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Comprehensive characterization of MHC class II haplotypes in Mauritian cynomolgus macaques

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Abstract

There are currently no non-human primate models with fully defined major histocompatibility complex (MHC) class II genetics. We recently showed that 6 common MHC haplotypes account for essentially all MHC diversity in cynomolgus macaques (*Macaca fascicularis*) from the island of Mauritius. Here we employ cDNA cloning and sequencing to comprehensively characterize full length MHC class II alleles expressed at the *Mafa-DPA*, *-DPB*, *-DQA*, *-DQB*, *-DRA*, and *-DRB* loci on the 6 common haplotypes. We describe 34 full-length MHC class II alleles, 12 of which are completely novel. Polymorphism was evident at all six loci, including *DPA* which is considered monomorphic in rhesus macaques. Similar to other old world monkeys, Mauritian cynomolgus macaques (MCM) share MHC class II allelic lineages with humans at the *DQ* and *DR* loci, but not at the *DP* loci. Additionally, we identified extensive sharing of MHC class II alleles between MCM and other non-human primates. The characterization of these full length expressed MHC class II alleles will enable researchers to generate MHC class II transferent cell lines and tetramers that can be used to explore CD4+ T-lymphocyte responses in MCM.

Keywords

MHC; Immunogenetics; *Macaca fascicularis*

Introduction

Macaques are valuable models for immunological research into disease pathogenesis, autoimmunity, and solid organ transplant rejection (Bontrop 2001; Patterson and Carrion 2005). The utility of these animals has fostered an interest in understanding their genetics, as evidenced by the recent completion of the rhesus genome draft sequence (The Rhesus Macaque Genome Sequencing and Analysis Consortium 2006).

Characterization of macaque major histocompatibility complex (MHC) genetics has proven particularly useful. The identification of MHC class I alleles in rhesus and cynomolgus macaques, for example, has revolutionized the study of simian immunodeficiency virus (SIV) pathogenesis. Investigators have utilized MHC class I sequences to develop reagents such as

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MHC:peptide tetramers, MHC-defined cell lines, and PCR-SSP primers that can be used to understand virus-specific CD8+ T-lymphocyte responses (Shimizu and DeMars 1989; Knapp et al. 1997; Allen et al. 1998; Kuroda et al. 1998; Ogg and McMichael 1998). These tools have allowed researchers to quantify naturally occurring and vaccine-elicited CD8+ T-lymphocyte responses, identify CD8+ T-lymphocyte responses with varying antiviral efficacy, and characterize subpopulations of animals with distinctive resistance or susceptibility to SIV disease progression (Altman et al. 1996; Allen et al. 2000; Allen et al. 2001; Carrington and Bontrop 2002; O'Connor et al. 2003; Loffredo et al. 2005).

Comparable molecular reagents for the study of MHC class II-restricted CD4+ T-lymphocyte responses are not widely available in macaques. Most previous studies examined only exon 2 of MHC class II genes, as this exon shows the greatest polymorphism and encodes the alpha 1 and beta 1 domains that are principally responsible for peptide binding (Kenter et al. 1992; Otting et al. 1992; Christ et al. 1994; Lekutis and Letvin 1995; Slierendregt et al. 1995b; de Groot et al. 1998; Otting et al. 1998; Otting et al. 2000; Otting et al. 2002; Vigon and Sauermann 2002; de Groot et al. 2004; Leuchte et al. 2004; Blancher et al. 2006; Sano et al. 2006). Without knowing the sequence encoding the entire open reading frame, it is impossible to generate transfectant cell lines for determining CD4+ T-lymphocyte epitope restriction or to construct MHC class II: peptide tetramers. Moreover, the genes encoding both the alpha and beta chains that comprise a functional MHC class II heterodimer are polymorphic, in contrast to MHC class I heterodimers in which the β_2 -microglobulin subunit is monomorphic. Additionally, in rhesus macaques, the combinations of cis-encoded MHC class II DQ alpha and beta subunits are stricter than DQ pairing in humans (Doxiadis et al. 2001). These observations suggest that in the absence of haplotype associations and full-length sequences, it is difficult to determine the MHC class II heterodimers that potentially restrict CD4+ T-lymphocyte responses in macaques. There are a few examples where the availability of full-length MHC class II allele sequences provided researchers with the tools to monitor CD4+ T-lymphocyte responses. Specifically, the relatively low degree of polymorphism at the *DRA* locus allowed researchers to express MHC *DRA*-*DRB* heterodimers and explore the presentation of specific SIV and HIV peptides (Dzuris et al. 2001). Additionally, Kuroda and colleagues developed an MHC class II tetramer that could detect antigen specific CD4+ cells in a rhesus macaque model of SHIV infection (Kuroda et al. 2000). These two studies illustrate that by continuing to define full-length MHC class II alleles, researchers will be able to develop more molecular reagents necessary to follow antigen-specific CD4+ responses in models of SIV infection.

Although non-human primates are often the best model system to study the pathology of certain infectious diseases including SIV, differences in host immunogenetics can complicate the interpretation of these studies. Until recently, it has been almost impossible to select animals that have identical host immunogenetics. We recently described a population of non-human primate Mauritian origin cynomolgus macaques (MCM) who have extremely simple MHC genetics. Six haplotypes account for essentially all of the diversity in these animals (Wiseman et al. 2007). Historical records suggest that these animals were introduced to the small island of Mauritius within the last 500 years (Sussman and Tattersall 1986). Additional molecular genetic studies suggest that they descended from a very small founder population (Lawler et al. 1995; Tosi and Coke 2006). The limited MHC haplotype diversity in these animals stands in stark contrast to all other macaques studied to date and makes MCM a particularly attractive model for characterizing the genes that encode MHC class II heterodimers.

Here we describe the full-length MHC class II alleles that are expressed on the six common haplotypes in the MCM. This study comprehensively examines the full length alleles expressed from all 6 MHC class II loci (*DPA*, *DPB*, *DQA*, *DQB*, *DRA*, and *DRB*) and links them to distinct MHC haplotypes. Our results provide the first complete analysis of the MHC class II region

of a non-human primate population that are a viable model for SIV pathogenesis and other infectious disease and transplantation studies.

Materials and Methods

Identification of animals to use for MHC class II cloning and sequencing

In a previous study (Wiseman et al. 2007), blood samples from feral MCM were purchased specifically for genetic analyses (Charles River BRF, Houston, TX). Microsatellite analysis identified animals that were homozygous for all of the 6 common haplotypes in MCM: CR108 (H1), CR012 (H2), CR011 (H3), A3M (H4), CR01 (H5), and CR079 (H6).

RNA isolation, cDNA synthesis, and cloning of MHC class II alleles

RNA was isolated from blood using the Roche Magnapure kit as described previously (Wiseman et al. 2007). cDNA was generated using the Superscript™ III First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA). MHC class II cDNAs were then amplified by PCR using the high fidelity polymerase Phusion (New England Biolabs). PCR primers were chosen for each locus based on conserved untranslated regions around MHC class II coding sequences identified in humans and other non-human primates (Table 1a). Multiple primers for several loci are listed in Table 1a because primer pairs for certain loci needed to be optimized for specific haplotypes. PCR reactions were run on the following program: 98°C for 30 seconds; 29 cycles of 98°C for 5 seconds, 63°C for 1 second, 72°C for 20 seconds; and a final extension at 72°C for 5 minutes on an MJ Research Tetrad Thermocycler (Bio-Rad Laboratories, Hercules, CA). The PCR product was subjected to agarose gel electrophoresis, the band was excised, and then it was purified using the Qiaquick PCR Gel Extraction Kit (Qiagen, Valencia, CA). Purified PCR products were ligated into pCR-Blunt vectors using the Zero Blunt Cloning kit (Invitrogen, Carlsbad, CA), and then transformed into *E. coli* Top10 (Invitrogen, Carlsbad, CA) competent cells.

DNA isolation of MHC class II alleles

Bacterial colonies were picked and placed in 1.4 mL of Circle Grow (Qbiogene, Irvine, CA) with 50 µg/mL kanamycin and incubated in a shaker for 17 to 24 hours. DNA was isolated using the Eppendorf® Perfectprep® Plasmid 96 VAC Direct Binding Kit (Brinkmann, Westbury, NY). DNA concentration was determined by absorbance using the Nanodrop 1000 (Wilmington, DE). Restriction digests of plasmids were performed using EcoRI (New England Biolabs, Ipswich, MA) to determine which clones contained MHC class II inserts of the appropriate size (~750–900bp).

Sequencing of MHC class II alleles

Each clone was bidirectionally sequenced using at least two primers per strand (Table 1b). Two of the primers, M13R and T7, anneal directly to the pCR-BLUNT vector. Two internal primers for each locus were designed using MHC Class II sequences of rhesus macaques. Sequencing reactions were performed with the DYEnamic ET Terminator Cycle Sequencing Kit (Amersham, Piscataway, NJ). Sequencing reactions were conducted under the following thermal cycling settings: 30 cycles of 95° C for 20 seconds, 50° C for 15 seconds, and 60° C for 1 minute. The products were purified with the Agencourt® CleanSEQ® dye-terminator removal kit (Agencourt Bioscience Corporation, Beverly, MA) and resolved on an ABI 3730 (Applied Biosystems, Foster City, CA). Sequences were analyzed using CodonCode Aligner software (CodonCode Corp, Dedham, MA) and Lasergene software (DNASTAR, Madison, WI). Approximately 15–30 clones were analyzed for the *DPA*, *DPB*, *DQA*, *DQB*, and *DRA* loci, while at least 48 clones were examined for the *DRB* loci. In order to avoid PCR artifacts, when three or more identical clones were found, the MHC class II sequence was considered

to be an actual transcribed allele. Novel MHC class II sequences were deposited in GenBank (Accession numbers are listed in Table 2).

Phylogenetic analyses

Sequences were aligned at the amino acid level using the CLUSTAL X program (Thompson et al. 1997) and the alignments were imposed on the DNA sequences. Phylogenetic trees were constructed by the neighbor-joining method (Saitou and Nei 1987) on the basis of the logdet nucleotide distance. The reliability of clustering patterns in phylogenetic trees was assessed by bootstrapping (Felsenstein 1985); 1000 bootstrap samples were used. In order to estimate divergence times of alleles, we used the linearized tree method (Takezaki et al. 1995) based on the number of synonymous nucleotide substitutions per synonymous site (Nei et al. 1986). A calibration was provided by Satta et al.'s estimate of the rate of synonymous substitution at primate class II MHC loci (Satta et al. 1993).

Results

Identification of transcribed MHC class II alleles

Recently, we described the limited MHC diversity of the MCM. We used microsatellite markers that spanned the entire 5-Mb MHC region of the genome and defined six common haplotypes (H1 through H6) shared among this population of animals (Wiseman et al. 2007). We also identified the transcribed MHC class I alleles associated with each haplotype. Here, we extended this approach to define the transcribed MHC class II alleles associated with haplotypes H1 through H6 at all six MHC class II loci (*DPA*, *DPB*, *DQA*, *DQB*, *DRA*, and *DRB*). We chose animals that were previously identified by microsatellite analysis as homozygous for the MHC class II region for cDNA cloning and sequencing (see Materials and Methods). For each locus, we designed PCR primers (Table 1a) against conserved untranslated regions based on MHC class II sequences from other primate species. Because homozygous animals were chosen for these analyses, cloning and sequencing unambiguously revealed specific alleles associated with each haplotype. Allele sequences were submitted to the IMGT (Robinson et al. 2003) for assignment of standard nomenclature.

Each haplotype contained unique *DQA* and *DQB* alleles. Four of the six haplotypes contained unique *DPA* and *DPB* loci, while one allele was shared between two haplotypes at these loci. Additionally, three *DRA* and nine *DRB* alleles were identified and there was some sharing of these alleles between haplotypes (Figure 1). By comparing these new alleles to previously described MHC class II alleles in other primate species (Das et al. 1983; Auffray et al. 1984; Wake 1986; Todd et al. 1987; Hatta et al. 2002), we could predict their domain structure (Figure 1). As seen in Figure 1, most of the sequence variation is found in the alpha 1 or beta 1 domains that correspond essentially to exon 2 of each allele. In addition to the extensive variation within these domains between alleles, there is also variation in the remaining coding sequences. Our analysis of full length allele sequences reveals this additional variability that is often not apparent when MHC class II alleles are defined solely on the basis of exon 2 sequences derived from genomic DNA. As researchers continue to generate full-length sequences of MHC class II alleles, this variation outside of exon 2 may prove to be useful for more thoroughly characterizing the phylogeny or the function of these alleles.

Since we could unambiguously associate specific alleles with specific haplotypes, we were able to construct a map linking alleles at all six class II loci to each individual haplotype (Figure 2). By linking these alleles to their haplotypes, we can now predict specific pairs of class II alpha and beta chains that most likely form functional heterodimers. Future experiments can use this data to determine whether class II alpha-beta pairing in cynomolgus macaques is haplotype-restricted, which would be similar to the strict pairing observed between DQ

molecules in rhesus macaques, or whether pairing is allowed between different haplotypes (Doxiadis et al. 2001).

Previous studies of the immunogenetics of the cynomolgus macaque have focused primarily on sequencing exon 2 of each allele from genomic DNA from animals of various origins (Kenter et al. 1992; Otting et al. 1992; Leuchte et al. 2004; Doxiadis et al. 2006; Sano et al. 2006). Only one previous report described full-length cDNA sequences of *DRB* alleles in cynomolgus macaques from the Philippines and Mauritius (Blancher et al. 2006). In the current study, we identified most of the full-length *DRB* alleles described by Blancher and colleagues in their cohort of MCM (Blancher et al. 2006), and our full-length *DRB* alleles also represent a subset of those identified by PCR-SSP in other studies (Leuchte et al. 2004; Wiseman et al. 2007). The observation that all three laboratories found a similar small number of expressed and genomic Mafa-*DRB* alleles in animals from the same geographic region further underscores the limited MHC diversity among this population. Since we characterized the MHC-*DRB* alleles expressed on the 6 most common haplotypes, the larger number of alleles identified by Leuchte et. al. and Blancher et. al. might reflect either pseudogenes identified by genomic PCR-SSP assays or differences between PCR primers used for cloning that might amplify a different set of alleles (Leuchte et al. 2004; Blancher et al. 2006). Importantly, our current study builds upon Blancher's study by extending the haplotype association to loci outside of the *DRB* region. Thus, by knowing the MHC haplotype of a MCM, the entire MHC class II region of these animals can now be predicted.

Our study greatly expands the repertoire of full-length expressed MHC class II alleles that have been reported for the entire cynomolgus macaque species, particularly at the *DPA*, *DPB*, *DQA*, *DQB*, and *DRA* loci. This comprehensive analysis of MHC class II alleles in cynomolgus macaques will be the foundation upon which to incorporate additional alleles from cynomolgus macaques of other geographic origins in the future.

MHC class II haplotype frequency

In our previous report, we defined the six MHC haplotypes present in the MCM, and we examined the frequencies of these haplotypes for the entire MHC region in a cohort of 117 feral MCM (Wiseman et al. 2007). Figure 3 illustrates the haplotype frequencies for the MHC class II region alone based on the microsatellite data from the same cohort of 117 feral MCM analyzed in our previous study. We found that 31% (72/234) of the chromosomes contained an intact H1 MHC class II haplotype (Figure 3). Not surprisingly, intact H1 MHC class II haplotypes are more common than intact H1 haplotypes of the entire 5 MB MHC region (Wiseman et al. 2007). Additionally, recombinant haplotypes account for only 8% of this total population of animals. Ultimately, this analysis suggests that recombination between the common class II haplotypes of MCM has been relatively infrequent.

MHC class II polymorphism

The degree of polymorphism of MCM is similar to other primates at the *DPB*, *DQA*, *DQB*, and *DRB* loci (Bontrop et al. 1999). While the *DRA* locus is fairly monomorphic in humans, rhesus macaques have 12 defined alleles in this locus (Lekutis and Letvin 1995; de Groot et al. 2004). Here, we found that the MCM are also polymorphic at the *DRA* locus, but more cynomolgus macaques from different geographic origins will need to be examined before determining the extent of polymorphism at this locus in this entire species. In contrast, it was reported that the *DPA* locus is monomorphic in rhesus macaques, although this locus does display some polymorphism in humans (Slenderregt et al. 1995b; Bontrop et al. 1999). Our identification and alignment of 5 *DPA* alleles (Figure 1) suggests that there is a greater degree of polymorphism in the MCM than in rhesus macaques at this locus. Overall, MHC class II

loci share similar degrees of polymorphism at certain loci with rhesus macaques, but the *DPA* locus appears to be exceptional in this regard.

Relationship of MHC class II alleles between primate species

Understanding the relationship of MHC class II alleles in primate species of different origins has garnered much attention among primate researchers (Bontrop et al. 1999; Doxiadis et al. 2006). In Table 2, we present the MCM class II alleles described here and previously reported alleles from other non-human primate species that we found to be identical either throughout the entire allele or across the exon 2 sequence. We found that alleles from the MCM fit into three categories: those that were previously described in the cynomolgus macaque, those that were previously described in other macaque species, and those that are newly described alleles. Unfortunately, only exon 2 sequences were available for many of the class II sequences we found to share identity with the alleles from MCM. While exon 2 sequences reveal key information about an allele, our data in Figure 1 clearly shows that variability can exist outside the exon 2 region. This overwhelming presence of exon 2 sequences listed in Table 2 further underscores the lack of complete MHC class II sequences in GenBank and the need to examine more full length sequences in future studies.

In a previous study, sharing of MHC class I alleles between MCM and cynomolgus macaques of other geographic origin was exceptionally rare (Krebs et al. 2005). In contrast, we now identify multiple MHC class II alleles in MCM that were also found in cynomolgus macaques of either East Asian or unknown origin. Identification of these shared class II alleles between cynomolgus macaques of different geographic origins provides support to the hypothesis that these animals were recently introduced from Indonesia to the island of Mauritius (Sussman and Tattersall 1986; Tosi and Coke 2006). Similar to previous observations made by others, we also found eight examples of MHC class II alleles identified in this study that are shared between MCM and rhesus macaques (Doxiadis et al. 2006). This additional evidence of allele sharing between cynomolgus and rhesus macaques lends further support to the hypothesis that there has been introgression between these macaque species in Indochina (Tosi et al. 2002; Tosi et al. 2003; Doxiadis et al. 2006).

Phylogeny of MCM MHC class II alleles

Previous studies have demonstrated evolutionary relationships between the MHC class II alleles found in rhesus macaques and humans (Kenter et al. 1992; Slierendregt et al. 1995b; Doxiadis et al. 2003). Thus, we conducted phylogenetic analyses of MCM and representative human MHC class II alleles (Figure 4). The phylogenetic analyses provided strong evidence that there are ancient allelic lineages shared between the MCM and humans at the *DQA* and *DQB* loci (Figure 4C–D). At both *DQA* (Figure 4C) and *DQB* (Figure 4D) loci, highly significant internal branches in the phylogenetic trees established clusters including both MCM and human sequences, providing strong support for the hypothesis of ancient allelic lineages. In the case of the *DRB* locus, the topology of the phylogenetic tree was consistent with the existence of ancient allelic lineages, but the internal branches of the tree did not receive significant bootstrap support (Figure 4F). On the other hand, at the *DPA*, *DPB*, and *DRA* loci, there was no evidence of lineage sharing between the two species. These results parallel other studies examining the sharing of allelic lineages between humans and other old world monkeys (Zhu et al. 1991; Kenter et al. 1992; Slierendregt et al. 1995b; Doxiadis et al. 2003). These observations from multiple primate species further indicate that the *DQA*, *DQB*, and probably *DRB* allelic lineages were present in a common ancestor, approximately 30 million years ago (Mya), prior to the separation of old world monkeys and hominids (Kenter et al. 1992; Bontrop et al. 1999; Otting et al. 2000; Otting et al. 2002; Bontrop 2006). Such long-term sharing of allelic lineages is only possible in loci subject to balancing selection acting to maintain polymorphism, and there is strong evidence of such selection at the *DQA*, *DQB*, and *DRB* loci

(Hughes and Nei 1989; Takahata and Nei 1990). The *DPB* locus is known to be less stable over time than other class II β chain loci (Gyllenstein et al. 1996; Bontrop et al. 1999; Bontrop 2006), but the basis of the difference in allelic stability is not well understood. On the other hand, it has long been known that *DRA* and *DPA* are not subject to long-term balancing selection, and that *DQA* is the only class II α chain locus subject to such selection (Hughes and Nei 1989).

At both the *DQA* and *DQB* loci, the alleles from the H2, H4, H5, and H6 haplotypes clustered together (Figure 4C–D). At each locus, this cluster received significant bootstrap support, although relationships were poorly resolved within the cluster (Figure 4C–D). Since these clusters did not include any human alleles, these alleles must have diverged from each other since human and Old World monkey lineages diverged. In order to estimate the time of the common ancestors of these loci, we applied the linearized tree method to a phylogenetic tree based on synonymous substitutions. Using the estimates of the synonymous substitution of Satta et al. (Satta et al. 1993) for primate class II MHC loci ($1.18\text{--}1.45 \times 10^{-9}$ substitutions/site/year), we estimated the age of the common ancestor of the four *DQA* alleles (*Mafa-DQA1*0104*, *Mafa-DQA1*0108*, *Mafa-DQA1*0106*, and *Mafa-DQA1*0107*) at 10–12 Mya; and that of the four *DQB* alleles (*DQBI*060101*, *DQBI*060102*, *DQBI*0611*, and *DQBI*0608*) at 8–10 Mya. These results thus suggest that the linked pairs of *DQA* and *DQB* alleles found in the H2, H4, H5, and H6 haplotypes arose during the same time period, approximately 8–12 Mya.

Discussion

Cynomolgus macaques are used for a wide variety of biomedical studies, including infectious disease, autoimmune, and transplantation research (Yoo et al. 1988; Walsh et al. 1996; Rimmelzwaan et al. 2001; Fouchier et al. 2003; Kuiken et al. 2003; Bosinger et al. 2004; Jahrling et al. 2004; Jonker et al. 2004; Rowe et al. 2004; Yoshioka et al. 2005). The immunogenetics of the animals used in these studies is rarely described, even though the MHC of an individual is known to contribute to the pathogenesis of various infectious diseases, the development of autoimmunity, and the success of a transplant (Bakker et al. 1992; Slierendregt et al. 1995a; Carrington and Bontrop 2002; Bontrop and Watkins 2005; Hale et al. 2005). Therefore, characterizing the immunogenetics of experimental animals is essential for interpreting the results of non-human primate studies.

Recently, we defined six MHC haplotypes and their associated MHC class I alleles in a large feral cohort of cynomolgus macaques from the island of Mauritius (Wiseman et al. 2007). In the current study, we identify the MHC class II alleles expressed at all six class II loci on all six of the MHC haplotypes found in MCMs. The combined data from these two studies provide a comprehensive analysis of the MHC alleles expressed in this exceptional population of cynomolgus macaques. Although Penedo and colleagues used STR typing and MHC exon 2 sequences to characterize MHC haplotypes in rhesus macaques (Penedo et al. 2005), our current study is the first study, to our knowledge, to characterize full-length MHC alleles expressed at all six class II loci on multiple haplotypes in any non-human primate. This unique population of animals with defined MHC genetics will be very useful to researchers who need to control for the immunogenetics of the animals in their study. Moreover, this report establishes a discovery process that can be extended to characterize the MHC class II genetics of other non-human primate populations.

Although exon 2 sequences of MHC class II alleles are useful for determining phylogenetic relationships among non-human primates, they fail to provide researchers with the molecular reagents needed to understand immunological responses. The full-length MHC class II sequences identified in the current study can now be used to generate cell lines expressing

functional class II proteins, develop class II tetramers, and define class II restricted epitopes for specific pathogens (DeMars et al. 1984; Erlich et al. 1986; Kwok et al. 2002). The development of these tools will parallel the previous development of class I tetramers and class I restricted epitopes upon discovery of full-length MHC class I sequences (Altman et al. 1996). These molecular reagents generated with full-length MHC class I sequences greatly improved the identification of pathogen-specific CD8 responses in non-human primate models of diseases such as SIV (Allen et al. 2001). Until now, only a limited number of tools have been developed to study antigen-specific CD4+ T lymphocytes (Kuroda et al. 2000; Dzuris et al. 2001). We anticipate that the increased generation of similar molecular reagents with MHC class II cDNA clones described here will enhance the ability of researchers to identify pathogen-specific CD4 cells during infection of cynomolgus macaques with pathogens such as SIV, SARS, and Ebola both during the natural course of infection and for vaccine studies (Sullivan et al. 2003; Reimann et al. 2005; Lawler et al. 2006).

SIV researchers, in particular, may benefit from the complete characterization of MHC class II genetics in MCM, as SIV infection of non-human primates is the best available model for studying the pathogenesis of HIV (Hu 2005). Previous studies in Indian rhesus macaques have been key to defining the relationship of MHC class I alleles and control of viremia (Carrington and Bontrop 2002; O'Connor et al. 2003; Bontrop and Watkins 2005; Yant et al. 2006). Unfortunately, the diversity of the MHC class II alleles in the Indian rhesus macaque and the limited supply of these animals has prevented researchers from thoroughly characterizing the relationship of MHC class II immunogenetics and control of viremia. Nonetheless, studies have suggested that HIV-specific CD4 cells are important in the control of viremia in patients infected with HIV (Rosenberg et al. 1997; Pitcher et al. 1999; Kaufmann et al. 2004; Norris et al. 2004). As an extension, several groups have identified SIV-specific CD4 cells in various non-human primate models of SIV or SHIV infection (Mills et al. 1991; Lekutis and Letvin 1997; Dittmer et al. 1998; Kuroda et al. 2000; Sarkar et al. 2002). Within these analyses, one group examined MHC class II DRB restriction of the HIV envelope in a SHIV model (Lekutis and Letvin 1997; Kuroda et al. 2000). These same DRB molecules were also shown to present SIV-derived peptides in another study (Dzuris et al. 2001). Unfortunately, however, unlike the detailed identification of MHC class I restricted SIV epitopes, the lack of the appropriate MHC class II molecular reagents has hindered the discovery of MHC class II epitopes. Importantly, we recently demonstrated that MCM are also susceptible to SIV infection with the highly pathogenic SIVmac239 viral isolate (Wiseman et al. 2007). Development of the appropriate molecular tools, based on the present study, will allow researchers to define class II restricted SIV epitopes in MCM and monitor SIV-specific CD4 responses.

In conclusion, we have successfully identified all of the MHC class II alleles expressed on all six of the most common MHC haplotypes in MCM. By combining this study with our previous work (Wiseman et al. 2007), we can now predict the entire repertoire of MHC alleles present in almost all cynomolgus macaques from the island of Mauritius. These studies position MCM as the only non-human primate population with completely characterized MHC class I and II immunogenetics.

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DPA Alleles

DPA Alleles

Signal peptide → Alpha 1 domain → Alpha 2 domain →

Majority MCPEDRMFQTRAVILRTLSLAFLLSLRGAGAKADHVSYTALFVQTHRPTEGYMFEFDEDEQFYVDLDKKETVWHLEEFGRAFSFEAQOGLLANIAALNNNINITTIQLSNHTQAAADPEVTVPFKPEVLGQPNTLTICH

+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+

10 20 30 40 50 60 70 80 90 100 110 120 130 140

Mafa-DPAl*0701F.....M.....TL.....P.....

Mafa-DPAl*0702 V.....A.....S.....M.....TL.....P.....

Mafa-DPAl*0401 .R.....L.FTT.....F.Y.L...I.F.....I.....IS....V.....T.....

Mafa-DPAl*0202S.....DD..T.KY..V.....R.....

Mafa-DPAl*0203 .R.....E.....L...G.....DD..T.KY..R.....

DKFFPPVLNVTLWCNGEPVTEGVASFLRLDPTDYSFHKFXHLYTFVPSAEDFYDCRVHHWGLDPLKHWEAQEIQMPETTEIVLCALGLVLGVIGIVCTVLIKSLSRSGRDPAQGPI-

+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+

150 160 170 180 190 200 210 220 230 240 250 260

Mafa-DPAl*0701RL.....SI..L.AM.....

Mafa-DPAl*0702RL.....R.....Q.....

Mafa-DPAl*0401Q.....T.....Y.....P.....

Mafa-DPAl*0202H.....

Mafa-DPAl*0203

DQA Alleles

DQA Alleles

Signal peptide → Alpha 1 domain → Alpha 2 domain

Majority MLINKALMLGALALTVMSPCGEDIVADHVASCGVNLVYSGYSPSQQTHTFDGDEQFYVDLGRKETAWRWPELSKFGGDFDQCALRNLAVGKHNILTKRYNSTAATNEVFEVTVFSKSPVTLGQNTLLCLVDNIFP

10 20 30 40 50 60 70 80 90 100 110 120 130 140

Mafa-DQA1*2403L.....+.....Y..M.....E.....E...V..QL..LF...RS.....+.....T..S.....+.....H.....

Mafa-DQA1*0503A.....E.....Y.....F...S.....E.....V..QL..V..Q..S..A..F..T..I.....Q...Y..M..QS.....+.....H.....

Mafa-DQA1*0106V..A.....+.....T...N.....E.....V..QL..V..Q..S..A..F..T..I.....AA.....Y..M..QS.....+.....H.....

Mafa-DQA1*0104+.....+.....+.....TV..Y...M.....+.....+.....+.....+.....

Mafa-DQA1*0107V..A.....+.....T...L...F.....R.....+.....+.....L...V.....+.....H.....

Mafa-DQA1*0108+.....I.....+.....V.....+.....+.....TA..Y...M.....+.....+.....+.....

Majority PVVNITWLSNGRSVTEGVSETSLFSKSDHSFFKISYLTFLPSADEIYDCRVEHWGLDEPLLKHWEPEIPAPMSELTEVTVYCALGLSVGLGVIGVVGVTVITQGLRSGASRRHQPL

150 160 170 180 190 200 210 220 230 240 250

Mafa-DQA1*2403+.....+.....+.....K.....+.....+.....M.....+.....L..R.....+.....

Mafa-DQA1*0503H.....+.....A.....K.....+.....+.....+.....+.....R.....+.....

Mafa-DQA1*0106H.....+.....+.....+.....+.....+.....+.....+.....R.....+.....

Mafa-DQA1*0104+.....I.....+.....+.....+.....+.....+.....+.....+.....

Mafa-DQA1*0107+.....+.....+.....+.....+.....+.....+.....+.....+.....

Mafa-DQA1*0108+.....+.....+.....+.....+.....+.....A.....+.....+.....+.....

DRA Alleles

DRA Alleles

Signal peptide → Alpha 1 domain → Alpha 2 domain

Majority: MAXSGVPVLGGFIIAIVLSAQSESNAIKKEHVIIQAEFYLNPDQSGEFMDFDGDGEIFHVDMAKKETVVRLEEFGRFASFEAQGALANIAVDKANLEIMTKRSNNTPIINVPPEVTVLNLSVPELGEPNVLICFIDKFSFP

Mafa-DRA*0102: .I.....

Mafa-DRA*0101: .E.....

Mafa-DRA*0103: .A.....T.....D.....Y.....L.....

CP → TM → Cyto

Majority: VVKVTLWLNKGPVTTGVSETVFLPREDHFLRKFHYLPFLPSTEDIYDCKVEHWGLDAPLLKHVEFDAPSPLPETTEMVVCALGLIVGLVGIIVGTFLIKGVKRSNAAEERGGL-

Mafa-DRA*0102:

Mafa-DRA*0101:

Mafa-DRA*0103:

Figure 1. MHC class II amino acid alignments in Mauritian cynomolgus macaques

Alignments of predicted amino acid sequences for all of the MHC class II alleles described in Mauritian cynomolgus macaques. Protein domains were predicted based on previously described predictions in other non-human primates. CP: connecting peptide, TM; transmembrane domain, and Cyto: cytoplasmic domain.

Figure 2. MHC class II alleles associated with each haplotype in Mauritian cynomolgus macaques

The alleles found to be associated with the H1 through H6 haplotypes are shown. Haplotypes have been color-coded to correspond to our previously published description of these haplotypes (Wiseman et al. 2007). The relative physical arrangement of loci was predicted from the alignment of MHC class II alleles recently described in rhesus macaques (Daza-Vamenta et al. 2004). The arrangement of the DRB alleles within the larger DRB locus, however, is arbitrary. Loci furthest to the left are predicted to be telomeric and closest to the MHC class I loci, while loci to the right are predicted to be centromeric and furthest from the MHC class I loci.

Figure 3. MHC class II haplotype frequencies in a cohort of 117 feral Mauritian cynomolgus macaques

In our previous study, microsatellites were used to examine the relative frequencies of different MHC haplotypes in a cohort of 117 feral MCM (Wiseman et al. 2007). Here, we re-evaluated the microsatellite data for the frequency of MHC class II haplotypes on the 234 chromosomes of this cohort.

Figure 4. Phylogenetic analysis of MCM and human MHC class II alleles

(a) DPA; (b) DPB; (c) DQA; (d) DQB; (e) DRA; (f) DRB. The haplotypes of MCM alleles are color-coded as in Figure 2. The numbers on the branches represent the percent of bootstrap samples supporting the branch; only values $\geq 50\%$ are shown.

Table 1**Table 1a: Primers used to amplify MHC class II alleles**

Locus Amplified	Primer Name	Primer Sequence (5' to 3')	Haplotype Amplified
DPA	5'MHCII-DPA-F	CGTAGTCATCAATTAGAGACCC	H1-H6
DPA	3'MHCII-DPA-R	TCCTAAGTCCTCTTCTGTTCAG	H1-H6
DPB	5'MHCII-DPB-F-1	GCAGCTCTTTTCATTTTGCCATCC	H1-H6
DPB	3'MHCII-DPB-R-1	CTTTTCAGTGAGCTCAGGAACCCTG	H1, H3-H5
DPB	3'MHCII-DPB-R-2	GTCCTGGAACCAGGTGCTAACG	H2, H6
DRA	5'MHCII-DRA-F-1	AGAGCACCCAAGAGGAAAATGGCC	H2, H3
DRA	3'MHCII-DRA-R-1	CTCTGGCCACACCTAACCCACC	H2, H3
DRA	5'MHCII-DRA-F-2	CCGAGCTCTACTGACTCCCAA	H1, H4-H6
DRA	3'MHCII-DRA-R-2	TGGGGTGGCTATAGGGCTGG	H1, H4-H6
DRB	5'MHCII-DRB-F-1	GCGGGATCCATGGTGTGTCTG	H1-H3, H5
DRB	3'MHCII-DRB-R-1	CGCGAATTCTCAGCTCAGGAGTCC	H1-H3, H5
DRB	5'MHCII-DRB-F-2	TGGTCCTGTCCTGTTCTCCAGCA	H1, H4, H6
DRB	3'MHCII-DRB-R-2	AGCTGGGGCAGAAGGTTCT	H1, H4, H6
DQA	5'MHCII-DQA-F-1	CTGAGGCTGCCTTGGGAAGAG	H3
DQA	3'MHCII-DQA-R-1	ACCTTCCCTTCCAGGATGGG	H3
DQA	5'MHCII-DQA-F-2	CTGAGGCTGCCTTGGGAAGAA	H1-2, H4-6
DQA	3'MHCII-DQA-R-2	TTAGGTAGCTGGGTGGCTTACT	H1-2, H4-6
DQB	5'MHCII-DQB-F-1	ACTTTCCCTTCGTCTCAATTAATG	H3
DQB	3'MHCII-DQB-R-1	AACCAATCCCAGTTAAATAGTCTCAGGAG	H3
DQB	5'MHCII-DQB-F-2	CCACTACTTTTCCCTTCGTCT	H1-2, H4-6
DQB	3'MHCII-DQB-R-2	GGCAGGGACAAGTAGGCATT	H1-2, H4-6

Table 1b: Primers used to sequence MHC class II alleles

Locus Sequenced	Primer Name	Primer Sequence (5' to 3')
All	T7	TAATACGACTCACTATAGGG
All	M13R	CAGGAAACAGCTATGAC
DPA	DPA-internal forward	TACAGACGCATAGACCAACAGGGGAG
DPA	DPA-internal reverse	AACACGGTCACCTCAGGGGGATC
DPB	DPB-internal forward	GTCCGGGGCAGGGCCACTCC
DPB	DPB-internal reverse	GGGCCCCTTCTTGGAGGGGG
DRA	DRA-internal forward	AAGAACACGTGATCATCCAGGC
DRA	DRA-internal reverse	GATTGGAGTATTGTTGGAGCGC
DRB	DRB-internal forward	GTGCTGAGCTCCCCACTGGC
DRB	DRB-internal reverse	GCCGCTGCACTGTGAAGCTC
DQA	DQA-internal forward	GTGGCTGACCACGTTGCCTCTT

Table 1a: Primers used to amplify MHC class II alleles

Locus Amplified	Primer Name	Primer Sequence (5' to 3')	Haplotype Amplified
DQA	DQA-internal reverse	TTGGTAGCAGCGGTGGAGTTG	
DQB	DQB-internal forward	TACCAGTTTAAGGGCCTGTGCTACT	
DQB	DQB-internal reverse	ACTGGTAGTTGTGTCTGCACACCGTGT	

MHC class II alleles were identified for each haplotype by cDNA cloning and sequencing. Primers listed in Table 1A were used to amplify class II alleles as described in Materials and Methods. The haplotypes whose alleles were successfully amplified with the specific primers are indicated. Primers listed in Table 1B were used to sequence individual clones at each locus.

Table 2

MHC class II identical alleles described previously

MCM allele name	Previously described identical allele	Previously described Acc. Number ¹	Reference Animal	MCM Accession number
<i>DPA alleles</i>				
Mafa-DPA1*0701	None	None	CR012 (H2)	EF208809
Mafa-DPA1*0702	None	None	CR108 (H1)	EF208810
Mafa-DPA1*0202	None	None	CR011 (H3)	EF208806
Mafa-DPA1*0401	None	None	A3M (H4)	EF208808
Mafa-DPA1*0203	None	None	CR01 (H5) and CR079 (H6)	EF208807
<i>DPB alleles</i>				
Mafa-DPB1*29	Mafa-DPB1: allele 29 (exon 2)	AB235884 (1)	CR011 (H3)	EF208813
Mafa-DPB1*12	Mafa-DPB1*11 (exon 2)	AM086069 (2)	A3M (H4)	EF208812
Mafa-DPB1*42	Mafa-DPB1: allele 37 (exon 2)	AB235892 (1)	CR108 (H1)	EF208814
Mafa-DPB1*21	Mafa-DPB1: allele 21 (exon 2)	AB235876 (1)	CR01 (H5) and CR079 (H6)	EF208811
	Mamu-DPB1*12 (exon 2)	Z32409 (3)		
Mafa-DPB1*43	None	None	CR012 (H2)	EF208815
<i>DQA alleles</i>				
Mafa-DQA1*2403	Mamu-DQA1*02 (exon 2)	M76194 (4)	CR108 (H1)	EF208820
Mafa-DQA1*0503	Mafa-DQA1*1-0503 (exon 2)	AM086055 (2)	CR011 (H3)	EF208819
	Mamu-DQA1*0502 (exon 2)	S76215 (5)		
Mafa-DQA1*0106	None	None	CR01 (H5)	EF208817
Mafa-DQA1*0104	Mamu-DQA1*05 (exon 2)	M76227 (4)	CR012 (H2)	EF208816
	Mafa-DQA1*0104 (exon 2)	AM086051 (2)		
Mafa-DQA1*0107	Mamu-DQA1*09 (exon 2)	M76200 (4)	A3M (H4)	EF208818
	Macaca arctoides-DQA1*01 (exon 2)	M76204 (4)		
Mafa-DQA1*0108	None	None	CR079 (H6)	
<i>DQB alleles</i>				
Mafa-DQB1*1601	Mamu-DQB1*1603 (exon 2)	AY053443 (6)	CR011 (H3)	EF208824
	Mafa-DQB1*1601 (exon 2)	AJ308064 (7)		
Mafa-DQB1*1801	Mamu-DQB1*1810 (exon 2)	AF071579 (8)	CR108 (H1)	EF208825
	Mafa-DQB1*1801 (exon 2)	AJ308068 (8)		
Mafa-DQB1*0611	Mafa-DQB1*0611 (exon 2)	AJ308059 (8)	CR01 (H5)	EF208823
Mafa-DQB1*060101	None	None	CR012 (H2)	EF208821
Mafa-DQB1*0608	Mafa-DQB1*0608 (exon 2)	AJ308056 (8)	A3M (H4)	EF208822
Mafa-DQB1*060102	None	None	CR079 (H6)	

MCM allele name	Previously described identical allele	Previously described Acc. Number ¹	Reference Animal	MCM Accession number
<i>DRA alleles</i>				
Mafa-DRA*0102	Mamu-DRA*01021 (complete cds)	AJ586874 (9)	CR012 (H2), CR011 (H3), A3M (H4)	EF208827
Mafa-DRA*0101	None	None	CR01 (H5)	EF208826
Mafa-DRA*0103	None	None	CR108 (H1)	EF208828
<i>DRB alleles</i>				
Mafa-DRB1*1001	Mafa-DRB1*1001 (exon 2)	AY340690 (10)	CR012 (H2)	EF208832
	Mafa-DRB*02 (complete cds)	AF492279 (11)		
	Mafa-DRB*cyn025 (exon 2)	AF492304 (11)		
Mafa-DRB*w402	Mafa-DRB*W402 (exon 2)	AY340695 (10)	CR012 (H2)	EF208829
	Mafa-DRB*03 (complete cds)	AF492277 (11)		
	Mafa-DRB*cyn020 (exon 2)	AF492334 (11)		
Mafa-DRB1*1002	Mafa-DRB1*1002 (exon 2)	AY340691 (10)	CR011 (H3)	EF208833
	Mafa-DRB*06 (complete cds)	AF492275 (11)		
	Mafa-DRB*cyn019a (exon 2)	AF492333 (11)		
Mafa-DRB5*0301	Mafa-DRB5*0301 (exon 2)	AY340698 (10)	CR01 (H5), A3M (H4)	EF208835
	Mafa-DRB*10 (complete cds)	AF492288 (11)		
	Mafa-DRB*cyn12ac (exon 2)	AF492325 (11)		
	Mamu-DRB5*0301 ² (exon 2)	Z26161 (12)		
Mafa-DRB*w2101	Mafa-DRB*W2101 (exon 2)	AY340688 (10)	CR108 (H1)	EF208831
	Mafa-DRB*04.0 (complete cds)	AF492295 (11)		
	Mafa-DRB*cyn021 (exon 2)	AF492301 (11)		
	Mane-DRB*02a (exon 2)	L76690 (13)		
Mafa-DRB*w501	Mafa-DRB*W501 (exon 2)	AY340689 (10)	CR108 (H1)	EF208830
	Mafa-DRB*12 (complete cds)	AF492305 (11)		
	Mafa-DRB*cyn016 (exon 2)	AF492330 (11)		
	Mane-DRB7*02a (exon 2)	L76689 (13)		
Mafa-DRB4*0101	Mafa-DRB4*0101 (exon 2)	AY340697 (10)	A3M (H4)	EF208834
	Mafa-DRB*08 (complete cds)	AF492276 (11)		
	Mafa-DRB*cy007a (exon 2)	AF492347 (11)		
Mafa-DRB1*0402	Mafa-DRB1*0402 (exon 2)	AY340694 (10)	CR079 (H6)	
	Mafa-DRB1*402 (complete cds)	DQ381741 (11)		
	Mafa-DRB*cyn003a (exon 2)	AF492337 (11)		
Mafa-DRB*w401	Mafa-DRB*w401 (exon 2)	AY340693 (10)	CR079 (H6)	
	Mafa-DRB*w401 (complete cds)	DQ381744 (11)		
	Mafa-DRB*cyn009a (exon 2)	AF492318 (11)		

¹ The reference for each previously described allele is shown in parentheses.

² Name inferred from previously published manuscript (Blancher et al. 2006)

MHC class II alleles identified in this study were compared with other known MHC alleles found in other macaque species. Previously named alleles with identical sequences are listed with their associated Accession numbers. The reference animal used in this study is shown. References listed are: (1) (Sano et al. 2006), (2) (Doxiadis et al. 2006), (3) (Sliereendregt et al. 1995b), (4) (Kenter et al. 1992), (5) (Christ et al. 1994), (6) (Vigon and Sauermann 2002), (7) (Otting et al. 2002), (8) (de Groot et al. 1998), (9) (de Groot et al. 2004), (10) (Leuchte et al. 2004), (11) (Blancher et al. 2006), (12) (Sliereendregt et al. 1992), (13) (Gaur and Nepom 1996)