Immunogenetics (2005) 57: 198–209
DOI 10.1007/s00251-005-0787-1

ORIGINAL PAPER

M. Cecilia T. Penedo · Ronald E. Bontrop · Corrine M. C. Heijmans · Nel Otting · Riet Noort · Annemiek J. M. Rouweler · Nanine de Groot · Natasja G. de Groot · Thea Ward · Gaby G. M. Doxiadis

Microsatellite typing of the rhesus macaque MHC region

Received: 8 December 2004 / Revised: 27 January 2005 / Published online: 5 April 2005

Abstract To improve the results gained by serotyping rhesus macaque major histocompatibility complex (MHC) antigens, molecular typing techniques have been established for class I and II genes. Like the rhesus macaque *Mamu-DRB* loci, the *Mamu-A* and *-B* are not only polymorphic but also polygenic. As a consequence, sequence-based typing of these genes is time-consuming. Therefore, eight MHC-linked microsatellites, or short tandem repeats (STRs), were evaluated for their use in haplotype characterization. Polymorphism analyses in rhesus macaques of Indian and Chinese origin showed high STR allelic diversity in both populations but different patterns of allele frequency distribution between the groups. Pedigree data for class I and II loci and the eight STRs allowed us to determine extended MHC haplotypes in rhesus macaque breeding groups. STR sequencing and comparisons with the complete rhesus macaque MHC genomic map allowed the exact positioning of the markers. Strong linkage disequilibria were observed between *Mamu-DR* and *-DQ* loci and adjacent STRs. Microsatellite typing provides an efficient, robust, and quick method of genotyping and deriving MHC haplotypes for rhesus macaques regardless of their geographical origin. The incorporation of MHC-linked STRs into routine genetic tests will contribute to efforts to improve the genetic characterization of the rhesus macaque for biomedical research and can provide comparative information about the evolution of the MHC region.

Keywords MHC · Non-human primates · Evolution · Microsatellites · Haplotype

M. C. T. Penedo · T. Ward
Veterinary Genetics Laboratory,
School of Veterinary Medicine,
University of California,
Davis, CA, 95616, USA

R. E. Bontrop · C. M. C. Heijmans · N. Otting · R. Noort · A. J. M. Rouweler · N. de Groot · N. G. de Groot · G. G. M. Doxiadis
Department of Comparative Genetics and Refinement,
Biomedical Primate Research Centre,
Lange Kleiweg 139,
2280, GJ, Rijswijk, The Netherlands

G. G. M. Doxiadis (✉)
Biomedical Primate Research Centre,
Lange Kleiweg 139,
2280, GJ, Rijswijk, The Netherlands
e-mail: doxiadis@bprc.nl
Tel.: +31-15-2842690
Fax: +31-15-2843999

Introduction

Rhesus macaques are widely used as preclinical models for human infectious and autoimmune diseases, of which HIV and multiple sclerosis are examples, as well as for transplantation and vaccine development research (Evans et al. 1999; Brok et al. 2001; Horton et al. 2001; Wood et al. 2001; Muhl et al. 2002; Newberg et al. 2002; Mothe et al. 2003; O'Connor et al. 2003; Friedrich et al. 2004; Knechtle and Burlingham 2004; Lee et al. 2004; Torrealba et al. 2004). Gene products of the major histocompatibility complex (MHC) play a key role in adaptive immunology, and a prominent feature of most of the genes in this region is their high degree of polymorphism. A well-characterized rhesus macaque (*Mamu*) MHC is a prerequisite for various aspects of biomedical research. By active immunization of rhesus macaques, mainly of Indian origin, 14 Mamu-A and 16 -B serotypes were defined (Bontrop et al. 1995; Otting et al. 2005). However, serological typing for non-Indian animals, such as those originating from China or Southeast Asia, is compromised by the lack of well-defined, specific antisera. Thus, comprehensive molecular typing methods, such as sequence-based or allele-specific amplification, have to be established for the *Mamu* system of rhesus macaques from different geographic sources.

In humans, class I genes *HLA-A*, *-B*, and *-C* and class II genes *HLA-DPA1*, *-DPB1*, *-DQA1*, *-DQB1*, and *-DRB1* exhibit a high degree of allelic variation. In the rhesus macaque, most of these loci are also present and known to be polymorphic. *Mamu-DQA1* and *-DQB1* are highly variable and segregate as stable *DQA1/DQB1* haplotypes (Khazand et al. 1999; Doxiadis et al. 2001). The most striking dif-

ference, however, can be observed for the class I *-A* and *-B* loci. Whereas in humans there is only one *HLA-A* and one *-B* locus per chromosome, each with a high degree of polymorphism, in cynomolgous and rhesus macaques there are multiple expressed *Mamu-A* and *-B* like loci on a single haplotype (Boyson et al. 1996; Erlich et al. 1996; Uda et al. 2004). Moreover, *Mamu-A* and *-B* region configurations display diversity with regard to the number and combination of loci transcribed per chromosome (Otting et al. 2005). The *Mamu-DRB* region is comparable to the class I, since more than 30 *-DRB* region configurations have been described that vary in loci number and content (Doxiadis et al. 2000, 2001). Each *Mamu-DRB* region configuration is composed of one to three transcribed *-DRB* genes, and up to five pseudogenes per chromosome. Contradictory chromosomal assignments have been published for the rhesus macaque MHC, first to Chr. 2 and later to Chr. 5 (Garver et al. 1980; Hirai et al. 1991). Recently, fluorescence in situ hybridization mapping of six rhesus macaque cosmid clones localized the MHC on the long arm of Chromosome 6 in 6q24, the orthologous region to human 6p21.3 (Huber et al. 2003).

The recent completion of the rhesus macaque MHC sequence (Daza-Vamenta et al. 2004) confirmed previous findings of variation in number and content for class I and II genes and revealed an overall similarity of organization with the human orthologue. This conserved organization was offset by internal expansions, most notably of *Mamu-A* and *Mamu-B* genes, which explained the difference in length of the region of 5.3 Mb in rhesus macaque and about 3.7 Mb in human.

Because the molecular typing of class I and II genes is complicated and time-consuming, an analysis of polymorphic microsatellites or short tandem repeats (STRs) spanning the MHC provides an alternative method for rapid and accurate characterization of the region. Such an approach has been used for MHC typing in humans for tissue matching and donor screening (Carrington and Wade 1996; Foissac et al. 2001). Hundreds of STRs are situated on human Chr. 6, and lists of markers mapping within or near the HLA region have been compiled and updated (Tamiya et al. 1999; Foissac et al. 2000; Matsuzaka et al. 2000, 2001; Cullen et al. 2003). Because STRs tend to be conserved among closely related species, especially between Old World monkeys and hominoids (Rubinsztein et al. 1995; Coote and Bruford 1996; Clisson et al. 2000; Rogers et al. 2000), HLA-linked STRs provide an abundant source of potential markers for use in rhesus macaques.

Among 37 STRs screened for robust amplification and polymorphism in rhesus macaque, eight markers, *D6S291*, *D6S2741*, *D6S2876*, *DRA-CA*, *MICA*, *MOG-CA*, *D6S1691*, and *D6S276*, were selected that spanned the HLA region (Martin et al. 1998; Foissac et al. 2000; Cullen et al. 2002, 2003). The remaining 29 markers were excluded, mainly because the human primers failed to amplify rhesus macaque DNA. This study sought to characterize the polymorphism of the eight STRs in rhesus macaques of Indian and Chinese origin and to evaluate the association of STR variants with alleles of the MHC class I and II genes. The ability to derive extended haplotypes for the MHC region provides additional relevant information that can be applied to experimental designs in biomedical, population, evolution, and cell biology research.

Material and methods

Animals

For haplotype analyses with STRs, class I, and class II loci, 118 rhesus macaques from the self-sustaining colony of the Biomedical Primate Research Centre (BPRC), with a breeding history of more than five generations, were tested. Most of the founder animals were from India, but animals from China and Burma were also present. The animals belonged to six breeding groups, each consisting of one alpha male, several females, and their offspring. The smallest group comprised three females and six offspring and the largest six females and 25 offspring. Four of these breeding groups had founders of Indian origin. One group of animals was of Burmese origin and all females of the last group originated in India, whereas the male was an Indian/Chinese crossbred macaque (Doxiadis et al. 2003).

Polymorphism of STR loci was analyzed in two groups of unrelated rhesus macaques from the breeding colony of the California National Primate Research Center (CNPRC), University of California, Davis, Calif., USA. These groups represented animals originating in India (n=51) and China (n=44). DNA samples were obtained from the archive of the Veterinary Genetics Laboratory (VGL), University of California.

For linkage analyses of STR markers, genotype data for seven paternal half- and full-sib families from the CNPRC colony with a total of 331 offspring–dam pairs were obtained from VGL's database.

Serological MHC typing

The BPRC rhesus macaques were serologically typed for MHC class I antigens, and 14 Mamu-A and 16 Mamu-B serotypes were defined. Serological assays were performed by a cytotoxicity test using specific antibodies produced by the active immunization of mainly Indian rhesus macaques (Bontrop et al. 1995).

DNA isolation and direct sequencing of *-DQA1*, *-DQB1*, and *-DPB1*

Genomic DNA was extracted from EDTA blood samples or from immortalized B lymphocytes by a standard salting out procedure. Partial sequences of exon 2 for *-DQA1*, *-DQB1*, and *-DPB1* were obtained by direct sequencing of PCR products according to procedures previously described (Doxiadis et al. 2003).

Cloning and sequencing of *-DRB*

Cloning and sequencing of *-DRB* exon 2 were performed as described earlier (Doxiadis et al. 2003) with the following modifications: The PCR program included a final step of 30 min at 72°C to produce a 3′-end extension by *Taq* polymerase, and the InsT/Aclone cloning kit (Fermentas, St. Leon-Roth, Germany) was used for direct cloning of PCR products. The PCR products were purified and ligated into the vector pTZ57R, which had been pre-cleaved by *Eco*321. After transformation in *Escherichia coli* Xl-blue, plasmid clones containing inserts were used to prepare DNA for cycle sequencing with the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction kit v3.1 (Applied Biosystems, Foster City, Calif., USA). Sequencing reactions were run on the ABI 3100 genetic analyzer (Applied Biosystems) and data analyzed using the Sequence Navigator program (Applied Biosystems) as previously described (de Groot et al. 2004).

STR genotyping

The STRs used were *D6S291*, *D6S2741* (alias *G2.56412*), *D6S2876* (alias *G51152*), *DRA-CA* (alias *D6S2883*), *MICA*, *MOG-CA* (alias *D6S2972*), *D6S276*, and *D6S1691*. Primer sequences, concentration in PCR reactions, fluorescence labels, and source references are shown in Table 1. The cycling parameters in PTC100 thermal cyclers (MJ Research, Waltham, Mass., USA) consisted of an initial denaturation for 5 min at 90°C of a mixture containing only DNA template and primers. After this step, the remaining reagents were added, and the program continued with four cycles of 1 min at 94°C, 30 s at 58°C, 30 s at 72°C, followed by 25 cycles of 45 s at 94°C, 30 s at 58°C, 30 s at 72°C. A final elongation step at 72°C was performed for 30 min. Multiplex PCR mixtures in a total volume of 12.5 µl contained 2.5 mM $MgCl_2$, 0.20 mM of each dNTP, PCR buffer II, and 0.5 U AmpliTaq polymerase (Applied Biosystems). PCR products were run on ABI PRISM 377 DNA sequencers (Applied Biosystems), and genotypes were determined using GeneScan-350 ROX size standard (Applied Biosystems) and the STRand computer software for fragment size analysis (available at http://www.vgl.ucdavis.edu/informatics/Strand/). Allele sizes were rounded to the nearest integer number.

Cloning and sequencing of STRs

Rhesus macaque sequences for each of the STRs were obtained by cloning PCR products from each of two heterozygous animals using a TOPO TA cloning kit and according to the manufacturer's recommendations (Invitrogen, Carlsbad, Calif., USA). To avoid sequencing of stutter bands, 12–18 colonies for each STR were first screened with fluorescence-labeled primers to select plasmid clones containing inserts corresponding to the alleles defined by the genotype of each animal. Sequencing of two to four alleles for each STR was done by cycle sequencing with an ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction kit, version 3.1 (Applied Biosystems). Sequencing reactions were run on ABI Prism 377 DNA sequencers (Applied Biosystems), and sequences were analyzed using the SeqMan module of the DNASTAR software suite (DNASTAR, Madison, Wis., USA). A representative sequence for each STR was deposited in GenBank (accession numbers AY786541–AY786548).

Table 1 Characteristics of major histocompatibility complex (MHC)-linked short tandem repeat (STR) markers and details for multiplex PCR amplification with fluorescence labeled primers

Locus	Label	Forward Primer	Reverse Primer	Repeat Unit	Number	Size (bp)	Multiplexed markers	Primer (uM)	Reference
D6S2741	VIC	AGACTAGATGTAG GGCTAGC	CTGCACTTGGCTA TCTCAAC	(CT)	18	249–289	1	0.163	Cullen et al. 2003
D6S2876	FAM	GGTAAAATTCCTG ACTGGCC	GACAGCTCTTCTT AACCTGC	(CA)	14	196–248	1	0.054	Mignot et al. 1997
DRA-CA	NED	TGGAATCTCATCA AGGTCAG	ACATTTGTATGCTT CAGATG	(CA)	19	234–280	1	1.350	Cullen et al. 2003
MICA	NED	CCTTTTTTTCAGG GAAAGTGC	CCTTACCATCTCCA GAAACTGC	(GCT)	5	191–206	1	0.096	Mizuki et al. 1997
MOG-CA	FAM	GAAATGTGAGAAT AAAGGAGA	GATAAAGGGGAAC TACTACA	(CA)	5	117–129	1	0.890	Roth et al. 1995
D6S276	NED	TTCCAGTGTATAC ATCAATCAAATCA	GGGTGCAACTTGT TCCTCCT[a]	(CA)	14	211–243	2	0.085	Weissenbach et al. 1992[a]
D6S291	VIC	CTCAGAGGATGCC ATGTCTAAAATA	GGGGATGACGAA TTATTCACTAACT	(CA)	12	196–224	2	0.037	Gyapay et al. 1994
D6S1691	FAM	AGGACAGAATTT TGCCTC	GCTGCTCCTGTATA AGTAATAAAC	(CA)	16	177–221	2	0.047	Dib et al. 1996

[a]Source of forward primer, reverse primer designed from rhesus sequence

STR polymorphism and linkage analyses

Animals from CNPRC representing unrelated Indian- and Chinese-origin rhesus macaques were used to characterize the polymorphism of STR loci. The computer program GENEPOP, version 3.1b (Raymond and Rousset 1995), was used to estimate allele frequencies and heterozygosities, and to test conformity to Hardy–Weinberg expectations (HWE). Polymorphism information content (PIC) was calculated according to Botstein et al. (1980).

Linkage analyses were performed with CRIMAP (Green et al. 1990), and the *BUILD* function with a LOD threshold of 3 was used to construct a map of the region based on genotype data of the eight STRs for seven paternal half-sib families.

Results and discussion

Characteristics of MHC-linked STRs

BLAST comparisons of rhesus macaque STR sequences with the GenBank database revealed high similarity to the human orthologues for *D6S276*, *D6S291*, and *D6S1691* (data not shown). These sequences were not represented in the published rhesus macaque MHC sequence (Daza-Vamenta et al. 2004) but, in agreement with predictions from the human genome map, we assumed that these STRs flank the MHC region of rhesus macaques, with *D6S291* located at the centromeric end and *D6S276/D6S1691* at the telomeric end. Sequences for *D6S2741*, *D6S2876*, *DRA-CA*, *MICA*, and *MOG-CA* had high similarity to sequences in rhesus macaques MHC bacterial artificial chromosome (BAC) clones, as well as to the human orthologues (data not shown).

Allele frequencies in Indian and Chinese rhesus macaques for the eight STRs are given in Table 2. The number of alleles per locus ranged from five (*MICA*) to 21 (*DRA-CA*). Chinese animals showed overall greater allelic diversity with a total number of alleles (TNA) of 102 and average heterozygosity of 0.82, whereas in Indian monkeys, TNA was 89 and average heterozygosity was 0.77. These results were in agreement with findings of higher allelic diversity and average heterozygosity in Chinese than in Indian rhesus macaques for other autosomal STRs (Morin et al. 1997). Differences between the two groups were characterized by distinct allele frequency distributions rather than by the presence of population-specific alleles in either group. Except for *MOG-CA* and *MICA*, all markers were as variable in the rhesus macaques as in humans, and some appeared to be even more polymorphic (Foissac et al. 2000).

The presence of a null allele in *D6S2741* and *D6S2876* in Indian and Chinese monkeys was identified through use of these markers for parentage analysis of CNPRC monkeys. A null allele for *D6S2741* was also identified among BPRC animals. Null alleles, caused by sequence amplification failure because of a mismatch in primer binding sequences, are more likely to occur when heterologous primers are used for PCR amplification. Therefore, this finding is not unexpected. Some of the animals included in the population samples were known to have a null allele at these loci, and this allowed us to obtain a minimum estimate of its frequency.

Genotypic distributions were in agreement with HWE except for *D6S2876* in Chinese rhesus monkeys, which showed statistically significant deviation ($P \leq 0.01$ after correction for multiple tests) explained by heterozygote deficiency. This result is most likely accounted for by undetected null alleles among the Chinese animals, which would cause an apparent deficit of heterozygous genotypes. The PIC values estimated for the eight markers (range 0.50–0.91) indicate that all loci will be highly informative for linkage- or association-based studies. The presence of null alleles in *D6S2741* and *D6S2876* justifies the development of rhesus macaques-specific primers for these markers to improve amplification of alleles in these loci.

MHC haplotypes

Haplotype analysis was done based on segregation of *Mamu-A* and *-B* serotypes; class II genotypes for *-DQA1*, *-DQB1*, and *-DRB* loci; and the eight STRs. The animals used for this analysis were members of BPRC breeding groups in which one alpha male was housed together with several females. Because each female had at least two offspring, extended parental MHC haplotypes could be defined for both parents as shown in Table 3.

Among 58 parental chromosomes, 44 distinct, extended haplotypes were identified. Only animals related to the same founder shared identical extended haplotypes, and this accounted for the remaining 15 chromosomes. The ancestor haplotypes are depicted in Table 3 with the same color. Among Indian rhesus macaques, STR typing provided additional information regarding configurations of the MHC region that might not be evident from comparisons of more limited typing of class I and class II genes. For example, ancestor haplotypes A2777 (purple) and haplotype B2957 (orange) share the same class I and class II *-DQB1*, *-DQA1*, and *-DRB* alleles but differ for *-DPB1* and *D6S2741*.

In contrast to the haplotype definition of Indian monkeys, MHC typing of non-Indian rhesus macaques is less developed. First, serotyping yields ambiguous results for lack of well-defined, specific antisera; second, molecular typing methods of MHC class I alleles are time consuming. Sequence-based class II typing, although informative and accurate, does not reflect the whole MHC. Therefore, STR typing provides a suitable method for the definition of extended haplotypes of non-Indian monkeys, as shown for Burmese animals of group 5 in Table 3. Haplotypes *c* and *i* of animals 4064 and 4050, for example, share the same *Mamu-DQA1-DQB1*, and *-DRB* alleles, whereas four STRs differentiate the two haplotypes. Another example is given by the haplotype *d* of monkey 4064 and *e* of monkey 4065, which can be distinguished only by two STR markers.

The extended haplotypes derived for BPRC animals indicated that, even in the absence of gene-specific typing, STR typing can be used to distinguish haplotypes that are

Table 2 Allele frequencies, observed (H_o) and expected (H_e) heterozygosity, and polymorphism information content (*PIC*) value of eight MHC-linked STRs in rhesus macaques of Indian (*In*=51) and Chinese (*Ch*=44) origin

D6S291			*D6S2741* (*G2.56412*)			*D6S2876* (*G51152*)			*DRA-CA* (*D6S2883*)			*MICA*			*MOG-CA* (*D6S2972*)			*D6S276*			*D6S1691*		
Alleles	In	Ch	Alleles	In	Ch	Alleles	In	Ch	Alleles	In	Ch	Alleles	In	Ch	Alleles	In	Ch	Alleles	In	Ch	Alleles	In	Ch
202	0.02	0.08	249	0.00	0.01	196	0.13	0.13	234	0.11	0.06	191	0.10	0.09	117	0.00	0.01	211	0.05	0.19	177	0.04	0.11
204	0.02	0.08	251	0.10	0.01	206	0.04	0.05	236	0.04	0.00	194	0.26	0.08	121	0.11	0.15	213	0.03	0.13	191	0.01	0.00
206	0.32	0.37	257	0.10	0.07	208	0.14	0.24	242	0.00	0.01	197	0.01	0.00	123	0.61	0.59	215	0.07	0.07	193	0.02	0.03
208	0.44	0.23	259	0.06	0.04	210	0.24	0.09	244	0.01	0.02	200	0.50	0.60	125	0.03	0.16	217	0.00	0.05	195	0.02	0.03
210	0.03	0.08	261	0.08	0.06	212	0.00	0.06	246	0.16	0.19	203	0.13	0.23	127	0.24	0.09	219	0.00	0.03	197	0.44	0.06
212	0.01	0.03	263	0.01	0.05	214	0.04	0.05	248	0.08	0.02				129	0.01	0.00	221	0.02	0.09	199	0.07	0.06
214	0.05	0.02	265	0.10	0.05	216	0.05	0.06	250	0.02	0.05							223	0.05	0.18	201	0.02	0.08
216	0.10	0.01	267	0.10	0.14	218	0.01	0.02	252	0.03	0.03							225	0.31	0.12	203	0.08	0.16
218	0.00	0.02	269	0.10	0.12	220	0.27	0.14	254	0.02	0.05							227	0.09	0.05	205	0.03	0.06
220	0.00	0.05	271	0.07	0.20	226	0.00	0.01	256	0.21	0.06							229	0.00	0.03	207	0.00	0.09
222	0.00	0.02	273	0.05	0.06	244	0.04	0.02	258	0.04	0.07							231	0.03	0.03	209	0.08	0.10
224	0.01	0.01	275	0.01	0.06	246	0.02	0.07	260	0.10	0.03							233	0.25	0.00	211	0.01	0.11
			277	0.08	0.02	250	0.01	0.00	262	0.00	0.03							235	0.08	0.00	213	0.07	0.03
			279	0.06	0.05	254	0.00	0.02	264	0.00	0.05							237	0.02	0.01	215	0.04	0.01
			281	0.02	0.01	256	0.00	0.04	266	0.11	0.09							239	0.00	0.01	217	0.01	0.05
			283	0.01	0.01	Null	0.01	0.00	268	0.05	0.11							243	0.00	0.01	219	0.02	0.02
			285	0.02	0.00				270	0.00	0.08										221	0.04	0.00
			287	0.01	0.02				272	0.00	0.02												
			289	0.00	0.01				274	0.00	0.02												
			Null	0.02	0.01				276	0.01	0.01												
									278	0.01	0.00												
No. of alleles[a]	9	12		17	18		11	14		15	19		5	4		5	5		11	14		16	15
H_o	0.59	0.84		0.78	0.89		0.75	0.57		0.82	0.84		0.73	0.50		0.55	0.61		0.88	0.80		0.80	0.91
H_e	0.69	0.80		0.93	0.92		0.83	0.89		0.89	0.93		0.66	0.58		0.56	0.60		0.82	0.89		0.78	0.91
PIC	0.64	0.76		0.92	0.89		0.80	0.87		0.87	0.91		0.60	0.52		0.50	0.55		0.79	0.87		0.76	0.90

[a]Null alleles not included in count

Table 3 Extended MHC haplotypes of rhesus macaques

Monkey		Haplotype	*D6S291*	*DPB1**	*D6S2741*	*D6S2876*	*DQB1*	*DQA1*	*DRB*	*DRA-CA*	*MICA*	*Serol.B*	*Serol.A*	*MOG-CA*	*D6S276*	*D6S1691*	Hapl.
Gr.1																	
C6	sire	a	204	*03*	265	196	*1811*	*2602*	*1*0406, 5*0301*	256	191	B32	A28	123	235	197	C/D2838
C6		b	206	*01*	271	208	*0601*	*0104*	*1*0309, 6*0101, W201*	260	194	B14	A5	123	235	197	B3441
9056	dam	c	206	*06*	259	220	*1801*	*2601*	*1*0303, 1*1007*	234	200	B18	A3	125	233	197	A2808
9056		d	208	*04*	279	244	*1808*	*2402*	*1*0403, *W501*	254	191	B32	A10	123	235	197	B2774
1DL	dam	e	204	*06*	269	196	*1811*	*2602*	*1*0406, 5*0301*	256	200	B13	A6	123	235	213	C426
1DL		f	206	*07*	261	216	*0605*	*0102*	*1*0306, 1*1003*	248	194	B11	A33	127	233	203	C2414
1RY	dam	g	206	*10*	283	204	*1502*	*2401*	*1*0313, *W604, *W605(8)*	268	200	B-	A5	125	235	203	C/D3159
1RY		h	206	*13*	261	196	*1811*	*2602*	*1*0406, 5*0301*	256	194	B11	A19	121	235	207	A2794
1KX	dam	i	206	*13*	261	210	*1802*	*2301*	*6*0114, *W303, *W401??*	256	194	B11	A6	123	225	197	D2837
1KX		j	208	*04*	279	244	*1808*	*2402*	*1*0403, *W501*	254?	191	B32	A10	123	235	197	B2774
1QK	dam	k	206	*04*	273	220	*1801*	*2601*	*1*0406, 5*0301*	256	200	B26	A22	123	225	199	C3005
1QK		l	206	*10*	267	196	*1810*	*2401*	*3*0403, *W305*	254	200	B26	A19	121	231	197	A2777
Gr. 3																	
BB66	sire	a	204	*07?*	279	220	*1801*	*2601*	*1*0303, 1*1007*	234	200	B13	A10	123	225	197	D3617
BB66		b	206	*01*	275	196	*1811*	*2602*	*6*0112, *W2501,*	266	200	B24	A22	123	233	197	A3070
8609	dam	c	206	*04*	275	220	*1801*	*2601*	*1*0303, 1*1007*	234	200	B26	A10	123	233	197	B1435
8609		d	206	*10*	0	208	*0601*	*0104*	*1*0309, 6*0101, *W201*	260	200	B24	A6	123	235	203	A600
1VA	dam	g	204	*06*	269	196	*1811*	*2602*	*1*0406, 5*0301*	256	200	B13	A6	123	235	213	C426
1VA		h	206	*11*	257	208	*0602*	*01051*	*6*0111, *W606, *W2104, *W2603*	236	200	B13	A9	127	215	197	B3026
D10	dam	i	206	*10*	267	196	*1810*	*2401*	*3*0403, *W305*	254	200	B26	A19	121	225	197	A2777
D10		j	206	*10*	285	208	*0601*	*0104*	*1*0309, 6*0101, *W201*	260	200	B26	A10	123	233	197	C3136
Gr.4																	
D55	sire	a	206	*10*	267	196	*1810*	*2401*	*3*0403, *W305*	254	200	B26	A19	121	225	197	A2777
D55		b	216	*07*	271	206	*1501*	*2603*	*3*0411 [a] , *W314 [a]*	254	203	B-	A-	121	213	193	C3946
8669B	dam	e	212	*06*	259	220	*1801*	*2601*	*1*0303, 1*1007*	234	203	B17	A6	123	225	197	DYusa
8669B		f	206	*07*	259	196	*1809*	*2602*	*1*0306, 1*1003*	248	200	B2	A10	123	225	199	D3837
8727	dam	c	206	*11*	257	220	*1801*	*2601*	*1*0306, 1*1007*	248	200	B2	A10	123	233	197	A2957
8727		d	208	*04*	279	244	*1808*	*2402*	*1*0403, *W501*	254	191	B32	A10	123	235	197	B2774
TA	dam	g	206	*12?*	259?	208	*0601*	*0104*	*1*0309, 6*0101, *W201*	260	200	B24	A6	123	235	203	A600
TA		h	206	*12*	269	220	*1804*	*2302*	*1*0310, *W101, *W602, *W609*	244	200	B34	A10	123	223	203	C2455
Gr. 5																	
4049	sire	a	202	*12*	285	208	*0601*	*0104*	*1*0321, 1*0323*	262	191	n.d.	n.d.	125	215	205	A4049
4049		b	200	*14*	287	210	*1801*	*2601*	*1*0309, *W2507*	268	200	n.d.	n.d.	127	225	195	B4049
4064	dam	c	206	*14*	287	208	*0601*	*0104*	*1*0321, 1*0322*	262	194	n.d.	n.d.	123	225	205	C4064
4064		d	214	*10*	271	244	*1808*	*2402*	*1*0403, *W502*	254	200	n.d.	n.d.	123	225	195	D4064
4065	dam	e	214	*10*	271	244	*1808*	*2402*	*1*0403, *W502*	254	200	n.d.	n.d.	125	215	195	C4065
4065		f	210	*10?*	259	208	*0602*	*01051*	*1*07032, 1*0306, *W2603*	234	191	n.d.	n.d.	123	223	203	D4065
4074	dam	g	218	*14*	287	210	*1801*	*2601*	*1*0309, *W2507*	268	200	n.d.	n.d.	127	235	197	C4074
4074		h	214	*14?*	259	204	*1710*	*2603*	*1*0407, 3*0409, 6*New*	254	200	n.d.	n.d.	123	223	207	D4074
4050	dam	i	214	*14*	287	208	*0601*	*0104*	*1*0321, 1*0322*	262	194	n.d.	n.d.	125	227	195	C4050
4050		j	206	*13*	261	244	*1503*	*0502*	*6*0111, *W606, *W2104, *W2603*	246	200	n.d.	n.d.	123	231	197	D4050
Gr. 7																	
C68	sire	a	204	*03*	265	248	*1501*	*2603*	*1*0701, 3*0405, 5*0303*	270	194	B14	A1	121	233	203	B2775
C68		b	206	*01*	275	196	*1811*	*2602*	*W2501, 6*0112*	266	200	B24	A22	123	233	197	A3070
9017	dam	c	208	*13*	261	220	*1801*	*2601*	*1*0303, 1*1007*	234	200	B29	A6	123	215	203	D2989
9017		d	206	*01*	275	196	*1811*	*2602*	*6*0112, *W2501*	266	200	B24	A22	123	233	197	A3070
9078	dam	e	206	*03*	265	196	*1811*	*2602*	*1*0406 ,5*0301*	256	194	B14	A3	127	235	197	C494
9078		f	206	*07*	261	216	*0605*	*0102*	*1*306, 1*1003*	248	194	B11	A33	127	233	203	C2414
9119	dam	k	204	*10*	275	208	*0601*	*0104*	*1*0309, 6*0101,*W201*	260	194	B11	A6	123	225	209	D584
9119		l	206	*10*	281	208	*0601*	*0104*	*1*0309, 6*0101,*W201*	260	200	B26	A9	127	231	197	AH34
9125	dam	m	206	*10*	0	208	*0601*	*0104*	*1*0309, 6*0101, *W201*	260	200	B24	A6	123	235	203	A600
9125		n	206	*12*	273	208	*0602*	*01051*	*6*0111, *W606, *W2104, *W2603*	236	194	B11	A19	123	233	199	B3836
2CP	dam	o	204	*10*	279	208	*0601*	*0104*	*1*0309, 6*0101, *W201*	260	194	B11	A19	127	225	197	A2774
2CP		p	206	*04*	273	196	*1811*	*2602*	*1*0406, 5*0301*	256	194	B26	A28	121	235	191	C2679
2CV	dam	h	206	*10*	0	208	*0601*	*0104*	*1*0309, 6*0101, *W201*	260	200	B24	A6	123	233	203	A600
2CV		g	206	*07*	261	216	*0605*	*0102*	*1*0306, 1*1003*	248	194	B11	A33	127	235	203	C2414
Gr. 8																	
8769	sire	a	206	*04*	279	196	*1810*	*2401*	*3*0403, *W305*	254	200	B26	A19	121	225	197	B2957
8769		b	206	*08*	267	206	*1803*	*2301*	*1*0405, 5*0304*	270	194	B11	A27	123	235	197	D1472
1OL	dam	c	214	*06*	277	220	*1801*	*2601*	*1*0303, 1*1007*	234	200	B26	A19	123	233	197	C3029
1OL		d	206	*11*	277	208	*0601*	*0104*	*1*0309, 6*0101, *W201*	260	200	B24	A21	123	211	197	A3441
2CK	dam	e=a	206	*04*	279	196	*1810*	*2401*	*3*0403, *W305*	254	200	B26	A19	121	225	197	B2957
2CK		f	206	*11*	257	220	*1801*	*2601*	*1*0306, 1*1007*	248	200	B2	A10	123	233	197	A2957

Loci/markers are listed according to their localization on the chromosome. Identical ancestor haplotypes are highlighted by the same colour. Pink colour indicates a possible crossover.

[a] *Mamu-DRB* alleles were deposited in GenBank (accession numbers AJ867581 and AJ867582; *Mamu-DRB1*0313 = Mamu-DRB1*0318).*

identical by descent and provide information about the MHC region useful in the selection of experimental animals or analysis of experimental data.

Linkage disequilibrium

Linkage disequilibrium (LD) describes the non-random association of alleles at nearby loci more often than would be expected if the loci were segregating independently in a population (Ardlie et al. 2002; Wall and Pritchard 2003). LD association analyses have become increasingly useful to map disease genes and phenotypes to provide insight into the biology of meiotic recombination and the evolution of MHC haplotypes in humans (Carrington 1999; Huttley et al. 1999). LD association studies in rhesus macaques, particularly with genes and markers in the MHC region, could provide critical information relating to several aspects of biomedical research and comparative data regarding the biology and evolution of the MHC in that species.

Blast comparisons of STR sequences that we obtained against the BAC clone data used to construct the complete rhesus macaque MHC sequence (Daza-Vamenta et al. 2004) placed *D6S2741* in clones 118H5 and 038L02 near *Mamu-DPB1*; *D6S2876* in clones 281E18, 63B15 and 007H18 near *Mamu-DQB/DQADRA-CA* in clones 370O021 and 240D05, near *Mamu-DRAMICA* in clones 24N14 and I88J04 near *MIC1*; and *MOG-CA* in clone 268P23 near *MOG*. Moreover, comparisons with other rhesus macaque sequences in GenBank confirmed that the *MICA* STR is located in exon 5 of *MIC1*, as it is in humans (Mizuki et al. 1997). These comparisons allowed the exact positioning of five markers on the genomic map of the rhesus macaque MHC (Fig. 1). The close proximity of these loci prompted us to investigate whether there were associations between *Mamu* alleles and adjacent STR markers that would be suggestive of LD.

Inspection of haplotypes showed association of alleles spanning the region *D6S2876* to *DRA-CA*, such as the blocks defined by [*D6S2876-220*; *DQB1*1801/DQA1*2601; DRB1*0303,DRB1*1007*; *DRA-CA-234*] and [*D6S2876-208*; *DQB1*0601/DQA1*0104; DRB1*0309, DRB6*0101,DRB*W201; DRA-CA-260*] found in Indian monkeys (Table 4). *Mamu-DRA* is polymorphic and alleles of this locus are associated with certain *DRB* region configurations (de Groot et al. 2004). The apparent LD between

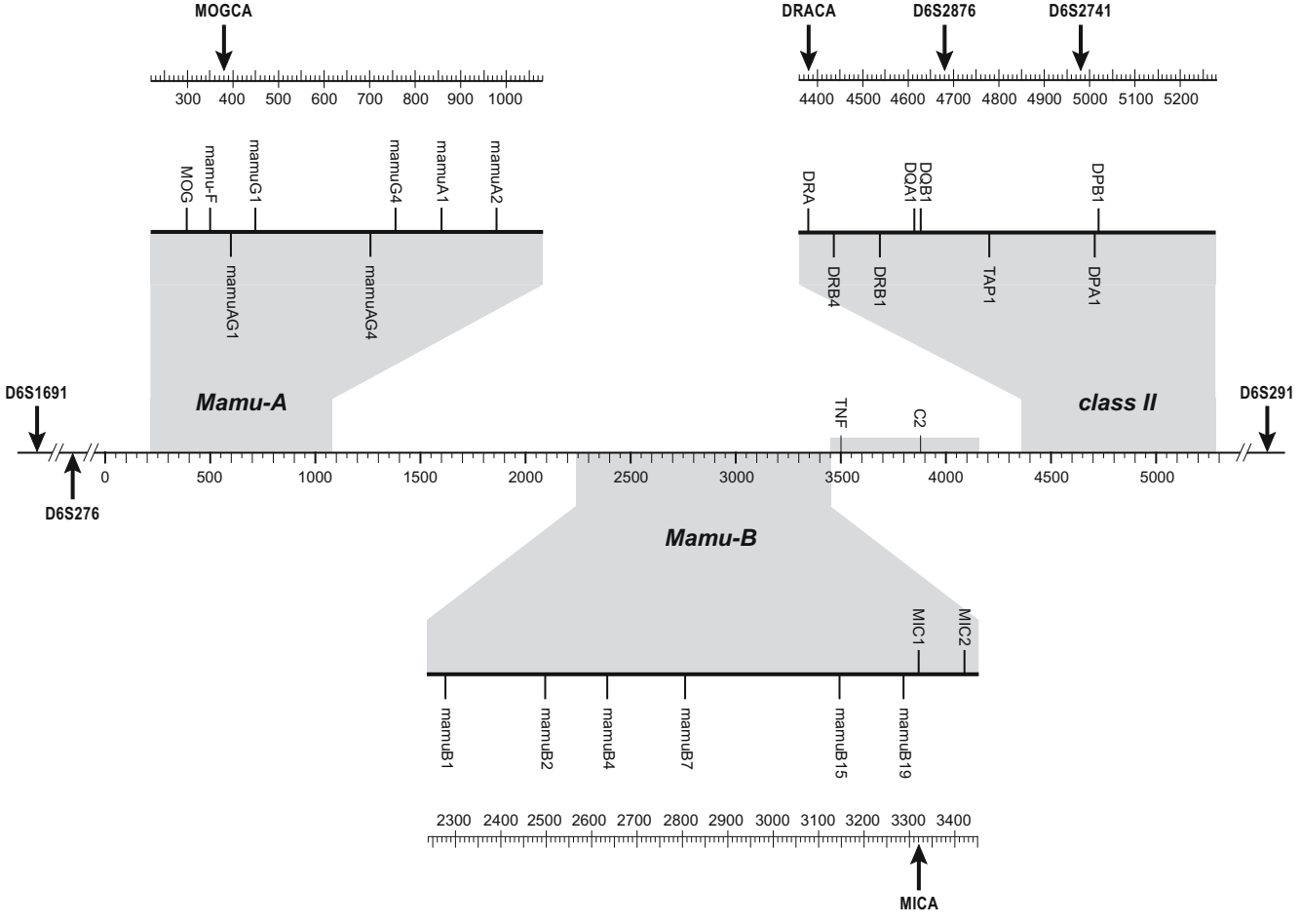

Fig. 1 Localization of short tandem repeat (STR) markers on the rhesus macaque major histocompatibility complex (MHC). The schematic map is drawn according to Daza-Vamenta et al. 2004, with the telomeric end to the *left* and centromeric to the *right* in a kilobase scale. The markers *D6S291*, *D6S276*, and *D6S1691* are localized outside the core MHC region. MHC class I and II regions are partly enlarged above or below the original scale, respectively

Table 4 Linkage disequilibria between rhesus macaque class II alleles and adjacent STR markers

Monkey	Origin	*D6S291*	*DPB1**	*D6S2741*	*D6S2876*	*DQB1*	*DQA1*	*DRB*	*DRA-CA*	*MICA*	*Serol.B*	*Serol.A*	*MOG-CA*	*D6S276*	*D6S1691*
D55	Ch	216	*07*	271	206	*1501*	*2603*	*3*0411, *W314*	254	203	B-	A-	121	213	193
C68	In	206	*01*	275	196	*1811*	*2602*	*6*0112, *W2501*	266	200	B24	A22	123	233	197
9125	In	206	*12*	273	208	*0602*	*01051*	*6*0111, *W606,*W2104, *W1603*	236	194	B11	A19	123	233	199
4065	Bu	210	*10?*	259	208	*0602*	*01051*	*1*07032, 1*0306, *W2603*	234	191	n.d.	n.d.	123	223	203
9017	In	208	*13*	261	220	*1801*	*2601*	*1*0303, 1*1007*	234	200	B29	A6	123	215	203
8609	In	206	*04*	275	220	*1801*	*2601*	*1*0303, 1*1007*	234	200	B26	A10	123	233	197
8669B	In	212	*06*	259	220	*1801*	*2601*	*1*0303, 1*1007*	234	203	B17	A6	123	225	197
1OL	In	214	*06*	277	220	*1801*	*2601*	*1*0303, 1*1007*	234	200	B26	A19	123	233	197
9056	In	206	*06*	259	220	*1801*	*2601*	*1*0303, 1*1007*	234	200	B18	A3	125	233	197
BB66	In	204	*07?*	279	220	*1801*	*2601*	*1*0303, 1*1007*	234	200	B13	A10	123	225	197
8669B	In	206	*07*	259	196	*1809*	*2602*	*1*0306, 1*1003*	248	200	B2	A10	123	225	199
9078	In	206	*07*	261	216	*0605*	*0102*	*1*0306,1*1003*	248	194	B11	A33	127	233	203
8727	In	206	*11*	257	220	*1801*	*2601*	*1*0306, 1*1007*	248	200	B2	A10	123	233	197
4049	Bu	200	*14*	287	210	*1801*	*2601*	*1*0309, *W2507*	268	200	n.d.	n.d.	127	225	195
4074	Bu	218	*14*	287	210	*1801*	*2601*	*1*0309,*W2507*	268	200	n.d.	n.d.	127	235	197
1OL	In	206	*11*	277	208	*0601*	*0104*	*1*0309, 6*0101, *W201*	260	200	B24	A21	123	211	197
C6	In	206	*01*	271	208	*0601*	*0104*	*1*0309, 6*0101, *W201*	260	194	B14	A5	123	235	197
9119	In	204	*10*	275	208	*0601*	*0104*	*1*0309, 6*0101, *W201*	260	194	B11	A6	123	225	209
9119	In	206	*10*	281	208	*0601*	*0104*	*1*0309, 6*0101, *W201*	260	200	B26	A9	127	231	197
9125	In	206	*10*	0	208	*0601*	*0104*	*1*0309, 6*0101, *W201*	260	200	B24	A6	123	235	203
2CP	In	204	*10*	279	208	*0601*	*0104*	*1*0309, 6*0101, *W201*	260	194	B11	A19	127	225	197
D10	In	206	*10*	285	208	*0601*	*0104*	*1*0309, 6*0101, *W201*	260	200	B26	A10	123	233	197
4064	Bu	206	*14*	287	208	*0601*	*0104*	*1*0321, 1*0322*	262	194	n.d.	n.d.	123	225	205
4050	Bu	214	*14*	287	208	*0601*	*0104*	*1*0321, 1*0322*	262	194	n.d.	n.d.	125	227	195
4049	Bu	202	*12*	285	208	*0601*	*0104*	*1*0321, 1*0323*	262	191	n.d.	n.d.	125	215	205
TA	In	206	*12*	269	220	*1804*	*2302*	*1*0310, *W101, *W602, *W609*	244	200	B34	A10	123	223	203
1RY	In	206	*10*	283	204	*1502*	*2401*	*1*0313, *W604, *W605(8)*	268	200	B-	A5	125	235	203
8727	In	208	*04*	279	244	*1808*	*2402*	*1*0403, *W501*	254	191	B32	A10	123	235	197
4064	Bu	214	*10*	271	244	*1808*	*2402*	*1*0403, *W502*	254	200	n.d.	n.d.	123	225	195
4065	Bu	214	*10*	271	244	*1808*	*2402*	*1*0403, *W502*	254	200	n.d.	n.d.	125	215	195
1KX	In	206	*13*	261	210	*1802*	*2301*	*6*0114, *W303, *W401??	256	194	B11	A6	123	225	197
8769	In	206	*08*	267	206	*1803*	*2301*	*1*0405, 5*0304*	270	194	B11	A27	123	235	197
2CP	In	206	*04*	273	196	*1811*	*2602*	*1*0406, 5*0301*	256	194	B26	A28	121	235	191
1VA	In	204	*06*	269	196	*1811*	*2602*	*1*0406, 5*0301*	256	200	B13	A6	123	235	213
9078	In	206	*03*	265	196	*1811*	*2602*	*1*0406, 5*0301*	256	194	B14	A3	127	235	197
C6	In	204	*03*	265	196	*1811*	*2602*	*1*0406, 5*0301*	256	191	B32	A28	123	235	197
1RY	In	206	*13*	261	196	*1811*	*2602*	*1*0406, 5*0301*	256	194	B11	A19	121	235	207
1QK	In	206	*04*	273	220	*1801*	*2601*	*1*0406, 5*0301*	256	200	B26	A22	123	225	199
4074	Bu	214	*14?*	259	204	*1710*	*2603*	*1*0407, 3*0409, 6*New*	254	200	n.d.	n.d.	123	223	207
C68	In	204	*03*	265	248	*1501*	*2603*	*1*0701, 3*0405, 5*0303*	270	194	B14	A1	121	233	203
D10	In	206	*10*	267	196	*1810*	*2401*	*3*0403, *W305*	254	200	B26	A19	121	225	197
2CK	In	206	*04*	279	196	*1810*	*2401*	*3*0403, *W305*	254	200	B26	A19	121	225	197
1VA	In	206	*11*	257	208	*0602*	*01051*	*6*0111, *W606, *W2104, *W2603*	236	200	B13	A9	127	215	197
4050	Bu	206	*13*	261	244	*1503*	*0502*	*6*0111, *W606, *W2104, *W2603*	246	200	n.d.	n.d.	123	231	197

DR/DRA-CA and/or *DQA1/DQB1/D6S2876* pairs are blocked, exceptions are marked yellow. *DPB1*10/D6S2741* combinations are highlighted in light blue, and the *DPB1*14/D6S2741-287* pair is highlighted in light green. Ch = China, Bu = Burma, In = India

DRA-CA and *DRB* alleles reflects these associations. In Burmese monkeys, however, [*D6S2876-208*; *DQB1*0601/DQA1* 0104*] was associated with another *-DRB* region configuration/*DRA-CA* allele, consistent with findings of specific *Mamu* haplotypes associated with the geographic origin of rhesus macaques (Doxiadis et al. 2003).

In contrast to the associations between *DQ*, *DQ*, *DR*, *D6S2876*, and *DRA-CA* alleles, strong LD could not be observed for all *Mamu-DPB1* and *D6S2741* alleles (Table 4). *DPB1*14*, an allele that to our knowledge has been found only in Burmese rhesus macaques, was exclusively associated with *D6S2741-287* (Table 4, light green). *DPB1*10*, the most frequent allele in Indian monkeys, however, was observed with various *D6S2741* alleles (Table 4, light blue). These observations are remarkable because of the close proximity of *D6S2741* to the *DPB1* locus in rhesus macaques. Although far more haplotypes need to be analyzed, one possible explanation for the lack of allelic association in Indian rhesus macaques could be the presence of a recombination hotspot between the *-DPB1* and the marker, as has been observed in humans (Cullen et al. 2002). Alternatively, a higher mutation rate for *D6S2741* could also be a factor in breaking down LD with *DPB1*. Further investigation is needed to determine whether such a hotspot exists in Indian but not in Burmese rhesus macaques, or whether *D6S2741-287* is a more recent mutation that arose on a *DPB1*14* chromosome.

No evidence of LD was found between serotypes of *Mamu-A*, *-B* and nearby STRs *MOG-CA* and *MICA*, respectively. This might be explained by the fact that only two STRs, localized in the more stable part of the MHC class I region, were analyzed. Additionally, these two STRs had the lowest level of allelic diversity and each contained one allele with frequency ≥0.50. These factors would make it difficult to detect allelic associations.

Recombination in the MHC region

Recombination in the MHC region was evaluated in two data sets. First, linkage analyses were performed with seven paternal families from the CNPRC colony with a total of

Table 5 Recombinations observed in rhesus macaque breeding groups

Monkey		Haplotype	*D6S291*	*DPB1**	*D6S2741*	*D6S2876*	*DQB1*	*DQA1*	*DRB*	*DRA-CA*	*MICA*	Serol.B	Serol.A	*MOG-CA*	*D6S276*	*D6S1691*
Gr.1																
C6	sire	a	204	*03*	265	196	*1811*	*2602*	*1*0406, 5*0301*	256	191	B32	A28	123	235	197
C6		b	206	*01*	271	208	*0601*	*0104*	*1*0309, 6*0101, W201*	260	194	B14	A5	123	235	197
9056	dam	c	206	*06*	259	220	*1801*	*2601*	*1*0303, 1*1007*	234	200	B18	A3	125	233	197
9056		d	208	*04*	279	244	*1808*	*2402*	*1*0403, *W501*	254	191	B32	A10	123	235	197
r01074	offspring	b	206	*01*	271	208	*0601*	*0104*	*1*0309, 6*0101, *W201*	260	194	B14	A5	123	235	197
r01074		d	208	*04*	279	244	*1808*	*2402*	*1*0403, *W501*	254	191	B32	A10	123	235	197
R00056	offspring	a	204	*03*	265	196	*1811*	*2602*	*1*0406, 5*0301*	256	191	B32	A28	123	235	197
R00056		c	206	*06*	259	220	*1801*	*2601*	*1*0303, 1*1007*	234	200	B18	A3	125	233	197
98039	offspring	a	204	*03*	265	196	*1811*	*2602*	*1*0406, 5*0301*	256	191	B32	A28	123	235	197
98039		d/c	208	*04*	279	244	*1808*	*2402*	*1*0403, *W501*	254	200	B18	A3	125	233	197
Gr.4																
D55	sire	a	206	*10*	267	196	*1810*	*2401*	*3*0403, *W305*	254	200	B26	A19	121	225	197
D55		b	216	*07*	271	206	*1501*	*2603*	*3*0411, *W314*	254	203	B-	A-	121	213	193
8669B	dam	e	212	*06*	259	220	*1801*	*2601*	*1*0303, 1*1007*	234	203	B17	A6	123	225	197
8669B		f	206	*07*	259	196	*1809*	*2602*	*1*0306, 1*1003*	248	200	B2	A10	123	225	199
r99007	offspring	a	206	*10*	267	196	*1810*	*2401*	*3*0403, *W305*	254	200	B26	A19	121	225	197
r99007		e	212	*06*	259	220	*1801*	*2601*	*1*0303, 1*1007*	234	203	B17	A6	123	225	197
r00035	offspring	b/a	216	*07*	271	206	*1501*	*2603*	*3*0411, *W314*	254	200	B26	A19	121	225	197
r00035		f	206	*07*	259	196	*1809*	*2602*	*1*0306, 1*1003*	248	200	B2	A10	123	225	199
98004	offspring	b	216	*07*	271	206	*1501*	*2603*	*3*0411, *W314*	254	203	B-	A-	121	213	193
98004		e/f	212	*06*	259	220	*1801*	*2601*	*1*0303, 1*1007*	234	200	B2	A10	123	225	199
Gr. 5																
4049	sire	a	202	*12*	285	208	*0601*	*0104*	*1*0321, 1*0323*	262	191	n.d.	n.d.	125	215	205
4049		b	200	*14*	287	210	*1801*	*2601*	*1*0309, *W2507*	268	200	n.d.	n.d.	127	225	195
4050	dam	i	214	*14*	287	208	*0601*	*0104*	*1*0321, 1*0322*	262	194	n.d.	n.d.	125	227	195
4050		j	206	*13*	261	244	*1503*	*0502*	*5*0111, *W606, *W2104, *W2603*	246	200	n.d.	n.d.	123	231	197
98022	offspring	a	202	*12*	285	208	n.d.	n.d.	*1*0321, 1*0323*	262	191	n.d.	n.d.	125	215	205
98022		i	214	*14*	287	208	n.d.	n.d.	*1*0321, 1*0322*	262	194	n.d.	n.d.	125	227	195
94018	offspring	a	202	*12*	285	208	n.d.	n.d.	*1*0321, 1*0323*	262	191	n.d.	n.d.	125	215	205
94018		i	214	*14*	287	208	n.d.	n.d.	*1*0321, 1*0322*	262	194	n.d.	n.d.	125	227	195
97030	offspring	b	200	*14*	287	210	*1801*	*2601*	*1*0309, *W2507*	268	200	n.d.	n.d.	127	225	195
97030		j/i	206	*14*	287	208	n.d.	n.d.	*1*0321, 1*0322*	262	194	n.d.	n.d.	125	227	195
96090	offspring	b/a	202	*14*	287	210	*1801*	*2601*	*1*0309, *W2507*	268	200	n.d.	n.d.	127	225	195
96090		j	206	*13*	261	244	*1503*	*0502*	*5*0111, *W606, *W2104, *W2603*	246	200	n.d.	n.d.	123	231	197
Gr. 5																
4049	sire	a	202	*12*	285	208	*0601*	*0104*	*1*0321, 1*0323*	262	191	n.d.	n.d.	125	215	205
4049		b	200	*14*	287	210	*1801*	*2601*	*1*0309, *W2507*	268	200	n.d.	n.d.	127	225	195
4065	dam	e	214	*10*	271	244	*1808*	*2402*	*1*0403, *W502*	254	200	n.d.	n.d.	125	215	195
4065		f	210	*?*	259	208	*0602*	*01051*	*1*07032, 1*0306, *W2603*	234	191	n.d.	n.d.	123	223	203
9312	offspring	a	202	*12*	285	208	*0601*	*0104*	*1*0321, 1*0323*	262	191	n.d.	n.d.	125	215	205
9312		e	214	*10*	271	244	*1808*	*2402*	*1*0403, *W502*	254	200	n.d.	n.d.	125	215	195
95005	offspring	b	200	*14*	287	210	*1801*	*2601*	*1*0309, *W2507*	268	200	n.d.	n.d.	127	225	195
95005		f/e	210	*10*	271	244	*1808*	*2402*	*1*0403, *W502*	254	200	n.d.	n.d.	125	215	195
97042	offspring	b	200	*14*	287	210	*1801*	*2601*	*1*0309, *W2507*	268	200	n.d.	n.d.	127	225	195
97042		f/e	210	*?*	259	208	*n.d.*	*01051*	*1*07032, 1*0306, *W2603*	234	191	n.d.	n.d.	123	215	195
Gr. 7																
C68	sire	a	204	*03*	265	248	*1501*	*2603*	*1*0701, 3*0405, 5*0303*	270	194	B14	A1	121	233	203
C68		b	206	*01*	275	196	*1811*	*2602*	*W2501, 6*0112*	266	200	B24	A22	123	233	197
9078	dam	e	206	*03*	265	196	*1811*	*2602*	*1*0406, 5*0301*	256	194	B14	A3	127	235	197
9078		f	206	*07*	261	216	*0605*	*0102*	*1*306, 1*1003*	248	194	B11	A33	127	233	203
r99020	offspring	a	204	*03*	265	248	*1501*	*2603*	*1*0701, 3*0405, 5*0303*	270	194	B14	A1	121	233	203
r99020		e	206	*03*	265	196	*1811*	*2602*	*1*0406, 5*0301*	256	194	B14	A3	127	235	197
97044	offspring	b	206	*01*	275	196	*1811*	*2602*	*W2501, 6*0112*	266	200	B24	A22	123	233	197
97044		f	206	*07*	261	216	*0605*	*0102*	*1*306,1*1003*	248	194	B11	A33	127	233	203
97012	offspring	a	204	*03*	265	248	*1501*	*2603*	*1*0701, 3*0405, 5*0303*	270	194	B11	A33	121	233	203
97012		e/f	206	*03*	265	196	*1811*	*2602*	*1*0406, 5*0301*	256	194	B14	A3	127	233	203
Gr. 7																
C68	sire	a	204	*03*	265	248	*1501*	*2603*	*1*0701, 3*0405, 5*0303*	270	194	B14	A1	121	233	203
C68		b	206	*01*	275	196	*1811*	*2602*	*6*0112, *W2501*	266	200	B24	A22	123	233	197
2CV	dam	g	206	*10*	261	216	*0605*	*0102*	*1*0306, 1*1003*	248	194	B11	A33	127	235	203
2CV		h	206	*07*	0	208	*0601*	*0104*	*1*0309, 6*0101, *W201*	260	200	B24	A6	123	233	203
98030	offspring	a	204	*03*	265	248	*1501*	*2603*	*1*0701, 3*0405, 5*0303*	270	194	B14	A1	121	233	203
98030		g	206	*10*	261	216	*0605*	*0102*	*1*0306, 1*1003*	248	194	B11	A33	127	235	203
97038	offspring	b	206	*01*	275	196	*1811*	*2602*	*W2501, 6*0112*	266	200	B24	A22	123	233	197
97038		h	206	*07*	0	208	*0601*	*0104*	*1*0309, 6*0101, *W201*	260	200	B24	A6	123	233	203
99016	offspring	a	204	*03*	265	248	*1501*	*2603*	*1*0701, 3*0405, 5*0303*	270	194	B14	A1	121	233	203
99016		h/g	206	*07*	0	208	*0601*	*0104*	*1*0309, 6*0101, *W201*	260	200	B24	A6	123	235	203

Paternal haplotypes involved in a recombination are marked blue/red, maternal haplotypes yellow/light green. Orange color indicates that the location of the crossover before or after the marker could not be determined. At least two offspring without a crossover are shown to verify the extended haplotype

331 offspring–dam pairs to obtain recombination distance for the STR markers. The average number of phase known, informative meioses was 171±49. The sex-averaged map constructed was *D6S291*—3.3 cM–*D6S2741*—1.2 cM–*D6S2876*—1.5 cM–*DRA-CA*—0.7 cM–*MICA*—0.5 cM–*MOGCA*—2.5 cM–*D6S276*—0.4 cM–*D6S1691*. These analyses allowed us to obtain the distance of the flanking markers *D6S291*, *D6S276*, and *D6S1691* relative to the core MHC STRs. Approximate estimates for location of the core MHC STRs, based on position in BAC clones and the *Mamu* region (Daza-Vamenta et al. 2004), placed *D6S2741* at ~4,980 kb (based on the position on BAC clone 118H5), *D6S2876* at ~4,680 kb (based on the position on BAC clone 281E18), *DRA-CA* at ~4,380 kb, *MICA* at ~3,320 kb, and *MOG-CA* at ~380 kb (Fig. 1). The distances between markers suggested recombination rates of about 0.005 cM/kb in the class II region between *D6S2741* and *DRACA*, 0.0007 cM/kb between *DRA-CA* and *MICA*, and 0.0002 cM/kb between *MICA* and *MOG-CA*. The average rate across a 4,600-kb span between D6S2741 and MOG-CA was 0.0009 cM/kb. Although these estimates are based on few markers and preliminary, the pattern of recombination distribution may be comparable to that found in humans (Cullen et al. 2002). However, the lower recombination rates determined in the *Mamu* class I in comparison to the class II region may be related to the lower informativeness of, and longer physical distance between, the two markers in the class I region (*MICA* and *MOG-CA*). The complete rhesus macaque MHC genomic map will provide ample source of markers for use in more rigorous studies of recombination across the region and comparison with what is known for humans.

To obtain additional information regarding recombination in the MHC, we also analyzed segregation data for the BPRC pedigrees. A total of nine recombinants were observed (Table 5) within and adjacent to a core MHC region of about 4.7 Mb spanning *DPB1* to *MOG-CA* (Daza-Vamenta et al. 2004). Two of these were localized between *DRA-CA* and *MICA*, a DNA segment of about 1.1 Mb separating class I and class II genes (Table 5, offspring 98004, group 4 and 98039, group 1). A third recombinant mapped between the *DR* loci and *MICA* (Table 5, offspring r00035, group 4). Since the *DRA-CA* allele was not informative in this offspring, positioning of the crossover before or after this marker was not possible. Because of the strong LD between this STR and the *-DRB* loci, the break most probably occurred between *DRA-CA* and *MICA*. Three recombinants separated marker *D6S291* from *DPB1* (Table 5, offspring 97030, 96090, and 95005, group 5). Three crossovers were observed telomeric of the class I region. One of these separated *Mamu-A* from *D6S276/D6S1691* (Table 5, offspring 97012, group 7) but was not informative for *MOG-CA*. Two crossovers separated *MOG-CA* from *D6S276/D6S1691* (Table 5, offspring 97042, group 5, and 99016, group 7). Similar to the results obtained for CNPRC families, the crossover events identified in the BPRC families occurred primarily at the ends of the MHC region. No recombinants were observed in the region spanning *DQ-DRA* loci, except perhaps for the one questionable case for which *DRA-CA* was not informative.

Conclusions

In this report, we characterized the polymorphism of eight STRs within or near the MHC and mapped their location in the rhesus macaque genomic sequence. Comparison of allelic diversity and frequency in Indian- and Chinese-origin rhesus macaques provided additional evidence of population differentiation that has been documented in the literature for STRs not linked to the MHC, blood proteins, and MHC class II genes. The observed differences for MHC-linked STRs between different rhesus macaque populations most likely underlie biological variation in adaptive and innate immunity and further justify efforts for more detailed characterization of the MHC region in this species. We also showed that these highly polymorphic markers were useful to help define extended haplotypes across the MHC, to identify haplotypes that were identical by descent, and to differentiate chromosome configurations that would otherwise appear identical, or nearly so, on the basis of limited gene-specific typing.

Segregation analyses suggested variable recombination rates across the MHC region in a pattern similar to that of humans. Because large pedigrees can be obtained from breeding colonies, more-detailed studies of recombination in rhesus macaques are possible and could provide comparative data regarding the evolution of the MHC within and between related species. The apparent LD between class II genes and adjacent STRs spanning the *DQ-DR* region suggested that inclusion of these and additional markers surrounding this region may be useful to define haplotypic blocks and may help in mapping genes or chromosomal segments associated with disease.

Genetic testing is increasingly used to establish or validate pedigree records and to manage breeding colonies of captive animals in primate centers around the world. The incorporation of MHC-linked STR typing as part of this routine will enhance the genetic characterization of captive-bred rhesus macaques and will help in the production and careful selection of experimental animals, particularly with respect to such an important genomic region as the MHC. The complete MHC sequence for rhesus macaques will make it possible to identify a plethora of other markers, STRs, or single nucleotide polymorphisms, that will further contribute to understanding the role of different MHC regions in immune-related processes.

Acknowledgements The work was financed in part by grant NIH/NCRR U24-RR18144-01, the European Union project INPRIMAT QLRI-CT-2002-01325, and NIH project 1-R24-RR16038-01 (CDFA no.93.306). The authors wish to thank D. Devine for editing the manuscript and Henk van Westbroek for preparing the figure.

References

Ardlie KG, Kruglyak L, Seielstad M (2002) Patterns of linkage disequilibrium in the human genome. Nat Rev Genet 3:299–309

Bontrop RE, Otting N, Slierendregt BL, Lanchbury JS (1995) Evolution of major histocompatibility complex polymorphisms and T-cell receptor diversity in primates. Immunol Rev 143:33–62

Botstein D, White RL, Skolnick M, Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 32:314–331

Boyson JE, Shufflebotham C, Cadavid LF, Urvater JA, Knapp LA, Hughes AL, Watkins DI (1996) The MHC class I genes of the rhesus monkey. Different evolutionary histories of MHC class I and II genes in primates. J Immunol 156:4656–4665

Brok HP, Bauer J, Jonker M, Blezer E, Amor S, Bontrop RE, Laman JD, 't Hart BA (2001) Non-human primate models of multiple sclerosis. Immunol Rev 183:173–185

Carrington M (1999) Recombination within the human MHC. Immunol Rev 167:245–256

Carrington M, Wade J (1996) Selection of transplant donors based on MHC microsatellite data. Hum Immunol 51:106–109

Clisson I, Lathuilliere M, Crouau-Roy B (2000) Conservation and evolution of microsatellite loci in primate taxa. Am J Primatol 50:205–214

Coote T, Bruford MW (1996) Human microsatellites applicable for analysis of genetic variation in apes and Old World monkeys. J Heredity 87:406–410

Cullen M, Perfetto SP, Klitz W, Nelson G, Carrington M (2002) High-resolution patterns of meiotic recombination across the human major histocompatibility complex. Am J Hum Genet 71:759–776

Cullen M, Malasky M, Harding A, Carrington M (2003) High-density map of short tandem repeats across the human major histocompatibility complex. Immunogenetics 54:900–910

Daza-Vamenta R, Glusman G, Rowen L, Guthrie B, Geraghty DE (2004) Genetic divergence of the rhesus macaque major histocompatibility complex. Genome Res 14:1501–1515

Dib C, Faure S, Fizames C, Samson D, Drouot N, Vignal A, Millasseau P, Marc S, Hazan J, Seboun E (1996) A comprehensive genetic map of the human genome based on 5,264 microsatellites. Nature 380:152–154

Doxiadis GG, Otting N, de Groot NG, Noort R, Bontrop RE (2000) Unprecedented polymorphism of Mhc-DRB region configurations in rhesus macaques. J Immunol 164:3193–3199

Doxiadis GG, Otting N, de Groot NG, Bontrop RE (2001) Differential evolutionary MHC class II strategies in humans and rhesus macaques: relevance for biomedical studies. Immunol Rev 183: 76–85

Doxiadis GG, Otting N, de Groot NG, de Groot N, Rouweler AJ, Noort R, Verschoor EJ, Bontjer I, Bontrop RE (2003) Evolutionary stability of MHC class II haplotypes in diverse rhesus macaque populations. Immunogenetics 55:540–551

Erlich HA, Bergstrom TF, Stoneking M, Gyllensten U (1996) HLA sequence polymorphism and the origin of humans. Science 274:1552–1554

Evans DT, O'Connor DH, Jing P, Dzuris JL, Sidney J, da Silva J, Allen TM, Horton H, Venham JE, Rudersdorf RA, Vogel T, Pauza CD, Bontrop RE, DeMars R, Sette A, Hughes AL, Watkins DI (1999) Virus-specific cytotoxic T-lymphocyte responses select for amino-acid variation in simian immunodeficiency virus Env and Nef. Nat Med 5:1270–1276

Foissac A, Salhi M, Cambon-Thomsen A (2000) Microsatellites in the HLA region: 1999 update. Tissue Antigens 55:477–509

Foissac A, Fort M, Clayton J, Abbal M, Raffoux C, Moine A, Bensa JC, Bignon JD, Mercier P, Cambon-Thomsen A (2001) Microsatellites in the HLA region: HLA prediction and strategies for bone marrow donor registries. Transplant Proc 33:491–492

Friedrich TC, Dodds EJ, Yant LJ, Vojnov L, Rudersdorf R, Cullen C, Evans DT, Desrosiers RC, Mothe BR, Sidney J, Sette A, Kunstman K, Wolinsky S, Piatak M, Lifson J, Hughes AL, Wilson N, O'Connor DH, Watkins DI (2004) Reversion of CTL escape-variant immunodeficiency viruses in vivo. Nat Med 10:275–281

Garver JJ, Estop AM, Meera Khan P, Balner H, Pearson PL (1980) Evidence of similar organization of the chromosomes carrying the major histocompatibility complex in man and other primates. Cytogenet Cell Genet 27:238–245

Green P, Falls K, Crooks S (1990) Documentation for CRI-MAP (version 2.4). Department of Genetics, Washington University School of Medicine, St.Louis

de Groot N, Doxiadis GG, De Groot NG, Otting N, Heijmans C, Rouweler AJ, Bontrop RE (2004) Genetic make up of the DR region in rhesus macaques: gene content, transcripts, and pseudogenes. J Immunol 172:6152–6157

Gyapay G, Morisette J, Vignal A, Dib C, Fizames C, Millasseau P, Marc S, Bernardi G, Lathrop M, Weissenbach J (1994) The 1993–94 Genethon human genetic linkage map. Nat Genet 7: 246–339

Hirai M, Takahashi E, Ishida T, Hori T (1991) Chromosomal localization of the major histocompatibility complex (MHC) in the rhesus monkey and chimpanzee by fluorescence in situ hybridization. Cytogenet Cell Genet 57:204–205

Horton H, Rehrauer W, Meek EC, Shultz MA, Piekarczyk MS, Jing P, Carter DK, Steffen SR, Calore B, Urvater JA, Vogel TU, Wilson NA, Watkins DI (2001) A common rhesus macaque MHC class I molecule which binds a cytotoxic T-lymphocyte epitope in Nef of simian immunodeficiency virus. Immunogenetics 53:423–426

Huber I, Walter L, Wimmer R, Pasantes JJ, Gunther E, Schempp W (2003) Cytogenetic mapping and orientation of the rhesus macaque MHC. Cytogenet Genome Res 103:144–149

Huttley GA, Smith MW, Carrington M, O'Brien SJ (1999) A scan for linkage disequilibrium across the human genome. Genetics 152:1711–1722

Khazand M, Peiberg C, Nagy M, Sauermann U (1999) Mhc-DQ-DRB haplotype analysis in the rhesus macaque: evidence for a number of different haplotypes displaying a low allelic polymorphism. Tissue Antigens 54:615–624

Knechtle SJ, Burlingham WJ (2004) Metastable tolerance in non-human primates and humans. Transplantation 77:936–939

Lee DM, Yeoman RR, Battaglia DE, Stouffer RL, Zelinski-Wooten MB, Fanton JW, Wolf DP (2004) Live birth after ovarian tissue transplant. Nature 428:137–138

Martin MP, Harding A, Chadwick R, Kronick M, Cullen M, Lin L, Mignot E, Carrington M (1998) Characterization of 12 microsatellite loci of the human MHC in a panel of reference cell lines. Immunogenetics 47:131–138

Matsuzaka Y, Makino S, Nakajima K, Tomizawa M, Oka A, Kimura M, Bahram S, Tamiya G, Inoko H (2000) New polymorphic microsatellite markers in the human MHC class II region. Tissue Antigens 56:492–500

Matsuzaka Y, Makino S, Nakajima K, Tomizawa M, Oka A, Bahram S, Kulski JK, Tamiya G, Inoko H (2001) New polymorphic microsatellite markers in the human MHC class III region. Tissue Antigens 57:397–404

Mignot EMCJ, Hallmayer J, Kimura A, Grumet FCS (1997) The natural history of a microsatellite located in the HLA-DQ region. In: Charron D (ed) Genetic diversity of HLA: functional and medical implications. EDK, Paris, pp121–124

Mizuki N, Ota M, Kimura M, Ohno S, Ando H, Katsuyama Y, Yamazaki M, Watanabe K, Goto K, Nakamura S, Bahram S, Inoko H (1997) Triplet repeat polymorphism in the transmembrane region of the MICA gene: a strong association of six GCT repetitions with Behçet disease. Proc Natl Acad Sci USA 94:1298–1303

Morin PA, Kanthaswamy S, Smith DG (1997) Simple sequence repeat (SSR) polymorphisms for colony management and population genetics in rhesus macaques (*Macaca mulatta*). Am J Primatol 42:199–213

Mothe BR, Weinfurter J, Wang C, Rehrauer W, Wilson N, Allen TM, Allison DB, Watkins DI (2003) Expression of the major histocompatibility complex class I molecule Mamu-A*01 is associated with control of simian immunodeficiency virus SIV mac239 replication. J Virol 77:2736–2740

Muhl T, Krawczak M, Ten Haaft P, Hunsmann G, Sauermann U (2002) MHC class I alleles influence set-point viral load and survival time in simian immunodeficiency virus-infected rhesus monkeys. J Immunol 169:3438–3446

Newberg MH, Kuroda MJ, Charini WA, Miura A, Lord CI, Schmitz JE, Gorgone DA, Lifton MA, Kuus-Reichel K, Letvin NL (2002) A simian immunodeficiency virus nef peptide is a dominant cytotoxic T lymphocyte epitope in Indian-origin rhesus monkeys expressing the common MHC class I allele mamu-A*02. Virology 301:365–373

O'Connor DH, Mothe BR, Weinfurter JT, Fuenger S, Rehrauer WM, Jing P, Rudersdorf RR, Liebl ME, Krebs K, Vasquez J, Dodds E, Loffredo J, Martin S, McDermott AB, Allen TM, Wang C, Doxiadis GG, Montefiori DC, Hughes A, Burton DR et al (2003) Major histocompatibility complex class I alleles associated with slow simian immunodeficiency virus disease progression bind epitopes recognized by dominant acute-phase cytotoxic-T-lymphocyte responses. J Virol 77:9029–9040

Otting N, Heijmans CMC, Noort R, de Groot NG, Doxiadis GGM, van Rood JJ, Watkins D, Bontrop RE (2005) Unparalleled complexity of the MHC class I region in rhesus macaques. Proc Natl Acad Sci USA 102:1626–1631

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J Heredity 86:248

Rogers J, Mahaney MC, Witte SM, Nair S, Newman D, Wedel S, Rodriguez LA, Rice KS, Slifer SH, Perelygin A, Slifer M, Palladino-Negro P, Newman T, Chambers K, Joslyn G, Parry P, Morin PA (2000) A genetic linkage map of the baboon (*Papio hamadryas*) genome based on human microsatellite polymorphisms. Genomics 67:237–247

Roth MP, Dolbois L, Borot N, Amadou C, Clanet M, Pontarotti P, Coppin H (1995) Three highly polymorphic microsatellites at the human myelin oligodendrocyte glycoprotein locus, 100 kb telomeric to HLA-F. Characterization and relation to HLA haplotypes. Hum Immunol 43:276–282

Rubinsztein DC, Amos W, Leggo J, Goodburn S, Jain S, Li SH, Margolis RL, Ross CA, Ferguson-Smith MA (1995) Microsatellite evolution—evidence for directionality and variation in rate between species. Nat Genet 10:337–343

Tamiya G, Shiina T, Oka A, Tomizawa M, Ota M, Katsuyama Y, Yoshitome M, Makino S, Kimura M, Inoko H (1999) New polymorphic microsatellite markers in the human MHC class I region. Tissue Antigens 54:221–228

Torrealba JR, Katayama M, Fechner JH Jr, Jankowska-Gan E, Kusaka S, Xu Q, Schultz JM, Oberley TD, Hu H, Hamawy MM, Jonker M, Wubben J, Doxiadis G, Bontrop R, Burlingham WJ, Knechtle SJ (2004) Metastable tolerance to rhesus monkey renal transplants is correlated with allograft TGF-beta 1+CD4+T regulatory cell infiltrates. J Immunol 172:5753–5764

Uda A, Tanabayashi K, Yamada YK, Akari H, Lee YJ, Mukai R, Terao K, Yamada A (2004) Detection of 14 alleles derived from the MHC class I *A* locus in cynomolgus monkeys. Immunogenetics 56:155–163

Wall JD, Pritchard JK (2003) Haplotype blocks and linkage disequilibrium in the human genome. Nat Rev Genet 4:587–597

Weissenbach J, Gyapay G, Dib C, Vignal A, Morissette J, Millasseau P, Vaysseix G, Lathrop M (1992) A second-generation linkage map of the human genome. Nature 359:794–801

Wood KJ, Jones ND, Bushell AR, Morris PJ (2001) Alloantigen-induced specific immunological unresponsiveness. Philos Trans R Soc Lond B Biol Sci 356:665–680