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Evolutionary stability of MHC class II haplotypes in diverse rhesus macaque populations

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Abstract A thoroughly characterized breeding colony of 172 pedigreed rhesus macaques was used to analyze exon 2 of the polymorphic *Mamu-DPB1, -DQA1, -DQB1,* and *-DRB* loci. Most of the monkeys or their ancestors originated in India, though the panel also included animals from Burma and China, as well as some of unknown origin and mixed breeds. In these animals, mtDNA appears to correlate with the aforementioned geographic origin, and a large number of *Mamu* class II alleles were observed. The different Mamu-*DPB1* alleles were largely shared between monkeys of different origin, whereas in humans particular alleles appear to be unique for ethnic populations. In contrast to *Mamu-DPB1*, the highly polymorphic *-DQA1/DQB1* alleles form tightly linked pairs that appear to be about two-thirds population specific. For most of the *DQA1/DQB1* pairs, *Mamu-DRB* region configurations present on the same chromosome have been ascertained, resulting in 41 different *-DQ/DRB* haplotypes. These distinct *DQ/DRB* haplotypes seem to be specific for monkeys of a determined origin. Thus, in evolutionary terms, the *Mamu-DP, -DQ*, and *-DR* regions show increasing instability with regard to allelic polymorphism, such as for *-DP/DQ,* or gene content and allelic polymorphism, such as for *-DR*, resulting in population-specific class II haplotypes. Furthermore, novel haplotypes are generated by recombination-like events. The results imply that mtDNA analysis in combination with *Mhc* typing is a helpful tool for selecting animals for biomedical experiments.

Keywords MHC · Non-human primate · Evolution

Introduction

Occupying habitats stretching from eastern Afghanistan and Pakistan in the west to the East China Sea in the east, the rhesus monkey has the widest geographic range of any non-human primate. The territories range from sea levels to >3,000 m and from semi-desert scrub to humid green forest (Melnick et al. 1993), and the monkeys vary widely in accordance with their extensive distribution (Fooden 2000). Despite this unusually large and ecologically heterogeneous range, however, rhesus macaques have been divided into very few morphological subspecies, and an unambiguous division of subspecies seems to be virtually impossible (Fooden 2000; Melnick et al. 1993). However, mtDNA analysis, has revealed the interpopulation and intraspecific diversity of the rhesus macaque species (Fooden 2000; Hayasaka et al. 1996; Melnick et al. 1993; Morales and Melnick 1998; Tosi et al. 2000). Phylogenetic trees based on mtDNA restriction enzyme analysis of rhesus macaques from Pakistan, India, China, and Burma reveal a major east-west division (Melnick et al. 1993), and indicate that Indian/Pakistan monkeys evolved away from the Chinese and Burmese animals. This division may have been caused by a glacial ice barrier in the Brahmaputra River valley during the Pleistocene. Comparable results have been reported from the sequence analyses of different fragments of mtDNA, whereas from Y-chromosome topology no or only slight evidence of paraphyly among rhesus macaques [*Macaca mulatta* (abbreviated as *Mamu*)] has been shown (Melnick et al. 1993; Tosi et al. 2000, 2002; Verschoor, personal communication). Based on the extreme philopatry of female rhesus macaques, the discrepancies between mtDNA and Y-chromosome topologies are possibly explained by either Y-chromosome introgression or

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mitochondrial differential lineage sorting (Tosi et al. 2000). It is also plausible that *SRY* gene mutations are rarely permitted, because this part of the Y chromosome is subject to differential selection forces and is therefore less variable than the mtDNA 12S region.

The rhesus macaque is the most commonly used non-human primate model in pre-clinical, immunological research on transplantation, as well as on chronic and infectious diseases. In humans, as in rhesus macaques, the major histocompatibility complex (MHC) gene products play an essential role in adaptive immunology. MHC class I and II molecules are cell surface glycoproteins that present peptides of intra- or extra-cellular origin to $CD8^+$ and $CD4^+$ T cells, respectively. The most striking feature of the vertebrate MHC complex is the extensive polymorphism of some of its genes, leading to an unprecedented variety of gene products. T cells recognize self- and foreign-antigens in the context of MHC class I and II molecules, and the T-cell repertoire is shaped by these peptide-MHC complexes. Differential peptide binding of distinct MHC molecules can lead to different T-cell activation and, as a consequence, particular MHC molecules/alleles are associated with susceptibility or resistance to infectious diseases (Balla-Jhagjhoorsingh et al. 1999; Bontrop 2001; Boyson et al. 1996; Evans et al. 1999; Furchner et al. 1999; Horton et al. 2001; Muhl et al. 2002; Reichstetter et al. 1999; Sauermann et al. 2000). On one hand, activation of T cells which recognize self-peptides especially in the context of MHC class II molecules seems to be a risk factor for autoimmune diseases (Brok et al. 2000; Nepom and Kwok 1998; Slierendregt et al. 1995a; t Hart et al. 2001). On the other hand, foreign allogeneic donor-peptides presented by host MHC class I and II molecules can lead to graft rejection or graft-versus-host reaction after transplantation (Balner and Van Rood 1971; Jonker et al. 1998; Reichstetter et al. 1999; Wood et al. 2001).

As in humans, the class II region of the rhesus macaque is divided into -DP, -DQ, and -DR. The classical class II molecules are transmembrane heterodimers, composed of an α- and β-chain subunit, encoded by *A* and *B* genes, respectively, most of which show an equal or even higher degree of polymorphism in rhesus monkeys than in humans. Most of the sequence variation is confined to exon 2 of *Mamu-DPB1, -DQA1, -DQB1,* and *-DRB* genes. So far, 16 *Mamu-DPB1*, 18 *-DQA1*, 40 *-DQB1*, and 128 *-DRB* alleles have been reported (IMGT/NHP sequence database; Robinson et al. 2003). In contrast to humans, the *Mamu-DPA1* gene is invariant and *-DPB1* only moderately variable (Slierendregt et al. 1995b), whereas the *HLA-DPA1* gene is oligomorphic and *-DPB1* highly polymorphic. *Mamu-DQA1/DQB1* pairs are tightly linked, even more than in humans, giving rise to only a small number of different *DQA1/DQB1* combinations (Doxiadis et al. 2001; Otting et al. 2002; Vigon and Sauermann 2002).

The most striking feature of the *Mamu* class II region, however, is its high level of *-DRB* region configuration polymorphism, displaying diversity with regard to number and combination of loci, but with only limited allelic polymorphism within a given configuration (Doxiadis et al. 2000; Khazand et al. 1999; Lobashevsky et al. 1999; Otting et al. 2000). In extended studies of pedigreed rhesus macaques, at least 31 *Mamu-DRB* region configurations could be described, coupled to only a few *DQA1/DQB1* pairs and vice versa (Doxiadis et al. 2001; Khazand et al. 1999). In biomedical studies, intensive use is made of rhesus macaques having widespread origins. Hence, the need exists for this animal's MHC to be well characterized for a comparison of differences in their polymorphisms, diversity, and haplotype composition. Accordingly, this study involving pedigreed rhesus macaques of different origins describes a comparative analysis of exon 2 of the polymorphic *Mamu-DPB1, -DQA1, -DQB1,* and *-DRB* class II loci.

Material and methods

Animals

In this study, 172 rhesus macaques from the self-sustaining colony at the Biomedical Primate Research Centre (BPRC) were analyzed. Most were born in captivity, but the founder animals came from different supplier centers and zoos. The origin of most of the rhesus macaques has been documented. All animals were serologically typed for their Mamu-A, -B, and -DR antigens.

DNA isolation and direct sequencing of *DQA1*, *DQB1*, and *DPB1* exon 2

Genomic DNA was extracted from EDTA blood samples or from immortalized B lymphocytes by a standard salting-out procedure. Samples were subjected to polymerase chain reaction (PCR) in a 50-μl reaction mixture containing 500 ng DNA, 250 μM each dNTP, 1×PCR buffer II (Applied Biosystems, Roche, N.J., USA), 2.5 mM $MgCl_2$, 1.25 units *Taq* polymerase (Applied Biosystems), and 0.4 μM of each primer. The following primers were used for amplifying exon 2:

5′DQA1(GH26 + *M13*)
*TGTAAACGACGCCAGT*GTGTAAACTTGTACCAG
3′DQA1 (GH27, *Bam*HI)
CAC*GGATCC*GGTAGCAGCGGTAGAGTTG
5′DQB1 (+ *M13*)
*TGTAAACGACGGCCAGT*TCCCCGCAGAGGATTTCGTG
3′DQB1(*Xba*I)
TGC*TCTAGA*GGGCGACGACGACGCCTCACCTC
5′DPB1(+ *M13*)
*TGTAAACGACGGCCAGT*GAGAGTGGCGCCTCCGCTCAT
3′DPB1 (*Xba*I)
CCC*TCTAGA*GCCCGGCCCAAAGCCCTCACTC.

The cycling parameters consisted of an initial denaturation step of 2 min, 94 °C, followed by three cycles of 90 s for each step at 94 °C, 56 °C (*DQA1*) or 60 °C (*DQB1/DPB1*), and 72 °C, then 32 cycles of 30 s for each step at the same temperatures. A final extension step was performed at 72 °C for 7 min.

The PCR products were purified using the QIAquick gel extraction kit (QIAGEN GmbH, Germany) according to the manufacturer's recommendations.

The purified DNA was sequenced on the ABI 3100 genetic analyzer (Applied Biosystems, Foster City, Calif.) in accordance with the manufacturer's guidelines, using 200–500 ng purified DNA, 0.2 μM primer, 1 μl BigDye terminator (Applied Biosystems), and 1×dilution buffer (400 mM Tris-HCl, 10 mM $MgCl_2$) in

a total of 10 μl. Sequences were analyzed using HETERO for heterozygote analysis based on sequence chromatograms and ALLELE for allele assignment (Rozemuller et al. 1993, 2001; Versluis et al. 1993).

Denaturing gradient gel electrophoresis (DGGE) of *DRB* exon 2 and sequencing

Separation of *Mamu-DRB* alleles with DGGE, dilution of DNA out of visualized bands, and subsequent direct sequencing of the alleles was performed as described earlier (Doxiadis et al. 2000; Knapp et al. 1997) with the sequencing primers 5′DRBseq and 3′DRBseq (Khazand et al. 1999). For cloning and sequencing purposes, exon 2 was amplified in a PCR reaction as described for *DQA1/DQB1/DPB1*, but in a final volume of 100 μl, with the following primers:

5′DRB (*Sal*I)	CCG*GTCGAC*TGTCCCCCCAGCACG-TTTC
3′DRB (*Xba*I)	*TCTAGA*TCACCTCGCCGCTGCACTGT.

Purification of PCR products, restriction enzyme digestion, cloning, and isolation of ssDNA were performed as described (Kenter et al. 1992). Cycle sequencing reactions were as summarized above. The data were analyzed using the program "Navigator" (Applied Biosystems, Roche, N.J.).

MtDNA analysis

Amplification of part of the mitochondrial 12S rRNA gene was performed essentially according to published methods (Kocher et al. 1989). Briefly, 200–500 ng of mtDNA, isolated from fresh EDTA blood, immortalized PBLs, or serum, was used as template in a final reaction volume of 50 μl, containing 1×PCR buffer + BSA (Applied Biosystems), 2 mM $MgCl_2$, 200 μM each dNTP, 2 units Ampli*Taq* Gold (Applied Biosystems). The cycling reaction started with an activation step at 95 °C for 15 min, followed by 35 cycles at 95 °C for 20 s, 55 °C for 20 s, 72 °C for 40 s, and a final elongation step at 72 °C for 5 min.

The PCR products were purified and sequenced as described above for *DQA1/DQB1/DPB1* and analyzed using the "Navigator" program.

Phylogenetic analysis

Multiple sequence alignments were created using the ClustalW Sequence Alignment program included in the MacVector 6.0 software package (Oxford Molecular). Phylogenetic analysis of the 12S rRNA nucleotide sequences was performed using PAUP version 4.0b10 (Swafford 2002). Pairwise distances were calculated using the Kimura-2 parameter, and the neighbor-joining method was used to create a phylogram. Confidence estimates of the groupings were calculated using the bootstrap method, generated from 1,000 replicates.

Results and discussion

Animal origin and mtDNA genotype

From a pedigreed rhesus macaque colony of approximately 900 animals, 172 individuals were selected. Indication of their origin or the origin of their ancestors was given by the respective import facilities. The founder animals showed morphological differences for pelage, body and head length and shape, and weight. However, as the intraspecies morphological differences of rhesus macaques can be greater than the differences between animals of different origins (Fooden 2000), morphological criteria are not sufficient to determine the origin of rhesus macaques. Therefore, mtDNA sequences were analyzed from nearly all animals to obtain further information about their origin. For the mtDNA analysis, we chose a segment of the 12S ribosomal RNA coding sequence, which is generally accepted to show species-related variability (van der Kuyl et al. 1995, 2000). For those animals of uncertain origin, paternal and maternal ancestors, if available, were additionally subjected to phylogenetic analysis.

The resulting phylogenetic tree has three main branches; one represents monkeys from the western part of the macaque habitat, namely, Indian monkeys, while the second represents sequences of animals from the eastern region (Fig. 1). Whereas the 12S mtDNA of Indian-origin monkeys form a nearly uniform cluster, the second branch is divided into at least two clades: one of Chinese-origin monkeys and the other of Burmese-origin macaques. Both clades are more diverse than the Indian cluster. In addition, the third branch forms an outgroup of all animals analyzed (Fig. 1, K96 with children L99 and 9176, as well as L93, L95, and L65). Considering that branching distance in a mtDNA phylogenetic tree is related to geographic distances (Zhang and Shi 1993), these rhesus macaques seem to have originated a considerable distance away from the habitats of the other rhesus macaques included in this study. Since an *SRY* gene sequence analysis examined so far shows no differences at all (data not shown), molecular data, which could be informative concerning monkey origins, are restricted, as mtDNA is inherited only via the maternal lineage. Thus, conclusions about the origin of the respective rhesus macaques must be drawn with caution. For example, whether monkeys L93 and L95 are thoroughbred remains uncertain, because the father was not available for testing. Rhesus macaques BB10 and BB113, however, were proven to be Chinese/Indian mixed-breed monkeys in keeping with the Chinese origin of the father, 3945, although in the phylogenetic tree their mtDNA cluster together with Indian monkeys (Fig. 1). With these reservations in mind, we designated 110 animals included in this study to be of Indian origin, 24 of Burmese, and three of Chinese, in addition to 26 Chinese/Indian and four Burmese/Indian mixed-breeds and five mixed-breeds of Indian and unknown origin. Animals of non-Indian origin included in the study are listed in Table 1.

MhcMamu haplotypes

Serologically defined Mamu-A, -B, -DR haplotypes

The 900 BPRC-bred rhesus macaques were pedigreed and 253 major histocompatibility (MHC) haplotypes could be defined based upon the combination of different Mamu-A, -B, and -DR serotypes. Since most of the animals originated in India, 231 of these MHC haplotypes were specific for Indian rhesus macaques. An additional 22

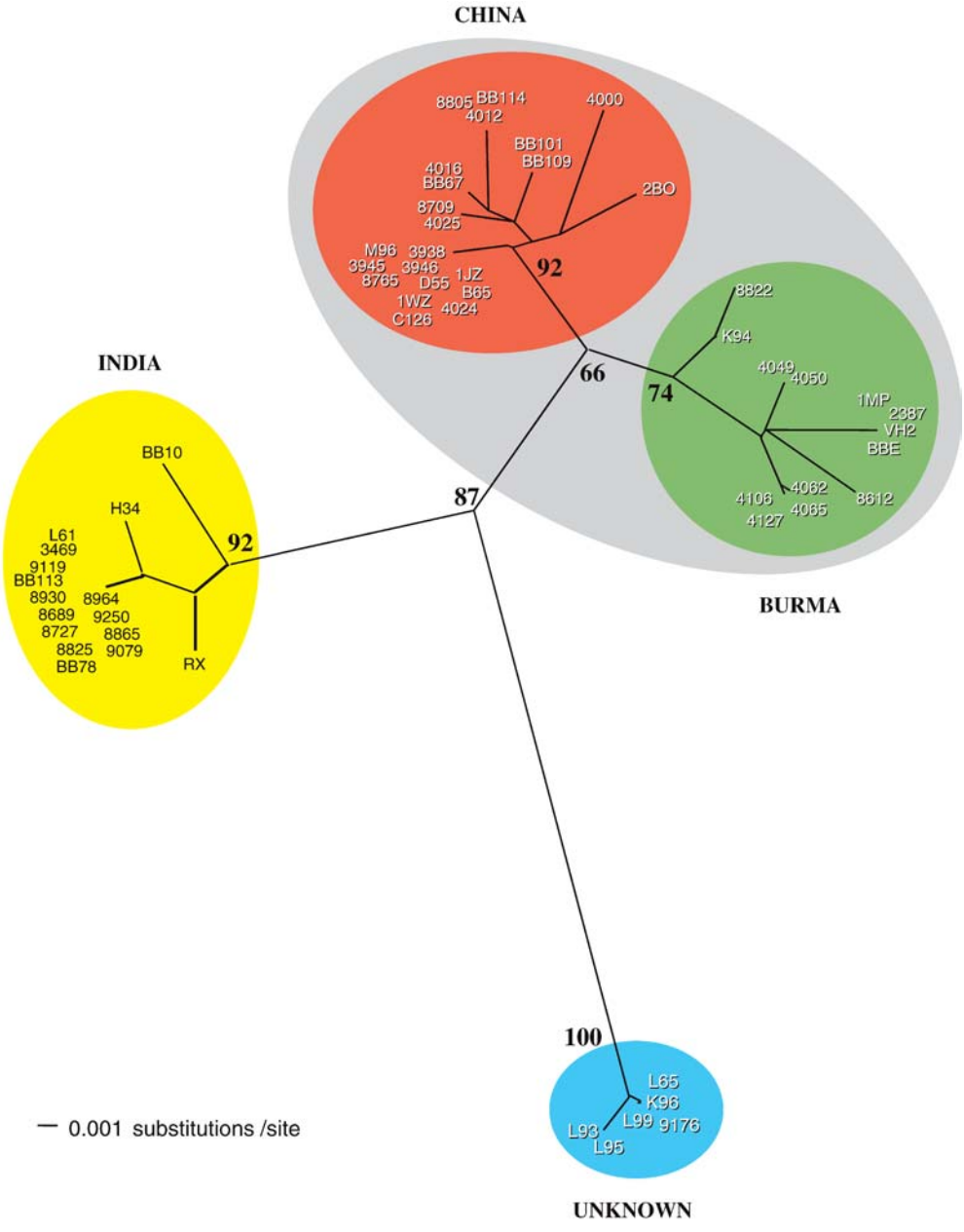

Fig. 1 Phylogenetic tree of mtDNA sequence coding for the 12S rRNA of selected rhesus macaques of different origins. Geographic clusters are marked by *colors*

unique Mamu-A, -B, and -DR haplotypes were observed only in monkeys of Chinese origin. Nearly all the serologically defined MHC haplotypes seem to be origin specific. For rhesus macaques originating in Myanmar (Burma) and of unknown origin, serological typing was hampered because the typing sera used were generated by active immunization against the founder rhesus macaque population, which was mostly of Indian origin.

MhcMamu class II haplotypes defined by molecular methods

Haplotype definition was performed by segregation analysis, with one exception: one *Mamu-DQ/DRB* haplotype was defined by comparing the combination of alleles with those of a homozygous same-origin animal. In the case of haplotype determination of rhesus macaques from India and China, the molecular typing data provided additional information to the known, serologically defined haplotypes.

DP region

Mamu-DPA1 and *-DPB1* genes encode the functional DP protein. Whereas the *Mamu-DPA1* gene is monomorphic, the *-DPB1* gene exhibits moderate allelic variation, with 16 alleles described so far (Slierendregt et al. 1995b; IMGT/NHP sequence database). From the selected panel,

Table 1 Rhesus macaques from Burma, China, and mixed-bred animals. (*In* India, *Bu* Burma, *Ch* China, *Un* Unknown; *f* father, *m* mother, *?* parents not known)

Monkey	Origin	Parents (f × m)	Monkey	Origin	Parents (f × m)
4062	Bu	?	8805	In × Ch	4032 × 4012
4070	Bu	?	BB101	In? × Ch	BB69 × ???
4097	Bu	?	D55	In × Ch	2777×3946
4094	Bu	?	BB10	Ch × In?	3945 × 2677
4105	Bu	?	BB36	Ch × In	3945 × 3617
4106	Bu	?	2BO	In × Ch	2774 × 4000?
4096	Bu	?	BB58	Ch × In	3945 × 3614
4051	Bu	?	94021	In × Ch	2BA × 4016
4049	Bu	?	BB117	In × Ch	BB66? × 4024
4050	Bu	?	1VV	In × Ch	3074 × 3939?
4064	Bu	?	BB114	In × Ch	BB66 × 4025
4065	Bu	?	BB77	Ch × In	3945 × 3621
4067	Bu	?	97011	Ch × In	D55 × TA
4072	Bu	?	98004	Ch × In	D55 × 8669B
4074	Bu	?	98060	Ch × In	D55 × TA
4077	Bu	?	r99007	Ch × In	D55 × 8669B?
9309	Bu	4049 × 4050	95030	In × Ch	C13 × M96
95043	Bu	4049 × 4074	BB113	Ch × In	3945 × ???
96047	Bu	4049 × 4064	BB78	Ch × In	3945 × 3649
96090	Bu	4049 × 4050	BB67	Ch × Ch	3945 × 4012?
97050	Bu	4049 × 4064?	M96	Ch	?
97030	Bu	4049 × 4050?	BB109	Ch × Ch	3945 × 4024
97042	Bu	4049 × 4065	8822	In? × Bi	H34 × K94
94018	Bu	4049 × 4050	8612	In × Bi	???? × 2387
1JZ	In × Ch	3074 × 3939	1MP	In × Bi	2957 × 3862
B65	In × Ch	2957 × 1OR	9145	In × Bi	3643 × 4095
1NS	In × Ch	2777 × 3938	L93	In? × Un	K10 × K98
1WZ	In × Ch	ME × 3942	L95	In? × Un	H34 × K96
8704	In × Ch	2777 × 3938	L99	In? × Un	H34 × K96
8709	In × Ch	4032 × 4012	9176	In? × Un	H34 × K96
8765	In × Ch	ME × 3942	L65	In? × Un	H34? × ?

Fig. 2 Frequencies of *-DPB1* alleles in rhesus macaques of different origins. Origin-specific alleles are highlighted by the same colors as used in Fig. 1. *N* number of haplotypes; *gf* gene frequency; [a]number of rhesus macaques was too small to calculate gene frequencies; *n.d.* not determined because of homozygosity or uncertainty of segregation

	India		Burma		China[a]	Unknown[a]
*Mamu-DPB1**	N=239	gf	N=52	gf	N=28	N=5
01	28	0.117				
02	12	0.050				
03	12	0.050				1
04	22	0.092	3	0.058	1	
05			1	0.019		
06	30	0.126	3	0.058	1	
07	1	0.004			2	
08	10	0.042	1	0.019		
09			2	0.038		
10	54	0.226	8	0.154	2	2
11	19	0.079	1	0.019		
12	8	0.033	5	0.096		
13	13	0.054	4	0.077	6	
14			6	0.115		
15					1	
n.d	30	0.126	18	0.346	15	2

	India		Burma		China[a]	Unknown[a]
*Mamu-DQB1**	(N=214)	gf	(N=50)	gf	(N=32)	(N=5)
0601	30	0.140	8	0.160		
0602	8	0.037	1	0.020		
0605	7	0.033	1	0.020		
0606	4	0.019	1	0.020		
0607					1	
0608						1
0609	7	0.033				
0610			1	0.020		
06112			1	0.020		
1501	13	0.061	1	0.020	7	1
1502	1	0.005	1	0.020		
1503			7	0.140		
1601			1	0.020	5	
1704					1	
1705	1	0.005				
1706					3	
1708	1	0.005				
1710			1	0.020		
1801	42	0.196	6	0.120		1
1802	12	0.056				
1803					1	
1804	14	0.065	1	0.020		
1808	16	0.075	4	0.080		
1809	4	0.019				
1810	6	0.028	1	0.020	5	
1811	33	0.154	4	0.080	2	
1815						1
2401			2	0.040	3	
n.d.	15	0.070	8	0.160	4	1

Fig. 3 Frequencies of *-DQB1* alleles in rhesus macaques of different origins. *N* number of haplotypes detected; *gf* gene frequency; [a]number of rhesus macaques was too small to calculate gene frequencies; *n.d.* not determined because of homozygosity or uncertainty of segregation

161 rhesus macaques were analyzed for their *Mamu-DPB1* alleles by oligotyping or direct sequencing (Doxiadis et al. 2001; Otting et al. 1998). Of the known 16 *-DPB1* alleles, 11 could be detected in monkeys originating from India. *Mamu-DPB1*05*, *-DBP1*09*, and *-DPB1*14*seem to be specific for Burmese monkeys, *-DPB1*15* for Chinese, and *-DPB1*01* and *-DPB1*02* for Indian animals (Fig. 2). The frequency of the specific *-DPB1* alleles varies in animals of Indian or Burmese origin, a phenomenon also observed for *HLA-DPB1* allele frequencies in human populations (Fig. 2). By far the most frequent *-DPB1* allele in Indian populations is *-DPB1*10*, whereas in Burmese rhesus macaques *-DPB1*14* is nearly as frequent. However, most of the *Mamu-DPB1* alleles are shared by animals of all different origins, which is in sharp contrast to humans, whose unique alleles are readily detected in novel ethnic populations. Moreover, these results confirm the low polymorphism and evolutionary stability of the *DPB1* locus in rhesus macaques (Doxiadis et al. 2001; Hashiba 2000; Otting et al. 1998).

DQ region

To date, 20 *Mamu-DQA1* and 40 *-DQB1* alleles have been described (Otting et al. 2002; Vigon and Sauermann 2002; Robinson et al. 2003). In the present study, we

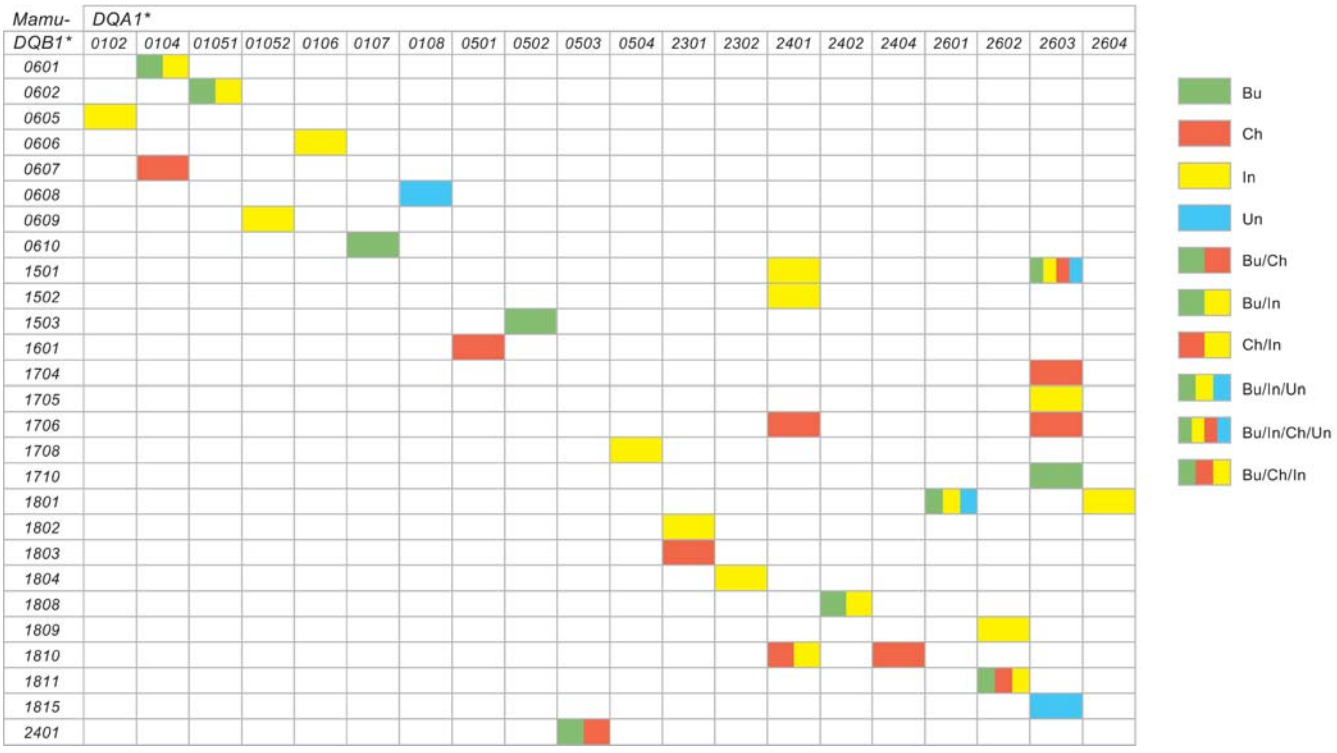

Fig. 4 *Mamu-DQA1/DQB1* combinations in rhesus macaques of different origins. *DQA1/DQB1* pairs specific for rhesus macaques of a certain origin are highlighted by the same bright colors as in Fig. 1 and Fig. 2. *Mamu-DQA1/DQB1* pairs present in monkeys of various origins are differentiated by composed colors (*In* Indian, *Bu* Burma, *Ch* China, *Un* Unknown)

analyzed approximately 150 animals by direct sequencing of exon 2 of their *Mamu-DQA1 and -DQB1* alleles, respectively. Nineteen *-DQA1* and 25 *-DQB1* alleles could be detected and, in addition, one unreported *-DQA1* and three *-DQB1* alleles were discovered. Of those 28 *Mamu-DQB1* alleles, 11 variants were observed in monkeys of both Indian as well as Burmese origin (Fig. 3). As observed for *-DPB1*, the *-DQB1* allele frequencies differ in the rhesus macaque populations. *DQB1*1801* is the most prominent allele in Indian rhesus macaques and the third most frequent in monkeys of Burmese origin, whereas for the *Mamu-DQB1*0601* frequency the opposite is observed. Both alleles, however, are absent in our rhesus macaques from China. Although only a few Chinese monkeys and very few monkeys of unknown origin were analyzed, six origin-specific *-DQB1* alleles could be detected in these populations. In contrast to *Mamu-DPB1* alleles, which are mostly shared by animals of all different origins, more than half of the *-DQB1* alleles, namely, 15 of 28 variants, are population specific (Fig. 3).

For 27 of the 28 *-DQB1* alleles observed in this study, the corresponding *-DQA1* allele, present on the same haplotype, was determined, resulting in only 31 *-DQA1/DQB1* combinations. This confirmed the extraordinarily strong linkage of *Mamu-DQA1* with *-DQB1* (Doxiadis et al. 2000) (Fig. 4). The population specificity observed for *-DQB1* alleles was increased when the presence and frequency of *DQA1/DQB1*pairs were analyzed. More than two-thirds, namely 23, of the 31 *DQA1/DQB1* combinations were seen in monkeys of one specific origin only. According to the frequency of Indian macaques within the panel, most of the origin-specific *-DQ* pairs were detected in rhesus macaques from India, but Chinese-, Burmese-, and unknown origin-specific combinations were present as well (Fig. 4). Members of the ancient lineage *Mamu-DQB1*06* all form unique and mostly population-specific combinations with the *-DQA1* lineage *-DQA1*01*. The same holds for the ancient *-DQA1*05,* which forms unique and mostly population-specific combinations with *-DQB1* alleles, although these belong to different lineages.

DQ/DR region

One hundred animals from the rhesus macaque panel were analyzed for their *-DRB* alleles by cloning and

Fig. 5 *Mamu-DQ/DRB* haplotypes in rhesus macaques of different origins. *DQ/DRB* haplotypes are listed according to *DQA1/DQB1* pairs. The origin of the rhesus macaques in which a certain *DQ/DRB* haplotype is detected is indicated by *colors*. [a]Only *-DRB6* alleles without the 63-bp deletion are summarized. [b]The presence of *DRB1*1003* on this haplotype has been proven only by segregation in one offspring of the family. Alleles mentioned *in parentheses* may also be present; + the presence of a specific allele of a certain lineage has not yet been confirmed (In India, Bu Burma, Ch China, Un unknown)

No	origin	DQA1*	DQB1*	DRB1*	DRB3*	DRB4*	DRB5*	DRB6*[a]	DRBW*	reference animal
1	In	0104	0601	0309				0101	201	3C
2	Bu	0104	0601	0321, 0322						4050
3	Bu	0104	0601	0321, 0323, 1003[b]						4049
4	In	01051	0602	0309				0101	201	1PV
5	In	01051	0602					0111	606, 2104, 2603	8862
6	Bu	01051	0602	0306, 07032					2603	4065
7	In	0102	0605	0306(5), 1003						1KM
8	In	0102	0605					112?	2002(1?), 2501	2Y
9	In	0106	0606	0317, 1008						1ZV
10	Un	0108	0608			0103			102, 101?	L95
11	In	01052	0609		0403				402, 2701	4041
12	Bu	0107	0610			0102	0306			8612
13	In	2603	1501	0701	0405		0303			1KM
14	Bu	2603	1501	0702	0406		0305			4094
15	Bu	0502	1503					0108, 0111[b]	606, 2104, 2603	4050
16	Bu	0502	1503	07032					403, 2702	8822
17	Ch	0501	1601	0306(5?), 07032				01+	2603(2)	BB113
18	In	2604	1801	0303, 1007						BB36
19	In	2601	1801	0303, 1007						1PS
20	In	2601	1801	0312, 1007						JY
21	In?	2601	1801	0406			0301			L93
22	Un	2601	1801						2003	L93
23	Bu	2601	1801	0309					2507	4049
24	In	2301	1802					0114	303, 401	4041
25	In	2302	1804	0310					101, 602, 609	2V
26	In	2402	1808	0403					501	2BZ
27	Bu	2402	1808	0403					502	4065
28	In	2402	1808	0313					604(6), 603(5)	P48
29	In	2602	1809	0306, 1003						1VK
30	In	2401	1810		0403(4?)				305	8845
31	Ch	2404	1810						3101	M96
32	Bu	2601	18+	0304, 1004				0110?	313	1MP
33	In	2601?	1811						2002(1?), 2501	BB113
34	Bu	2602	1811	0306, 1003						4062
35	In	2602	1811	0406			0301			1SA
36	In	2602	1811					0112	2501	BB24
37	In	2602	1811						702, 2002?	9055
38	In	2602	1811	0404					307, 702	BB103
39	Un	2603	1815	0406			0301			L99
40	Ch	0503	2401		0408				404	BB36
41	Bu	0503	2401	07031		0103			2505	4096

origin	DQA1*	DQB1*	DRB1*	DRB5*	DRB6*	DRBW*
In	0102	0605	0306,1003			
In	2602	1809	0306,1003			
Bu	2602	1811	0306,1003			
In	0104	0601	0309		0101	201
In	01051	0602	0309		0101	201
In	0102	0605			0112?	2002(1), 2501
In	2601?	1811			?	2002(1), 2501
In	01051	0602			0111	606, 2104, 2603
Bu	0502	1503			0111	606, 2104, 2603
In?	2601	1801	0406	0301		
In	2602	1811	0406	0301		
Un	2603	1815	0406	0301		
Bu	01051	0602	0306, 07032		?	2603
Ch	0501	1601	0306, 07032		01+	2603

Fig. 6 Identical *Mamu-DRB* region configurations in combination with different *-DQA1/DQB1* pairs. Alleles *in parentheses* may also be present; + the presence of a specific allele of a known lineage has not yet been confirmed; *?* the presence of a specific allele or lineage has not yet been confirmed (In India, Bu Burma, Ch China, Un unknown)

sequencing of exon 2 (Otting et al. 2000). The *-DRB* alleles of one additional animal were deduced by comparison with known alleles in DGGE (Doxiadis et al. 2001; Otting et al. 2000). Of the *Mamu-DRB* alleles known thus far (IMGT/NHP sequence database), 65 were detected and an additional four could be described. For 22 of the 31 different *-DQ* combinations (Fig. 4) the 32 accompanying *-DRB* configurations were determined, resulting in 41 distinct *DQ/DRB* haplotypes (Fig. 5). For monkeys from a certain geographic region, 29 of the 32 *-DRB* haplotypes are specific; however, all 41 of the *DQ/DRB* haplotypes are origin specific (Fig. 5). Most of the *-DQ* pairs are observed in combination with one or two different *-DRB* configurations. Only two *DQA1/DQB1* pairs, *DQA1*2601/DQB1*1801* and *DQA1*2602/DQB1*1811,* are seen with more than three *-DRB* region configurations. Twenty-three *DQ/DRB* haplotypes were detected in rhesus macaques originating in India, 12 in animals from Burma, three in those from China, and three in those of unknown origin. Some of these haplotypes are combinations of *DQ* pairs with unique, population-specific *DRB* region configurations. For example, the *-DQA1*0503, -DQB1*2401* pair is linked to the *-DRB* region configuration *-DRB3*0408*, *-DRB*W404* specifically in Chinese monkeys, whereas the same *-DQ* pair is coupled to the *-DRB* region configuration *-DRB1*07031, -DRB4*0103*, *-DRBW*2505* in Burmese rhesus macaques (Fig. 5, nos. 40 and 41). Other origin-specific *DQ/DRB* combinations differ from those observed in monkeys from different habitats only by allelic *-DRB* variations (Fig. 5, nos.13 and 14). A third possibility for creating a variant *DQ/DRB* haplotype is by recombination of a given *-DRB* haplotype with different *DQA1/DQB1* pairs (Fig. 6). Four *-DRB* region configurations are observed which combine with two different *Mamu-DQ* pairs. Only two *-DRB* haplotypes, *Mamu-DRB1*0306, -DRB1*1003,* and *-DRB1*0406, -DRB5*0301,* were observed in combination with more than two *DQA1/DQB1* pairs.

Class II haplotype stability

The polymorphic *Mamu-DPB1* locus differs from its human counterpart in the degree and type of polymorphism (Bontrop et al. 1999; Doxiadis et al.2000, 2001; Hashiba 2000; Otting et al. 2000). The level of allelic variation with only 16 *Mamu-DPB1* alleles described is much lower in rhesus macaques than in humans, whereas the situation is *vice versa* for variability indices. Therefore it is not surprising that rhesus macaques of origins as varied as India, China, and Burma show only small differences in the presence and frequency of *-DPB1* alleles. Furthermore, the *Mamu-DPA1* locus is described as being monomorphic, so the *-DP* region in rhesus macaques seems relatively stable. With the *Mamu-DQ* region, however, the situation is different. First, the amount of polymorphism for *-DQA1*, as well as *-DQB1*, is in rhesus macaques at least as high as in humans. Second, the number of *Mamu-DQB1* alleles, and even more distinct, those of *DQA1/DQB1* pairs that are specific for monkeys of a specific origin, is much higher than in the case of the *-DPB1* locus, namely, two-thirds. This

origin	DQA1*	DQB1*	DRB loci/lineages					
In	2401	1810	*W305	3*0403				
In	01052	0609		3*0403	*W402	*2701		
In	0104	0601		1*0309	*W201	*0101		
Bu	2601	1801	*W2507	1*0309				
Bu	0503	2401		4*0103	1*07031	*W2505		
Un	0108	0608	*W102	4*0103				
In	2602	1811	*W702	*W2002				
In	0102	0605		*W2002	*W2501	*0112?		
In	0102	0605	1*1003	1*0306				
Ch	0501	1601		1*0306	1*07032	*W2603	6*01+	
In	01051	0602				*W2603	6*0111	*W606 *W2104

Fig. 7 Possible generation of new *Mamu* class II haplotypes. A *question mark* indicates that the presence of a specific allele has not yet been ascertained; + the presence of a certain allele of a known lineage has not yet been determined (In India, Bu Burma, Ch China, Un unknown)

population specificity is increased when in addition the -*DRB* region is observed; nearly all of the -*DRB* haplotypes are specific for rhesus macaques of a certain origin. Unfortunateley, *Mamu* class II haplotype data of non-Indian rhesus macaques are restricted in this study because of the presence of mostly Indian monkeys within the BPRC's breeding colony. As a result, only a limited number of haplotypes based on segregation studies have been deduced. However, analysis of exon 2 of -*DRB* from several Chinese monkeys by DGGE revealed, for most of the animals, unknown banding patterns. These preliminary data indicate that *Mhc* class II analysis of breeding colonies of non-Indian rhesus macaques will result in the definition of a large number of undescribed *Mamu-DRB* configurations and class II haplotypes. Furthermore, the combination of *DQA1/DQB1* and -*DRB* alleles leads to 41 haplotypes, all of them population-specific. Recombination events not only between the *DP* and *DQ/DR* region, but also between *DQ* and *DR* seem to be one of the possibilities for the formation of a new haplotype (Fig. 6). Furthermore, the existence of the same -*DRB* alleles in two or more different *DQ/DRB* region configurations makes it plausible that new haplotypes were generated by unequal crossing-over events (Fig. 7).

Therefore, simple allotyping, such as for -*DRB1* alone in humans, is not sufficient for rhesus macaques. A genetically well-characterized rhesus macaque colony will help to breed and define animals carefully for biomedical research in case of known susceptibility or resistance *MHC* alleles. Examples are given by the *Mamu-A*01* allele, the gene product of which is associated with an improved control of a simian immunodeficiency virus infection, and the Mamu-A26 molecule that is highly protective against collagen-induced arthritis (CIA), the experimental form of rheumatoid arthritis (Allen et al. 2001; Mothe et al. 2003; Bakker et al. 1992). In addition, class II molecules are described in rhesus macaques as being involved in susceptibility to experimental autoimmune encephalomyelitis (EAE) in rhesus macaques, the experimental form of multiple sclerosis (MS) (Slierendregt et al. 1995a). The gene product of *Mamu-DPB1*01,* which was demonstrated to function as restriction element in myelin basic protein (MBP) peptide presentation, was in this study detected only in Indian monkeys. As well as in disease association studies, *Mhc* typing data are also crucial for transplantation studies. Furthermore, definition of the *MHC* class II haplotypes in rhesus macaques of different origins can help to sustain an outbred rhesus macaque colony, which will ensure by its diversity that the appropiate animals are available to solve future questions. Thus, armed with knowledge of the MHC, researchers can make an informed choice about which animals to use to elicit the most relevant answer to a specific biomedical question. The approach described will lead to a refinement of the studies and to the need for fewer animals in biomedical research.

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