Unparalleled complexity of the MHC class I region in rhesus macaques

Nel Otting[†‡], Corrine M. C. Heijmans[†], Riet C. Noort[†], Natasja G. de Groot[†], Gaby G. M. Doxiadis[†], Jon J. van Rood[§], David I. Watkins[¶], and Ronald E. Bontrop[†]

[†]Department of Comparative Genetics and Refinement, Biomedical Primate Research Centre, P.O. Box 3306, 2280 GH Rijswijk, The Netherlands; [§]Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, E3-Q, P.O. Box 9600, 2300 RC Leiden, The Netherlands; and [¶]Department of Pathology and Laboratory Medicine, University of Wisconsin, Madison, WI 53715

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The highly polymorphic gene products of the classical MHC class I genes in humans (*HLA-A*, *HLA-B*, and *HLA-C*) play a critical role in the immune defense against intracellular infections. Because nonhuman primates are important models for AIDS vaccine research, rhesus monkeys from a thoroughly pedigreed and serotyped colony were subjected to full-length cDNA analysis of MHC class I gene transcripts. Rhesus macaques express multiple dominant *Mamu-A* and *Mamu-B* transcripts (majors) per chromosome, which are characterized by high expression levels. The presence of additional cDNAs with low levels of expression (minors) suggests evidence for transcriptional control of MHC class I genes. Moreover, phylogenetic analyses illustrate that most of the *Mamu-A* and *Mamu-B* loci/lineages identified display no or only limited levels of allelic polymorphism. Thus, MHC class I diversity in rhesus macaques is typified by the existence of an unmatched high number of *Mamu-A and Mamu-B* region configurations that exhibit polymorphism with regard to the number and combination of transcribed loci present per chromosome.

AIDS | non-human primate

MHC class I glycoproteins are expressed on nucleated cells, and their biological function is to present foreign peptides of intracellular origin to cytotoxic T cells, which may result in the destruction of infected cells (1). In addition, inhibitory and stimulatory receptors on natural killer cells scan for the presence and absence of MHC class I molecules (2, 3). The polymorphism of the *HLA-A*, *HLA-B*, and *HLA-C* genes in the human population has been studied extensively, and hundreds of alleles have been identified (4). In contrast, the gene products of the nonclassical *HLA-E*, *HLA-F*, and *HLA-G* genes show low levels of polymorphism and a restricted tissue distribution and are thought to exert specialized functions (5, 6).

Considerable research has been conducted on the rhesus macaque (*Macaca mulatta*) MHC, because this species is widely used as a model for human diseases and organ transplantation. Simian immunodeficiency virus infection of macaques, for instance, is an important model for the study of AIDS (7–9). The rhesus macaque equivalents of the classical *HLA-A* and *HLA-B* genes (10–12) and the nonclassical *HLA-E* (13), *HLA-F* (14), and *HLA-G* (15) genes were identified and designated *Mamu-A*, *Mamu-B*, *Mamu-E*, *Mamu-F*, and *Mamu-G*, respectively. The latter appears to be a pseudogene, and its function may have been taken over by *Mamu-AG*, which is expressed on the rhesus monkey placenta and shares unique features with *HLA-G* (16, 17). Rhesus macaques possess an additional oligomorphic *Mamu-B*-like gene designated *Mamu-I*, which shares classical and nonclassical characteristics (18). The ortholoques of the *HLA-C* gene were found neither in rhesus macaques nor in any other species of Old World monkeys.

The organization of the rhesus macaque MHC class I region may be complex, because initial studies suggested that the *Mamu-A* and *Mamu-B* genes may have been duplicated (11, 19). The main question to be answered, however, revolves around the reported *Mamu-A* and *Mamu-B* sequences; the complex locus/allele relationships are not yet understood. The aim of this study was to shed light on the complexity of the class I region by providing a thorough inventory of the number of expressed *Mamu-A* and *Mamu-B* loci per chromosome by using a large panel of serotyped and pedigreed animals.

Materials and Methods

Animals and Cell Lines. The Biomedical Primate Research Centre houses a self-sustaining outbred colony of ≈1,000 rhesus macaques that have been pedigreed based on the segregation of serologically defined MHC haplotypes. Serotyping is performed by polyclonal sera raised by active immunizations. Serotypes are defined by a cluster of positive typing reactions. A blank serotype means that the typing reactions are not unambiguously interpretable. An inbreeding program resulted in a group of Mamu-A, Mamu-B, and Mamu-DR homozygous animals of consanguineous origin (20). The present Herpes papiotransformed B cell line cohort (≈100 individuals) consisted of samples originating mainly from Indian animals, as well as a few of Chinese and Burmese origin. Cell lines were selected in such a way that the panel covered all known Mamu-A and Mamu-B serotypes multiple times.

cDNA Cloning and Sequencing. RNA was isolated from B cells (RNeasy kit, Qiagen, Valencia, CA) and subjected to a One-Step RT-PCR kit, as recommended by the supplier. In these reactions, we used the primer sets 5′MAS/3′MAS and 5′MBS/3′MBS, which are specific for *Mamu-A* and *Mamu-B* transcripts, respectively (11). The final elongation step was extended to 30 min to generate a 3′dA overhang. The RT-PCR products were cloned by using the InsT/Aclone kit (Fermentas, St. Leon-Rot, Germany). After transformation colonies were picked for plasmid isolations (16–32 colonies for the *Mamu-A* transcript and 32–64 colonies for the *Mamu-B* transcript). Sequencing reactions were performed by using the BigDye terminator cycle sequencing kit, and samples were run on automated capillary sequencing systems (Applied Biosystems). All unreported *Mamu-A* and *Mamu-B* sequences and their corresponding accession numbers are depicted in Table 1. The sequences were named according to the proposal published in ref. 21.

Locus-Specific PCR and Phylogenetic Analysis. To establish the presence of *Mamu-A2*(*A*05*) or *Mamu-A4*(*A*14*) alleles within cDNA samples, the following primer sets were used: (5′A*05) GCCCCCAGGCTCGCACTCCTTGAGA and (3′A*05) CTSGCCCTCCAGGTAGGCTCTCCA; (5′A*14) GGGAC-

Freely available online through the PNAS open access option.

Data deposition: The sequences reported in this paper have been deposited in the EMBL database (accession nos. AJ542567–AJ542580, AJ551315–AJ551321, AJ556875–AJ556908, AJ620415, AJ620416, AJ844596–AJ844602, and AJ849330).

‡To whom correspondence should be addressed. E-mail: otting@bprc.nl.

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Table 1. Unreported *Mamu-A* and *Mamu-B* sequences

Alleles	Accession nos.	Reference animals
Mamu-A		
**0602*	AJ542567	KM, 8653
**0505*	AJ551315	1VJ, 1IH
**0506*	AJ551316	BB58, BB10
**0507*	AJ551317	8745
**0509*	AJ551318	2B, 2G
**0510*	AJ551319	Ri260
**0511*	AJ551320	1KM, 1VV
**0703*	AJ542568	C77, 9222
**1305*	AJ551321	3238, 8813
**1306*	AJ542570	98049
**1602*	AJ542572	98049
**19*	AJ542573	1VJ, 1IH
**21*	AJ542574	9133, 8813
**23*	AJ542575	1JT, 9222
**24*	AJ542576	9133, 8813
**25*	AJ542577	2B, 2G
**26*	AJ542578	8827, 8769
**27*	AJ542579	9151, 1ZA
**28*	AJ542580	C77
Mamu-B		
**0602*	AJ844596	9178, KP
**0702*	AJ556875	1GG, 8884
**0703*	AJ556876	BB10, BB113
**19*	AJ556877	1RK, 1JT
**20*	AJ556878	1VJ, B21
**21*	AJ556879	1VJ, B21
**22*	AJ556880	1VJ, 9151
**24*	AJ556881	1RK, 1JT
**26*	AJ844602	MR
**27*	AJ556882	MR, 3019
**28*	AJ556883	1VJ, B21
**29012*	AJ556884	2AK, 1IH
**3002*	AJ556885	1GG, 8884
**3601*	AJ556886	2BZ, 1QA
**3602*	AJ556887	BB36, BB78
**37*	AJ556888	2BZ, 2QA
**38*	AJ556889	8827, 1GG
**39*	AJ556890	BB10, BB58
**40*	AJ556891	2B, 2G, 2V
**41*	AJ556892	1OX, 2CA
**43*	AJ556893	MR, 3019
**44*	AJ556894	2B, 2G, 2V
**4501*	AJ556895	2BZ, 1QA
**4502*	AJ556896	BB36, BB78
**46*	AJ556897	1RK, 8822
**4701*	AJ556898	8822, 8827
**4702*	AJ556899	B65
**48*	AJ556900	1OX, 2CA
**49*	AJ844603	3C, 96084
**5002*	AJ620415	3238, EKK
**5301*	AJ556901	M14
**5302*	AJ844604	3C, MR
**54*	AJ556902	1PV
**55*	AJ556903	1PV, 1GX
**57*	AJ844605	MR, 96084
**5802*	AJ556904	1PV, 1GX
**6002*	AJ556905	8745
**61*	AJ556906	8745
**63*	AJ556907	1PV, 3019
**64*	AJ556908	1OX, 9202
**65*	AJ620416	3238, EKK
**66*	AJ844597	95044, 8704
**67*	AJ844598	95044, 8704
**68*	AJ844599	95044, 8704
**69*	AJ844601	1PH, 9208
**70*	AJ844600	3C, MR
**71*	AJ849330	9178

Names, EMBL accession numbers, and reference animals are provided.

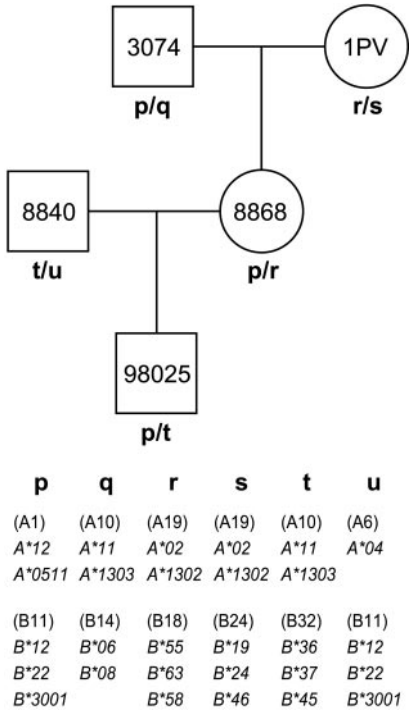

Fig. 1. Segregation of serotypes and *Mamu-A* and *Mamu-B* cDNAs in a Mendelian fashion in a rhesus macaque family. The maternal and paternal chromosomes are designated p–u. Serotypes are shown in brackets, and cDNAs are depicted in italics.

CCGACGGGCGCCTCCAA and (3′A*14) GGCCCTCCAG-GTAGACTCTGTC. The 5′A*05 primer annealed in exon 2 and the 3′-primer in exon 3, respectively. The A*14 primers both have annealing sites in exon 3. Amplifications were carried out starting with 2 min at 94°C, followed by 25 cycles of 94°C, 65°C, and 72°C, for 1 min each. The PCR products were subjected to direct sequencing, as described above. Neighbor-joining trees were constructed as published in refs. 20 and 22. Bootstrap values based on 1,000 replications are indicated.

Results and Discussion

Definition of *Mamu-A* and *Mamu-B* Region Configurations and Identification of *Mamu-A1*. The cDNAs and the serologically defined Mamu-A and Mamu-B specificities segregate in a Mendelian manner (Fig. 1). In analogy to *Mamu-DR*, the *Mamu-A* and *Mamu-B* region configurations display variation with regard to the number and combination of *Mhc* class I loci present per haplotype. Serotype names are used for the identification of different region configurations.

In contrast to the single *HLA-A* locus in humans, rhesus macaques express multiple *Mamu-A*-like cDNAs, illustrating the presence of compound loci segregating on a single chromosome (Fig. 1). Because of the extent and quality of the B cell line panel, it was possible to unravel the complex relationships that exist between serotypes and cDNAs. As can be seen, for all A serotypes, an allele of a polymorphic locus can be defined (Table 2). One-dimensional isoelectric focusing analyses illustrated that the corresponding gene products are characterized by relatively high expression levels (data not shown). For most of these structures, simian immunodeficiency virus epitopes have been defined (23–29), indicating that they act as bona fide MHC class I molecules.

In the large outbred colony studied, most serotypes are represented by one allele of the polymorphic *A* locus (Table 2). A few exceptions to this rule were observed. For instance, the

Table 2. *Mamu-A* serotypes and major cDNA sequences detected

Serotype	cDNAs				Configuration
	Mamu-A1	*Mamu-A2*	*Mamu-A3*	*Mamu-A4*	
A1	*A*12*	*A*0511*	—	*A*14*	I
A3	*A*19*	*A*0505*	—	*A*14*	I
A5	*A*27*	*A*05*	—	*A*14*	I
A9	*A*01*	*A*0504*	—	*A*14*	I
A22	*A*0701/02/03*	*A*0507*	—	*A*14*	I
A23	*A*0601/02*	*A*0501*	—	*A*14*	I
Blank	*A*25*	*A*0509*	—	*A*14*	I
A28	*A*21*	*A*24*	*A*1305*	*A*14*	II
A10	*A*11*	*A*0503*	*A*1303*	*A*14*	II
A21	*A*1602*	—	*A*1306*	*A*14*	III
A19	*A*02*	—	*A*1302*	*A*14*	III
A6	*A*04*	—	—	*A*14*	IV
A33	*A*23*	—	—	*A*14*	IV
A27	*A*26*	—	—	*A*14*	IV
Blank	*A*28*	—	—	*A*14*	IV

The *Mamu-A*05* allele depicted by only two digits has not yet been assigned unambiguously. Proposed locus names and configuration definitions are indicated. —, Indicates the absence of cDNAs.

A22 and A23 serotypes are represented by three different *Mamu-A*07* and two *Mamu-A*06* alleles, respectively, whereas an earlier reported allele (11) differs slightly from *Mamu-A*1602*. It is concluded that, in the colonies analyzed thus far (mostly Indian animals), the lineages encoded by the locus controlling the serotype (proposed name *Mamu-A1*) display low degrees of allelic variation. Similar to the human system, however, phylogenetic comparisons illustrated that the various lineages controlled by this locus are characterized by large genetic distances (Fig. 2).

The *Mamu-A2*(*05/*24) Locus. In addition to the polymorphic *Mamu-A* transcript, some region configurations express a *Mamu-A*05* cDNA (Table 2). In our panel, at least eight highly related alleles are observed, each of which seems to segregate with a particular serotype. For example, *Mamu-A*0501* appears to be unique for the A23 serotype, whereas the *Mamu-A*0504* allele is found only in A9-positive animals (Table 2).

Urvater *et al*. (29) reported that the *Mamu-A*05* and *Mamu-A*07* genes are characterized by a 162-bp insertion in intron 2, which was confirmed for *Mamu-A*05*, but the *Mamu-A*07* genes in our panel seem to lack the insert. The *Mamu-A*24* genes, however, possess this intron modification. Based on the presence of the shared insert and the supporting phylogenetic tree (Fig. 2), *Mamu-A*05* and *Mamu-A*24* are considered to represent two lineages controlled by one locus (Table 2). It is highly probable that the *Mamu-A*05/*24* locus arose from a duplication/recombination in which the *Mamu-A*07* gene was involved, subsequently followed by the intron 2 insertion. This alteration is also observed in *Mafa-A*05* genes of cynomolgus macaques (*Macaca fascicularis*), indicating that this locus is at least 2.5 million years old. The *Mamu-A*05/*24* locus (proposed name *Mamu-A2*), characterized by differential distribution, might encode a nonclassical class I protein with a specialized function in the immune response. A similar situation may exist for the *Patr-AL* locus in chimpanzees (30).

The *Mamu-A3*(*13) and *Mamu-A4*(*14) Loci. Four *Mamu-A*13* alleles were reported in ref. 31. In our panel, two alleles designated *Mamu-A*1305* and *Mamu-A*1306,* were detected, and they segregate with the A28 and A21 serotypes, respectively (Table 2). Based on the presence of two other *Mamu-A* loci in, for instance, A10-positive animals, *Mamu-A*13* must represent a third locus (proposed name *Mamu-A3*) present in only two region configurations (Table 2).

*Mamu-A*14* sequences, which are characterized by a unique two-triplet deletion in the first exon, are present in all animals

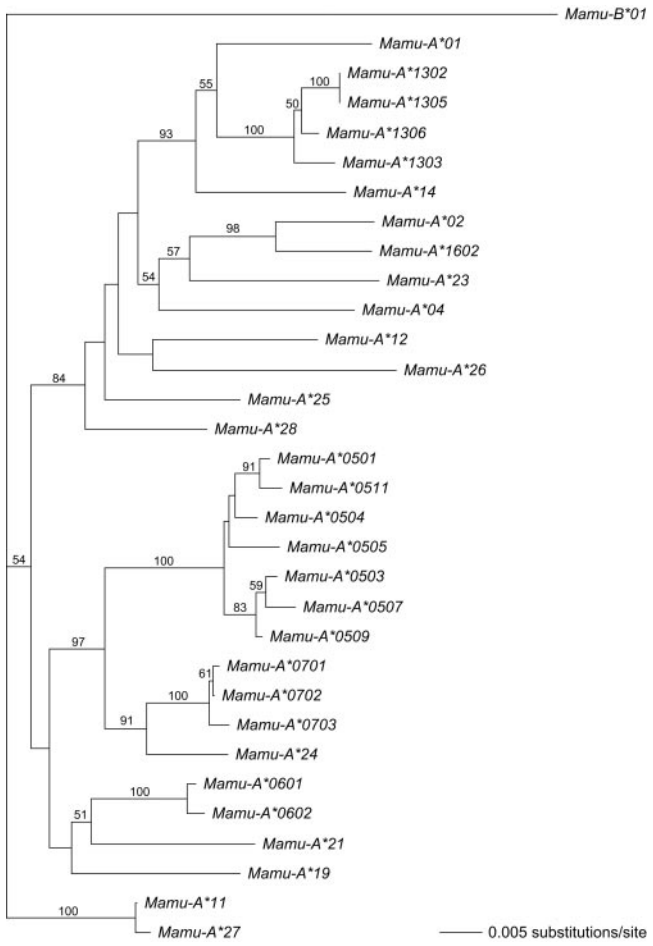

Fig. 2. Phylogenetic tree of full-length *Mamu-A* sequences. The tree was constructed according to the neighbor-joining method, and bootstrap values based on 1,000 replications are indicated.

Table 3. *Mamu-B* serotypes and corresponding major cDNA sequences

Serotypes	cDNAs			
B2[a]	*B*24*	*B*38*	*B*4701/02*	—
B2[b]	*B*38*	*B*46*	*B*4701*	—
B11[a]	*B*38*	*B*3001*	*B*12*	*B*22*
B11[b]	—	*B*3001*	*B*12*	*B*22*
B13	*B*48*	*B*64*	*B*41*	—
B14	*B*08*	—	*B*06*	—
B17	*B*17*	—	*B*2901*	—
B18	*B*55*	*B*63*	*B*5802*	—
B20	*B*21*	*B*28*	*B*20*	—
B24	*B*24*	*B*46*	*B*19*	—
B25	*B*5002*	*B*65*	*B*69*	—
B26[a]	*B*01*	*B*3002*	*B*0701/02*	—
B26[b]	*B*01*	*B*6002*	*B*61*	—
B26[c]	*B*01*	*B*46*	*B*0702*	—
B29	*B*44*	—	*B*40*	—
B31	*B*66*	*B*67*	*B*68*	—
B32	*B*3601/02*	*B*37*	*B*4501/02*	—
B34	*B*27*	*B*43*	—	—
B35	*B*0602*	*B*71*	—	—
blank[a]	*B*01*	*B*39*	*B*0703*	—
blank[b]	*B*01*	*B*53*	*B*0702*	—

Sequences are listed in a random order and are represented by four digits when polymorphism for the respective lineage has been documented. —, Indicates the absence of cDNAs.

(Table 2). Apparently this locus displays no polymorphism in our population of Indian animals. The situation for this locus is very similar to the *Mamu-I* locus that arose from a duplication event and, as a consequence, shares unique features with *Mamu-B* (18). A proposed name for this locus is *Mamu-A4*.

***Mamu-A* Region Configurations.** The four *Mamu-A* region configurations identified express not only the invariant *Mamu-A4* gene but also one *Mamu-A1* gene controlling the serotype and, in most cases, one or two additional genes belonging to either the *Mamu-A2* or *Mamu-A3* loci (Table 2). In contrast to humans, who may express maximally two distinct HLA-A molecules, a heterozygous rhesus macaque may express maximally up to seven different Mamu-A-like molecules. In general, however, the different *Mamu-A* loci and/or lineages display low levels of allelic polymorphism. Recently, a rhesus macaque *Mhc* class I region was sequenced (32), and the data are fully compatible with the *Mamu-A* region configuration IV as is present in A6 serotyped animals (Table 2).

***Mamu-B* Serotypes Are Defined by Combinations of cDNAs with High Expression Levels.** For each B serotype, at least one unique combination of cDNAs was detected (Table 3). As can be seen, most B serotypes are typified by the presence of two, three, and in one case even four cDNAs. For the corresponding gene products of these cDNAs, simian immunodeficiency virus epitopes recognized by cytotoxic T lymphocyte have been defined (8, 12, 27, 29, 33, 34). In some samples, cDNAs with low expression levels were detected next to the major cDNAs with high expression levels. These "minors" are not listed in Table 3.

For some serotypes, at least two different combinations of cDNAs were observed. For example, the *Mamu-B*01*, *Mamu-B*3002*, and *Mamu-B*0701* sequences were found in Indian animals with the B26 serotype, whereas, in Chinese animals, the *Mamu-B*01*, *Mamu-B*6002,* and *Mamu-B*61* sequences were observed (Table 3). A few highly related sequences are situated on different *Mamu-B* region configurations. For instance, the

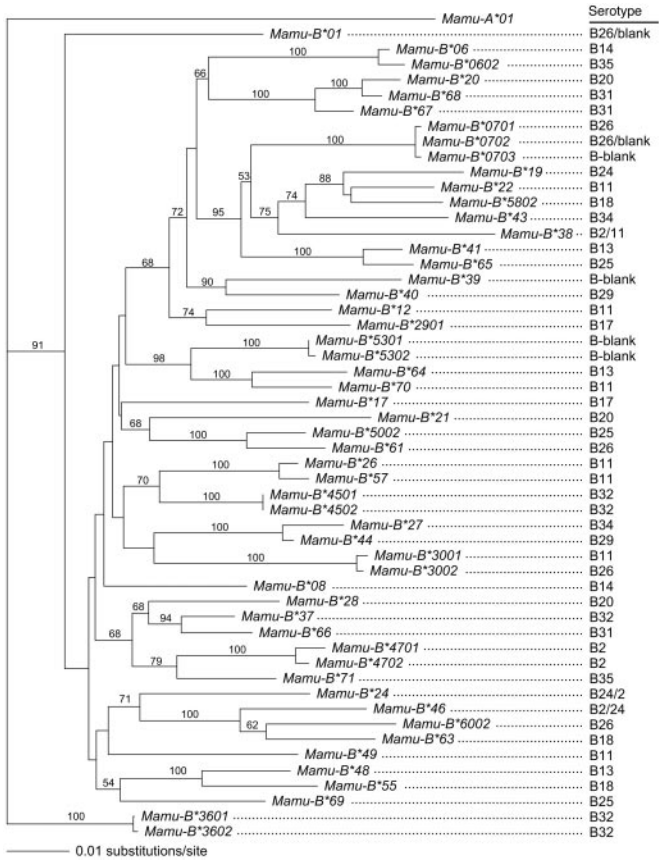

Fig. 3. Phylogenetic tree of full-length *Mamu-B* cDNAs. The tree was constructed according to the neighbor-joining method, and bootstrap values based on 1,000 replications are indicated. Serotypes have been provided.

*Mamu-B*3001* and *Mamu-B*3002* alleles, differing for three base pairs, were detected in the case of the B11 and B26 serotypes, respectively (Table 3). Furthermore, different *Mamu-B* region configurations may share identical sequences, as was the case for the *Mamu-B*46* (Table 3). In general, the different *Mamu-B* loci/lineages display no or limited levels of allelic variation and are characterized by large genetic distances (Fig. 3). In total, 21 unique cDNA combinations were identified (Table 3) that appear to segregate in families with the relevant serotype (Fig. 1).

***Mamu-B* Region Diversity Is About Loci.** In contrast to *Mamu-A*, the definition of loci appears to be difficult for the *Mamu-B* region.

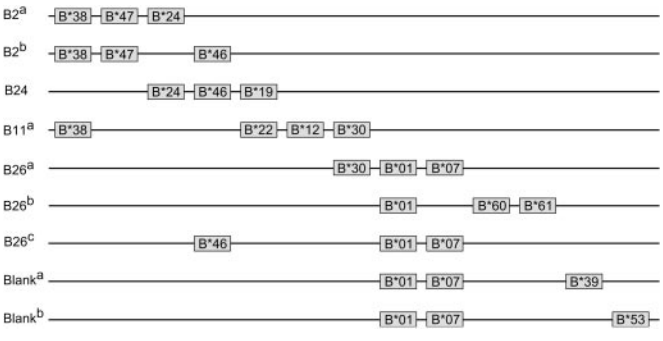

Fig. 4. Schematic representation of nine *Mamu-B* region configurations. The physical order and distance of the genes responsible for the major cDNA transcripts have not yet been defined. Vertical alignment of cDNAs reflects identical locus designation at the genomic level.

Table 4. MHC class I transcription level analysis of B11 serotyped cells

Animal	Serotype	*B*12*	*B*3001*	*B*38*	*B*22*	*B*53*	*B*49*	*B*57*	*B*70*	*B*26*
3C	11,11	++	++	—	+	+/−	+/−	—	+/−	—
MR	34,11	++	++	—	+	+/−		+/−	+/−	+/−
96084	11,11	++	++	++	+	+/−	+/−	+/−	+/−	—

The plus and minus signs are an indication of the level of expression. Major cDNAs are indicated by ++ (more expressed) or + (less expressed), and the minors are indicated by +/−. —, Indicates the absence of cDNA.

A schematic representation of nine selected *Mamu-B* region configurations (differing in number and combination of genes) was made to illustrate the complex locus/allele relationships (Fig. 4). For animals with the B2 serotypes, two distinct *Mamu-B* region configurations were observed sharing the *Mamu-B*38* and *Mamu-B*47* genes that, by definition, represent different loci (Fig. 4). The $B2^a$ serotype possesses an additional *Mamu-B*24* sequence, whereas the other harbors *Mamu-B*46*. These genes represent a third and fourth *Mamu-B* locus, as is supported by B24-seropositive animals, where those loci are found on the same configuration together with *Mamu-B*19*. Hence, only these three *Mamu-B* region configurations define five *Mamu-B* loci, which apparently display no polymorphism (Table 3).

For the $B11^a$ serotype, the *Mamu-B*38*, *Mamu-B*3001*, *Mamu-B*12*, and *Mamu-B*22* sequences were found to segregate on one chromosome and, thus, must represent four distinct loci. The *Mamu-B*19*, *Mamu-B*22*, and *Mamu-B*58* cDNAs share a unique one-codon deletion in exon 5 and cluster tightly together in the phylogenetic tree (Fig. 3). For that reason, the *Mamu-B*19* sequence (B24) and *Mamu-B*22* cDNA ($B11^a$) are considered to represent one locus (Fig. 4). The *Mamu-B*38* locus is shared with the B2 serotyped cells, whereas the *Mamu-B*12* and *Mamu-B*30* sequences are considered to represent two additional *Mamu-B* loci.

All cells with the B26 and blank serotypes share the *Mamu-B*01* cDNA, and its large phylogenetic distance from known *Mamu-B* sequences (Fig. 3) is an indication that it represents yet another *Mamu-B* locus (Fig. 4). The *Mamu-B*30* locus in B11-serotyped cells is shared with the $B26^a$ region configuration, whereas the $B26^c$, $B2^b$ and B24 serotypes share the *Mamu-B*46* locus. *Mamu-B*07*, which lacks the characteristic one-codon deletion present in *Mamu-B*19* and *Mamu-B*22* (Fig. 3), is considered to define a separate locus. Neither the sequences nor the phylogenetic analyses provide any argument for grouping the *Mamu-B*39*, *Mamu-B*53*, *Mamu-B*60*, or *Mamu-B*61* sequences together with earlier defined loci. Hence, this small sample provides evidence that at least as many as 13 different *Mamu-B*-like loci can be defined in the case of only five serotypes. In total, 21 different *Mamu-B* region configurations are defined (Table 3), indicating that the number of *Mamu-B* loci may be much higher.

Recently, it was documented that the rhesus MHC class I region contains at least 18 apparently functional *Mamu-B*-like genes (32). The reported *Mamu-B* region is in agreement with our B11-like configurations; however, the genomic data documented the presence of additional functional genes such as *Mamu-B*26*, *Mamu-B*49*, *Mamu-B*53*, *Mamu-B*57*, and *Mamu-B*70*. RT-PCR studies indeed demonstrated that for some B11 region configurations additional minor class I cDNAs can be detected in small numbers (Table 4). This finding indicates that the various *Mamu-B* genes exhibit differential transcription levels. The present report defines for a considerable number of *Mamu-A* and *Mamu-B* configurations the dominant cDNAs characterized by the highest expression levels. Knowledge of the exact genetic order and gene content of a substantial number of *Mamu-B* region configurations is an absolute prerequisite to define a sensible nomenclature system.

Duplications and Crossing-Over Events. The present results strongly suggest that diversity within the *Mamu-B* region is maintained by duplication and reshuffling of *Mamu-B* loci. This model is in sharp contrast to the situation in humans, where only one *HLA-B* gene is present per haplotype, and this locus displays a high degree of allelic polymorphism. Allelic polymorphism is virtually absent for the *Mamu-B* region. The differential gene number observed for the *Mamu-A* and *Mamu-B* regions is probably maintained by unequal crossing-over events. In this respect, the MHC class I region may resemble the human KIR loci (2, 3) and the *HLA-DR* and *Mamu-DR* regions (35). Before the unequal crossing over, several rounds of duplications must have expanded the number of class I genes in macaques. Such an expanded MHC class I gene repertoire is not unique and has been reported for some rodents (36), cattle (37), birds (38), and macaques (5, 11, 39). This study, however, illustrates that within a given population, individuals may display huge differences in the number of expressed class I genes and in the combinations of those genes as they segregate on a chromosome.

Differential Strategies to Cope with Parasites: Polymorphism Versus Diversity. The *HLA-A*, *HLA-B*, and *HLA-C* genes display a high degree of polymorphism in the human population. Because of this allelic polymorphism, individual variation is thought to reduce the chance that one pathogen can sweep through the entire population. Rhesus macaque populations seem to have banked on an alternative strategy, because allelic polymorphism appears to be virtually absent for the MHC class I genes. In contrast to humans, who normally have a fixed set of classical HLA genes, rhesus macaque populations are characterized by the presence of an abundance of *Mamu-A* and *Mamu-B* region configurations displaying polymorphism with regard to the number and combination of expressed loci present per chromosome. Diversity in gene number, but also gene combinations, is a bona fide strategy to ensure that different individuals mount distinct responses against the same pathogen. Moreover, the *Mamu-A* and *Mamu-B* region configurations themselves seem to be subject to frequent crossing-over processes, even further maximizing and maintaining diversity in the population. Additional layers of polymorphism may be added by differential expression levels.

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