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Identification of MHC class I sequences in Chinese-origin rhesus macaques

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Abstract

The rhesus macaque (*Macaca mulatta*) is an excellent model for human disease and vaccine research. Two populations exhibiting distinctive morphological and physiological characteristics, Indian- and Chinese-origin rhesus macaques, are commonly used in research. Genetic analysis has focused on the Indian macaque population, but the accessibility of these animals for research is limited. Due to their greater availability, Chinese rhesus macaques are now being used more frequently, particularly in vaccine and biodefense studies, although relatively little is known about their immunogenetics. In this study, we discovered major histocompatibility complex (MHC) class I cDNAs in 12 Chinese rhesus macaques and detected 41 distinct *Mamu-A* and *Mamu-B* sequences. Twenty-seven of these class I cDNAs were novel, while six and eight of these sequences were previously reported in Chinese and Indian rhesus macaques, respectively. We then performed microsatellite analysis on DNA from these 12 animals, as well as an additional 18 animals, and developed sequence specific primer PCR (PCR-SSP) assays for eight cDNAs found in multiple animals. We also examined our cohort for potential admixture of Chinese and Indian origin animals using a recently developed panel of single nucleotide polymorphisms (SNPs). The discovery of 27 novel MHC class I sequences in this analysis underscores the genetic diversity of Chinese rhesus macaques and contributes reagents that will be valuable for studying cellular immunology in this population.

Keywords

Rhesus macaque; MHC class I; *Mamu-A*; *Mamu-B*

Introduction

Rhesus macaques (*Macaca mulatta*) are a commonly used animal model for studying a variety of human diseases and vaccines. Two populations based primarily on geographical origin, the Chinese and the Indian rhesus macaques, are commonly used today in biomedical research. Chinese rhesus macaques differ from their Indian counterparts in morphology and physiology (Champoux et al. 1997, Clarke and O'Neil 1999), as well as disease course when challenged under the same protocols (Ling et al. 2002, Marcondes et al. 2006, Reimann et al. 2005, Trichel et al. 2002). Historically, Indian-origin rhesus macaques have been used more frequently in research; thus, more genetic analysis has been performed on this population (Boyson et al. 1996, Otting et al. 2005, Urvater et al. 2000a,b, Voss and Letvin 1996, Yasutomi et al. 1995). Since the 1978 ban on export of rhesus macaques from India, however, they are more difficult to obtain, leading to a greater utilization of Chinese rhesus macaques.

Simian immunodeficiency virus (SIV) studies using rhesus macaques have shown marked differences in disease progression between Chinese and Indian macaques. Chinese macaques tended toward better control of viral replication, stronger antibody responses, and less depletion of intestinal effector cells when challenged with SIVmac239 (Joag et al. 1994, Ling et al. 2002). Chinese macaques also had lower viral loads 6 weeks postinfection in a mucosal challenge with SIVmac251 (Marthas et al. 2001). A study challenging rhesus macaques with SIV/DeltaB670 likewise showed lower viral set points and less severe symptoms in the Chinese macaque cohort (Trichel et al. 2002). Similar results (lower virus levels, greater numbers of CD4⁺ T cells, increased survival) were reported in a challenge of Chinese macaques with the simian-human immunodeficiency virus SHIV-89.6P (Reimann et al. 2005).

Despite increasing utilization of Chinese rhesus macaques in SIV and other biomedical research, relatively little is known about the major histocompatibility complex (MHC) immunogenetics of Chinese rhesus macaques. Prior studies have reported exon 2 sequences and frequencies for class II alleles at the DR, DQ, and DP loci and examined Chinese rhesus macaques for the frequency of *Mamu-A*01* (Doxiadis et al. 2003, Viray et al. 2001, Vogel et al. 1995). Recently, 59 *Mamu-A* partial alleles (lacking approximately 30 bases from the 5' end of the coding region) were reported for Chinese rhesus macaques (Otting et al. 2007). It is not clear whether these 59 alleles represent a small or large percentage of MHC allelic diversity in this population. The limited data on Chinese rhesus macaque MHC sequences corresponds to a lack of reagents and techniques that have proved vital to immunology research in Indian rhesus macaques, such as MHC-defined cell lines and MHC: peptide tetramers (Allen et al. 2001; Shimizu and DeMars 1989). Genotyping of Chinese rhesus macaques is currently limited to microsatellite analysis, which reveals animals with similar haplotypes but does not indicate which genes are present on these haplotypes (Penedo et al. 2005). Without knowledge of gene sequences it is impossible to construct immunological reagents or genotyping tests, as is currently possible in Indian rhesus macaques (Kaizu et al. 2007, Knapp et al. 1997).

To further characterize the MHC region of Chinese rhesus macaques, we identified MHC class I sequences using cDNA cloning and sequencing techniques on a cohort of 12 Chinese rhesus macaques. We also performed microsatellite typing and developed cDNA-specific PCR genotyping using sequence specific primers (PCR-SSP) for eight of the new *Mamu-A* and *Mamu-B* sequences identified in this study.

Materials and methods

Animals

Peripheral blood mononuclear cell (PBMC) samples were obtained from 12 Chinese rhesus macaques (ChRh01–12) from Covance (Alice, TX, USA). Limited pedigree information was

available for six of these animals, although none were known to be closely related to each other. Eighteen additional animals from the same source were examined by microsatellite and PCR-SSP analysis (ChRh13–30). From the available pedigree information, two animals from this expanded cohort are closely related to ChRh01-12 or to each other (e.g., half siblings in one case).

RNA/DNA isolation, cDNA synthesis, and cloning of MHC class I cDNAs

Nucleic acids from the 12 PBMC samples were isolated using the Qiagen AllPrep DNA/RNA Mini purification kit (Qiagen, Valencia, CA, USA). Synthesis of complementary DNA (cDNA) was performed using the Superscript[™] III First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA, USA). Polymerase chain reaction (PCR) was performed to amplify the MHC class I cDNAs using high-fidelity Phusion[™] polymerase (New England Biolabs, Ipswich, MA, USA) and primers specific for the untranslated regions around MHC class I transcripts. For each sample analyzed, two different primer pairs were used: a common 5' primer 5' MHC_UTR (5'-GGACTCAGAATCTCCCCAGACGCCGAG) and either 3' MHC_U TR_A (5'-CAGGAACAYAGACACATTCAGG) or 3' MHC_UTR_B (5'-GTCTCTCCACCTCCTCAC). 3' MHC_UTR_A had a higher degree of specificity for *Mamu-A* transcripts; likewise, 3' MHC_UTR_B favors amplification of *Mamu-B* transcripts (Wiseman et al. 2007). The following PCR program was used for all cDNA amplifications on an MJ Research Tetrad Thermocycler (Bio-Rad Laboratories, Hercules, CA, USA): initial denaturation at 98°C for 30 s; between 25 and 35 cycles of 98°C for 5 s, 63°C for 1 s, 72°C for 20 s, and a final extension of 72°C for 5 min. Aliquots of each reaction were checked for amplification after 25 cycles, with 3–10 additional PCR cycles for reactions showing undetectable amplification at that stage. A maximum of 35 cycles were carried out for each reaction. When reactions exhibited strong amplification, they were subjected to agarose gel electrophoresis. PCR products of 1.2 kb were excised and purified using the MinElute PCR Purification Kit from Qiagen. The purified products were ligated into pCR-Blunt vectors using the Invitrogen Zero Blunt Cloning kit (Invitrogen). Ligations were transformed into *Escherichia coli* Top10 (Invitrogen) chemically competent cells. Forty-four bacterial colonies per primer pair (88 per animal) were picked and incubated with shaking for 17–24 h in 1.3 ml of Circle Grow (Qbiogene, Irvine, CA, USA) containing 50 mg/ml kanamycin. The Eppendorf[®] Perfectprep[®] Plasmid 96 VAC Direct Binding Kit (Brinkmann, Westbury, NY) was used for DNA isolation. Concentration was determined using the Nanodrop 1000 (Wilmington, DE). *EcoRI* (New England Biolabs) restriction digests of the plasmids were performed to establish which clones contained the desired ~1.2 kb MHC class I insert.

Sequencing of MHC class I cDNAs

Bidirectional sequencing for each clone was performed using four primers: T7 (5'-TAATACGACTCACTATAGGG) and M13 (5'-CAGGAAACAGCTATGAC) which bind to the pCR-Blunt vector, and 5'Refstrand (5'-GCTACGTGGACGACACGC) and 3'Refstrand (5'-CAGAAGGCACCACCACAGC) internal primers (Wiseman et al. 2007). Sequencing reactions were performed using the DYEnamic ET Terminator Cycle Sequencing Kit (Amersham, Piscataway, NJ, USA) and the following thermocycling conditions: 30 cycles of 95°C for 20 s, 50°C for 15 s, and 60°C for 1 min. Purification of sequencing products was performed with the Agencourt[®] CleanSEQ[®] dye-terminator removal kit (Agen-court Bioscience Corporation, Beverly, MA, USA). Purified products were run on an ABI 3730 (Applied Biosystems, Foster City, CA, USA), and sequence analysis was performed using the CodonCode Aligner (CodonCode, Dedham, MA, USA) and Lasergene (DNASTAR, Madison, WI, USA) software packages. Between 50 and 88 MHC class I clones were analyzed for each animal. To minimize errors inherent in the PCR process, a sequence was only considered to be authentic when three or more identical clones were observed. Our goal was to survey the most highly expressed cDNAs within each animal. Therefore, it is likely that there are

additional class I sequences within these animals expressed at lower levels that were missed due to the limited number of clones examined and our stringent requirement for three independent clones per sequence. All full-length cDNA sequences were submitted to GenBank (accession numbers EF580136-EF580176) as well as the IMGT/MHC Non-human Primate Immuno Polymorphism Database-MHC (IPD-MHC) and NHP Nomenclature Committee.

Phylogenetic analysis

Sequences were aligned by the CLUSTAL X program (Thompson et al. 1997). A phylogenetic tree was reconstructed by the neighbor-joining method (Saitou and Nei 1987) based on the Tamura three-parameter distance (Tamura 1992), which takes into account both nucleotide content and transitional bias. Any site at which the alignment postulated a gap in any sequence was excluded from all pairwise comparisons. The reliability of clustering patterns in the tree was assessed by bootstrapping (Felsenstein 1985); 1,000 bootstrap samples were used.

PCR-SSP assays

Sequence-specific primers were designed for eight representative *Mamu-A* and *Mamu-B* sequences discovered in the cohort of 12 Chinese rhesus macaques. Alignments were generated using MegAlign software (DNASTAR), and potential primers were evaluated using Primer3 (v. 0.4.0; Rozen and Skaletsky 2000). PCR-SSP reactions were performed using cDNA templates, 0.5 μ M primers, and AmpliTaq Gold PCR Master Mix (Applied Biosystems) with the following PCR program: denaturation at 96°C for 5 min; 35 cycles of 94°C for 30 s, optimal annealing temperature (65–69°C) for 45 s, 72°C for 45 s, and extension at 72°C for 10 min. The optimal annealing temperature for each primer pair was established to be 65°C except for *Mamu-B*1902*, where 69°C was required for discrimination. PCR products were examined by agarose gel electrophoresis. Positive products were isolated and purified for sequence verification using Qiagen's MinElute PCR Purification Kit (Qiagen). Sequencing reactions were performed using the appropriate PCR-SSP primers with the same thermal cycling conditions and analysis procedures described above for MHC class I cDNA characterization. The pair of primers for *Mamu-A1*5702*, in addition to amplifying the sequence of interest, also occasionally amplified *Mamu-B*8701*. Although the amplification of *Mamu-B*8701* was generally not as robust, sequence verification of positive products was necessary when using this primer pair.

Microsatellite analysis

Microsatellite analysis of both cohorts of Chinese-origin rhesus macaques was performed as previously described (Wiseman et al. 2007). Three new primer pairs flanking short tandem repeats identified in rhesus genomic sequences were incorporated to improve resolution of the *Mamu-B* and *Mamu-DRB* regions (Daza-Vamenta et al. 2004). These three primer pairs are P03-193435 (forward primer—5'-CAGGAGTGCTGAAGTCAT; reverse primer—5'-CACTCCAGAACCTGGTGACA) in the *Mamu-B* region (BAC MMU244P03; AC148695) and D05-104184 (forward primer—5'-CCGGCAGGAAAATATTCTGAG; reverse primer—5'-AGGACGGCAGTTTGAGTTTG) and D05-144699 (forward primer—5'-CTCCACCCACATTCCAAATC; reverse primer—5'-GGAGGTAAAGGCTGCAATGA) in the class II *DRB* region (BAC MMU240D05; AC148693). The P03-193435 and D05-104184 forward primers were fluorescently labeled with HEX (6-carboxy-2', 4, 4', 5', 7, 7'-hexachlorofluorescein) and the D05-144699 forward primer was labeled with FAM (6-carboxyfluorescein).

SNP analysis

Single nucleotide polymorphism (SNP) analysis was performed as previously described using 18 Chinese rhesus-specific, 20 Indian rhesus-specific, and 18 shared SNP markers (Ferguson et al. 2007).

Results and discussion

Summary of MHC class I cDNAs identified

We began by sequencing 864 MHC class I cDNA clones from 12 Chinese rhesus macaques. We identified 41 distinct class I sequences in this cohort. Of these 41 sequences, 8 had been previously reported in rhesus macaques of Indian origin with at least partial cDNA sequences already available in GenBank. In addition, six were identical to alleles recently reported in Chinese rhesus macaques (Otting et al. 2007). The remaining 27 cDNAs described in this study have not been reported previously. Table 1 lists all 41 distinct sequences, with GenBank accession number and reference animal(s) as well as any identities to previously reported cDNAs.

The phylogenetic relationship between the identified sequences is shown in Fig. 1. As expected, there are two major branches to the tree containing the *Mamu-A* and *Mamu-B* loci (the *Mamu-B*-like *Mamu-I* sequences group amongst the *Mamu-B* cDNAs). Class I sequences previously reported in Indian macaques do not group independently from the Chinese sequences, indicating no obvious separation of lineages between the Chinese and Indian populations.

Three cDNAs identified are identical at the nucleic acid level to sequences in related nonhuman primate species. *Mamu-AI*1001* and *Mamu-B*0703* are identical to the cynomolgus macaque (*Macaca fascicularis*) sequences *Mafa-AI*1002* and *Mafa-B*400101*, respectively. Likewise, *Mamu-I*0106* is identical to the pigtail macaque (*Macaca nemestrina*) sequence *Mane-I*0101*.

Interestingly, one of the alleles identified in this study, *Mamu-B*1702*, is nearly identical to the *Mamu-B*17* allele which has been associated with control of SIVmac239 replication (Yant et al. 2006). There are only six nucleotide differences between these two alleles, which result in only three conservative amino acid substitutions (K27R, L269V, and V310A).

Sequence sharing, microsatellite analysis and PCR-SSP

Seventeen of the cDNAs identified by cloning and sequencing are shared between two or more of the twelve animals used for cDNA identification; a summary of these sequences is shown in Table 2. Since there are several instances of animals sharing two or more sequences, such as ChRh10, ChRh11, and ChRh12 (sharing both *Mamu-AI*2601* and *Mamu-B*9001*), we hypothesized that animals within our cohort may share haplotypes. We typed the cohort of 12 Chinese rhesus macaques with a panel of microsatellite markers spanning the MHC region. One inferred microsatellite haplotype was shared in animals ChRh10, ChRh11, and ChRh12 (Fig. 2a). Another shared microsatellite haplotype was seen in animals ChRh03, ChRh05, and ChRh07 (Fig. 2b). Despite similarities in microsatellite profiles for the second haplotypes of ChRh05 and ChRh07 (same microsatellite allele sizes at 6 of 7 markers analyzed), these animals appear to have multiple distinct *Mamu-A* and *Mamu-B* cDNAs, as there was only one additional sequence shared between them (*Mamu-B*8701*). While it was unexpected that these animals do not share additional *Mamu-A* and *Mamu-B* sequences, the linkage between microsatellite profiles and MHC loci is not always perfect. Microsatellite profiles were also generated for an additional cohort of 18 Chinese rhesus macaques, and there is evidence for 8 additional shared MHC class I haplotypes in this expanded cohort which were not observed in the original cohort of 12 animals (data not shown).

We developed PCR-SSP primers for eight cDNAs identified by cloning and sequencing in animals sharing common *Mamu-A* and *Mamu-B* sequences: *Mamu-A1*2601*, *Mamu-A1*5702*, *Mamu-B*1702*, *Mamu-B*1902*, *Mamu-B*3901*, *Mamu-B*8901*, *Mamu-B*9001*, and *Mamu-B*9101*. The primer sequences and PCR-SSP results for each cDNA are shown in Fig. 3. PCR-SSP assays for these eight cDNAs were then run on both the original cohort of 12 cloning and sequencing animals, as well as the additional cohort of 18 animals. In addition to showing the microsatellite profiles, Fig. 2 also illustrates the identified sequences predicted to be associated with two shared haplotypes based on cloning and sequencing and/or PCR-SSP analysis.

Potential admixture of Chinese- and Indian-origin rhesus macaques

Eight of the sequences identified in this study were identical to cDNAs previously reported in rhesus macaques of Indian origin (*Mamu-A1*2601*, *Mamu-A4*1403*, *Mamu-B*0703*, *Mamu-B*2401*, *Mamu-B*3901*, *Mamu-B*4002*, *Mamu-B*4301*, and *Mamu-B*6901*). One of these alleles, *Mamu-A1*2601*, was also recently detected in Chinese rhesus macaques by Otting et al. (2007). The *Mamu-A4*1403* allele reported here is consistent with being an Indian origin macaque allele, lacking the stop codon in exon 5 that was present in the only previously reported Chinese *Mamu-A4* allele (Otting et al. 2007). The degree of *Mamu-B* cDNA sharing between rhesus macaques of different geographic origin is not currently known, and there is only limited information on *Mamu-A* allele sharing. However, polymorphism at MHC loci is maintained for long periods of time by balancing selection and trans-specific polymorphism of MHC-A and MHC-B alleles has been previously observed (Takahata and Nei 1990). Thus, sharing of class I MHC sequences between Chinese and Indian rhesus macaques is not entirely unexpected. Nevertheless, an additional factor besides the age of sequences that might contribute to cDNA sharing would be admixture of Chinese and Indian rhesus macaques in our cohort of animals.

To explore potential admixture, we performed SNP analysis as recently described (Ferguson et al. 2007) to examine the geographic origin of our animals (Fig. 4). The SNPs examined are derived from 3' untranslated and flanking genomic sequences rather than coding regions. Although we do not know the selective pressures on these regions, it is unlikely that the majority of these SNPs are functional or under selection and thus are more likely to reflect recent population history than are MHC sequences. Results of the SNP analysis indicate that eight of the animals used in cloning and sequencing are of Chinese origin (ChRh01, ChRh02, ChRh03, ChRh04, ChRh05, ChRh06, ChRh08, and ChRh09). The remaining four animals contained SNP alleles associated with Indian-origin animals at two different loci, suggesting that they are either Chinese/Indian hybrids or are derived from a population of Chinese rhesus macaques originating from a previously unsampled region of China. Interestingly, three of the putative Indian MHC class I sequences identified in this study were isolated from animals of definitive Chinese origin (*Mamu-B*6901* from ChRh01, *Mamu-B*4002* from ChRh02, and *Mamu-B*2401* from ChRh03 and ChRh04). Therefore, even if a subset of the animals used in our study are admixtures between Indian and Chinese rhesus macaques, there is still evidence for sharing of MHC class I sequences between rhesus macaques of different geographic origins.

Implications for vaccine and biodefense research

As previously mentioned, *Mamu-B*1702* identified in this study is nearly identical to the Indian rhesus macaque allele *Mamu-B*17*. Based on PCR-SSP typing, this allele was identified in two of the 30 animals screened, or approximately 7% of the expanded cohort. The amino acid substitutions in *Mamu-B*1702* are not expected to alter the specificity of peptide binding since the only substitution within any of the peptide binding domains is a conservative lysine to arginine change at amino acid residue 6 of the alpha 1 domain. Therefore, *Mamu-B*1702* is likely able to present the previously identified *Mamu-B*17* SIV-derived peptides (Mothé et al.

2002). Thus, reagents such as MHC: peptide tetramers that have been developed for this interesting allele could be useful for Chinese rhesus macaques that express *Mamu-B*1702*.

The sharing of MHC class I sequences between Indian and Chinese rhesus macaques noted in this study (eight of the 41 total cDNAs identified) suggests a potential for additional shared sequences between these two populations. There may be other Indian MHC class I sequences of interest, or cDNAs with close homology to well-characterized Indian sequences, present in Chinese-origin animals. While a previous study by Vogel et al. (1995) did not identify any animals containing the *Mamu-A*01* allele in a cohort of 37 Chinese rhesus macaques, PCR-SSP analysis of other MHC class I sequences identified in Indian macaques could be performed on cohorts of Chinese rhesus macaques followed by confirmation of positive products by direct sequencing. Such an analysis may identify biologically important Indian genes in the Chinese rhesus population, such as alleles that restrict SIV CD8⁺ T cell epitopes (Kaizu et al. 2007).

This study, in addition to discovering 27 novel MHC sequences in Chinese-origin rhesus macaques, also describes the first methods for MHC genotypic analysis of Chinese rhesus macaques and provides a workflow for additional Chinese macaque MHC resource development. The conditions described here for cDNA cloning and sequencing can be used for MHC class I sequence discovery and to search for shared MHC class I cDNAs. Genotyping by PCR-SSP and microsatellite analysis provide a method for prospective establishment of genetically directed studies.

The sequences identified in this study are an important addition to the limited MHC immunogenetic information available for rhesus macaques of Chinese origin. As more Chinese-origin cDNAs are identified, additional reagents and techniques developed for and vital in immunological research in Indian rhesus macaques can be adapted for studies utilizing Chinese origin rhesus macaques. This will expand the tools available to researchers using Chinese rhesus macaques and help to alleviate the current shortage of Indian macaques.

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Fig. 1. Phylogenetic analysis of 41 MHC class I sequences identified in Chinese-origin rhesus macaques

Neighbor-joining tree based on 1068 aligned nucleotide sites. Numbers on branches are percentages of 1,000 bootstrap samples supporting the branch; only values $\geq 50\%$ are shown

a

Marker		ChRh10	ChRh11	ChRh12	
D6S2972	Class IA	120	120	120	
D6S2970		297	298	297	
D6S2704		156	150	156	
D6S2691		273	219	273	
P03-193435	Class IB	256	185	256	
MICA		192	204	192	
D6S2793		257	243	na	
			276	na	
Shared Haplotype		cDNAs Mamu-A1*2601 <i>Mamu-B*9001</i>	cDNAs Mamu-A1*2601 Mamu-B*9001	cDNAs Mamu-A1*2601 Mamu-B*9001	
Distinct Haplotype		Mamu-A1*1102 Mamu-B*4301	Mamu-A1*0403 Mamu-B*9101	Mamu-A1*0308 Mamu-B*0703 Mamu-B*3901	

b

Marker		ChRh03	ChRh05	ChRh07	
D6S2972	Class IA	120	122	120	
D6S2970		278	285	278	
D6S2704		156	150	156	
D6S2691		241	299	241	
P03-193435	Class IB	141	135	141	
MICA		201	195	201	
D6S2793		280	259	280	
Shared Haplotype		cDNAs <i>Mamu-A1*1103</i> Mamu-B*4802 <i>Mamu-B*8901</i>	cDNAs <i>Mamu-A1*1103</i> Mamu-B*4802 <i>Mamu-B*8901</i>	cDNAs Mamu-A1*1103 Mamu-B*4802 Mamu-B*8901	
Shared cDNAs		Mamu-A1*2202 Mamu-A7*0103 <i>Mamu-B*8102</i>	Mamu-A1*2202 Mamu-A7*0103 Mamu-B*8102 Mamu-B*8701	Mamu-B*8701	
Distinct cDNAs		Mamu-B*1902 Mamu-B*2401	Mamu-B*6902 Mamu-B*8801	Mamu-A2*050202 Mamu-A4*1403 Mamu-I*0106	

Fig. 2. Putative shared microsatellite haplotypes and *Mamu-A* and *Mamu-B* cDNAs for six animals
a. ChRh10, ChRh11, and ChRh12; **b** ChRh03, ChRh05, and ChRh07. The microsatellite profiles of each set of animals with a shared haplotype are shown with their respective microsatellite allele sizes. Designation of MHC cDNAs to a shared haplotype is inferred. For Fig. 2b, one or more of the shared cDNAs could be associated with the shared haplotype, despite not being observed in all three animals due to the limited number of cDNA clones examined. cDNAs listed in *bold* were identified in three or more clones while cDNAs listed in *grey* were present in only one or two clones

Fig. 3. PCR-SSP results for eight Chinese rhesus macaque MHC class I sequences

The primer sequences and gel images for the eight cDNAs selected for PCR-SSP assay development are shown. Numbers above each lane represent the animal name (ChRh01 through ChRh12). The *plus* signs indicate animals shown to be positive for each sequence by cDNA cloning and sequencing. The *minus* sign indicates an animal positive for *Mamu-B*8701*, which is occasionally amplified by the primers for *Mamu-A1*5702*. The brightest band of the ladder corresponds to 500 bp

Fig. 4. SNP analysis of ChRh01-ChRh12

SNPs used for the analysis are identified on the *x*-axis and animal IDs are on the *y*-axis. Animals shaded in blue are of known Chinese origin and those in orange are of known Indian origin. Rhesus macaques from our cohort that are either Chinese/Indian hybrids or are derived from a population of Chinese rhesus macaques originating in a previously unsampled region of China are shaded in gray. SNP genotypes are color-coded with green representing homozygous major alleles, red representing homozygous minor alleles, and yellow representing heterozygous alleles. A single Indian allele in a putative Chinese animal falls within our definition of pure ancestry, which allows for the presence of a single allele that may be extremely rare in the defined ancestry group (minor allele frequency <0.02). However, statistical likelihood of having two such rare alleles is low (<0.0004) and suggests that the animal is from an uncharacterized geographic region or is a hybrid. It is also possible that all animals in the cohort may be from the same geographic region, explaining the presence of the rare alleles in multiple animals

Table 1MHC *Mamu-A* and *Mamu-B* sequences identified in Chinese-origin rhesus macaques ChRh01-12

Allele	Accession no.	Animal ID	Identical to
<i>Mamu-A1*0308</i>	EF580157	ChRh12	
<i>Mamu-A1*0403</i>	EF580156	ChRh11	
<i>Mamu-A1*1001</i>	EF580139	ChRh02	AM295894 (Ch)
<i>Mamu-A1*1102</i>	EF580140	ChRh10	AM295896 (Ch)
<i>Mamu-A1*1103</i>	EF580154	ChRh07, 08	
<i>Mamu-A1*1804</i>	EF580152	ChRh02	
<i>Mamu-A1*2201</i>	EF580141	ChRh08	AM295904 (Ch)
<i>Mamu-A1*2202</i>	EF580153	ChRh03, 05	
<i>Mamu-A1*2601</i>	EF580136	ChRh10, 11, 12	AJ542578 (In)
<i>Mamu-A1*3201</i>	EF580142	ChRh06	AM295908 (Ch)
<i>Mamu-A1*5201</i>	EF580143	ChRh01	AM295917 (Ch)
<i>Mamu-A1*5702</i>	EF580151	ChRh01, 04	
<i>Mamu-A2*0102</i>	EF580155	ChRh09	
<i>Mamu-A2*050202</i>	EF580137	ChRh07, 08	
<i>Mamu-A4*1403</i>	EF580138	ChRh07	AY707077 (In)
<i>Mamu-A7*0103</i>	EF580144	ChRh03, 04, 05	AM295952 (Ch)
<i>Mamu-B*0703</i>	EF580149	ChRh12	AJ556876 (In)
<i>Mamu-B*1702</i>	EF580162	ChRh02, 09	
<i>Mamu-B*1902</i>	EF580169	ChRh03	
<i>Mamu-B*2401</i>	EF580145	ChRh03, 04	AJ556881 (In)
<i>Mamu-B*3901</i>	EF580146	ChRh12	AJ556890 (In)
<i>Mamu-B*4002</i>	EF580150	ChRh02	EF362448 (In)
<i>Mamu-B*4301</i>	EF580147	ChRh10	AJ556893 (In)
<i>Mamu-B*4503</i>	EF580167	ChRh04	
<i>Mamu-B*4802</i>	EF580168	ChRh03, 05, 07	
<i>Mamu-B*6502</i>	EF580163	ChRh01, 02	
<i>Mamu-B*6901</i>	EF580148	ChRh01	AJ844601 (In)
<i>Mamu-B*6902</i>	EF580158	ChRh01, 05	
<i>Mamu-B*8102</i>	EF580159	ChRh01, 05	
<i>Mamu-B*8201</i>	EF580160	ChRh02	
<i>Mamu-B*8301</i>	EF580161	ChRh02, 09	
<i>Mamu-B*8401</i>	EF580164	ChRh04	
<i>Mamu-B*8501</i>	EF580165	ChRh06	
<i>Mamu-B*8601</i>	EF580166	ChRh06	
<i>Mamu-B*8701</i>	EF580170	ChRh05, 07	
<i>Mamu-B*8801</i>	EF580171	ChRh05	
<i>Mamu-B*8901</i>	EF580172	ChRh07	
<i>Mamu-B*9001</i>	EF580173	ChRh11, 12	
<i>Mamu-B*9101</i>	EF580174	ChRh11	
<i>Mamu-I*0106</i>	EF580176	ChRh07	

Allele	Accession no.	Animal ID	Identical to
<i>Mamu-I*0118</i>	EF580175	ChRh01	

Summary of 41 cDNAs identified in a cohort of 12 Chinese-origin rhesus macaques. GenBank accession numbers and reference animal(s) are listed for each sequence. All cDNAs were also checked for identity with sequences in GenBank; accession numbers are listed for any sequences identical to any GenBank entries

In, indicates a GenBank entry from an Indian-origin rhesus macaque; *Ch*, indicates Chinese-origin.

Table 2

Summary of shared MHC class I *Mamu-A* and *Mamu-B* cDNAs

Allele	Animal											
	ChRh 01	ChRh 02	ChRh 03	ChRh 04	ChRh 05	ChRh 06	ChRh 07	ChRh 08	ChRh 09	ChRh 10	ChRh 11	ChRh 12
<i>Mamu-AI*1103</i>												
<i>Mamu-AI*2202</i>												
<i>Mamu-AI*2601</i>												
<i>Mamu-AI*5702</i>												
<i>Mamu-A2*050202</i>												
<i>Mamu-A4*1403</i>												
<i>Mamu-A7*0103</i>												
<i>Mamu-B*1702</i>												
<i>Mamu-B*2401</i>												
<i>Mamu-B*4802</i>												
<i>Mamu-B*6502</i>												
<i>Mamu-B*6902</i>												
<i>Mamu-B*8102</i>												
<i>Mamu-B*8301</i>												
<i>Mamu-B*8701</i>												
<i>Mamu-B*8901</i>												
<i>Mamu-B*9001</i>												

Shared sequences identified by cDNA cloning and sequencing from 12 Chinese-origin rhesus macaques. Black boxes indicate the presence of the cDNA in three or more identical clones. Grey boxes indicate the presence of the cDNA in one or two clones in that particular animal (not meeting our stringent criteria for sequence discovery, but identified during retrospective sequence analysis). Animal ChRh06 did not share any sequences with the other 11 animals.