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Molecular typing of major histocompatibility complex class I alleles in the Indian rhesus macaque which restrict SIV CD8$^+$ T cell epitopes

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Abstract The utility of the rhesus macaque as an animal model in both HIV vaccine development and pathogenesis studies necessitates the development of accurate and efficient major histocompatibility complex (MHC) genotyping technologies. In this paper, we describe the development and application of allele-specific polymerase chain reaction (PCR) amplification for the simultaneous detection of eight MHC class I alleles from the rhesus macaque (*Macaca mulatta*) of Indian descent. These alleles were selected, as they have been implicated in the restriction of CD8$^+$ T cell epitopes of simian immunodeficiency virus (SIV). Molecular typing of *Mamu-A*01*, *Mamu-A*02*, *Mamu-A*08*, *Mamu-A*11*, *Mamu-B*01*, *Mamu-B*03*, *Mamu-B*04*, and *Mamu-B*17* was conducted in a high throughput fashion using genomic DNA. Our amplification strategy included a conserved internal control target to minimize false negative results and can be completed in less than 5 h. We have genotyped over 4,000 animals to establish allele frequencies from colonies all over the western hemisphere. The ability to identify MHC-defined rhesus macaques will greatly enhance investigation of the immune responses, which are responsible for the control of viral replication. Furthermore, application of this technically simple and accurate typing method should facilitate selection, utilization, and breeding of rhesus macaques for AIDS virus pathogenesis and vaccine studies.

Keywords Rhesus macaque · SIV · MHC · Genotyping

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Introduction

With the number of individuals globally infected with human immunodeficiency virus (HIV) at greater than 40 million and rising (UNAIDS/WHO AIDS Epidemic Update, December 2006), few biomedical priorities are more urgent than the effort to produce an effective AIDS vaccine. Induction of vaccine-induced broadly reactive neutralizing antibody response has proven very difficult due primarily to the diversity of the viral envelope protein (Burton et al. 2004; Gilbert et al. 2005). Consequently, current vaccine initiatives are focused on engendering potent cellular immune responses capable of controlling viral replication (Koff et al. 2006).

Certain major histocompatibility complex (MHC) class I alleles have been associated with control of HIV replication (Kaslow et al. 1996; McNeil et al. 1996; Carrington et al. 1999; Migueles et al. 2000; Carrington and O'Brien 2003; Kiepiela et al. 2004). However, the diversity of HIV and the dramatic polymorphism in the human MHC class I loci

make it very difficult to define those immune responses which are beneficial. Simian immunodeficiency virus (SIV) infection of macaques is the best available animal model for defining those MHC-restricted immune responses critical to control of HIV pathogenesis (Nathanson et al. 1999; Hirsch and Lifson 2000; Sibal and Samson 2001).

As the genomic organization of the MHC is similar in rhesus macaques (*Macaca mulatta*) and humans (Kelley et al. 2005; Bontrop 2006), the rhesus macaque has been used extensively in biomedical research. While rhesus macaques express orthologues of MHC class I loci homologous to the *HLA-A* and *HLA-B* loci (Miller et al. 1991; Boyson et al. 1996), phylogenetic analysis clearly indicates that the great majority of rhesus classical MHC class I alleles cluster outside of and do not appear to be related to any of the classical human class I lineages (Boyson et al. 1996). Moreover, in contrast to the limited three highly polymorphic *HLA-A*, *HLA-B*, and *HLA-C* loci per haplotype in humans, any given rhesus chromosome can contain from 4 up to 14 functional class I loci (Daza-Vamenta et al. 2004; Otting et al. 2005; Shiina et al. 2006). Presently, there is no evidence for a locus similar to *HLA-C* in the rhesus macaque, suggesting that the *HLA-C* locus is of a fairly recent origin in humans (Watkins et al. 1988; Boyson et al. 1996). While much is yet to be elucidated about the MHC of the rhesus macaque relative to the human, it appears that each species has evolved different, yet effective, strategies to ensure that the critical function of the MHC is maintained in the face of numerous pathogens (Otting et al. 2005). Humans display extensive class I allelic polymorphism, whereas the rhesus macaque relies on many configurations with regard to the number and combination of loci.

To support the most efficient utilization of animal resources and to maximize the understanding of immune responses in SIV vaccine and viral pathogenesis studies, MHC typing technologies must be developed. To that end, we have directed our initial development efforts in MHC typing to those class I alleles that have been shown to functionally present viral epitopes. The Supplemental Table lists the eight MHC class I molecules of the rhesus macaque, Mamu-A*01, Mamu-A*02, Mamu-A*08, Mamu-A*11, Mamu-B*01, Mamu-B*03, Mamu-B*04, and Mamu-B*17, and summarizes information regarding the $CD8^+$ T cell epitopes derived from various SIV and SIV/HIV (SHIV) strains reported to be presented by these molecules. These restricted epitopes are derived from amino acid sequences encompassing the Gag, Pol, Env, Tat, Nef, Vif, Vpx, and Vpr proteins of these SIV and SHIV strains. In this present study, we describe a molecular genotyping method that simultaneously and specifically identifies *Mamu-A*01*, *Mamu-A*02*, *Mamu-A*08*, *Mamu-A*11*, *Mamu-B*01*, *Mamu-B*03*, *Mamu-B*04*, and *Mamu-B*17* from rhesus macaques of Indian descent. This allele-specific polymerase chain reaction (PCR) amplification is accurate, efficient, cost effective, and relatively straightforward, facilitating its adoption in other laboratories. This typing methodology has permitted the determination of frequencies for these eight MHC class I alleles from a significant number of rhesus animals from multiple cohorts around the western hemisphere. This technique will facilitate a greater understanding of the immune responses engendered by SIV infection during vaccine and viral pathogenesis studies, along with the better selection, utilization, and breeding of Indian rhesus macaques.

Materials and methods

Animals and samples

Animals were maintained at the Wisconsin National Primate Research Center (University of Wisconsin–Madison, Madison, WI), an Association for Assessment and Accreditation of Laboratory Animal Care International (AAALACI) accredited facility. The animals were cared for according to the US animal welfare regulations and guidelines of the University of Wisconsin–Madison Institutional Animal Care and Use Committee.

Animals from all other institutions for which cell or blood samples were sent with requests for MHC class I typing were maintained and cared for according to the regulations and guidelines established at their respective facilities.

DNA extraction

Genomic DNA was isolated from a maximum of 3.0×10^6 peripheral blood mononuclear cells or 500 μl ethylenediaminetetraacetic acid (EDTA) anti-coagulated whole blood or buffy coat using the MagNA Pure LC system (Roche Applied Science, Indianapolis, IN) and the MagNA Pure LC DNA Isolation–Large Volume protocol (version 3.0) according to manufacturers guidelines. The elution volume of extracted DNA was 200 μl of MagNA Pure LC DNA Isolation–Large Volume elution buffer. DNA concentrations (ng/μl) and Abs 260 nm/Abs 280 nm ratios were determined using a NanoDrop UV Spectrophotometer (NanoDrop Technologies, Wilmington, DE). Extracted genomic DNA with Abs 260 nm/Abs280 nm ratios ranging from 1.7–2.1 were diluted to15 ng/μl in a 40-μl volume using DNAse-, RNAse-free water (Invitrogen, Carlsbad, CA) for genotyping.

MHC genotyping by allele-specific amplification

All PCR primers were synthesized and provided salt-free by Operon Biotechnologies (Huntsville, AL). Allele-specific

primer sequences and their location within the complementary DNA (cDNA) sequences of *Mamu-A*01*, *Mamu-A*02*, *Mamu-A*08*, *Mamu-A*11*, *Mamu-B*01*, *Mamu-B*03*, *Mamu-B*04*, and *Mamu-B*17* are noted in Table 1; primer locations within the cDNA sequences were determined by aligning *Mamu-A*02*, *Mamu-A*08*, *Mamu-A*11* to *Mamu-A*01* and *Mamu-B*03*, *Mamu-B*04*, *Mamu-B*17* to *Mamu-B*01*. Primers targeting highly conserved sequences of *Mamu-DRB* exon 2 (forward: 5′ GCC TCG AGT GTC CCC CCA GCA CGT TTC 3′; reverse: 5′ GCA AGC TTT CAC CTC GCC GCT G 3′), yielding a ~300-base-pair amplification product, were included as an internal control in all typing reactions (Knapp et al. 1997a, b). Working stocks of primer mixes specific for the individual rhesus class I alleles and the *Mamu-DRB* internal control, at the concentrations listed in Table 1, were pre-aliquoted in a volume of 12 μl into Low Profile 96 well polycarbonate trays (Continental Lab Products, San Diego, California). Working primer mixes for *Mamu-A*02* and *Mamu-A*11* with the *Mamu-DRB* internal control primers also contained 500 mM of molecular grade betaine (Sigma-Aldrich, St. Louis, MO). Typing trays containing working primer mixes were sealed with SilverSeal I foil plate seals (Continental Lab Products) and stored at 4°C for up to 2 weeks.

A 5× PCR reaction buffer at pH 9.5 consisting of 300 mM TRIS hydrochloride, 10 mM magnesium chloride, and 75 mM ammonium sulfate was prepared with molecular grade reagents (Fisher Scientific, Pittsburgh, PA) and DNAse-, RNAse-free Water (Invitrogen). Once in solution, 5× PCR reaction buffer was filter sterilized, divided into 8 ml aliquots in sterile 15 ml Corning conical tubes (Fisher Scientific) and stored at −20°C for up to 12 months. The 5× PCR reaction buffer was thawed at 50–60°C with vigorous vortexing. A PCR master mix was made by combining an 8.0-ml aliquot of thawed 5× PCR reaction buffer with 1,640 μl of DNAse-, RNAse-free Water (Invitrogen), 2.0 ml of glycerol (Sigma-Aldrich), 320 μl of 25-mM deoxynucleotide triphosphates (Promega, Madison, WI),

Table 1 Allele-specific PCR amplification primers for genotyping of *Mamu-A*01*, *Mamu-A*02*, *Mamu-A*08*, *Mamu-A*11*, *Mamu-B*01*, *Mamu-B*03*, *Mamu-B*04*, and *Mamu-B*17* alleles from the Indian rhesus macaque

MHC class I allele	GenBank accession number	Forward primer sequence	Reverse primer sequence	Concentration of allele-specific primers (μM)	Concentration of internal control primers (μM)	Theoretical product size (bp)
*A*01*	U50836	5′ GGG CGG GCT CTC ACT CCA TGA A 3′ (56–77)	5′ TCC GCC GCC TCC CAC TTG 3′ (512–495)	0.75	0.30	706
*A*02*	U50837	5′ GGG GCC CTG GCC CTG ACT 3′ (31–48)	5′ CTC GCC CTC CAG GTA GGT 3′ (549–532)	0.50	0.50	1,018
*A*08*	AF243179	5′ TTG GGA CCG GAA CAC ACG GAT CTA 3′ (237–260)	5′ TGC GCT GGG TGT TCT GAG CA 3′ (496–477)	0.10	0.30	509
*A*11*	AF199357	5′ CGG GGA GCC CCG CTT CTT CA 3′ (111–130)	5′ CTC GCC CTC CAG GTA GGT 3′ (549–532)	0.25	0.80	688
*B*01*	U42837	5′ CAG CGA CGC CGA GAG TCG 3′ (206–223)	5′ CCG CGG CGG TCC AGG AGT 3′ (504–487)	0.30	0.30	548
*B*03*	U41825	5′ TTC GTG CGG TTC GAC AGT 3′ (192–209)	5′ GTT CCA TCT CCT CCT GGC CTA 3′ (814–794)	0.75	0.30	1,445
*B*04*	U41826	5′ GCG CGA AAC GCC CAA AGA CAG 3′ (637–657)	5′ CTG GAC GCA GCC TGA GAG TAG 3′ (1,082–1,062)	1.0	0.30	1,368
*B*17*	AF199358	5′ GCC GGC TCG CAC TCC ATG AA 3′ (93–112)	5′ GCG CGC TGC AGC GTC TCC 3′ (640–623)	0.50	0.30	797

Accession numbers of the GenBank sequences corresponding to *Mamu-A*01*, *Mamu-A*02*, *Mamu-A*08*, *Mamu-A*11*, *Mamu-B*01*, *Mamu-B*03*, *Mamu-B*04*, and *Mamu-B*17* alleles are listed. Both forward and reverse primers used for allele-specific PCR amplification are defined. Beneath the primer sequence are the locations of the primer in the GenBank cDNA sequence for each of the alleles; these locations are relative and were determined by aligning *Mamu-A*02*, *Mamu-A*08*, and *Mamu-A*11* to *Mamu-A*01* sequence and *Mamu-B*03*, *Mamu-B*04*, *Mamu-B*17* relative to the *Mamu-B*01* sequence. The working concentrations of allele-specific and internal control primers listed represent concentrations before the addition of an equivalent volume of the mixture of PCR reaction buffer, Platinum *Taq* DNA polymerase and diluted genomic DNA. The sizes of the amplification products were calculated from the locations of primers within the allele cDNA sequences and the addition of 250, 250, 573, 100, and 250 bp for the standard size of each intron between exons 1–2, 2–3, 3–4, 4–5, and 5–6 respectively.

and 40 μl of 10 mg/ml cresol red (Sigma-Aldrich). For each set of eight rhesus MHC class I allele typing reactions to be conducted for a given sample, 40 μl of extracted genomic DNA diluted to 15 ng/μl was added with gentle mixing to 64 μl PCR master mix to which 10 U of Platinum *Taq* DNA polymerase (5 U/μl; Invitrogen) had been added. Twelve microliters of PCR master mix with Platinum *Taq* DNA polymerase, and diluted genomic DNA was added to each primer mix pre-aliquoted into a Low Profile 96 well polycarbonate trays (Continental Lab Products). Trays were covered and sealed with SilverSeal I foil plate seals (Continental Lab Products) before thermal cycling.

Thermal cycling was conducted on a Tetrad™ thermal cycler (BioRad, Hercules, CA; formerly MJ Research, Cambridge, MA) under the following conditions: an initial 1-min denaturation at 96.0°C, followed by six cycles of 96.0°C denaturation for 25 s, 67.9°C annealing for 50 s and 45 s of elongation at 72.0°C; six cycles of 96.0°C denaturation for 25 s, 66.4°C annealing for 50 s and a 45-s elongation at 72.0°C; five cycles of 96.0°C denaturation for 25 s, 66.0°C annealing for 60 s (during which the annealing temperature was decreased 1.0°C for each of the five cycles) and a 45-s extension at 72.0°C; and finally 16 cycles of 96.0°C denaturation for 25 s, 63.0°C annealing for 50 s and 45 s of elongation at 72.0°C, followed by a final extension at 72.0°C for 10 min and a terminal hold at 25.0°C.

Subsequently, products resulting from PCR amplification were electrophoresed on 2.0% agarose (low EEO, molecular grade, Fisher Scientific) gels containing ethidium bromide (molecular grade, Fisher Scientific) on Owl Centipede separation systems (Fisher Scientific) at a constant voltage (230 V) for 17 min in 0.5× sodium borate buffer (Brody and Kern 2004). Gels were visualized and electronically documented using a fluorescent imaging system (Alpha Innotech, San Leandro, CA). Amplification products detected after electrophoresis were analyzed relative to a 100-bp DNA ladder (Invitrogen) for the presence of *Mamu-A*01*, *Mamu-A*02*, *Mamu-A*08*, *Mamu-A*11*, *Mamu-B*01*, *Mamu-B*03*, *Mamu-B*04*, and *Mamu-B*17* allele-specific amplicons (sizes are listed in Table 1). In addition to allele-specific amplicons, genotyping reactions were considered valid by the presence of the required internal control product. Additionally, known positive control DNA was included for each allele-specificity with each batch of genotyping reactions.

Results

PCR amplification utilizing sequence-specific priming (PCR-SSP) is currently the method of choice for intermediate (specificity for multiple alleles sharing primer targeted polymorphisms) or high (allele level) resolution HLA typing in many clinical histocompatibility laboratories (Olerup and Zetterquist 1991, 1992). This allele-specific PCR amplification methodology involves designing at least one or preferably both primers so that they will permit amplification based on the 3′-mismatch principle (Welsh and Bunce 1999). A single mismatch at the 3′ terminus of a primer will prevent enzymatic extension by DNA polymerase. We have aligned MHC class I cDNA sequences derived from the rhesus macaque of Indian descent submitted to GenBank (alignments not shown; see restricting allele accession numbers referenced in Table 1). Using these MHC class I allele alignments, primers targeting polymorphisms that are unique to and would provide specific amplification of the *Mamu-A*01*, *Mamu-A*02*, *Mamu-A*08*, *Mamu-A*11*, *Mamu-B*01*, *Mamu-B*03*, *Mamu-B*04*, and *Mamu-B*17* alleles were designed and are listed in Table 1. The DNA sequences targeted by the forward and reverse primers are located in exons 2, 3, or 4 because these exons contain highly variable regions capable of discriminating among class I alleles of the rhesus macaque. Whenever possible, the 3′ terminal region of each primer targets a specific nucleotide polymorphism unique to the MHC class I allele to maximize the specificity of the reaction. Furthermore, DNA sequences targeted by allele-specific primers were deliberately selected to amplify a broad range of intervening sequence. This strategy facilitates verification of allele-specificity by DNA sequencing of amplification products and allows for the detection of new alleles. In addition, the allele-specific product size minimizes any potential conflict in interpretation of these amplification products relative to non-specific products.

To maximize the throughput and efficiency of MHC genotyping, PCR-SSP typing reactions for the *Mamu-A*01*, *Mamu-A*02*, *Mamu-A*08*, *Mamu-A*11*, *Mamu-B*01*, *Mamu-B*03*, *Mamu-B*04*, and *Mamu-B*17* alleles were conducted under one set of universal thermal cycling parameters. These were optimized collectively under parallel reaction conditions such as buffer pH, magnesium ion concentration, concentrations of allele-specific and internal control primers, amount of input genomic DNA, and amount of *Taq* DNA polymerase (data not shown). PCR-SSP genotyping of these eight alleles has been authenticated by the production and sequencing of cDNA libraries and the direct sequencing of various allele-specific amplicons from a representative variety of both related and unrelated animals. Although not all allele-specific genotyping reactions are equally robust, amplification of these eight alleles has been optimized to provide specific and definitive results under these universal conditions (Fig. 1a–d). Each of the pedigrees in this representative genotyping includes multiple family members from three successive generations. In Fig. 1a, the segregation of haplotypes containing

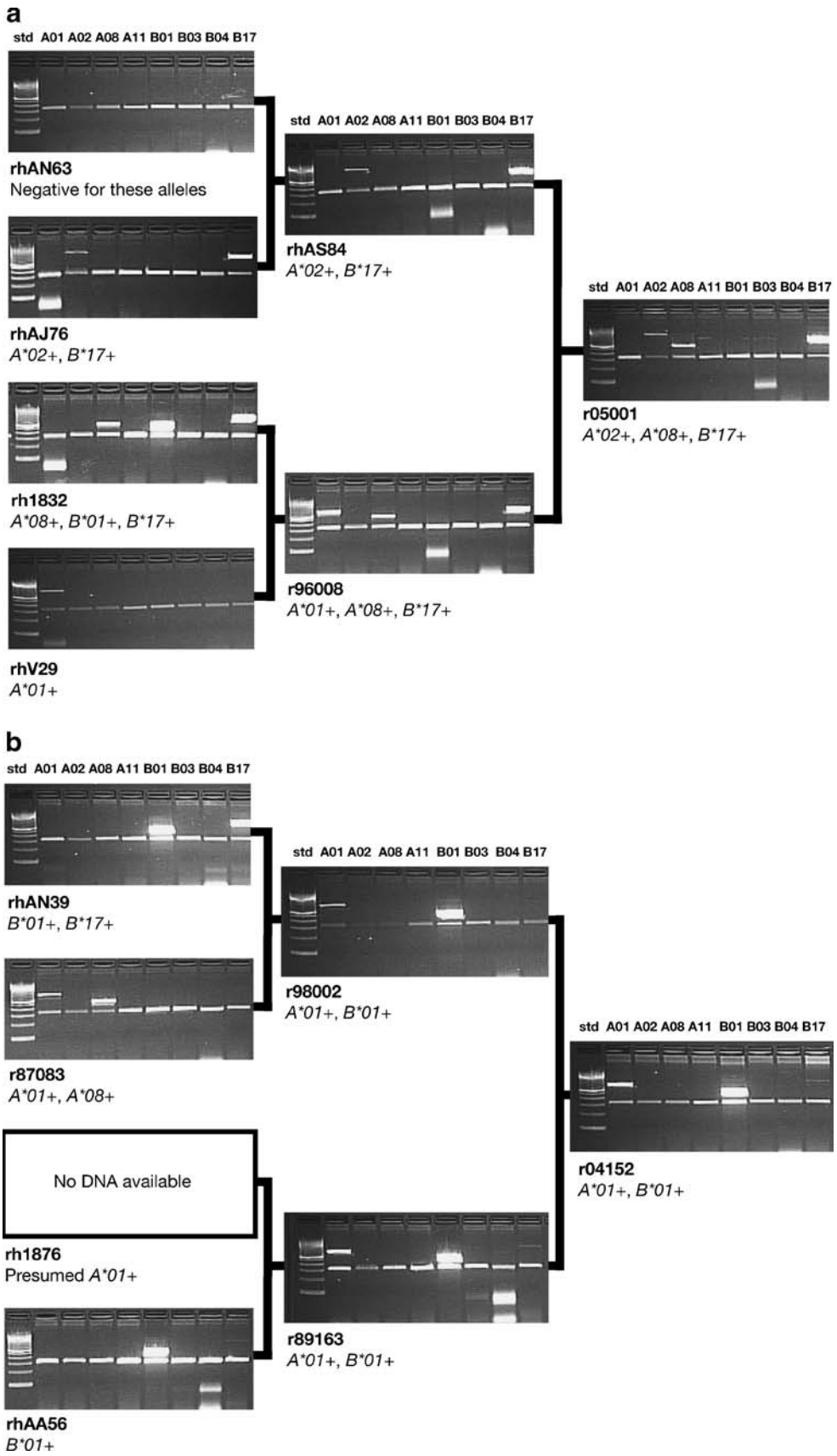
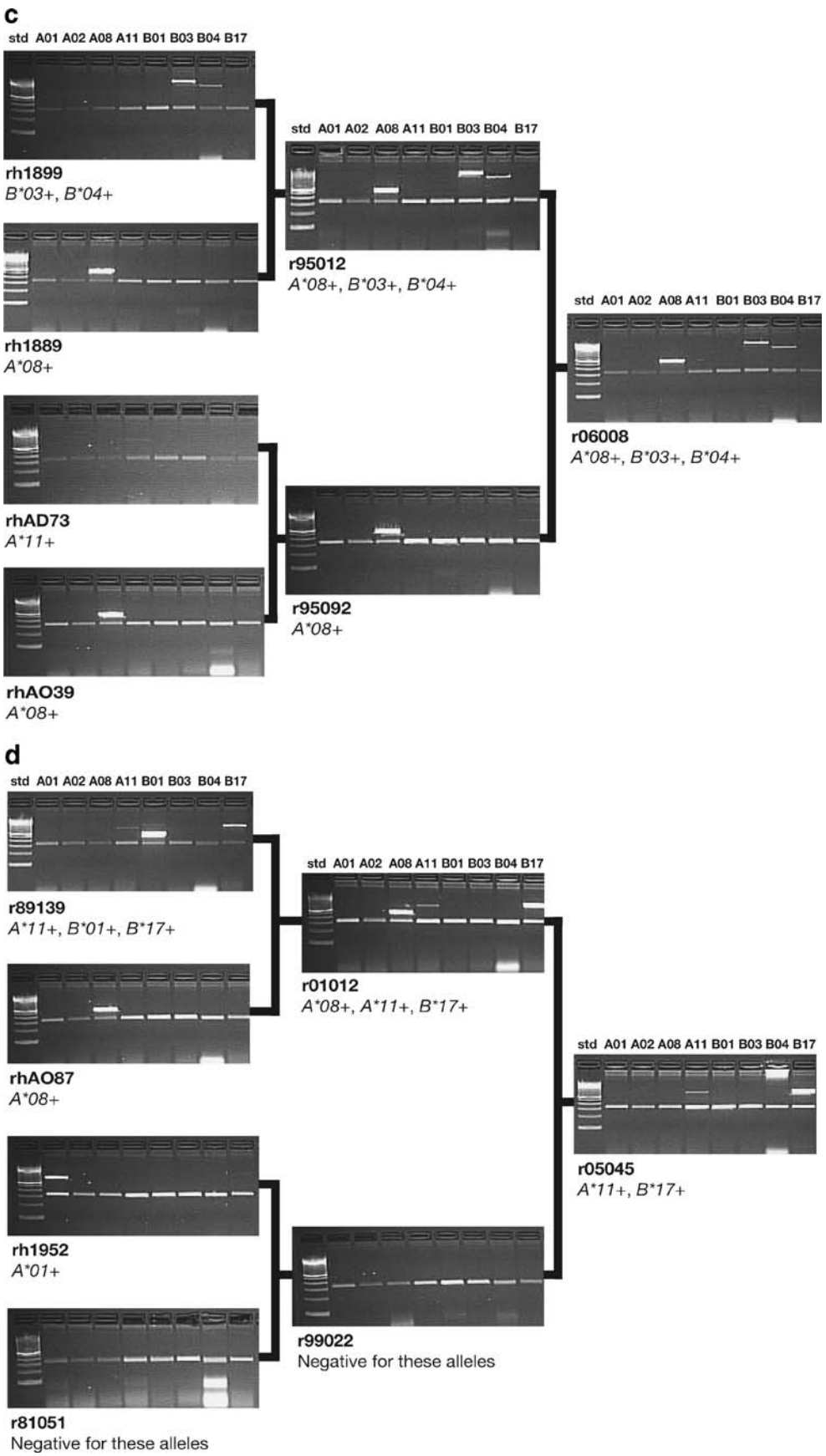


Fig. 1 **a**–**d** Representative MHC genotyping by allele-specific PCR amplification of multiple members of four unrelated families across three generations that collectively have the *Mamu-A*01*, *Mamu-A*02*, *Mamu-A*08*, *Mamu-A*11*, *Mamu-B*01*, *Mamu-B*03*, *Mamu-B*04*, and *Mamu-B*17* alleles. Positive genotyping results are shown under each animal ID. The internal control amplification product is present in each typing reaction, and each gel image contains a standard DNA molecular weight marker (*std*)

Fig. 1 (continued)



*Mamu-A*01*, *Mamu-A*08*/*Mamu-B*17*, and *Mamu-A*02*/*Mamu-B*17* is demonstrated. Similarly, the inheritances of *Mamu-A*01* and *Mamu-B*01*, *Mamu-A*08*, and *Mamu-B*03*/*Mamu-B*04*, and *Mamu-A*11*/*Mamu-B*17* are illustrated in Fig. 1b–d, respectively. The sizes of each of the allele-specific amplification products (Fig. 1a–d) correspond to that predicted based on the location of primers (Table 1). A set of primers yielding a ~300-bp product corresponding to conserved sequences of the rhesus DRB class II alleles was present in all PCR-SSP reactions as an internal control to detect the presence of an inhibitor of PCR amplification, minimizing the potential for false negative results.

As summarized in Fig. 2, we have typed for *Mamu-A*01*, *Mamu-A*02*, *Mamu-A*08*, *Mamu-A*11*, *Mamu-B*01*, *Mamu-B*03*, *Mamu-B*04*, and *Mamu-B*17* using allele-specific PCR amplification and established allele frequencies for Indian rhesus macaques from a variety of cohorts around the western hemisphere. Interestingly, while similarities among these different populations were apparent, disparities in allele frequencies both within and among colonies were also evident. *Mamu-A*01*, *Mamu-A*02*, *Mamu-A*08*, and *Mamu-B*01* were all detected at a high frequency, having an average frequency ranging from 20–30% across all the cohorts. However, there were some exceptions to these observations. The frequencies of *Mamu-A*01* at the Ohio State facility and *Mamu-B*01* at the Caribbean facility were approximately twofold lower than the average. At the California National Primate Research Center, the *Mamu-A*08* frequency was threefold below the average of all colonies at only 9%. In general, *Mamu-A*11* and *Mamu-B*17* were present at only moderate levels with means of 4.0 and 11.3%, respectively. It is interesting to note that in the case of both *Mamu-A*11* and *Mamu-B*17*, the Caribbean frequency falls to less than 2%. Moreover, the frequency of *Mamu-B*17* was at least twofold higher at the Oregon National Primate Center relative to the average (11.3%) of all the groups. In all cases, *Mamu-B*03* and *Mamu-B*04* allele frequencies were consistently low, at 3% or less.

Discussion

Elucidation of the role of cytotoxic T-lymphocytes in controlling HIV and SIV requires the definition of MHC class I molecules and the HIV and SIV peptides that they bind. The significance of MHC matching to transplantation has also promoted the development of HLA typing methodologies. Unfortunately, genotyping techniques for the MHC of the rhesus macaque have lagged far behind HLA typing in humans. In contrast to the almost 1,600 alleles described for the human class I *HLA-A*, *HLA-B*, and *HLA-C* loci as of December 2006 (Robinson et al. 2006), currently GenBank contains sequences for only about 130 macaque MHC class I alleles, primarily derived from

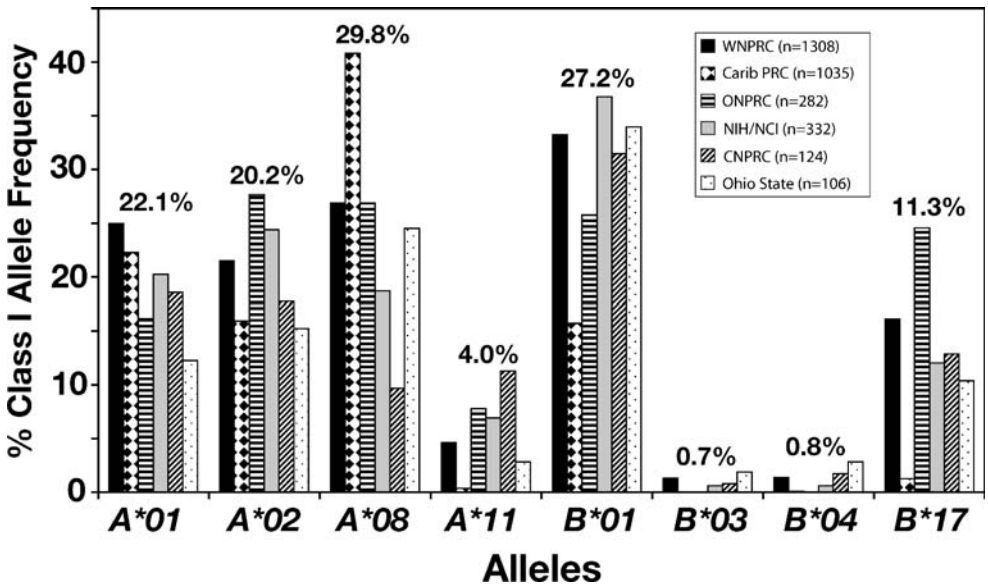


Fig. 2 Frequencies of the *Mamu-A*01*, *Mamu-A*02*, *Mamu-A*08*, *Mamu-A*11*, *Mamu-B*01*, *Mamu-B*03*, *Mamu-B*04*, and *Mamu-B*17* alleles based on allele-specific amplification from genomic DNA of Indian rhesus macaques from various colonies. The institutions included are as follows; Wisconsin National Primate Research Center (*WNPRC*), Caribbean Primate Research Center (*Carib PRC*), Oregon National Primate Research Center (*ONPRC*), National Institutes of Health and National Cancer Institute (*NIH/NCI*), California National Primate Research Center (*CNPRC*), and The Ohio State University (*Ohio State*). Average frequency for each of the Indian rhesus class I alleles across all six institutions is shown above the *bar graphs*

captive-bred animals of Indian origin. Given the broad significance of the rhesus animal model and the fundamental role of the MHC in the immune response, it is not surprising that molecular-based typing assays using allele-specific amplification (Knapp et al. 1997a; Lobashevsky and Thomas 2000; Muhl et al. 2002) and reference strand conformational analysis (Tanaka-Takahashi et al. 2007) for various rhesus class I alleles have been previously described.

In this study, we described the development of unified thermal cycling conditions for the simultaneous PCR-SSP-based genotyping of eight MHC class I alleles, *Mamu-A*01*, *Mamu-A*02*, *Mamu-A*08*, *Mamu-A*11*, *Mamu-B*01*, *Mamu-B*03*, *Mamu-B*04*, and *Mamu-B*17*, from the rhesus macaque of Indian descent. All of these alleles have been implicated in the restriction of SIV CD8+ T cell epitopes. We previously reported primer sequences for these eight alleles (Horton et al. 2001; Schramm et al. 2002; Vogel et al. 2002; Loffredo et al. 2005). To improve the original PCR-SSP for *Mamu-A*01* (Knapp et al. 1997b), primers were redesigned in a subsequent study (Schramm et al. 2002), followed by modification of the forward primer here to further increase amplification efficiency. However, it should be noted that these new primers have occasionally exhibited cross reactivity in amplifying the non-classical *Mamu-AG*01*. *Mamu-A*01* is easily distinguished from *Mamu-AG*01* by differences in product size. Elevated frequencies of *Mamu-B*03* have caused us to redesign the reverse primer here to minimize false positive results. As demonstrated in our current genotyping design (Fig. 1a–d), this platform is specific, robust, and straightforward. Additionally, PCR-SSP enables high throughput analysis for all the eight or any combination of these alleles, requires inexpensive equipment, is cost effective, and is less technically demanding and labor intensive than other molecular methods. Our standard procedure using PCR-SSP amplification for the detection of these eight alleles requires little input genomic DNA (approximately 70 ng). In fact, depending on the allele-specificity, lower amounts of high-quality input genomic DNA (down to approximately 25 ng per reaction) yielded valid genotyping results (data not shown). Over the last 5 years, using these allele-specific PCR primers, we have performed over 60,000 reactions and have provided MHC class I typing to more than 60 different investigators at over 30 public and private institutions around the western hemisphere.

The cellular immune responses restricted by certain MHC class I molecules, specifically HLA-B*27 and HLA-B*57, have been associated with a protective benefit and control of HIV replication (Kaslow et al. 1996; McNeil et al. 1996; Migueles et al. 2000; Carrington and O'Brien 2003; Kiepiela et al. 2004). The MHC-defined Indian rhesus macaque infected with SIV provides an excellent model for understanding the influence of MHC class I alleles on the replication of this pathogenic virus. Infection of Indian rhesus macaques with SIVmac239, a molecularly cloned AIDS virus, has been the most widely evaluated animal model for HIV pathogenesis and vaccine studies (Bontrop and Watkins 2005). MHC typing of macaques is critical in identifying class I alleles, which restrict epitopes that engender cytotoxic T-cell lymphocytes (CTL) and in facilitating analyses of CTL responses after vaccination and/or during the course of SIV pathogenesis. With the exception of *Mamu-B*17* at the Caribbean Primate Research Center, *Mamu-A*01*, *Mamu-A*02*, and *Mamu-B*17* alleles were all present at moderate to high (10–27.5%) frequencies across various colonies (Fig. 2). The ability to type for these alleles has been beneficial to numerous studies. *Mamu-A*01* was the first MHC class I allele described in rhesus macaques (Miller et al. 1991) and was subsequently found to be associated with moderate control of SIV replication (Zhang et al. 2002; Mothe et al. 2003). Mamu-A*01 SIV-derived epitopes have been thoroughly investigated (Allen et al. 1998, 2001). Among these epitopes, $Gag_{181–189}$CM9 and $Tat_{28–35}$SL8 are of particular interest. The immunodominant CTL response against $Tat_{28–35}$SL8 selects for rapid escape mutants of SIV during the acute phase, while CTL against $Gag_{181–189}$CM9 select for mutants during the chronic phase (Allen et al. 2000). Presentation of epitopes by Mamu-A*02 has been thoroughly elucidated (Vogel et al. 2002; Loffredo et al. 2004), with $Nef_{159–167}$YY9 and $Gag_{71–79}$GY9 having a similar relationship with $Tat_{28–35}$SL8 and $Gag_{181–189}$CM9 respectively, in terms of selection for rapidly or slowly escaping SIV mutants (Vogel et al. 2002). While *Mamu-A*08* and *Mamu-B*01* are also expressed at relatively high frequencies (Fig. 2), their role in SIV vaccination and pathogenesis studies remains unclear. Knowledge of epitope presentation by Mamu-A*08 has been limited thus far to a single epitope derived from envelope in SHIV, a chimeric SIV that contains the envelope of HIV_{HXBC2} (Voss and Letvin 1996). Although Mamu-B*01-restricted epitopes were initially reported (Yasutomi et al. 1995; Su et al. 2005), it has been difficult to make tetramers for these previously reported epitopes (CJ Miller, personal communication), and no immunogenic SIV-derived epitopes were identified for Mamu-B*01 in a subsequent analysis (Loffredo et al. 2005). Collectively, these observations suggest that Mamu-B*01 does not bind SIV-derived epitopes and has no effect on SIV disease progression. *Mamu-A*11* and *Mamu-B*17* both occur at average frequencies two- to fivefold lower than *Mamu-A*01*, *Mamu-A*02*, *Mamu-A*08*, and *Mamu-B*01* (Fig. 2). *Mamu-B*17* is associated with control of SIV replication (O'Connor et al. 2003; Yant et al. 2006), although inheritance of *Mamu-B*17*-containing haplotypes does not predict control of SIV (Wojcechowskyj

et al. 2007). Mamu-A*11-presented epitopes have been defined systematically with three of them being cross-reactive with the mouse H-2 class I molecule Kk (Sette et al. 2005). *Mamu-B*03* and *Mamu-B*04* were linked to a small number of slow progressors (Evans et al. 1999a). The low frequency of *Mamu-A*11*, *Mamu-B*03*, and *Mamu-B*04* may preclude them from being practical in future SIV studies.

While MHC-defined macaques are useful in vaccine development and pathogenesis studies, MHC genotyping may also provide some very valuable information in additional arenas. MHC typing will facilitate colony management, selective inbreeding of animals, in vitro fertilization programs, production of monozygotic twins, and selected full- or half-siblings. These developments will enhance and expand rhesus macaque resources for critical vaccine studies so that the correlates of immunity can be dissected.

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