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MHC class I characterization of Indonesian cynomolgus macaques

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Abstract

Cynomolgus macaques (*Macaca fascicularis*) are quickly becoming a useful model for infectious disease and transplantation research. Even though cynomolgus macaques from different geographic regions are used for these studies, there has been limited characterization of full-length Major Histocompatibility Complex (MHC) Class I immunogenetics of distinct geographic populations. Here, we identified 48 MHC class I cDNA nucleotide sequences in eleven Indonesian cynomolgus macaques, including 41 novel *Mafa-A* and *Mafa-B* sequences. We found seven MHC class I sequences in Indonesian macaques that were identical to MHC class I sequences identified in Malaysian or Mauritian macaques. Sharing of nucleotide sequences between these geographically distinct populations is also consistent with the hypothesis that Indonesia was a source of the Mauritian macaque population. In addition, we found that the Indonesian cDNA sequence *Mafa-B*7601* is identical throughout its peptide binding domain to Mamu-B*03, an allele that has been associated with control of SIV viremia in Indian rhesus macaques. Overall, a better understanding of the MHC class I alleles present in Indonesian cynomolgus macaques improves their value as a model for disease research and it better defines the biogeography of cynomolgus macaques throughout Southeast Asia.

Keywords

MHC; Immunogenetics; Indonesia; *Macaca fascicularis*

Introduction

Indian rhesus macaques (*Macaca mulatta*) have been widely used as an animal model for studying human diseases because of their extensive similarities to humans (Gibbs et al. 2007). However, the limited availability of rhesus macaques due to high demand and the 1978 ban on exportation from India (Southwick and Siddiqi 1994) has led to a reduced supply of these animals for important scientific research. Investigators have thus turned to closely related macaque species as viable scientific models, including the cynomolgus macaque (*Macaca fascicularis*). These animals are becoming increasingly valuable as models for the study of infectious diseases, such as AIDS, tuberculosis, and severe acute respiratory syndrome (SARS)

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(Capuano et al. 2003; Lawler et al. 2006; Wiseman et al. 2007). In addition, cynomolgus macaques are used as models of Alzheimer's disease, Parkinson's disease, and human reproduction (Conlee et al. 2004; Emborg 2007; Shively et al. 2007; Wang et al. 2007), and they are often used for transplantation and immunotherapy research (Liu et al. 2007; Wiseman and O'Connor 2007). With the recent increase in demand for cynomolgus macaques in biological research, there is a need to define Major Histocompatibility Complex (MHC) genetics in this species.

Cynomolgus macaques are found throughout Southeast Asia and extensive MHC polymorphism among these geographically-determined populations is evident (Kondo et al. 1993; Bonhomme et al. 2007). Unfortunately, researchers often overlook these differences between regionally defined populations of macaques. Previous studies of MHC class I alleles in both rhesus and cynomolgus macaques from different geographic origins have demonstrated that only a small subset of alleles are shared, while most alleles are unique to animals from a particular region (Krebs et al. 2005; Otting et al. 2007; Karl et al. 2008). In addition, it is reasonable to predict that rare MHC class I alleles present in macaques from different geographic regions are either derived from a common ancestor or arose during secondary contact, while the occurrence of numerous alleles unique to each population reflects the loss of ancestral alleles through genetic drift subsequent to geographic isolation and/or the effects of geographically variable selection pressures.

To our knowledge, there are only a handful of published studies on MHC class I allele sequences in cynomolgus macaques, and animals of Indonesian origin have not been well-examined to date (Uda et al. 2004; Krebs et al. 2005; Uda et al. 2005; Otting et al. 2007; Wiseman et al. 2007). The location of Indonesia, however, makes this population of cynomolgus macaques particularly interesting. The variable sea levels associated with glaciation and deglaciation during the Pleistocene Age in Southeast Asia led to a variety of land mass configurations that could have promoted macaque migration and thus greatly diversified the macaque populations in this region (Voris 2000). Studies of mitochondrial DNA sequences among cynomolgus macaques from five different geographic origins support the notion that this species originated in Southeast Asia (Smith et al. 2007). Therefore, a comparison of the MHC class I cDNA sequences between cynomolgus macaques of Indonesia and other areas in Southeast Asia may foster a better understanding of the biogeography of these animals.

Recent studies of MHC class I and II alleles in cynomolgus macaques of Mauritian origin have revealed uniquely simple MHC immunogenetics (Leuchte et al. 2004; Blancher et al. 2006; O'Connor et al. 2007; Wiseman et al. 2007). The limited MHC diversity observed in this geographically isolated population makes these animals extremely valuable for pathogenesis and transplantation research (Wiseman and O'Connor 2007; Wiseman et al. 2007). It has been suggested that cynomolgus macaques were introduced to Mauritius by European travelers approximately 400 years ago (Sussman and Tattersall 1986), and that both founder effects and subsequent inbreeding led to extensive allele sharing among Mauritian cynomolgus macaques in the polymorphic MHC loci (Krebs et al. 2005). Furthermore, analyses of mitochondrial and Y-chromosomal DNA suggest that the most probable origin of Mauritian macaques is Indonesia, or more specifically, Java or Sumatra (Tosi and Coke 2007). Therefore, we hypothesized that *Mafa-A* and *Mafa-B* cDNA sequences would be shared between Indonesian and Mauritian cynomolgus macaques.

To examine whether MHC class I genes found in Indonesian cynomolgus macaques are a central source for other geographic populations of cynomolgus macaques, we characterized 48 full length *Mafa-A* and *Mafa-B* cDNA sequences in 11 cynomolgus macaques of Indonesian origin. We found several nucleotide sequences that are shared between Indonesian and

Mauritian or Malaysian cynomolgus macaques. In addition, we found that the predicted amino acid translation of one nucleotide sequence would generate an MHC class I molecule with a peptide binding domain identical to Mamu-B*03, an allele associated with low SIV plasma viremia in Indian rhesus macaques (Evans et al. 1999; Loffredo et al. 2007b). This study provides further evidence that Mauritian cynomolgus macaques originated, at least in part, in Indonesia, and it further expands the repertoire of full length MHC class I nucleotide sequences found in cynomolgus macaques, improving the value of this species as a model for biomedical research.

Materials and Methods

Animals

The Washington National Primate Research Center (Seattle, Washington) provided peripheral blood mononuclear cell (PBMC) samples for 12 Indonesian cynomolgus macaques (IN01-IN12). Two of these animals (IN02 and IN04) were obtained from Jakarta, while the remaining animals were bred in captivity at a natural habitat breeding colony on Tinjil Island located off the south coast of west Java in Indonesia. In addition, the Cerus Corporation (Concord, CA) supplied thirty PBMC samples from cynomolgus macaques of Indonesian origin (CE01-CE30). These animals were originally imported from purpose-bred colonies stocked primarily with animals from Sumatra by Primate Products (Miami, FL) and Worldwide Primates, Inc. (Miami, FL). Although knowledge regarding the relatedness of these animals is limited, microsatellite analysis suggests that they are not closely related (data not shown).

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

RNA and DNA were isolated from IN01-IN12 PBMC using the Qiagen AllPrep DNA/RNA Mini purification kit (Valencia, CA), while RNA and DNA from CE01-CE30 PBMC were obtained using the MagNA Pure LC Instrument (Roche Applied Science, Indianapolis, IN). Complementary DNA (cDNA) was synthesized using the Invitrogen Superscript™ III First-Strand Synthesis System for RT-PCR (Carlsbad, CA). To amplify the MHC class I cDNAs, PCR was executed with high-fidelity Phusion™ polymerase (New England Biolabs, Ipswich, MA) using primers specific for the MHC class I untranslated regions. To analyze both *Mafa-A* and *Mafa-B* sequences, different primer combinations were used. We used a common 5′ primer [5′MHC_UTR (5′-GGACTCAGAATCTCCCCAGACGCCGAG)] and locus specific 3′ primers [3′MHC_UTR_A (5′-CAGGAACAYAGACACATTCAGG)] or [3′MHC_UTR_B (5′-GTCTCTCCACCTCCTCAC)]. All cDNA amplifications were run under the same PCR program on an MJ Research Tetrad Thermocycler (Bio-Rad Laboratories, Hercules, CA): initial denaturation at 98°C for 30 seconds; 21–29 cycles of 98°C for 5 seconds, 63°C for 1 second, 72°C for 20 seconds; and a final extension period of 72°C for 5 minutes. Agarose gel electrophoresis was used to determine the lowest cycle number yielding a detectable PCR product. The 1.2 kilobase pair band was excised and purified using the QIAquick PCR Purification Kit (Qiagen, Valencia, CA).

Using either the Invitrogen Zero Blunt® Cloning Kit (Carlsbad, CA) or the Invitrogen Zero Blunt® TOPO® PCR Cloning Kit (Carlsbad, CA), the purified PCR products were ligated into pCR-Blunt vectors. Ligated samples were transformed into chemically competent *Escherichia coli* Top10 cells (Invitrogen, Carlsbad, CA). Twenty-four to ninety-six colonies per locus (48–192 per animal) were picked and incubated in a shaker for 17–24 hours at 37°C in 1.3 mL of Circle Grow (Qbiogene, Irvine, CA) containing kanamycin (50 μg/mL). DNA was isolated using the Eppendorf® Perfectprep® Plasmid 96 VAC Direct Binding Kit (Brinkmann, Westbury, NY). Sample concentrations were determined using the Nanodrop 1000 (Wilmington, DE). In order to ascertain which clones contained an MHC class I cDNA insert, *Eco*RI (New England Biolabs, Ipswich, MA) restriction digests were performed.

Sequencing of MHC Class I Alleles

Clones were sequenced in the forward and reverse directions using five primers: T7 (5′-TAATACGACTCACTATAGGG), M13rev (5′-CAGGAAACAGCTATGAC), and internal primers 5′Refstrand_v2 (5′-GCTACTACAACCAGAGCGAGG), 5′Transmembrane (5′-GGAACCTTCCAGAAGTGGG), and 3′Refstrand (5′-CAGAAGGCACCACCACAGC). The DYEnamic ET Terminator Cycle Sequencing Kit (Amersham, Piscataway, NJ) was used to perform sequencing reactions under the following conditions: 30 cycles of 95°C for 20 seconds, 50°C for 15 seconds, and 60°C for 1 minute. Sequencing products were purified using the Agencourt® CleanSEQ® dye-terminator removal kit (Beverly, MA). The purified products were sequenced on an ABI 3730xl (Applied Biosystems, Foster City, CA), and analyzed using CodonCode Aligner (Dedham, MA) and Lasergene (DNASTAR, Madison, WI) software. To ensure authenticity, sequences were given unique names if three or more identical full-length clones could be observed for that particular sequence in a single animal. The identified *Mafa-A* and *Mafa-B* nucleotide sequences were submitted to GenBank (accession numbers EU203680-EU203726, and EU046324) and to the IMGT/MHC Non-human Primate Immuno Polymorphism Database-MHC (IPD-MHC) and NHP Nomenclature Committee (Robinson et al. 2003).

MHC class I genotyping

Sequence-specific PCR assays for *Mafa-B*4501* and *Mafa-B*5101* were designed using MegAlign software (DNASTAR, Madison, WI) and Primer3 (v. 0.4.0) (Rozen and Skaletsky 2000). The primers were optimized for use with the Amplitaq Gold PCR Master Mix at 5 μM B*450101-F (5′-GGTCTCACACAGTCCAGACA), B*450101-R (5′-CTCTGTCCTTCTCCGCTG), B*510101-F (5′-CAAGGACGCCGCACAGT), B*510101-R (5′-GCACCGGCCCTCCAC)]. A set of positive control primers designed to amplify exon 2 and 3 sequences of nearly all MHC class I alleles were run in parallel with identical PCR conditions [5′RefStrand (5′-GCTACGTGGACGACACGC), 3′Short-RSCA (5′-TTCAGGGCGATGTAATCC)]. Samples were run at the following conditions: 96°C for 5 min; 30 cycles of 94 °C for 30s, 65°C for 45s, 72°C for 45s; and a final extension at 72° for 10 min. PCR products were resolved on a 1.8% agarose gel and visualized with SYBR Safe DNA gel stain (Invitrogen, Eugene, OR). PCR products were then excised and purified using Ultrafree-DA Centrifugal Devices (Millipore, Billerica, MA). Purified PCR products were sequenced bidirectionally with the same primers used for the PCR-SSP reaction using the DYEnamic ET Terminator Cycle Sequencing Kit (Amersham, Piscataway, NJ) under the same conditions described above for sequencing MHC class I alleles.

Sequence-specific PCR assays for *Mamu-B*03* were performed as described previously (Kaizu et al. 2007).

Microsatellite analysis

Microsatellite analysis of DNA from all 42 Indonesian cynomolgus macaques was performed with a panel of markers spanning the MHC class I B region as previously described (Wiseman et al. 2007; Wojcechowskyj et al. 2007; Karl et al. 2008). Two new primer pairs were used to further define this region of the MHC, P03-129952F (5′-TGGGCAACAACAGTGAAACT), P03-129952R (5′-GTGGGTTAGGGGTCCTGTTT), L13-1494F (5′-AGCAGGTCCTCAGAATCCAA), and L13-1494R (5′-CAGCTACTCGGGACGCTAAG). P03-129952F was labeled with HEX (6-carboxy-2, 4, 4, 5, 7, 7-hexachlorofluorescein) and L13-1494F was labeled with FAM (6-carboxyfluorescein).

Phylogenetic Analysis

The phylogenetic analysis was based on 1031 nucleotide sites from coding regions, aligned by the Clustal X program (Thompson et al. 1997). A neighbor-joining tree (Saitou and Nei 1987) was constructed on the basis of the Maximum Composite Likelihood (MCL) distance using the MEGA 4.0 program (Tamura et al. 2007). The reliability of clustering patterns in the tree was assessed by bootstrapping (Felsenstein 1985); 1000 bootstrap pseudo-samples were used.

Results and Discussion

Summary of MHC class I cDNA nucleotide sequences identified

Indonesia consists of approximately 13,000 islands (Loudon et al. 2006), and it is likely that animals from distant sites have distinct MHC genetic repertoires. Therefore, we chose to identify MHC class I nucleotide sequences from cynomolgus macaques that came from independent sources and different regions within Indonesia, including Jakarta, Tinjil Island, and Sumatra. We sequenced 1,760 MHC class I cDNA clones from eleven cynomolgus macaques of Indonesian origin and identified 48 distinct MHC class I nucleotide sequences, including 19 *Mafa-A* and 29 *Mafa-B* cDNAs. Of these 48 sequences, seven were previously reported in GenBank, while the other 41 nucleotide sequences are newly described in this manuscript. The diversity of *Mafa-A* and *Mafa-B* cDNA sequences found in such a small cohort of animals is not surprising, since microsatellite analysis of the MHC region showed very little relatedness between these individuals (data not shown). Table 1 lists all 48 cDNA sequences, their accession number, reference animal, and identity to other previously described MHC class I genes. Allele names were designated by the Non-human Primate Immuno Polymorphism Database-MHC (IPD-MHC) nomenclature committee (Robinson et al. 2003).

During the process of *Mafa-A* and *Mafa-B* sequence discovery, nucleotide sequences were defined as authentic if we could identify three identical full-length clones from a single animal. After we completed the discovery process, we re-examined the nucleotide sequences from each animal to determine if any defined Indonesian MHC class I cDNAs could be detected in two or fewer clones in the other animals examined. This analysis led to the recognition of three additional *Mafa-B* alleles in IN10, thus identifying 4 *Mafa-B* alleles shared between IN04 and IN10. All animals and the nucleotide sequences we detected are summarized in Table 2.

Surprisingly, of the 19 identified *Mafa-A* sequences, 18 sequences were named based on similarity to alleles previously described for the *Mafa-A1* locus. Only one *Mafa-A* sequence identified in this study was categorized as an allele from the *Mafa-A2* locus. These results are in stark contrast to the observation by Otting and colleagues who found that cynomolgus macaques express a diverse set of *Mafa-A* alleles derived from at least six different loci (Otting et al. 2007). The reduced diversity observed in the current study may be a consequence of different PCR primers used for each study, or it may suggest that selection for high expression of *Mafa-A1* alleles has occurred in Indonesian cynomolgus macaques.

Additionally, we found that the number of distinct *Mafa-B* cDNA nucleotide sequences expressed in each animal ranged from one to six. Unlike the recently characterized *Mafa-A* loci, distinct *Mafa-B* loci have not yet been defined in macaques (Otting et al. 2007). Although it has been suggested that the dominant *Mafa-B* cDNAs would be most highly expressed, knowledge of gene order and gene number of *Mafa-B* alleles in macaques is still lacking (Otting et al. 2005). Therefore, the range of distinct *Mafa-B* cDNAs per animal that we observed was likely a consequence of the limited number of clones examined per animal, differential gene expression, and variable gene number per haplotype in macaques.

Phylogenetic analysis of MHC class I cDNA sequences

In the phylogenetic tree of MHC class I nucleotide sequences, the *Mafa-A* and *Mafa-B* cDNAs formed distinct clusters, separated by a branch that received highly significant (99%) bootstrap support (Figure 1). Within each of these clusters, sequences identified in Indonesian cynomolgus macaques and Mauritian cynomolgus macaques were intermingled (Figure 1). Indeed, in nine cases, the closest relative of a Mauritian allele was an Indonesian cDNA sequence; and these clustering patterns were generally well supported by bootstrapping (99% in the case of six pairs and >70% for all nine pairs of alleles; Figure 1). The phylogenetic analysis was thus consistent with the hypothesis that Mauritian MHC class I cDNA sequences represent a sample from the Indonesian population of MHC class I cDNA sequences.

Sharing of MHC cDNA sequences between different macaque species

Two of the seven previously described Indonesian cynomolgus macaque MHC class I nucleotide sequences were identical to alleles described in other macaque species. *Mafa-B*7901* was previously identified *as Mamu-B*05* in a rhesus macaque of Indian origin, while *Mafa-A1*1002* was previously identified in a rhesus macaque of Chinese origin, a pig-tail macaque of unknown origin, and a cynomolgus macaque from the University of Utrecht of unknown geographic origin (Table 1) (Boyson et al. 1996;Lafont et al. 2007;Otting et al. 2007). In addition, we identified *Mafa-B*5601*, a sequence that deviates from *Mane-B*03* by a single nucleotide synonymous substitution (Lafont et al. 2003). Our observation that approximately 6% (3/48) of the *Mafa-A* and *Mafa-B* nucleotide sequences identified in this population of Indonesian cynomolgus macaques are shared with other macaque species is similar to the frequency of MHC class I sequence sharing in other studies (Otting et al. 2007). This infrequent sharing of cDNA sequences between macaque species supports studies suggesting that certain rare MHC class I alleles were likely present in a common ancestor. In contrast, the observation that >90% of the observed MHC class I cDNAs are unique to cynomolgus macaques further suggests that MHC class I alleles are largely species specific, a circumstance that is likely attributable to pathogen-driven selection (Cooke and Hill 2001;Piertney and Oliver 2006). Ultimately, this observation further highlights the importance of a thorough characterization of the MHC of macaques from varying species and geographic origins.

Interestingly, we found that the predicted gene product of *Mafa-B*7601* was identical to the Indian rhesus macaque allele *Mamu-B*03* throughout the entire polymorphic region of the molecule (Figure 2). The only amino acid differences between these two predicted molecules reside in the leader peptide. Studies have found that *Mamu-B*03* is associated with improved control of SIV viremia in Indian rhesus macaques, but the rarity of this allele makes it difficult to study in large cohorts of animals (Evans et al. 1999;Evans et al. 2000;Loffredo et al. 2007b). Additionally, the predicted peptide binding motif of Mamu-B*03 is similar to HLA-B*27, an allele in humans that has been associated with slower disease progression in HIV-infected individuals (Dzuris et al. 2000;Carrington and O'Brien 2003). The predicted alpha 2 domain of Mamu-B*03 is also identical to another Indian rhesus macaque allele associated with improved control of SIV viremia, Mamu-B*08, and it has been hypothesized that these two alleles may restrict similar peptides (Loffredo et al. 2007a;Loffredo et al. 2007b). Therefore, it is reasonable to predict that *Mafa-B*7601* may be associated with control of SIV viremia in cynomolgus macaques since it likely binds peptides identical to those bound by Mamu-B*03.

Because Mafa-B*7601 may be an important molecule for understanding the role of CD8+ cell-mediated immune control of SIV viremia in macaques, we decided to genotype our expanded cohort of 42 Indonesian cynomolgus macaques for the presence of the *Mafa-B*7601* cDNA nucleotide sequence. The methods for typing *Mamu-B*03* by PCR-SSP have been established,

and we expected this assay would detect *Mafa-B*7601*. We found that 2/42 (4.8%) animals in our cohort possessed this allele, and these results were confirmed by sequence verification of the positive PCR products (data not shown). The frequency of *Mafa-B*7601* is comparable to the frequency of *Mamu-B*08* (5.8%), but greater than *Mamu-B*03* (0.7%) in Indian rhesus macaques (Kaizu et al. 2007). The higher frequency of *Mafa-B*7601* in Indonesian cynomolgus macaques implies that this population of macaques may provide a valuable model for understanding the role of this protective MHC class I molecule in control of SIV viremia.

Sharing of MHC class I cDNA sequences between Indonesian and Malaysian cynomolgus macaques

In this study, we found MHC class I sequences that are identical to or closely related to *Mafa-A* and *Mafa-B* sequences previously found in cynomolgus macaques of Malaysian origin. The nucleotide sequence *Mafa-B*1201* is identical to *Mafa-B*12*, an allele that was previously found in Malaysian macaques (Uda et al. 2005). In addition, the Malaysian MHC class I allele *Mafa-A*08* differs from the Indonesian allele *Mafa-A1*780102* by a single synonymous nucleotide substitution and it differs from the Indonesian allele *Mafa-A1*7802* by a single amino acid substitution in the leader peptide (Uda et al. 2004). These three nearly-identical *Mafa-A* alleles, therefore, are likely capable of presenting identical peptide epitopes to T cells.

Previous studies have identified the co-existence of Y-chromosomal lineages in Malaysian and Sumatran macaques and provided support to the hypothesis that a land bridge existed between Malaysia and Indonesia during the Late Pleistocene age that led to gene flow between these two populations (Voris 2000; Tosi and Coke 2007). Here, our observation that three Indonesian MHC class I nucleotide sequences are identical or nearly-identical to MHC class I nucleotide sequences previously found in Malaysian macaques further supports the notion of gene flow between these two populations.

Sharing of MHC class I cDNA sequences between Indonesian and Mauritian cynomolgus macaques

Based upon phylogenetic studies of Mauritian and Indonesian cynomolgus macaques, we hypothesized that these two geographically distinct populations of macaques would also share MHC class I cDNA sequences (Tosi and Coke 2007). In our analysis, we found three Indonesian cynomolgus macaque MHC class I cDNA sequences that were previously identified in Mauritian cynomolgus macaques; an observation that is consistent with our hypothesis.

We found that *Mafa-B*1201* in Indonesian macaques was identical to *Mafa-B*12* in Mauritian macaques. From this study, we can now conclude that *Mafa-B*1201* is actually shared between three geographically distinct populations of cynomolgus macaques: Indonesian, Mauritian, and Malaysian. This observation is surprising, as MHC class I alleles are rarely found in multiple geographically distinct populations (Krebs et al. 2005; Otting et al. 2007; Karl et al. 2008). In Mauritian macaques, *Mafa-B*12*, is found on the relatively rare H5 Mauritian MHC haplotype which comprises approximately 3% of the feral population (Wiseman et al. 2007). Interestingly, this allele was found in two animals, IN04 and IN10. In both of these animals, we also identified *Mafa-B*5002*, which is closely related to *Mafa-B*500101* (Figure 1), an allele that is also found on the H5 Mauritian MHC haplotype (Wiseman et al. 2007). This data suggests that these two *Mafa-B* sequences may be carried on the same ancestral MHC haplotype in Indonesian macaques and it further supports the hypothesis that some Mauritian cynomolgus macaques originated in Indonesia.

We also identified *Mafa-B*4501* and *Mafa-B*5101*, a pair of MHC class I nucleotide sequences that are identical to *Mafa-B*450101* and *Mafa-B*510101* found in Mauritian cynomolgus macaques. These two *Mafa-B* sequences are found on the same common H3 MHC haplotype

found in feral Mauritian cynomolgus macaques at a frequency of approximately 15% (Wiseman et al. 2007). To further investigate the frequency of these *Mafa-B* sequences, we developed PCR-SSP assays to screen the entire larger cohort of 42 Indonesian cynomolgus macaques, and we then sequenced the positive PCR products to verify the identity of each *Mafa-B* sequence. Surprisingly, we found that six animals express *Mafa-B*4501*, but only three of these same animals express *Mafa-B*5101* (Figure 3A). Unlike feral Mauritian cynomolgus macaques, where the overall presence of recombinant H3 MHC class I haplotypes is fairly low, we found that half of the Indonesian macaques expressing *Mafa-B*4501* did not concomitantly express *Mafa-B*5101*. In contrast, we found no animals expressing *Mafa-B*5101* without *Mafa-B*4501*.

We further explored whether Indonesian cynomolgus macaques share MHC haplotypes with Mauritian cynomolgus macaques by typing them with an expanded panel of microsatellite markers that spans the *Mafa-B* region of the genome (Wojcechowskyj et al. 2007; Karl et al. 2008). Interestingly, we found identical allele sizes for microsatellite markers (P03-193435 and L13-1494) shared between H3-homozygous Mauritian macaques and the Indonesian animals who express both *Mafa-B*4501* and *Mafa-B*5101* (Figure 3B). The microsatellite allele size of 135bp for marker P03-193435 is only detected in our Indonesian cohort in animals that express *Mafa-B*5101*. This observation suggests this microsatellite locus is tightly linked to *Mafa-B*5101* and may be useful, in addition to the PCR-SSP assay, when screening for animals who possess this *Mafa-B* cDNA sequence.

Overall, our observation that three *Mafa-B* sequences are shared between cynomolgus macaques of Indonesian and Mauritian origin provides further evidence that Indonesia is likely a source of the Mauritian cynomolgus macaques. Because we defined MHC class I sequences from a relatively small cohort of Indonesian macaques, it is not surprising that we found a limited number of cDNAs shared between these two geographic populations. It is certainly possible that further exploration in additional Indonesian cynomolgus macaques may reveal a greater extent of *Mafa-A* and *Mafa-B* sequence sharing. While there are multiple lines of evidence suggesting that Indonesia is the most likely origin of the Mauritian macaques, other possible geographic origins have been suggested. Specifically, certain MHC class II *DRB* alleles have been identified in both Filipino and Mauritian cynomolgus macaques. Likewise, it has been observed that variants of non-MHC proteins are shared between cynomolgus macaques of Mauritian and Southeast Asian origin (Kondo et al. 1993; Blancher et al. 2006). Therefore, it is certainly possible that MHC class I cDNA sequences found in Mauritian cynomolgus macaques may also be identified in cynomolgus macaques from other regions in Southeast Asia.

Implications for SIV vaccine research

Several studies in rhesus macaques have demonstrated that CD8+ T cells are important in the immune response to SIV (Schmitz et al. 1999; McMichael and Hanke 2002). To best understand the role that CD8+ T cells play in SIV pathogenesis, it is critical that researchers are aware of the MHC molecules expressed in their experimental animals so that CD8+ T cell responses can be monitored during disease progression. Unfortunately, few studies have examined CD8+ T cell biology in SIV-infected cynomolgus macaques because, until recently, very few MHC class I cDNA sequences were described in this species. In contrast to the well-defined SIV epitopes in Indian rhesus macaques, there have only been two studies published that define SIV-derived CTL peptide epitopes in cynomolgus macaques (Geretti et al. 1997; Negri et al. 2006). Although these studies identify specific SIV peptide sequences, they fail to identify the MHC class I molecule responsible for the epitope restriction. By characterizing full length MHC class I cDNA sequences in cynomolgus macaques of specific geographic

origins, SIV researchers can now begin to develop reagents to better study CD8+ T cell responses in SIV-infected cynomolgus macaques.

The presence of certain *Mamu-B* and *HLA-B* alleles has also been associated with control of SIV/HIV viremia in Indian rhesus macaques and humans (Carrington and O'Brien 2003; Bontrop and Watkins 2005). Therefore, it seems likely that *Mafa-B* alleles also exist in cynomolgus macaques that may afford protection from SIV disease progression. Here, we provide data suggesting that the Indonesian MHC class I molecule Mafa-B*7601 may be capable of presenting a similar set of peptides as Mamu-B*03, a MHC class I molecule associated with control of SIV viremia in Indian rhesus macaques. Because *Mafa-B*760*1 is present at a frequency of about 5% in Indonesian cynomolgus macaques and these animals are known to be susceptible to infection with SHIV89.6p (Shiu-Lok Hu, personal communication), this animal model may be useful for better understanding the role of this MHC molecule in SIV disease.

In addition, an ongoing study examining the association of MHC genetics in Mauritian cynomolgus macaques with resistance to SHIV89.6p infection provides evidence suggesting that the *Mafa-B* alleles present on the H3 Mauritian MHC haplotype associate with low plasma viremia, while the *Mafa-B* alleles present on the H5 Mauritian MHC haplotype associate with high plasma viremia (Florese et. al., *submitted*). Because the Indonesian and Mauritian cynomolgus macaques share these potentially protective and susceptible *Mafa-B* alleles, the Indonesian population may also be a useful model for examining the influence of MHC genetics on SIV pathogenesis.

In conclusion, the MHC class I cDNA sequences described in this manuscript provide an important addition to the limited immunogenetic information available for Indonesian cynomolgus macaques. Moreover, the identification of *Mafa-A* and *Mafa-B* sequences that have been associated with protection and susceptibility to SIV increase the value of Indonesian cynomolgus macaques as an animal model for SIV pathogenesis and vaccine research. The results of this study underscore the importance of additional MHC class I sequence discovery in non-human primates. Identification of shared and unique MHC class I cDNA sequences may be critical for disease research and may help better understand the biogeography of non-human primates.

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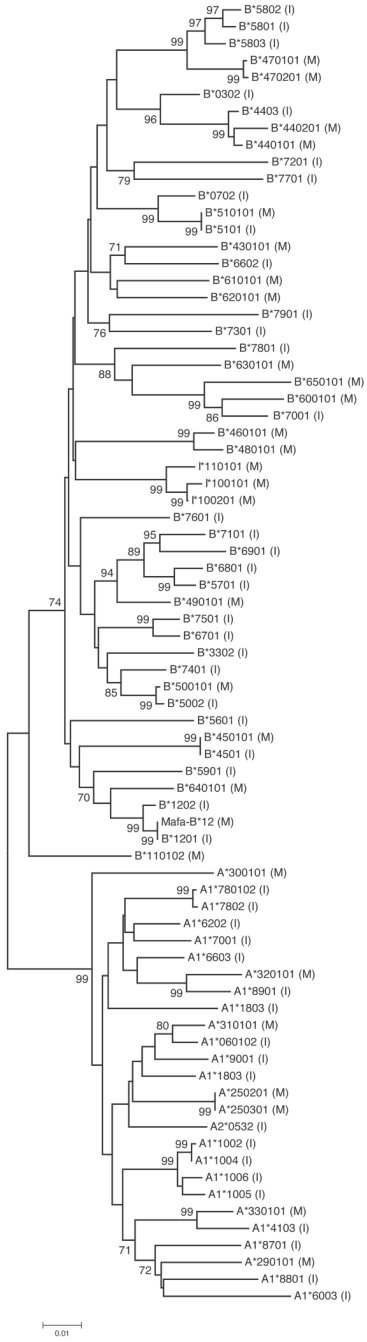

Figure 1. Neighbor-joining tree of MHC class I cDNA sequences from cynomolgus macaques from Mauritius (M) and Indonesia (I)

Numbers on the branches represent the percent of bootstrap samples supporting a given branch; only values ≥ 70% are shown.

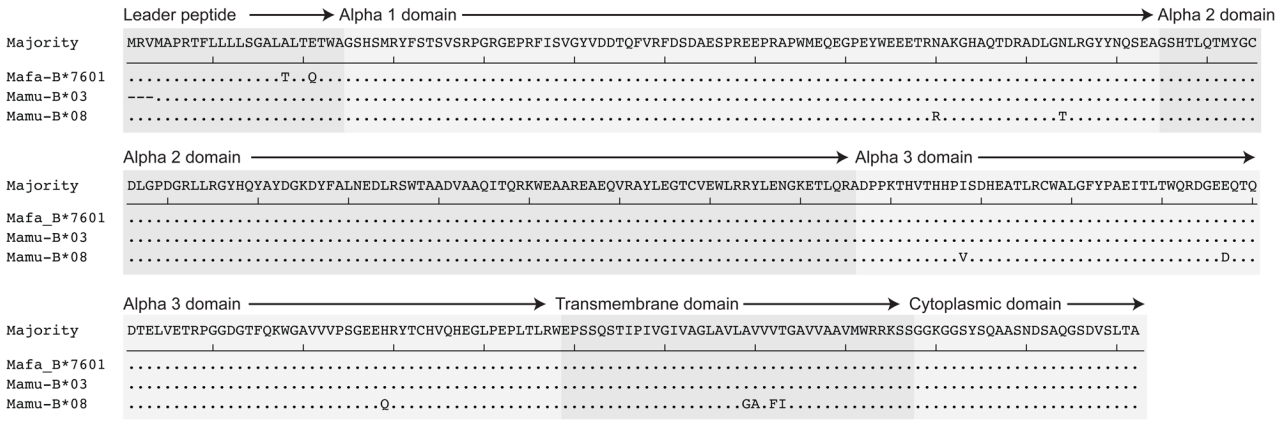

Figure 2. Mafa-B*7601 and Mamu-B*03 share identical peptide binding domains

Predicted amino acid translations of Mafa-B*7601, Mamu-B*03, and Mamu-B*08 were aligned with MegAlign software (DNASTAR, Madison, WI). Peptide binding domains were predicted based on previous studies of MHC class I alleles in Indian rhesus macaques (Boyson et al. 1996).

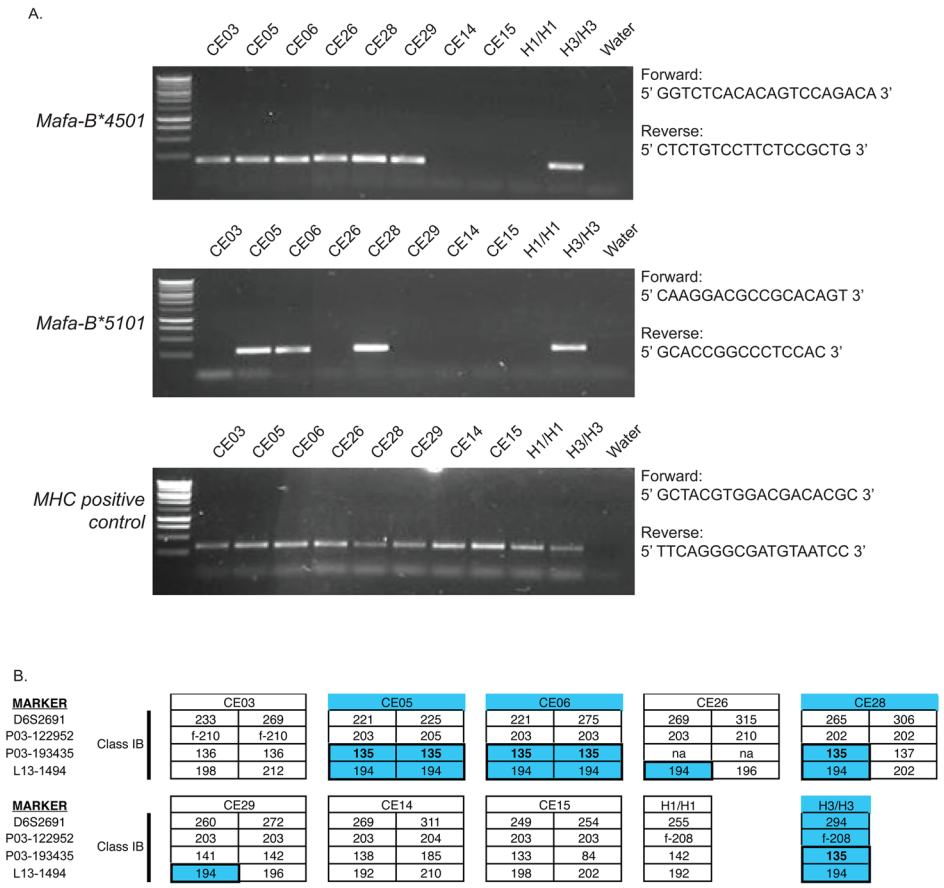

Figure 3. *Mafa-B*4501* and *Mafa-B*5101* are shared between Indonesian and Mauritian cynomolgus macaques

(A) PCR-SSP analysis of *Mafa-B*4501* and *Mafa-B*5101* in 8 Indonesian cynomolgus macaques is shown. In parallel, primers based on sequences in exons 2 and 3 that are conserved in nearly all MHC class I alleles were used as a positive control to verify the cDNA integrity. Samples from H3/H3 and H1/H1 Mauritian cynomolgus macaques were used as positive and negative controls, respectively, for the amplification of *Mafa-B*4501* and *Mafa-B*5101*. (B) Microsatellite analysis of the same 8 Indonesian cynomolgus macaques and two control Mauritian macaques is shown. Microsatellite allele sizes matching those found in an H3/H3 homozygous animal are highlighted in blue. The names of the animals who express both *Mafa-B*4501* and *Mafa-B*5101* are also highlighted in blue.

Table 1

MHC class I cDNA sequences identified in Indonesian-origin cynomolgus macaques (ICM).

ICM allele name	ICM Accession #	Reference animal	Previously described identical nucleotide sequence[1]
Mafa-A alleles			
Mafa-A1*060102	EU203689	IN04 (02367)	
Mafa-A1*1002	EU203687	IN02 (02326)	Mane-A*19 (pig-tail, EF010520);Mafa-A1*1002 (unknown cynomolgus, AM295831); Mamu-A1*1001 (Chinese rhesus, AM295894) (1,2)
Mafa-A1*1004	EU203706	CE19 (13659)	
Mafa-A1*1005	EU203707	CE28 (13668)	
Mafa-A1*1006	EU203699	IN12 (04146)	
Mafa-A1*1803	EU203709	CE29 (13670)	
Mafa-A1*2202	EU203696	IN07 (04132)	Mafa-A1*2202 (unknown cynomolgus, AM295835) (2)
Mafa-A1*4103	EU203713	CE16 (13655)	
Mafa-A1*6003	EU203698	IN10 (04141)	
Mafa-A1*6202	EU203711	CE12 (13651)	
Mafa-A1*6603	EU203712	CE12 (13651)	
Mafa-A1*7001	EU203708	CE28 (13668)	Mafa-A1*7001 (unknown cynomolgus, AM295858) (2)
Mafa-A1*780102	EU203685	IN01 (01095), IN02 (02326)	
Mafa-A1*7802	EU203705	CE19 (13659)	
Mafa-A1*8701	EU203710	CE29 (13670)	
Mafa-A1*8801	EU203686	IN01 (01095)	
Mafa-A1*8901	EU203697	IN10 (04141)	
Mafa-A1*9001	EU203700	IN12 (04146)	
Mafa-A2*0532	EU203688	IN04 (02367)	
Mafa-B alleles			
Mafa-B*0302	EU203720	CE29 (13670)	
Mafa-B*0702	EU203704	IN12 (04146)	
Mafa-B*1201	EU203690	IN04 (02367), IN10 (04141)	Mafa-B*12 (Malaysian and Mauritian cynomolgus, AB195442) (3,4)
Mafa-B*1202	EU203682	IN02 (02326)	
Mafa-B*3302	EU046324	CE16 (13655), CE19 (13659)	
Mafa-B*4403	EU203715	CE19 (13659)	
Mafa-B*4501	EU203717	CE28 (13668)	Mafa-B*450101 (Mauritian cynomolgus, AY958143) (5)
Mafa-B*5002	EU203693	IN04 (02367)	
Mafa-B*5101	EU203718	CE28 (13668)	Mafa-B*510101 (Mauritian cynomolgus, AY958150) (5)
Mafa-B*5601	EU203714	CE16 (13655)	
Mafa-B*5701	EU203719	CE28 (13668)	
Mafa-B*5801	EU203722	CE12 (13651)	
Mafa-B*5802	EU203683	IN02 (02326)	
Mafa-B*5803	EU203721	CE29 (13670)	
Mafa-B*5901	EU203723	CE12 (13651)	
Mafa-B*6602	EU203716	CE28 (13668)	
Mafa-B*6701	EU203724	CE12 (13651)	
Mafa-B*6801	EU203725	CE12 (13651)	
Mafa-B*6901	EU203726	CE16 (13655)	
Mafa-B*7001	EU203680	IN01 (01095)	
Mafa-B*7101	EU203681	IN02 (02326)	
Mafa-B*7201	EU203684	IN02 (02326)	
Mafa-B*7301	EU203701	IN07 (04132)	
Mafa-B*7401	EU203702	IN07 (04132)	
Mafa-B*7501	EU203703	IN12 (04146)	
Mafa-B*7601	EU203691	IN04 (02367)	
Mafa-B*7701	EU203692	IN04 (02367)	
Mafa-B*7801	EU203694	IN04 (02367), CE28 (13668)	
Mafa-B*7901	EU203695	IN04 (02367)	Mamu-B*05 (Indian rhesus, U41827) (6)

[1] The references for the previously described alleles are shown in parentheses.

MHC class I cDNA sequences identified in this study were compared with other known MHC nucleotide sequences previously identified in other macaque species. Previously named alleles whose sequences are identical to the Indonesian cynomolgus macaque MHC class I cDNA sequences are shown with

their accession number. The reference animal used for allele discovery is shown. References listed are: (1) (Lafont et al. 2007), (2) (Otting et al. 2007), (3) (Uda et al. 2005), (4) (Wiseman et al. 2007), (5) (Krebs et al. 2005), (6) (Boyson et al. 1996).

Table 2

Summary of MHC class I cDNA sequences identified in each Indonesian-origin cynomolgus macaque

Official name	IN01	IN02	IN04	IN07	IN10	IN12	CE12	CE16	CE19	CE28	CE29
Mafa-A1*060102			■								
Mafa-A1*1002		■									
Mafa-A1*1004									■		
Mafa-A1*1005										■	
Mafa-A1*1006							■				
Mafa-A1*1803											■
Mafa-A1*2202				■							
Mafa-A1*4103								■			
Mafa-A1*6003					■						
Mafa-A1*6202							■				
Mafa-A1*6603							■				
Mafa-A1*7001										■	
Mafa-A1*780102	■	■									
Mafa-A1*7802									■		
Mafa-A1*8701											■
Mafa-A1*8801	■										
Mafa-A1*8901					■						
Mafa-A1*9001						■					
Mafa-A2*0532			■								
Mafa-B*0302											■
Mafa-B*0702						■					
Mafa-B*1201			■		■						
Mafa-B*1202		■									
Mafa-B*3302								■	■		
Mafa-B*4403									■		
Mafa-B*4501										■	
Mafa-B*5002			■								
Mafa-B*5101										■	
Mafa-B*5601								■			
Mafa-B*5701										■	
Mafa-B*5801							■				
Mafa-B*5802		■									
Mafa-B*5803											■
Mafa-B*5901							■				
Mafa-B*6602										■	
Mafa-B*6701							■				
Mafa-B*6801							■				
Mafa-B*6901								■			
Mafa-B*7001	■										
Mafa-B*7101		■									
Mafa-B*7201		■									
Mafa-B*7301				■							
Mafa-B*7401				■							
Mafa-B*7501						■					
Mafa-B*7601			■		■						
Mafa-B*7701			■								
Mafa-B*7801			■							■	
Mafa-B*7901			■		■						

MHC class I cDNA sequences identified in each animal are shown. MHC class I cDNAs identified in three or more clones in a single animal are highlighted in black. MHC class I cDNAs identified in only one