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Comparison of the Frequencies of Major Histocompatibility (MHC) Class-II DQA1 and DQB1 Alleles in Indian and Chinese Rhesus Macaques (*Macaca mulatta*)

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The major histocompatibility complex (MHC) comprises related gene families, some of which are highly polymorphic, whose protein products mediate immune response. Rhesus macaques (*Macaca mulatta*) are a vital animal model for research in human diseases and are native to regions extending from Afghanistan in the west to the Eastern Plains of China and from Peking to the north, southward through islands of Southeast Asia. The distributions of MHC class-II *Mamu* DQA1 and *Mamu* DQB1 alleles in two groups of domestically bred rhesus macaques of Indian and Chinese origin and the *Mamu* DQA1 genotypes of a small number of Burmese rhesus macaques were compared. Major allelic differences were observed between the Indian and Chinese rhesus macaques, and gene diversity decreased from east to west. These and other intra-specific genetic differences among regional populations of rhesus macaques might influence the outcome of biomedical research in which they are used as subjects, and illustrate the importance of completely genetically characterizing subjects used as animal models in biomedical research.

Recently, increasing attention has focused on identifying genetic factors contributing to the variable expression of disease susceptibility and resistance. The major histocompatibility complex (MHC), a 700,000-base pair (bp) region located on the short arm of chromosome 6 in humans (and chromosome 2 in rhesus macaques [*Macaca mulatta*]), encodes dimeric integral membrane proteins that mediate immune response. The MHC is divided into two classes with distinct immunologic roles. The MHC class-I molecules are peptides which, when complexed with intracellular processed antigens, activate the response of $CD8^+$ cytotoxic T lymphocytes (CTL) (1, 2).The highly polymorphic MHC class-II molecules, generally found on the surface of white blood cells, are peptide receptors that bind extracellular antigens stimulating either $CD4^+$ CTL function or antibody production (3, 4). The MHC class-II region is further divided into three sub-regions: DQ, DR, and DP. Each sub-region encodes multiple α- and β-glycoprotein chains that form functional dimers (5) whose products, two α-helices and a β-pleated sheet, form a peptide-binding groove (6). With the exception of DQA, all class-II α-genes are monomorphic (7), and the most polymorphic sites are located in the second exons of the β-genes (5).

Since the ban on importation of rhesus macaques from India began in 1978 (8), use of macaques of Indian origin bred domestically as animal models of human diseases has been the mainstay of biomedical research in the United States (9, 10). Because the MHC complexes of the rhesus macaque and human are analogous (11-13), rhesus macaques play a vital role in research on the relationship between the MHC class-II genes and immune diseases, such as acquired immune deficiency syndrome (AIDS) (3, 4, 14). Although rhesus macaques occupy an extensive geographic range that includes most of India, China, and the southeast Asian mainland, the National Institutes of Health (NIH) has designated Indian rhesus macaques with at least one MHC class-I *Mamu* A*01 allele as the animal of choice for subjects in AIDS-related research because the reagents necessary to study cell-mediated immunity in such animals are already available (14, 15). The genetic homogeneity of such an animal model should reduce the influence of inter-subject genetic heterogeneity on experimental outcomes, but it cannot reflect the full range of immune responses among humans.

More recently, rhesus macaques of Chinese origin have become available and are currently the only source for maintaining genetic heterogeneity in the domestic supply of rhesus macaques bred for biomedical research. Maintenance of genetic heterogeneity is especially crucial in members of specific-pathogen-free (SPF) rhesus colonies (16) used as subjects in AIDS-related research, because they have been carefully derived, descend from a few founders, and consequently, can experience genetic bottlenecks that reduce fitness and lead to genetic subdivision of the domestic supply of SPF rhesus macaques (17). Additionally, use of Chinese rhesus macaques to supplement existing populations of domestic Indian rhesus ensures an adequate supply of rhesus macaques for future research. However, low frequencies of the *Mamu* A*01 allele have been reported in rhesus macaques of Chinese origin (18). Even captive Indian rhesus macaques bred at different facilities and whose founder populations derive from different regions of India have marked differences in the frequency (e.g., as low as 0% and as high as 25%) of this and other MHC alleles (19). Genetic subdivision resulting from processes, such as founder effect, inter-generational genetic drift, genetic bottlenecks, and remote consanguineous inbreeding undoubtedly contribute to these genetic differences (17). Although the relevance of these differences is unclear, the choice of suitable characteristics of an animal model for AIDS-related re-

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search will strongly influence the availability of subjects meeting these criteria for use in such research. Moreover, we cannot be certain that future sources for our foreign supply of rhesus macaques, should they change, will meet these criteria.

Numerous parameters have been used to compare Indian and Chinese rhesus populations in captive settings, including morphometric measurements (20), infant temperament and reaction to social separation and reunion (21, 22) and physiologic/biochemical parameters (22, 23). Substantial genetic differences between Indian and Chinese rhesus macaques at protein coding (17, 24, 25), microsatellite (26-28), and mitochondrial DNA (29-32) loci also have been documented. We have reported less pronounced differences in frequencies of genes at protein coding (17) and microsatellite (26) loci between rhesus macaques from eastern and central India. If any genetic differences among regional populations of rhesus macaques influence response to infection, such differences should be detectable at the MHC loci that mediate immune response. The single most appropriate animal model that is genetically homogeneous should be selected for studies of infectious diseases, such as simian immunodeficiency virus (SIV) infection. Recent studies of clinical responses of Chinese and Indian rhesus macaques to experimentally induced infection with SIV_{mac} suggest that Chinese rhesus macaques might actually provide a better model for AIDS-related research than do Indian rhesus macaques.

In a study involving use of Indian and Chinese rhesus macaques, Joag and co-workers (33) observed that Chinese rhesus were more resistant to SIV_{mac} infection than were Indian rhesus macaques. Consistent with this outcome, Ling and co-workers (15) found that, after experimentally induced infection with SIV_{mac} 239, plasma virus loads in Chinese rhesus macaques were significantly lower than those in Indian rhesus macaques. Sauermann and co-workers (34) documented that certain class-II *Mamu* DQB1 and *Mamu* DRB genes also influence the rate of progression of SIV infection in rhesus macaques. Genetic differences between Indian and Chinese rhesus macaques at MHC class-II loci might be responsible for some of the observed clinical differences between their respective responses to SIV infection.

Frequencies of MHC class-II *Mamu* DQA1 and DQB1 alleles in Indian rhesus macaques have been described (35-38). Humans and rhesus macaques share at least one lineage of alleles at both loci, which selectively pair to form dimers that are homologous and, therefore, have been highly conserved for at least 35 million years. Using polymerase chain reaction (PCR) methods followed by restriction analysis to identify restriction fragment length polymorphism (RFLP), we characterized the MHC class-II *Mamu* DQA1 and *Mamu* DQB1 allele frequencies of rhesus macaques of Chinese origin, and genotyped the MHC class- II *Mamu* DQB1 alleles of Indian rhesus macaques that had been typed for the *Mamu* DQA1 locus (35, 39).

Materials and Methods

Macaques and blood samples. Blood samples from 21 Chinese rhesus macaques that were randomly selected from a much larger number provided by COVANCE Inc. (The Texas Primate Center) in Alice, Texas, and those from 40 rhesus macaques of Indian origin that were acquired from the Laboratory Animal Breeders Services (LABS) of Virginia in Yemassie, South Carolina were studied. These animals represent random samples from large SPF colonies established approximately 15 years ago with the support of the National Center for Research Resources of the National Institutes of Health (16), and were studied, using protocols approved by the campus Animal Care and Use Committee. The 40 Indian rhesus macaques descend from a highly diverse population of founders trapped at a wide range of longitudes across the central portion of northern India (40), and are a subset of 65 Indian rhesus macaques that were previously genotyped for the second exon of the *Mamu* DQA1 gene (35). The genetic structure of this population is broadly representative of that of the domestic breeding colonies of Indian rhesus macaques in the United States (17). The Chinese rhesus macaques studied were infants born into three captive multimale-multifemale breeding groups between 1993 and 1998. Their sires and dams were imported in two separate shipments and, like most Chinese rhesus macaques in the United States, are believed to have originated in China's southwestern-most province, Yunnan. In addition, a small sample of Burmese rhesus macaques (n = 10) from the California Regional Primate Research Center at Davis was typed for the *Mamu* DQA1 locus. These Burmese animals also were screened for six hypervariable microsatellite, or STR, loci (D1s550, D2s1328, D5s1470, D6s501, D7s794 and D8s1106), using previously described methods (26-28), to ensure that the small numbers studied were sufficiently representative to avoid sampling errors.

Gene amplification. The methods used for *Mamu* DQA1 amplification are those outlined in Scharf and co-workers (41) and Sauermann and co-workers (36, 37), with a few minor modifications.The Indian rhesus macaque samples were extracted as described by Rolfs and co-workers (35).The DNA from all other samples was extracted from whole blood or plasma, using a Qiagen QIAmp Blood Mini Kit (Valencia, Calif.). Primers GH26 (5' GTGAAACTTGTACCAG 3') and GH 27 (5' CACGGATCCG GTAGCGGTAGAGTT 3') (34-36, 39) were used to amplify a 242-bp fragment of the second exon of the *Mamu* DQA1 locus. A 303-bp fragment of the second exon of the *Mamu* DQB1 locus was amplified, using primers DQB SalI (5' TCCCCGCAGAGGATT TCGTG 3') and DB131 (5' TCCTGCAGGGCGACGACGCTCAC CT 3') (34, 35).

Using a Perkin Elmer Applied Biosystems GeneAmp PCR Core Reagent kit (Foster City, Calif.), 1 μl of DNA template (4 to 12 μg) was added to a 100-μl PCR cocktail consisting of 1X PCR Buffer II, 200 μ*M* each dNTP, 2.5 U of Ampli*Taq* DNA polymerase/100 μl, 25 μ*M* each GH26 and GH 27, and 2.5 m*M* $MgCl_2$. Forty cycles of PCR amplification were conducted in a Perkin Elmer 9700 GeneAmp thermal cycler under the following conditions: initial hold at 95°C for 2 min, denaturation at 94°C for 30 sec., annealing at 55°C for 30 sec., and extension at 73°C for 30 sec., with a final extension of 72°C for 5 min. The DQB1 amplification was done as described previously, except with at an annealing temperature of 51°C and with 1.0 m*M* $MgCl_2$.

Successful amplification of the second exon of *Mamu* DQA1 was confirmed by screening PCR products together with a size standard, pBR322 digested by use of the *Msp* I restriction enzyme (New England Biolabs, Beverly, Mass.) on an 8% non-denaturing polyacrylamide gel to identify a 242-bp fragment. Alleles *0501, *0502, and *0503 could be distinguished from all other DQA alleles by presence of a 3-bp deletion resulting in amplification of a 239-bp (rather than a 242-bp) PCR product. The DNA samples were digested by use of a panel of the following nine restriction enzymes (New England Biolabs) to distin-

guish the *Mamu* DQA1 alleles: *Dde* I, *Bsm*A I, NlA III, *Bsp*H I, *Sfc* I, *Scr*F I (*Bss*k I), *Mnl* I, *Fnu* 4HI, and *Mse* I. Seven microliters of amplified template were used for each digest, with reaction volumes ranging from 9.0 to 24.0 µl.All restriction digests were done at 37°C for three to four hours, with the exception of *Bsm* AI (55°C). Allele assignments were based on the restriction enzyme patterns described by Christ and co-workers (38).

Amplification of the second exon of the *Mamu* DQB1 gene was also confirmed by use of polyacrylamide gel electrophoresis and sizing of the expected 303-bp fragment. The *Mamu* DQB1 fragment was then subjected to a minimum of six digests, as described by Sauermann and co-workers (34), using the following restriction enzymes: *Hae* III, BSiHKAI, *Hin*f I, *Sau* 96, *Scr*f I, *Bsr* I, *Mnl* I, NlA III, *Pst* I, and *Xmn* I. All digests were incubated at 37°C for three to four hours, except for *Bsi*HKAI and *Bsr* I (65°C). The *Mamu* DQA1 and *Mamu* DQB1 digests were resolved electrophoretically on 8% polyacrylamide gels run at 400 V for one and a half to two hours. Gels were stained with SyBrGreen 10000 X (Molecular Probes, Eugene, Oreg.) for 30 min and were visualized, using an AlphaImager 2000 system (Alpha Innotech, San Leandro, Calif.).

Analysis of data. Estimates of gene diversity were calculated by use of the method of Nei (42, 43).The allele distributions of the Indian and Chinese rhesus samples were compared, using estimates of Fisher's exact probability in the Genepop software program (44), bootstrapping each pairwise comparison with 1,000 iterations. The null hypothesis for each comparison was that the two populations being compared derive from the same gene pool. To minimize the influence of numerous alleles with low frequencies on the estimates of Fisher's exact probability, all *Mamu* DQA1 alleles other than *0102, *0104, *0105, *240X, *2501, and *2503 and all *Mamu* DQB1 alleles other than *0601, *0605, *0607, *1801, and *1808 were collapsed into an "other" genotype category for statistical comparisons of Indian and Chinese rhesus macaques. The sample size of Burmese rhesus macaques was deemed too small, especially given the large number of *Mamu* DQA1 alleles they exhibit (i.e., 13), to provide meaningful statistical comparisons with the other two regional rhesus populations.

Results

The *Mamu* DQA1 and *Mamu* DQB1 haplotypes of 40 Indian, 21 Chinese, and ten Burmese rhesus macaques are given in Table 1. Seven of the *Mamu* DQB1 alleles (one in the Chinese and six in the Indian rhesus macaques) and one *Mamu* DQA1 allele (in a Burmese rhesus macaque), denoted by a question mark, could not be resolved by use of the available restriction enzyme panel, and the genetic parameters reported in this study

Table 1. DQA1 and DQB1 genotypes in 40 Indian and 21 Chinese rhesus macaques, and DQA1 genotypes in 10 Burmese rhesus macaques

	Indian					Chinese					Burmese		
	DQA1		DQB1			DQA1		DQB1			DQA1	
ID	Alelle 1	Allele 2	Allele 1	Allele 2	ID	Alelle 1	Allele 2	Allele 1	Allele 2	ID	Allele 1	Allele 2
96Y	*0102	*0104	*0601	?	93B 647	*0108	*0108	*0607	*0607	23277	*0102	*0502
92T	*2501	*0106	*1801	*1703	93B 634	*240X	*240X	*1805	*1805	23276	*2501	*0503
6UC	*0105	*0105	*1802	*0609	93B 654	*0502	*0503	*1703	*1808	23278	*0106	*2502
96R	*240x	*2501	*1801	*1801	93B 594	*2501	*2501	*1801	*1801	23269	*0107	*0104
7KM	*0104	*0104	*1808	*0601	93B 645	*0503	*240X	*1802	*1503	23270	*2502	?
4X1	*0102	*0104	*0605	*0601	93B 641	*0502	*2302	*1804	*1804	23271	*240x	*0103
6BD	*240x	*0104	*1806	*0601	97E 029	*2501	*2302	*1801	*1804	23272	*0101	*1052
7K9	*2402	*2402	*1808	*1808	97E 114	*240X	*0108	*0607	*1702	23273	*2402	*0103
4H4	*2501	*0104	*1801	*0601	97E 060	*0108	*240X	*0607	*1503	23274	*0104	*0104
84Y	*2501	*0101	*1801	*1805	97E 085	*1051	*1052	*0602	*0609	23275	*0107	*1052
8MN	*0104	*0104	*0601	*0601	97E 082	*0106	*2501	*1801	*0606			
9SM	*2503	*0104	*0601	*1502	97E 094	*0101	*1051	*0602	?[b]			
AH70	*240x	*0104	*1808	*1805	97E 114	*0108	*240x	*0607	*1702			
5SG	*0105	*0104	*1803	*0601	98E 059	*0106	*2501	*0606	*1804			
57U	*0102	(*2302)	*0605	*1804	98E 017	*2503	*2503	*1501	*1501			
4XE	*240x	*0102	*1808	*0605	98E 043	*2503	*240x	*1805	*1703			
9S8	*240x	*2501	*1502	*1801	98E 139	*2502	*0502	*1808	*1808			
9SS	*0104	*0104	*0601	*0601	98E 084	*0503	*2402	*1808	*0607			
4UJ	*2501	*0104	*0601	*1801	98E 146	*2503	*240x	*1501	*1503			
4UH	*2501	*0104	*1801	*0601	98E 050	*240x	*2402	*1805	*1808			
4SJi	*2501	*0102	*0605	?	98E 098	*2301	*2503	*1804	*1801			
6NSi	*0104	*0104	*1805	?								
C4N	*0104	*0104	*1804	*0601								
4PFi	*0102	*0106	*0605	*0605								
C1A	*2501	*0104	*1801	*0601								
569	*2501	*2502	*1801	*1808								
5FY	*2501	*2501	*1801	*1801								
88T	*240x	*2502	*1806	*1503								
92I	*0104	*0104	*0601	*1803								
56Pi	*0103	*0104	*0601	?								
C1D	*0102	*0102	*0605	*0605								
50N	*2501	*0104	*1801	*0601								
5G9	*2501	*2503	*1801	*1808								
5G7	*240x	*2501	*1801	*1502								
8QL	*0104	*0104	*0601	*1802								
9SD	*2501	*2503	*1801	*1801								
8QD	*0104	*0104	*0601	*1804								
5LF	*0102	(*2302)	*0605	*1804								
4WPi	*240x	*0102	*1802	?[a]								
6AL	*240x	*2502	*1806	?								

? Unable to resolved allele identity.
[a] Allele is either *0606 or *1802.
[b] Allele is either *0602 or *0603.

were based on frequencies recalculated after excluding these unassignable alleles.

Twelve *Mamu* DQA1 alleles and 13 *Mamu* DQB1 alleles were observed in the sample of Chinese rhesus macaques, comparable to the number of alleles previously reported in Indian rhesus macaque samples of far greater size. Thirteen *Mamu* DQA1 alleles were detected in the smaller sample of Burmese rhesus macaques. Although the Mamu DQA1 restriction enzyme panel used is incapable of distinguishing among *2401, *2403, and *2404, which Rolfs and co-workers (35) designated the 240x phenotype, the identification of the *Mamu* DQB1 allele whose linkage to a particular *Mamu* DQA1 allele is known (Table 2), often allows assignment of the missing allele at one of the two loci when the identity of the allele at the other locus for the same animal is either known or can be deduced. For example, the Indian rhesus macaques AH70, 4XE, and 88T have the *Mamu* DQA1 240x phenotype, together with *Mamu* DQB1 *1805, *1808 and *1503, respectively, which Sauermann and co-workers (36, 37) reported to be linked to *Mamu* DQA1 *2401, *2402, and *2404. By use of similar reasoning, the *Mamu* DQA1 *240X phenotypes in Chinese rhesus macaques 93B634 and 98E050 probably represent *2401, and those in Chinese rhesus macaques 93B645 and 97E060 probably represent *2404. The unassigned *Mamu* DQB1 alleles (designated by "?" in Table 1) for the Indian rhesus macaques 96Y and 4Sji are probably *0605 and 1801, respectively. Since the only DQB1 allele of 96Y that is known,*0601, should be linked to DQA1 allele *0104, which 96Y also carries, its missing DQB1 allele is probably *0605, which is typically linked to DQA1 allele 0102, which 96Y also carries. Similarly, since one DQA1/DQB1 haplotype of 4SJi should be *0102/*0605, because the two are typically linked and both are present in 4SJi, the missing DQB1 allele of 4SJi is probably *1801, which is linked to *2501, the remaining known DQA1 allele carried by 4SJi. In addition, two of the 40 Indian rhesus, 57U and 5LF, had an unidentified *Mamu* DQA1 allele (35), but both animals had the corresponding *Mamu* DQB1 allele *1804 that is linked to the *Mamu* DQA1 allele *2302 (36, 37), which for both animals, appears in parentheses in Table 1. Therefore, knowledge of the *Mamu* DQB1 alleles, although not used for estimating the population genetic parameters described in Table 3 and the statistics based on them, clarified the identity of some *Mamu* DQA1 alleles that could not otherwise be assigned.

The other Indian rhesus macaques also exhibited many of the linkages cited in Table 2. Linkages with the highest frequencies were *Mamu* DQA1-DQB1 *0104-*0601, *0102-*0605, and *2501-*1801, not surprising given that alleles *0104, *0102, and *2501 were the most frequently observed *Mamu* DQA1 alleles in the study by Rolfs and co-workers (35), and alleles *0601, *0605, and *1801 were the most frequently observed *Mamu* DQB1 alleles in the study reported here, which includes 40 of the rhesus macaques studied by Rolfs and co-workers (35, 39).

Comparison of the *Mamu* DQA1 allele frequencies described in the Chinese rhesus macaques of this study with those of previous studies where Indian rhesus macaques were predominantly or exclusively used is provided in Table 3. The *Mamu* DQA1 allele frequencies of the Indian rhesus macaques exhibited marked similarity to that of the larger sample of which it is a subset and whose *Mamu* DQA1 haplotypes were studied by Rolfs and coworkers (35), and, albeit less so, than that estimated in the most recent study of Sauermann (36), about half the samples from which were selected from the same captive breeding colony as

Table 2. Linkages for *Mamu* DQA1-DQB1 alleles

MHC lineage	DQA allele	DQB allele	MHC lineage
DQA1 *01	*0102	*0605	DQB1 *06
	*0104	*0601/*1805	DQB1 *06/*18
	*1051	*0602	DQB1 *06
	*1052	*0609	DQB1 *06
	*0106	*0606	DQB1 *06
	*0107	*0604	DQB1 *06
	*0108	*0607	DQB1 *06
DQA1 *23	*2301	*0605/*1802/*1804	DQB1 *06/*18/*18
	*2302	*1804	DQB1 *18
DQA1 *24	*2401	*1805	DQB1 *18
	*2402	*1808	DQB1 *18
	*2404	*1503	DQB1 *15
DQA1 *25	*2501	*1801	DQB1 *18
	*2502	*1806/*1809	DQB1 *18
	*2503	*1501	DQB1 *15

MHC = Major histocompatibility complex.

those studied here, suggesting that sampling error did not influence the selection of Indian rhesus macaques to be genotyped for the *Mamu* DQB1 locus. The frequencies of *Mamu* DQB1 *0605 and *Mamu* DQA1 *0102, to which it is linked, were higher, and those of *Mamu* DQB1 *1806 and *Mamu* DQA1 *2502, to which it is linked, were lower in the Indian rhesus macaques chosen for study than in those reported in earlier studies. This is probably because about half the latter samples were acquired from rhesus macaques whose ancestors originated near Lucknow, India, east of the origin location of rhesus macaques sampled for this study. Nevertheless, the *Mamu* DQB1 alleles shared in common between the present and earlier studies of Indian rhesus macaques account for between 89 and 98% of the alleles in the present study. In contrast, alleles shared between the Indian and Chinese rhesus macaques included in the present study account for fewer than half (46%) the *Mamu* DQB1 alleles of the Indian rhesus macaques but more than 60% (62%) of those of the Chinese rhesus macaques.

The *240X phenotype (21.4%) and *2503 (11.9%) and *0108 (11.9%), the most frequently observed *Mamu* DQA1 alleles in Chinese rhesus macaques, were either absent or less common in Indian and Burmese rhesus macaques. In contrast, the most common *Mamu* DQA1 allele in Indian and Burmese rhesus macaques, *0104, was completely absent in Chinese rhesus macaques. Indian rhesus macaques shared fewer *Mamu* DQA1 alleles in common with Chinese (about 50%) than with Burmese (80 to 90%) rhesus macaques; the *Mamu* DQA1 alleles shared in common between the Indian and Burmese rhesus macaques accounted for 94 and 75%, respectively, of the *Mamu* DQA1 alleles in each population. The hypothesis that the *Mamu* DQA1 allele distributions of the Indian and Chinese rhesus macaques were sampled from the same gene pool was rejected at the 0.00001 level of probability (with SE < 0.00001).

The Chinese and Indian rhesus macaques in the study reported here had 13 *Mamu* DQB1 alleles, only eight of which are shared by members of both of these regional rhesus populations. The most common *Mamu* DQB1 allele in Indian rhesus macaques, *0601, was completely absent in Chinese rhesus macaques, whereas the most common *Mamu* DQB1 allele in Chinese rhesus macaques, *0607, was completely absent in Indian rhesus macaques. Just as for the *Mamu* DQA1 locus, the hypothesis that the *Mamu* DQB1 allele distributions of Indian and Chinese rhesus macaques were sampled from the same gene pool was rejected at the 0.00001 level of probability (SE < 0.00001).

Gene diversity, the theoretical estimate of the average frequency of heterozygous genotypes expected under conditions of genetic

Table 3. Frequencies of DQA1 alleles in Indian, Chinese, and Burmese rhesus macaques (A) and of DQB1 alleles in Indian and Chinese rhesus macaques (B)

A.

	Earlier studies of Indian rhesus macaques				Present study		
Allele	Christ et al., 1994 n=38	Rolfs et al., 2001 n=65	Sauermann et al., 1995 n=184[c]	Sauermann, 1998 n=258	Indian n=40	Chinese n=21	Burmese n=10
*2301	0	0.015	0.111	0.085	0	0.023	0
*2302	0	0	0	0.05	0.025	0.071	0
*240x[a]	0	0.108	0	0.091	0.1125	0.214	0.05
*2402	0	0.015	0.025	0.033	0.025	0.047	0.05
*2501	0.263	0.192	0.32	0.19	0.2	0.095	0.05
*2502	0.197	0.062	0.147	0.135	0.0375	0.048	0.1
*2503	0	0.046	0	0.008	0.0375	0.119	0
*0101	0	0.012	0	0	0.025	0	0.05
*0102	0.013	0.169	0.019	0.064	0.1125	0	0.05
*0103	0.04	0.019	0	0	0.0125	0	0.1
*0104	0.316	0.277	0.353	0.236	0.35	0	0.15
*0105[e]	0	0.031	0	0.061	0.0375	0.071	0.1
*0106	0	0.031	0.025	0.021	0.025	0.071	0.05
*0107	0	0	0	0.006	0	0	0.1
*0108	0	0	0	0	0	0.119	0
*0501	0	0	0	0	0	0	0
*0502	0	0	0	0.006	0	0.048	0.05
*0503	0	0	0	0	0	0.071	0.05
?[b]	0.171	0.023	0	0.014	0	0	0.05
No. of alleles	5	12	7	13	12	12	13
Proportion (%) shared with Chinese	0.46	0.5	0.63	0.6	0.5	1	0.5
Proportion (%) shared with Burmese	0.83	0.92	0.89	0.84	0.94	0.67	1
Gene diversity	0.62	0.81	0.74	0.85	0.81	0.89	0.87

B.

Allele	Sauermann et al, 1996 n=184[c]	Sauermann, 1998 n=258	Indian n=40	Chinese n=21
*0601	0.316	0.236	0.25	0
*0602[d]	0	0.047	0	0.048
*0604	0	0.006	0	0
*0605	0.019	0.064	0.112	0
*0606	0.025	0.021	0	0.048
*0607	0	0	0	0.142
*0609	0	0.014	0.012	0.024
*1501	0	0.008	0	0.071
*1502	0	0	0.038	0
*1503	0	0.058	0.012	0.071
*1702	0	0	0	0.048
*1703	0	0.014	0.012	0.048
*1801	0.32	0.19	0.212	0.119
*1802	0.062	0.085	0.038	0.024
*1803	0	0	0.025	0
*1804	0.049	0.05	0.05	0.119
*1805	0.037	0.033	0.038	0.095
*1806	0.147	0.114	0.038	0
*1808	0.025	0.033	0.088	0.119
*1809	0	0.021	0	0
?[b]	0	0.006	0.075	0.024
No. of alleles	9	16	13	13
Proportion (%) shared with Chinese	0.52	0.55	0.46	1
Proportion (%) shared with Indian	0.98	0.89	1	0.62
Gene diversity	0.77	0.87	0.79	0.88

[a] Alleles *2401,*2403,*2404 were combined into allele *240X, as in Christ et al (1994) and Rolfs et al (2001).
[b] Indicates the frequency of alleles that were not resolved.
[c] Includes 12 rhesus macaques of Chinese origin.
[d] Represents *0602/*0603.
[e] Includes alleles *1051 and *1052.

equilibrium, was strongly correlated with the number of alleles detected. However, in studies detecting a dozen or more alleles, including that reported here, gene diversity was higher in Chinese than Indian rhesus macaques. The earlier studies of Indian rhesus macaques reported allele frequencies the gene diversity of which exceeded that of Indian rhesus macaques studied here, probably because their sample sizes were larger, the geographic distribution of sites in India from which the animals they studied were sampled is much larger, and they included a few Chinese rhesus macaques in their studies. Nonetheless, the *Mamu* DQA1 and *Mamu* DQB1 allele distributions of the Chinese rhesus macaques were dominated by fewer alleles of high frequency and, consequently, yielded a higher level of gene diversity than did those of all the studies of Indian rhesus macaques reported in Table 3.

Despite their low sample size, the Burmese rhesus macaques had an average gene diversity value for the six STR loci studied (0.73; data not shown) which, similar to that for the *Mamu* DQA1 locus, is intermediate between that reported for larger samples of Indian and Chinese rhesus, using these same six STR loci (26). Thus, it is unlikely that sampling error substantially influenced the estimates of their gene diversity in this study.

Discussion

Results of this study support the preliminary (albeit unpublished) results of an earlier study in which DQ alleles found in Indian rhesus macaques were reported to be absent in a Chinese monkey cohort studied (34). Chinese rhesus macaques of our study had higher levels of genetic diversity at the DQA1 and DQB1 loci than did Indian rhesus macaques. Since Chinese rhesus macaques also have been reported to have higher levels of gene diversity at microsatellite (STR) loci as well (26), higher levels of heterozygosity might be a general characteristic of Chinese rhesus macaques. Alternatively, this might reflect diversity in wild Chinese rhesus macaque populations that have only recently been introduced to captive settings and which, therefore, still retain most of their native genetic heterogeneity. In contrast, Indian rhesus macaques might have experienced loss of genetic diversity during generations of captivity and genetic management of a closed domestic population (17). However, the high gene diversity and allele number observed in the small sample of Burmese rhesus macaques also suggested that higher levels of gene diversity are more characteristic of rhesus populations in the eastern part of the species' range than of those in India. In support of this argument, rhesus macaques from Kashmir, near the far western extreme of the geographic range of *M. mulatta,* have an even lower level of gene diversity than do the Indian rhesus of this study (26). Moreover, compared with Chinese rhesus macaques, Indian rhesus macaques of this study had lower frequency of the alleles that the two regional populations share in common, suggesting that Indian rhesus macaques are more genetically unique than are Chinese rhesus macaques. One possible explanation for these differences is that *M. mulatta* first evolved in the east region of their present range, then spread westward. Indian rhesus macaques, especially

those from the far northwestern frontier (i.e., Kashmir) are more highly derived evolutionarily than are rhesus macaques in the eastern region of the species' range and have probably lost some alleles, resulting in low gene diversity due to sequential founder effects after their geographic dispersal westward. Studies of rhesus macaques sampled throughout the geographic range of their natural habitat are needed to further characterize and assess the cause of regional differences in gene diversity within *M. mulatta*.

These results also confirm previous observations (36) of close linkage between the *Mamu* DQA1 and *Mamu* DQB1 loci, in Indian and Chinese rhesus macaques. The conservation of linkages between alleles belonging to the DQA*01 and DQB*06 lineages in humans and non-human primates that have been described, must predate the divergence between the hominoids and cercopithecinae (7, 45-48). This homology between DQ haplotypes of human and rhesus macaques suggests the potential usefulness of rhesus macaques with such haplotypes as an animal model for study of some infectious human diseases. Although linkages between members of *Mamu* DQA1 lineage *01 and *Mamu* DQB1 lineage *06 were highly conserved in the macaques of this study, a one-to-one correspondence between a particular *Mamu* DQA1 allele and a particular *Mamu* DQB1 allele was not always observed. For example, in five of eight instances in which an Indian rhesus macaque was typed homozygous for the *Mamu* DQA1 allele *0104, that animal was assigned a heterozygous *Mamu* DQB1 genotype including the expected *0601 allele and a second allele of the DQB*18 lineage other than *1805, with which it is typically linked. It is conceivable that this absence of fixed linkage resulted from our use of PCR conditions (e.g., annealing temperatures) that preferentially amplified the most common *0104 allele or from misinterpretation of the complex *Mamu* DQB1 restriction digest patterns. Our somewhat higher estimates of the frequency of the *0104 allele (0.35) than those reported in earlier studies (0.28 to 0.35) favors the former explanation. If so, the frequencies of alleles in the DQA23 lineages, which are linked to alleles of the DQB*18 lineage, assigned to the rhesus macaques in question, will have been slightly underestimated. Additional studies of a more geographically diverse sample of rhesus macaques and/or sequencing multiple clones of the 242-bp fragment of the DQA1 gene might clarify this issue.

Although previous studies of mtDNA have reported that rhesus macaques from Burma resemble rhesus macaques from China more closely than those from India (30), allele *0104, the most frequently observed *Mamu* DQA1 allele in the Burmese and Indian samples, was absent in the Chinese rhesus macaques studied here. The single homozygous Burmese rhesus macaque in this study was homozygous for the *Mamu* DQA1 *0104 allele. Although the Burmese rhesus macaques of this study shared 94% of their *Mamu* DQA1 alleles in common with Indian rhesus macaques, they shared only 67% of their *Mamu* DQA1 alleles in common with the Chinese rhesus macaques. Since substitutional changes in mtDNA are generally considered to be selectively neutral (49), it is conceivable that unknown selective forces on the MHC region that are related to the environment or geography are responsible for this curious difference in inter-regional genetic distances between the mtDNA genome and the MHC region. Alternatively, sampling error might have led to uncharacteristic similarities between the genotypes of the Indian and Burmese samples and/or uncharacteristic differences between those of the Chinese and Burmese samples.

Rhesus macaques are widely used in biomedical research of AIDS-related human diseases, the courses of which can be influenced by differences in the class-II MHC genotypes of research subjects. The major genetic differences between Indian and Chinese rhesus macaques that we have documented might be related to the clinical differences reported in their responses to SIV_{mac} infection and, consequently, might influence their relative suitability as animal models for such studies. Moreover, genetic differences in MHC allele frequencies between populations of macaques that derive from different regions of north central India have been reported (19, 36), including those in frequencies of the class-I *Mamu* A*01 allele, which has been designated a desirable marker in subjects of AIDS-related research. It is equally likely that comparable genetic differences occur among various regional populations of Chinese rhesus macaques. Although most domestically bred Chinese rhesus macaques are believed to have originated in Yunnan province, breeding centers in China from which rhesus macaques might be imported in the future extend as far east as Shanghai. Thus, it would be prudent to characterize genetic differences within and among regional populations of Chinese and Indian rhesus macaques, who cannot be trusted to respect international political boundaries, to minimize their influence on the outcome of research using rhesus macaques as an animal model. Care should be taken to ensure that genetic differences construed as regional variation actually reflect not local or idiosyncratic variation nor sampling error. This sampling scheme will be difficult to arrange since few breeding centers know, and most lack the means to determine, the exact location of origin of their breeding stock.

When the geographic ranges of genetic variation among rhesus macaques at the DQA1 and DQB1 loci are fully understood, comparisons of the influences of those DQ haplotypes, such as *0102/*0605, *0104/*0601, and *0108/*0607, that contribute most to this regional variation can be made. It is important that highly conserved haplotypes of the DQA*01 and DQB*06 lineages, such as the aforementioned three, represent the most tightly linked of all MHC loci and are among the few that are homologous between rhesus macaques and humans. Studies of immune response of rhesus macaques with specific DQA*01/DQB*06 haplotypes which represent maximal geographic variability in exposure to a wide range of pathogens might lead to especially useful animal models of human disease.

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