CCT CCT 	CTT GAG ATT ATT 	GTG ACC TAT TAT 	GAC AGC GAC GAC 	AAC 140 TTC TGC CTC 	ATC CTC AAG ACA 	TTT TCC GTG GTG GAG GAG 	CCT AAG GAG GAG 	CCT AGT CAC CAC CAC GTG 	GTG GAT TGG TGG GTC 	120 GTC T T T CAT T CAT 	AAC 	ATC TTC GAC G G G 2000 CTG 2000	ACA TTC CAG G G G G G	TGG AAAG CCT TTG TTG 	CTG T T T T ATC CTT CTT CTT CTT CTT	AGC AGT CTG GTG GTG 	AAT TAC TAC AAA GGC	GGG CTC 180 CAC CTC CTC	CAG C C C ACC C ACC TGG TGG GTG 	TCA TTC TTC G AG G AG G GGC GGC	GTC 	ACA 	GAA TCT
HLA-DQA1*0101 Aona-DQA1*2701 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2704 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2701 Aona-DQA1*2702 Aona-DQA1*2703 Aona-DQA1*2704 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2704 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2705	GGT (ATT C C C GTT GAT GAT GCC 	TGT C C C TCT C TCT GAG GAG CCT 	CTT GAG GAG ATT ATT ATG 	GTG ACC TAT 	GAC AGC GAC GAG GAG GAG	AAC 140 TTC TGC -	ATC CTC AAG AAG 	TTT TCCC GTG GTG GAG GAG GAG	CCT AAG GAG GAG ACT 	CCT AGT CAC GTG GTG 	GTG GAT GAT GAT GAT GTG GTC GTC GTC	120 GTC T T CAT T CAT T GGC TGC 	AAC T T A CTG CTG GCCC GCCC	ATC 	ACA TTC CAG G G G G G G G G G G G G G G G G G	TGG AAAG CCT TTG TTG 	CTG T T T T ATC CTT CTT TCT TCT 	AGC AGT CTG GTG GTG 	AAT TAC AAA AAA GGGC GGGC	GGG CTC 180 CAC CTC CTC	CAG C C C ACC C ACC C TGG C TGG C GTG GTG 	TCA TTC TTC G AG GGC GGC GGC	GTC CTC SXON GCCT ATT 	ACA	GAA TCT
HLA-DQA1*0101 Aona-DQA1*2701 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2704 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2702 Aona-DQA1*2702 Aona-DQA1*2703 Aona-DQA1*2704 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2705 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2705 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703	GGT () GGT () GGT () 160 GCT () 160 GCT () CCA ()	ATT C C C GTT GAT GAT GGC GCC	TGT C C C TCT C TCT GAG GAG CCT CCT 	CTT GAG ATT ATG 	GTG TAT 	GAC AGC GAC GAG GAG GAG	AAC 140 TTC TGC TGC CTC 	ATC CTC AAG AAG AAG AAG AAG 	TTT TCC GTG GAG GAG 	CCT AAG GAG GAG ACT	CCT AGT CAC CAC GTG GTG 	GTG GAT TGG 	120 GTC T T T CAT T CAT T GGC TGC 	AAC T 	ATC TTC 	ACA TTC CAG G G G G G G G G G G	TGG AAG 	CTG T T T ATC ATC CTT CTT CTT CTT CTT CTT 	AGC AGT CTG GTG GTG 	AAT TAC TAC AAA AAA GGGC GGGC	GGG CTC 180 CAC 180 CAC CTC 	CAG C C C ACC C ACC C TGG TGG GTG GTG 	TCA TTC G[AG GGC GGC GGC	GTC -	ACA 	GAA TCT GTG GTG
HLA-DQA1*0101 Aona-DQA1*2701 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2704 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2701 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2705 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2704 Aona-DQA1*2704 Aona-DQA1*2704	GGT () GGT ()) GGT ()) 160 GCT ()) CCA ())	ATT 	TGT C C C C TCT C C C -	CTT GAG ATT ATG ATG 	GTG ACC TAT 	GAC GAC 	AAC 1400 TTC TGC CTC 	ATC AAG 	TTT TCC GTG GAG GAG GAG	CCT GAG 	CCT AGT CAC GTG GTG GTG 	GTG GAT GAT TGG GTC GTC GTC 	120 GTC T T CAT T CAT T GGC TGC 	AAC 	ATC TTC GAC G G G 2000 CTG CTG CTG 	ACA TTC CAG G	TGG 	CTG T T T T T T CTT CTT CTT CTT CTT CTT CTT 	AGC AGT CTG GTG 	AAT TAC AAAA GGGC	GGG 180 CAC CAC CAC -	CAG C C C C ACC C TGG TGG GTG GTG 	TCA	GTC CTC XON G CCT ATT 	ACA 	GAA
HLA-DQA1*0101 Aona-DQA1*2701 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2704 Aona-DQA1*2704 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2704 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2704 Aona-DQA1*2705	GGT (ATT C C C GTT GAT GAT GAT 	TGT C C C TCT C TCT C C TCT C 	CTT GAG ATT ATT 	GTG ACC TAT TCA 	GAC AGC GAC GAG GAG GAG	AAC 140 TTC TGC CTC 	ATC CTC AAG ACA ACA 	TTT TCC GTG GAG GAG	CCT AAG GAG GAG GACT ACT	CCT AGT CAC GTG GTG 	GTG GAT GAT TGG GTC GTC	120 GTC T T T CAT T GGC GGC TGC 	AAC 	ATC 	ACA TTC G G G	TGG AAG AAG CCT CCT TTG TTG	CTG T T T T T T CTT CTT C	AGC AGT CTG CTG GTG 	AAT TAC TAC AAAA GGGC GGGC	GGG 180 CAC CTC CTC 	CAG C C C C C C C 	TCA	GTC 	ACA	GAA
HLA-DQA1*0101 Aona-DQA1*2701 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2704 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2702 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2704 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2704 Aona-DQA1*2704 Aona-DQA1*2704 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2705 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2704 Aona-DQA1*2705	CTC	ATT 	TGT C C C C TCT GAG CCT 	CTT GAG ATT ATG 	GTG 	GAC AGC GAC GAG GAG 	AAC 140 TTC TGC CTC 	ATC CTC AAAG ACA 	TTT TCCC GTG GTG GAG 2220	CCT AAG GAG GAG GACT ACT	CCT AGT CAC CAC GTG 	GTG GAT GAT GAT GTC GTC GTC	120 GTC T 	AAC	ATC TTC GAC 	ACA TTC TTC CAG G G G G G G	TGG AAAG AAAG CCT CCT TTG TTG	CTG T T T T T T CTT CTT CTT CTT CTT 	AGC AGT CTG GTG GTG GTG	AAT TAC TAC AAAA GGGC GGGC	GGG CTC 180 CAC CTC -	CAG C C C C ACC C C TGG GTG 	TCA TTC	GTC CTC SXON G CCT 	ACA	GAA TCT
HLA-DQA1*0101 Aona-DQA1*2701 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2704 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2702 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2705 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2704 Aona-DQA1*2705 HLA-DQA1*0101	GGT (GGT (GG	ATT 	TGT C C C TCT C C C GAG GAG GAG GTC	CTT GAG ATT ATT ATG TTC	GTG ACC TAT TAT 	GAC AGC GAC GAC GAG ATC	AAC 140 TTC TGC CTC 	ATC CTC AAAG ACA GGC	TTT TCC GTG GAG GAG 2220 CTG	CCT AAAG GAGG GAG GAGT CGT	CCT AGT CAC GTG GTG 	GTG GAT GAT GAT GAT GTC GTC GTC GTC GTT	120 GTC T T CAT T CAT GGC GGC TGC GGT	AAC 	ATC TTC GAC G G 2000 CTG CTG CTG TCC	ACA 	TGG AAAG CCCT TTG TTG 	CTG T T T T T T T CTT CTT	AGC AGT CTG CTG GTG GTG GGG	AAT TAC TAC AAAA AAAA GGC GGC CCA	GGG 180 CAC CTC -	CAG C C C C ACC C C 	TCA TTTC G[AG GGC	GTC CTC 	ACA	GAA TCT
HLA-DQA1*0101 Aona-DQA1*2701 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2704 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2702 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2701 Aona-DQA1*2701 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2704 Aona-DQA1*2703 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2705	GGT (GGT (GG	ATT 	TGT C C C C TCT GAG GAG CCT GTC 	CTT GAG ATT ATT ATG 	GTG ACC TAT 	GAC AGC GAC GAG GAG 	AAC 140 TTC TGC TGC CTC 	ATC AAAG AAAG AAAG ACA 	TTT TCC GTG GAG GAG 2220 CTG CTG	CCT AAG AAG GAG GAG ACT ACT CGT	CCT AGT CAC GTG GTG GTG GTCA A	GTG GAT GAT GAT GTC GTC GTC GTC GTC	120 GTC T T CAT T CAT GGC GGC TGC TGC 	AAC 	ATC TTC GAC 	ACA TTC TTC CAG G G G G G G	TGG AAG CCT TTG 	CTG T T T T T T CTT CTT C	AGC AGT CTG CTG GTG GTG GTG GTG	AAT TAC TAC AAA GGGC GGGC GGGC CCCAC	GGG 180 CAC CTC -	CAG C C C C ACC C C TGG GTG GTG GTG 	TCA TTC G[AG GGC GGC GGC	GTC CTC ATT 	ACA	GAA
HLA-DQA1*0101 Aona-DQA1*2701 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2704 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2702 Aona-DQA1*2702 Aona-DQA1*2703 Aona-DQA1*2704 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2704 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2705	GGT (GGT (ATT 	TGT C C C TCT C C C 	CTT GAG ATT ATT ATG TTC 	GTG TAT TCA 	GAC AGC GAC GAG GAG GAG ATC 	AAC 140 TTC TGC CTC 	ATC CTC AAG ACA GGC GGC 	TTT TCCC GTG GAG GAG 2220 CTG 2220	CCT AAG AAG GAG GAG GAG	CCT CAC CAC GTG TCA A A	GTG GAT GAT GAT TGG GTC GTC GTC GTT GTT	120 GTC T T CAT T CAT T GGC GGC TGC 	AAC 	ATC TTC GAC G G 	ACA TTC G G G G G G G G G G G G AGA	TGG CCT TTG 	CTG T T T T T T CTT C C	AGC AGT CTG CTG GTG GTG GTG GGG GGGG	AAT TAC TAC	GGG CTC 180 CAC CTC TTG TTG 	CAG C C C ACC C C TGG TGG GTG 	TCA TTC	GTC CTC SXON G CCT 	ACA	GAA GTG GTG
HLA-DQA1*0101 Aona-DQA1*2701 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2704 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2702 Aona-DQA1*2703 Aona-DQA1*2704 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2705 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2703 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2705	GGT (GGT (GG	ATT 	TGT C C C TCT C C C GAG GAG 	CTT GAG ATT ATT ATG 	GTG ACC TAT TCA ATC 	GAC AGC GAC GAC GAC GAC ATC 	AAC 140 TTC TGC CTC 	ATC CTC AAAG ACA GGC 	TTT TCCC GTG GTG 	CCT AAG AAG GAG GAG GAG CGGT	CCT AGT CAC CAC GTG TCA A A	GTG GAT GAT GAT GAT GTG GTC GTC GTC GTT GTT	120 GTC T T CAT T CAT GGC GGC GGC GGT 	AAC 	ATC TTC GAC G G 200 CTG CTG CTG CTG 	ACA TTTC TTTC CAG G G G G G G G G AGA AGA AGA AGA	TGG CCT TTG 	CTG T T T T T T C C	AGC AGT CTG CTG GTG GGG GGGG 	AAT TAC TAC AAAA GGC GGC CCACC	GGG CTC 180 CAC CTC TTG 	CAG C C C C ACC C C TGG 	TCA TTC TTCC G[A0 GGC GGC	GTC 	ACA	GAA
HLA-DQA1*0101 Aona-DQA1*2701 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2704 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2704 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2704 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2705 HLA-DQA1*0101 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703 Aona-DQA1*2703	GGT (GGT (GGT (ATT 	TGT C C C C TCT GAG 	CTT GAG ATT ATT ATG 	GTG TAT TCA 	GAC AGC GAC GAC GAG ATC ATC	AAC 140 TTC CTC CTC 	ATC CTC AAAG AAAG GGC 	TTT TCC GTG GAG GAG GAG 2220 CTG 2220	CCT GAG GAG GAG GACT CCGT	CCT AGT CAC CAC GTG GTG GTG TCA A A A	GTG GAT GAT GAT GAT GTC GTC GTC GTC GTC GTC	120 GTC T T CAT T CAT T GGC GGC TGC 	AAC 	ATC TTC 	ACA TTC TTC CAG G G G G G G G G G G A A A A A A A A A A A A A A	TGG AAAG AAAG CCCT TTG TTG CACGGGGG	CTG T T T T T T CTT CTT C	AGC AGT CTG CTG GTG GTG GTG GTG 	AAT TAC TAC AAA GGC GGC CCAC	GGG 180 CAC CTC -	CAG C C C ACC C ACC C TGG GTG 	TCA TTC G AG - GGC GGC	GTC CTC	ACA	GAA TCT GTG

Fig. 1. A Nucleotide sequence of *Aona-DQA1* alleles. In the numbering system used, codon 1 specifies the first amino acid residue of the mature protein. The *top line* represents the sequence of HLA-DQA1*0101. Identity with the top sequence is indicated by *dashes* (--). The boundaries of the exons 1–4 are marked. **B** Alignment of *Aona-DQA1* amino acid sequences

obtained by the translation of the nucleotide sequences given in **A**. The *top line* gives the amino acid sequence of *HLA-DQA1*0101*. Identity with the top sequence is indicated by *dashes* (--). A *back-slash* (/) marks gaps inserted to maximize the alignment

В 20 40 1 60 80 ${\tt MILNKALLLGALALTTVMSPCGGEDIVADHVASCGVNLYQFYGPSGQYTHEFDGDEEFYVDLERKETAWRWPEFSKFGGFDPQGALRNMAVAKHNLNIMIKRYNSTAATN$ HLA-DQA1*0101 HLA-DOA1*0201 -S----F---V-KL-L-HRLR/----F--T-I--L----L---S--M--Y-HLA-DQA1*03011 -V-OL-L-RR-RR--м -5---F--T-I--L-S. HLA-DQA1*0401 ---V-CL-VLRQ-R/--G--s -0 ---F--T-I--T· -s HLA-DQA1*05011 ---V-CL-VLRQ-R/-·s ·G· -F--T-I--L HLA-DQA1*06011 -V-CL-VLRQ-R/--F--T-I--T Aona-DQA1*2701 -AY-T T-I-AG-_м - C. -S--T -V--T.-V--- n --D-T Aona-DQA1*2702 AY-T -8--v--t.-v--T-I-AG--м--S -T. -M--A--D-L Aona-DQA1*2703 AY-TT T -37 -TS--TET-TG-Y--D-T--05 -M .c - 9 .c. Aona-DQA1*2704 -L-V--T-TS--I--T-I-TG-Y--D-L--M--т -м -N -AY-I--S -GK--v--S-S-Aona-DQA1*2705 -T-I-TG-YT-D-L--AY-I -M--т -S -т. -0 -G--T.-V--- A--S-S-100 120 140 160 180 HLA-DOA1*0101 HLA-DOA1*0201 -H-HLA-DQA1*03011 -H HLA-DQA1*0401 -H HLA-DQA1*05011 --L---E-S ·Н -ĸ HLA-DQA1*06011 -н Aona-DQA1*2701 - E E -н-Aona-DQA1*2702 -R--EE--H Aona-DQA1*2703 -EE--H-Aona-DQA1*2704 --ĸ--EE -н--A Aona-DQA1*2705 -EE 200 220 CALGLSVGLVGIVVGTVFIIQGLRSVGASRHQGPL* HLA-DOA1*0101 HLA-DOA1*0201 -L--R-HLA-DQA1*03011 -R HLA-DQA1*0401 HLA-DQA1*05011 HLA-DQA1*06011 -R Aona-DOA1*2701 --R--HT-

Fig. 1. B

-R---T-

-R---T

-R--T

Table 1. Distribution of the Aona-DQA1 and -DQB1/2 alleles found in 19 A. nancymaae monkeys. Alleles were detected by nucleotide sequence analysis after PCR amplification. Aona-DQB1*22012, Aona-DQB1*2301, Aona-DQB1*2302 and Aona-DQB1*2312 have been amplified from one animal. These sequences are confirmed by two independent PCR reactions with 4-10 independent clones sequenced from each allele. The other Aona-DQB1/2 alleles were derived from more than one animal and therefore from independent PCR reactions and up to 19 clones were sequenced per allele. ND Not determined

Aona-DQA1 Aona-DQB1 Aona-DQB2 Monkey code number *2308 3026 ND 7208 ND *2306 *0101; 0102 8078 ND *2308 *0101 8105 ND *2311 8138 ND *2305; *2308; *2309; *2310; *2311 *2310 8183 ND 8149 *2307; *2308 ND *2305 8222 ND 8230 *2311 ND 8232 *2308; *2311 ND 8290 ND *2307; *2308 *0101 *22011; *2312 8294 ND 9191 ND *22012; *2301; *2307, *2308 *2302; *2304 9200 ND 9452 ND *22011 *0102 9472 ND *22011; *2303; *2308; *2309 11145a *2701; *2702 *2306 *2701; *2703 *2303; *2304; *2308; *2309 11190a *2704 ; *2705 11192a *2308 *0102

^aAlleles identified in these animals were derived from cDNA

Aona-DOA1*2702

Aona-DOA1*2703

Aona-DQA1*2704

Aona-DQA1*2705

532

Aona-DQB1*2312

Aona-DQB2*0101

Aona-DQB2*102

T--- C

T--- C T--- C

Α		-	10										0									2	0				
Consensus	CAG T	rt 2	AAG	GGC	ATG	TGC	TAC	TTC	ACC	AAC	GGG	ACG	GAG	CGC	GTG	CGG	GGT	GTG	ACC	AGA	TAC	ATC	TAT	AAC	CGA	GAG	GAG
Aona-DQB1*22011	G								-GG							C	CT-			C	-T-						
Aona-DQB1*22012	G	·							-GG							C	CT-			C	- T -						
Acro DOB1+2201				m	~ ~												7			C M		c					
Aona-DOB1*2301 Aona-DOB1*2302				T	C-C												A		-A-	CAT		G					
Aona-DQB1*2303				T	c-c											T	CT-			-A-		G					
Aona-DQB1*2304		·		т	C-C											т	CA-			-A-		G					
Aona-DQB1*2305		·		T	C-C					T						T	CT-		A	GA-	C	G					
Aona-DQB1*2306		·		T	C-C							A				T	CT-		A	GA-	C	G					
Aona-DQB1*2307		:		TTT 	C-C							A				T	CT-			-A-		G					
Aona-DOB1*2309				T	c-c							A				T	CA-			-A-		G					
Aona-DQB1*2310				TTT	c-c							A				T	CT-			-A-		G					
Aona-DQB1*2311		·		TTT	c-c							A				T	CT-			-A-		G					
Aona-DQB1*2312		·		AAT	т–с							A				т	CA-	т	-A-	-A-		G					
None DOD2+0101																			~						~		
$Aona-DQB2 \times 0101$:						 ∆											G						C		
																			•								
				40										50										60			
Consensus	TAC G	IG (CGC	TTC	GAC	AGC	GAC	GTG	GGG	GAG	TAC	CGG	GCG	GTG	ACG	CCG	CTG	GGG	CGG	CCT	GTC	GCC	GAG	TAC	TGG	AAC	AGC
Aona-DQB1*22011	C											-T-	C-C						-0-	C	-A-				-T-		G
AOMA-DOBI-22012	0											-1-	C-C						-c-	C	-A-				-1-		G
Aona-DQB1*2301								с			T										-A-				-AC		
Aona-DQB1*2302	-T										с-т										-A-						
Aona-DQB1*2303		·						C			T										-A-			с	-AC		
Aona-DQB1*2304	-T- A	·	T								T										CA-				-TC		G
Aona-DQB1*2305	-T- A	`	T								T	 T								-A-	A-T				-TC		G
Aona-DOB1*2307								c			T										-A-				-TC		G
Aona-DQB1*2308								c			T										-A-				-TC		G
Aona-DQB1*2309		·						C			\mathbf{T}										-A-				$-\mathbf{TC}$		G
Aona-DQB1*2310	-T	·						C			T										-A-						
Aona-DQB1*2311		:						с			T								-c-		-A-						
Aona-DQB1*2512	- <u>r</u>	,	A						0		T.										TA-						
Aona-DQB2*0101	T -0	c- ·									-T-				C	GA-				111	AG-	A		G			-A-
Aona-DQB2*0102	(c- ·									- T -				C	GA-				111	AG-	A		G			-A-
							70										80										90
Consensus	CAG A	AG (GAC	GTC	CTG	GAG	AGG	ACC	CGG	GCG	GAG	TGG	GAC	ACG	GTG	TGC	AGA	CAC	AAC	TAC	GAG	GTG	GCG	TAC	CGC	GGG	ATC
<i>Aona-</i> DQB1*22011				т								CT-					G				с	c	-A-	-т-	-c-	-c-	
Aona-DQB1*22012		·		т								CT-									с—-	с	-A-	- T -	-c-	-C-	
				_			-							_							~	_		~-	_		~
Aona-DQB1*2301	/	· · ·		A				ст-				ACA		A							C	T	-A-	CT-	-T-	AC-	-0-
Aona-DOB1*2303	/			A			c					CT-									c	т	-A-	CT-	-T-	AC-	-c-
Aona-DQB1*2304				A								ATA									c	-G-	-A-	CT-	- T -	AC-	-c-
Aona-DQB1*2305		·		A			C				-C-	GT-									C	C	-A-	CT-	$-\mathbf{T}-$	AC-	-C-
Aona-DQB1*2306		·		A				GT-				ATA									C	т	-A-	CT-	- T -	AC-	-C-
Aona-DQB1*2307		·		A			c					CT-									с—-	C	-A-	CT-	-C-	AC-	-C-
Aona-DOB1*2308				A			C					CT-									C	C	-A-	CT-	-1-	AC-	-c-
Aona-DQB1*2310				A			c					CT-									c	c	-A-	CT-	-T-	AC-	-c-
Aona-DQB1*2311	/.	11 -		A								CT-									с	с	-A-	ст-	$-\mathbf{T}-$	AC-	-c-
Aona-DQB1*2312		·		A				GT-			-T-	GT-									c	-c-	-A-	ст-	-т-	AC-	-c-
1					-		~ ~	~~~		~	~		_									~		~			~
AOna-DQB2*0101	T-T	`		T	T		CA-	CAG			-0-	GT-	T									-0-	-A-	CT-		AC-	-0-
ACMA-DQB2.0102	1-1 -			1	1		CA-	CAG		C	- C -	G1-	1									- C -		C1-		AC-	-0-
Consensus	CTG C	AG J	A																								
Aona-DQB1*22011	TC	(C C																								
NOUG-DÜDT.77017	10	- 1	-																								
Aona-DQB1*2301	т	(с																								
Aona-DQB1*2302	т	(с																								
Aona-DQB1*2303	т	(с									Fig	g. 2.	Al	Nucl	eoti	de s	eque	nce	of 2	Aond	ı-DC) <i>B1/</i>	2 al	leles	. In	the
Aona-DQB1*2304	T	(C									nu	mbe	ring	syst	tem	used	l, co	don	1 s	peci	fies	the	first	ami	no a	acid
Aona-DQB1*2305	T	(C C									res	sidue	e of	the	mat	ure	prote	ein.	The	ton	line	rep	rese	nts f	he c	con-
Aona-DOB1*2300	T	(č									set	isus	sem	ienc	e of	prir	nate	MH	ICI	OOR	gen	es. 1	[dent	titv	with	the
Aona-DQB1*2308	- T	(c									tor) sec	nien	ce i	s ind	licat	ed b	v da	shes	()	Ā	hack	k-sla	sh ()) m:	arks
Aona-DQB1*2309	т	(с									021	ns in	1901 Sert	ed t	$n m^{\circ}$	avim	ize t	he a	lion	men	t R	Alio	nme	nt o	$f \Delta c$	na_
Aona-DQB1*2310	т	(С									ga] ת	$\frac{1}{2}$	$\frac{10010}{12}$	min/	2 20	id e	ize t eque	ncor	ob	taine	u D A b	v th	,i€ ⊳ t≁	anel	ation	nu-
Aona-DQB1*2311	T	(c									$\frac{D}{th}$	201	i∠ di alaat	uuu Hida		10 30	Que	Non	; 00	TL	Ju U	y ui n liv			tho	

sensus sequence of primate MHC DQB genes. Identity with the top sequence is indicated by dashes (--). A back-slash (/) marks gaps inserted to maximize the alignment. **B** Alignment of Aona-DQB1/2 amino acid sequences obtained by the translation of the nucleotide sequences given in \mathbf{A} . The top line gives the consensus amino acid sequence of primate MHC-DQB alleles. Identity with the top sequence is indicated by dashes (--). A backslash (/) marks gaps inserted to maximize the alignment. The amino acid positions contributing to pockets 4 and 9 are shaded in gray

В	10	20	30	40	50	60	70	80	90		
	1	I	I	1	1	I	1	1	1		
Consensus	YQFKGM	CYFTNGTERV	TGVTRYIYNR	EEYVRFDSDV	GEYRAVTPLG	RPVAEYWNSQ	KDVLERTRAE	LDTVCRHNYE	VAYRGILQ		
Aona-DQB1*22011	v	R	RLF	L	LP	P-DL-G-	F	Q	LEFPA-S-		
Aona-DQB1*22012	v	R	RLF	L	LP	P-DL-G-	F	Q	LEFPA-S-		
Aona-DQB1*2301	L		RS-NH-V	L		DY	IS	тQ	LELLTT		
Aona-DQB1*2302	L		RS-NH-V	F	н	D	/-IV	IQ	LELLTT		
Aona-DQB1*2303	L		RLK-V	L		DHY	IS	Q	LELLTT		
Aona-DQB1*2304	L		RHK-V	FM		HF-G-		IQ	GELLTT		
Aona-DQB1*2305	L		RLEHV	FM		-HIF-G-		vQ	LELLTT		
Aona-DQB1*2306	L		RLEHV		FW	-HT		IQ	LELLTT		
Aona-DQB1*2307	FL		RLK-V	L		DF-G-	IS	Q	LELPTT		
Aona-DQB1*2308	L		RHK-V	L		DF-G-	IS	Q	LELLTT		
Aona-DQB1*2309	L		RHK-V	L		DF-G-	IS	Q	LELPTT		
Aona-DQB1*2310	FL		RLK-V	FL		D	IS	Q	LELLTT		
Aona-DQB1*2311	FL		RLK-V	L		P-D	/-I	Q	LELLTT		
Aona-DQB1*2312	NF		RHLNK-V	F-S	R	ұ	vv	vQ	AELLTT		
Aona-DQB2*0101			RA	A	FE	-/st-dny	FQQA	VY	AEL-TT		
Aona-DOB2*0102		I	RA	A	FE	-/ST-DNY	F00A	VY	AEL-TT		

Fig. 2. B

Monkey 11190 shared the *Aona-DQA1**2701 allele with animal 11145 (Table 1). For comparison, several human alleles are also depicted in Fig. 1B. Amino acid polymorphism observed in *Aotus* can in most cases also be found in *HLA-DQA1* alleles. *DQA2*-like genes were not identified.

Polymorphism of Aona-DQB1 alleles

The second exon of *Aona-DQB* genes was amplified from cDNA or genomic DNA of 19 monkeys. A total of 122 sequences were sequenced which identified 14 *Aona-DQB1* alleles. These alleles belong to two lineages, namely *Aona-DQB1*22* and *Aona-DQB1*23* (Fig. 2A). The deduced amino acid sequences are given in Fig. 2B. Five of these alleles were amplified from both genomic DNA and cDNA, namely *Aona-DQB1*2303*, *Aona-DQB1*2304*, *Aona-DQB1*2306*, *Aona-DQB1*2308* and *Aona-DQB1*2309*.

A phylogenetic tree constructed for the exon 2 sequences by the neighbor-joining method (Saitou and Nei 1987) shows that the majority of *Aotus* alleles are closely related to each other. The *Aona-DQB1*22011* allele forms a clade with the *Caja-DQB1*2201* allele. Sequences of representative *HLA-DQB1* and *Caja-DQB1* alleles are located on different branches than the *Aona-DQB1*23* alleles (Fig. 3).

Presence of Aona-DQB2 sequences

The evolutionary equivalents of *HLA-DQB2* alleles were detected in *Aotus* (Fig. 2A, B). The two alleles *Aona-DQB2*0101* and *Aona-DQB2*0102* showed 96% and 94% identity to *Caja-DQB2* (AF004746) and 91% and 92% identity to *HLA-DQB2* (X87344), respectively. Amino acids contributing to Aona-DQB1 peptide binding pockets

Using the HLA-DR structure as a model for class II peptide binding, 15 amino acid positions in DQB1 were classified as participating in pockets: 11, 13, 28, 47, 57, 61, 67, 70, 71, 74, 78, 85, 86, 89 and 90 (Brown et al. 1993; Stern et al. 1994). The variability at these positions was assessed from the identified DOB1 alleles. As demonstrated in the variability plot of Fig. 4, the vast majority of amino acid positions participating in the peptide binding region are polymorphic. Position 57 of the $DQ \beta$ chain is particularly interesting in humans because it is associated with autoimmune diabetes (Todd et al. 1987). In different HLA-DOB1 alleles, this position is occupied either by Asp or non-Asp (Ala, Val or Ser) residues. In Aona-DOB1 sequences, position $\beta 57$ is occupied predominantly by Asp and alternatively by Ile, Thr, Tyr and Ser.

Discussion

T cells recognize complexes of MHC class II molecules and bound peptide. The understanding of the specificity of peptide-MHC class II interactions was greatly facilitated by the elucidation of the three-dimensional structure of MHC class II molecules (Madden 1995). The crystal structures of HLA-DR1 suggest that the general principles of peptide binding to class II proteins are very similar for different species and different class II molecules (Brown et al. 1993; Stern et al. 1994). Indeed, residues forming hydrogen bonds to the peptide backbone in HLA-DR1 are conserved in all class II proteins, implying similar polyproline type II helical conformation, N-to-C terminal orientation, and spacing of anchor residues in the bound peptides (Stern et al. 1994). The HLA-DR1 structure revealed pockets in the peptide binding grooves accommodating several anchor residues of a peptide. Fig. 3. Phylogenetic tree constructed according to the neighbor-joining method (Saitou and Nei 1987). The relationship between the newly described Aona-DQB1 alleles and selected alleles of Callithrix jacchus and human is shown. Bootstrap values are indicated at the branches. The sequences included: HLA-DÔB1*0201 (L40179), HLA-DÕB1*03011 (L34096), HLA-DÕB1*0401 (L34099), HLA-DQB1*05011 (L34101), HLA-DÕB1*06011 (L34104), Caja-DQB1*2301 (AF004744), Caja-DQB1*2302 (AF004745) and Caja-DQB1*2201 (AF004743)



The specificity of the peptide-binding pockets is influenced by polymorphic amino acid residues of the MHC molecules resulting in allele-specific class II binding motifs.

In the present study, we analyzed the nucleotide sequences of the *DQA1* and *DQB1/DQB2* genes of *A. nancymaae.* The *Aona-DQA1* alleles sequenced have been characterized as cDNAs. Five alleles, all belonging to the same lineage of *DQA1*, namely *Aona-DQA1*27*, were identified after sequencing of several independent clones. This lineage has been assigned according to the second exon sequences and it has not been described in other Catarrhini and Platyrrhini so

far (Bontrop et al. 1999). Because of the limited numbers of animals analyzed, we cannot draw conclusions on how many other *DQA* lineages might be present in the *A. nancymaae* population. Polymorphic amino acid residues in the *Aona-DQA1* alleles are also present in *HLA-DQA1* alleles (Fig. 1B). Most variation seen within the *Aona-DQA1*27* lineage can currently be explained by point mutations, whereas intra-allelic exchanges of motifs were not evident.

Functional expression of *DQB2* instead of *DQB1* has been described in a cell line of *A. trivirgatus* (Gaur et al. 1992). However, using a different set of primers from those used by Gaur and collegues



amino acid position

Fig. 4. Amino acid variability plot (Wu and Kabat 1970) for derived amino acid sequences of *Aona-DQB1* exon 2. *Filled* and *empty bars* indicate the amino acid residues involved in the peptide binding region (PBR) and non-PBR, respectively (Brown et al. 1993). *Numbers* along the *X* axis denote amino acid position based on a complete exon 2 sequence and "n" at the *Y* axis denotes the number of different amino acids found at the corresponding positions

(1992), we could amplify DQB1 alleles from both genomic DNA and cDNA. The number of different amino acids occurring at the same position in the identified DQB1 alleles is highest at positions that are likely to be part of the peptide binding region (PBR) of Aona-DQB1 molecules (Fig. 4) (Brown et al. 1993). Hence, positive selection appears to promote variability at the PBR sites. This feature is characteristic for genes that encode proteins with antigen-presenting function and indicates that DQB1 alleles are functional in A. nancymaae (Bergstrom and Gyllensten 1995). Variation seen within Aona-DQB1 lineages could be explained by point mutations, whereas intraallelic exchanges of motifs seem to play a marginal role. This is in contrast to our findings with Aona-DRB alleles where intra-allelic exchange of motifs is common (Nino-Vasquez et al. 2000). Family data will be required to assign genes to haplotypes.

One monkey (animal 8138) was investigated in greater detail than the others. Sequence analysis of a great number of cloned PCR products from genomic DNA of this monkey showed that the *Aona-DQB1*2305*, *Aona-DQB1*2308*, *Aona-DQB1*2309*, *Aona-DQB1*2310*, and *Aona-DQB1*2311* alleles could be identified. Four different *Aona-DQB1* alleles could be identified in the monkeys 9191, 9472 and 11190. These results suggest that more than two *Aona-DQB1*23* alleles are present at the genomic DNA and cDNA level. Duplication events like at the *Aona-DQB1* locus may thus also have occurred at the *Aona-DQB1* locus. A detailed contig mapping study would be needed to confirm how many *Aona-DRB* and *Aona-DQB* loci are present in the *Aotus MHC*.

In *Aotus*, as well as in the cotton-top tamarin and common marmoset, the evolutionary equivalents of *HLA-DQB2* were detected (Bontrop et al. 1999). This locus is regarded as a pseudogene locus in humans. The high homology of the *Aona-DQB2*0102* and *Aona-DQB2*0202* to *HLA-DQB2* sequences underlines the old age of this locus and may reflect the lack of positive selection also in *Aotus*.

While peptide-binding motifs have been elucidated for many HLA-DR alleles, the peptide binding specificity of human HLA-DQ is still controversial (Reizis et al. 1998). Based on the crystallographic structure of murine I-A, the equivalent of HLA-DO, four essential pockets named P1, P4, P6 and P9 could interact with the amino acid side chains of bound peptides (Fremont et al. 1998). Pocket P4 is described as one major determinant of peptide binding (Fu et al. 1995). It could be shown that, in particular, the polymorphic residues at positions β 70, β 71 and β 74 contributing to pocket P4 strongly influence the peptide binding specificity (Stern et al. 1994; Ou et al. 1998). Peptides carrying a negative charge at relative position 4 favorably bind to class II molecules with a positively charged pocket P4, but not to class II molecules with a negatively charged pocket P4 (Djoulah et al. 1999). The positions β 70, β 71 and β 74 are occupied in humans by motifs RKA (positive), RTE (neutral), EDS (negative), GTE (negative) and RTA (positive), with the resulting overall charges given in brackets. Aona-DQB1 alleles carry the motifs RTE (neutral), STE (negative), STA (neutral), RVE (neutral) and RVV (positive). The motif RTE is present in humans and Aotus. Hence, both species have P4 pockets which are charged (positive and negative) or neutral.

The Asp/non-Asp dimorphism found at position $\beta 57$ of *HLA-DQB1* alleles contributes to the peptide binding specificity to HLA-DQ molecules as demonstrated by peptide binding studies (Nepom et al. 1996). According to protein crystal structure analysis, residue $\beta 57$ contributes to the ninth peptide binding pocket and forms an ion pair with the Arg present at position 79 on the *DQ* α chain (Brown et al. 1993; Stern et al. 1994). The amino acid positions $\beta 57$ and $\alpha 79$ are also occupied by Asp and Arg, respectively, in the *Aotus* monkeys. Therefore, similar rules might govern the binding of certain amino acid side chains in pocket 9 in *Aotus* and humans.

In humans, the combination of DQA1 and DQB1alleles is highly restricted by haplotype and there is evidence for structural restriction on the α and β chains forming functional heterodimers (Bergstrom and Gyllensten 1995). This phenomenon could indicate that the narrow repertoire of DQA1 lineages in *Aotus* is imposed by the limited repertoire of DQB1alleles or vice versa.

The Saoe-DQA1 and Saoe-DQB1 loci of the cottontop tamarin appear to be essentially monomorphic (Gyllensten et al. 1994). Furthermore, in the common marmoset the diversity of DQA and DQB alleles is also very limited (Antunes et al. 1998). The results of the analysis of MHC DQB1 sequences in a randomly selected population of monkeys indicates that A. nancymaae is the first example of a New World monkey species displaying a polymorphic Aona-DQB1 repertoire.

In summary, we present sequences of five Aona-DQA1 alleles present in three randomly selected animals. Fourteen alleles of *Aona-DQB1* and two alleles of *Aona-DQB2* could be identified in 19 animals. Taken together, the *DQA1* and *DQB1* polymorphism in the New World monkey *A. nancymaae* seems to be more limited than in Old World Simians (Bergstrom and Gyllensten 1995), but functionally important residues for peptide binding are conserved between *Aotus* and humans.

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