ABSTRACT

MHC class I allele characterization in Indonesian cynomolgus macaques

There has recently been increased interest in the use of cynomolgus macaques for the study of infectious diseases. However, little focus has been given to studying the immunogenetics of these animals. To improve researcher's understanding and utilization of these model organisms, we characterized 48 full length MHC (Major Histocompability Complex) alleles in Indonesian cynomolgus macaques via cloning and sequencing. Furthermore, we found that three of these alleles were originally found in cynomolgus macaques of Mauritian origin, supporting the hypothesis that Mauritian animals originally came from Indonesia. The Mauritian alleles, *Mafa-B*4501*,*Mafa-B*5101*, and *Mafa-B*1201* present in 19%, 7% and 4% of our cohort respectively according to PCR-SSP results.

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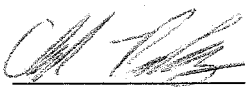

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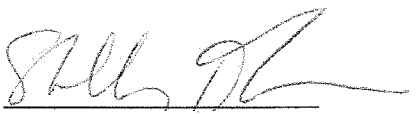

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COVER SHEET

TITLE: MHC class I allele characterization in Indonesian cynomolgus macaques.

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Abstract

*There has recently been increased interest in the use of cynomolgus macaques for the study of infectious diseases. However, little focus has been given to studying the immunogenetics of these animals. To improve researcher's understanding and utilization of these model organisms, we characterized 48 full length MHC (Major Histocompability Complex) alleles in Indonesian cynomolgus macaques via cloning and sequencing. Furthermore, we found that three of these alleles were originally found in cynomolgus macaques of Mauritian origin, supporting the hypothesis that Mauritian animals originally came from Indonesia. The Mauritian alleles, Mafa-B*4501,Mafa-B*5101, and Mafa-B*1201 present in 19%, 7% and 4% of our cohort respectively according to PCR-SSP results.*

Introduction

In the study of various infectious diseases and immunological responses, non-human primates have proven to be invaluable as a model organism for research. Recently, one of the most common primate models, the Indian rhesus macaque, has become increasingly difficult to obtain due to export restrictions placed by the Indian government (Marshall 2004). Fearing that this may delay or hinder current research, several institutions such as the National Institution for Health (NIH) have become actively involved in finding alternative non-human primates for research (ILAR Journal 2003).

One non-human primate that has been receiving increased attention is the cynomolgus macaque (*Macaca fascicularis)*. This species is becoming more widely used as a model for SIV, SARS infection, autoimmunity and anthrax studies around the world due to its abundant availability (Lawler et al. 2006; Negri et al. 2006; Vasconcelos et al. 2003). However, the use of a new model organism for research has drawbacks. The genetics behind immunological responses for cynomolgus macaques is relatively unknown. More specifically, the genetics of the major histocompatability complex (MHC) in these animals is not well understood.

The MHC is a receptor protein that presents peptide fragments from both internal and foreign proteins on the surface of cell membranes. CD8+ cytotoxic T lymphocytes recognize MHC class I proteins presenting peptides potentially derived from microbes and can stimulate the lysis of infected cells (Bontrop & Watkins 2005). Furthermore, the MHC region of the genome is characterized by a high degree of polymorphism, which allows cells to present a wider variety of peptides (Rudolph et al. 2006). The integral role of the MHC in immune responses makes it an important topic for immunogenetic studies.

The cynomolgus macaque has also proven to be a valuable animal model in the development of a human immunodeficiency virus vaccine due in part to its ability to develop AIDS-like symptoms after infection with simian immunodefienciy virus (SIV)(Reimann et al. 2005). In macaque SIV vaccine trials, there appears to be a significant role for CD8+ T Cells in suppressing SIV viral loads (Schmitz et al. 1999), and these responses are dependent on class I MHC-peptide complex interactions with CD8+ T cells. In SIV infected macaques, the MHC-peptide complex often restricts antigen presentation to T cells, a concept that is referred to as MHC class I restricted epitopes. The defining of class I restricted T cells helps researchers to better characterize and examine T cell responses during SIV infection. However, without sufficient knowledge of the MHC class I alleles in macaques, it is difficult to describe these restricted epitopes. To our knowledge, there are only a handful of published studies on MHC Class I alleles in cynomolgus macaques (Krebs et al. 2005, Otting et al. 2007, Uda et al. 2004, Uda et al. 2005, Wiseman et al. 2007). Due in part to the lack of information on the MHC of cynomolgus macaques, there has been only two studies published on defining SIV derived CTL epitopes in cynomolgus macaques (Negri et al. 2006, Geretti

et al. 1997). Thus, the characterization of MHC class I alleles in Indonesian cynomolgus macaques will progress the understanding of T cell responses in SIV research.

Our lab recently identified that cynomolgus Macaques from the island of Marutius have uniquely simple MHC immunogenetics, making these animals useful as model organisms in immune response studies (Wiseman et al. 2007). It has been suggested that the cynomolgus macaques were introduced to Mauritius by European travelers approximately 400 years ago (Sussman and Tattersal 1986), and that the small colonizing population led to extensive allele sharing among Mauritian cynomolgus macaques in the polymorphic MHC loci (Krebs et al. 2005). The origin of these macaques may provide insight into the regional differences in the MHC between subpopulations of cynomolgus macaques, an observation that is often overlooked by researchers when chosing animals for their studies. Recent analysis of mitochondrial and Y-chromosomal DNA suggests that the most probable origin of Mauritian macaques is Java or Sumatra (Tosi & Coke, 2006).

We hypothesized that due to a common ancestry, Mauritian and Indonesian cynomolgus macaques would share MHC class I alleles. To test this hypothesis, we characterized 48 full length MHC Class I alleles in cynomolgus macaques of Indonesian origin. Through cloning and sequencing methods, we found three alleles that were shared between Indonesian and Mauritian cynomolgus macaques. Furthermore, PCR-SSP genotyping revealed a more extensive sharing of the alleles, *Mafa-B*4501* and *Mafa-B*5101*, both of which are found in 15% of Mauritian cynomolgus macaques according to STR data. This study provides further evidence that Mauritian cynomolgus

macaques most likely originated in Indonesia in addition to providing researchers with the means to more efficiently study immune responses in cynomolgus macaques.

Materials and Methods

RNA was isolated from the processed blood via magnetic particles using the Roche Magnapure kit. cDNA was generated using the Invitrogen Superscript 3 Kit. The MHC alleles were amplified from the cDNA using PCR with primers specific for conserved sequences just outside the MHC region. The PCR products were gel purified, and the excised bands purified using the Qiagen Qiaquick PCR Gel Extraction kit. The blunt purified PCR product was ligated into pcr-BLUNT vectors using the Invitrogen Zero Blunt Cloning kit, and the plasmids were transformed into E. coli via heat shock. The E. coli containing complete plasmids are selected for by growing the E. coli on LB Agar plates with 50 μg/mL kanamycin.

After the transformation, bacteria was plated on LB Agar plates with kanamycin, and incubated for 17 to 24 hours. Following the incubation, the colonies were picked and grown in Circle Grow with kanamycin for 17 to 24 hours. The plasmid was then vacuum purified using the Perfectprep Plasmid 96 Vac, Direct Bind kit. A restriction digest was performed using the EcoRI restriction enzyme, and the digested DNA appeared as two bands when run on a 1.3% agarose gel with the insert around 800-900 bp and the vector around 3.5 Kb. 96 clones with correct inserts were sequenced from each animal, 48 MHC class I A clones, and 48 MHC Class I B clones.

When sequencing the MHC clones, four primers were utilized. Two of the primers, M13 and T7, are located in the vector. Additionally, both consensus forward and reverse internal primers were used. Sequencing reactions will be performed with the

DYEnamic ET Terminator Cycle Sequencing Kit. Sequences were run on an ABI 3730 DNA Analyzer, and analyzed with CodonCode.

Sequence-specific PCR assays for *the Mafa-B*4501*, *Mafa-B*5101*, and *Mafa-B*1201* alleles were designed using MegAlign software (DNASTAR, Madison, WI) and Primer3 (v. 0.4.0). The primers were optimized for use with the Amplitaq Gold PCR Master Mix at 5 μM. A set of positive control primers designed to bind non-specifically to the MHC locus was run simultaneously with identical conditions. Samples were run on MJ Research PTC-225 thermocyclers at 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. All primers were designed to have an optimal annealing temperature of 65 ˚C. PCR products were resolved on a 1.8% agarose gel and visualized with SYBR Safe DNA gel stain (Invitrogen, Eugene, OR).

Results and Discussion

After completing the cloning and sequencing of 11 Indonesian cynomolgus macaques, we characterized 48 full length MHC Class I alleles. To better understand the MHC in these animals, we chose to characterize alleles from animals from various regions of Indonesia, including Jakarta, Tinjil Island and Sumatra. Of these 48 alleles, 41 were classified as novel according to MHC class I sequences available in Genbank. These alleles are represented in Table 1. Due to the lack of MHC class I alleles reported for Indonesian cynomolgus macaques, we expected to find that a large fraction of our alleles would be novel. Approximately 85% of our characterized alleles were determined to be unique, confirming this prediction.

Of the seven previously identified alleles, three were identified as identical to alleles found in Mauritian origin cynomolgus macaques (*Mafa-B*120101, Mafa-B*4501 and Mafa-B*5101)* (Krebs et al. 2005). *Mafa-B*4501* and *Mafa-B*5101* are found on a common haplotype that presents in 15% of Mauritian cynomolgus macaques. B*1201 on the other hand, is found on a more rare Mauritian MHC haplotype, but has also been characterized in cynomolgus macaques of Malaysian origin. To examine the extent of sharing of these alleles between Mauritian and Indonesian macaques, PCR-SSP primers were designed in order to screen our entire cohort of 42 animals. The results indicate that of these 42 Indonesia animals, eight animals share *Mafa-B*4501,* three animals share Mafa-*B*5101* and two animals share *Mafa*-B*1201. These results suggest that *Mafa-B*4501* appears at a frequency of 19% and *Mafa-B*5101* appears at a frequency of 7% in our representative sample of Indonesian animals. With our limited sample population, it is difficult to discover multiple alleles shared between Mauritian and Indonesian cynomolgus macaques. It is possible that a further investigation into the MHC genetics of Indonesian cynomolgus macaques will reveal additional allele sharing. However, our results still support the hypothesis proposed by Tosi and Coke that Mauritian and Indonesian cynomolgus macaques share a common ancestry.

This study provides additional immunogenetic information for Indonesian cynomolgus macaques. With this data, researchers will be more capable of generating tools to more effectively study immune responses in cynomolgus macaques. In addition, the sharing of alleles between Mauritian and Indonesian cynomolgus macaques further supports the notion that Mauritian animals most likely originated from an Indonesian population.

ICM allele name	ICM Accession #	Reference animal	Previously described identical allele[1]
Mafa-A alleles			
Mafa-A1*060102	EU203689	IN03 (02347)	
Mafa-A1*1002	EU203687	IN02 (02326)	Mane-A*19 (pig-tail, EF010520);Mafa-A1-1002 (unknown cynomolgus, AM295831); Mamu-A1*1001 (Chinese rhesus, AM295894)
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)
Mafa-A1*4103	EU203713	CE16 (13655)	
Mafa-A1*6003	EU203698	IN10 (04141)	
Mafa-A1*6202	EU203711	CE12 (13651)	
Mafa-A1*6603	EU203712	CD12 (13651)	
Mafa-A1*7001	EU203708	CD28 (13668)	Mafa-A1*7001 (unknown cynomolgus, AM295858)
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	IN03 (02347)	
Mafa-B alleles			
Mafa-B*0302	EU203720	CE29 (13670)	
Mafa-B*0702	EU203704	IN12 (04146)	
Mafa-B*1201	EU203690	IN03 (02347), IN10 (04141)	Mafa-B*12 (Malaysian and Mauritian cynomolgus, AB195442)
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)
Mafa-B*5002	EU203693	IN03 (02347)	
Mafa-B*5101	EU203718	CE28 (13668)	Mafa-B*510101 (Mauritian cynomolgus, AY958150)
Mafa-B*5601	EU203714	CE16 (13655)	
Mafa-B*5701	EU203719	CD28 (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	IN03 (02347)	
Mafa-B*7701	EU203692	IN03 (02347)	
Mafa-B*7801	EU203694	IN03 (02347), CE28 (13668)	
Mafa-B*7901	EU203695	IN03 (02347)	Mamu-B*05 (Indian rhesus, U41827)

Table 1 The allele name, as given by the Non-human Primate Immuno Polymorphism Database-MHC (IPD-MHC) nomenclature committee, and genbank accession number are described. The reference animal for each allele is listed as well as previously named alleles with identical sequences.

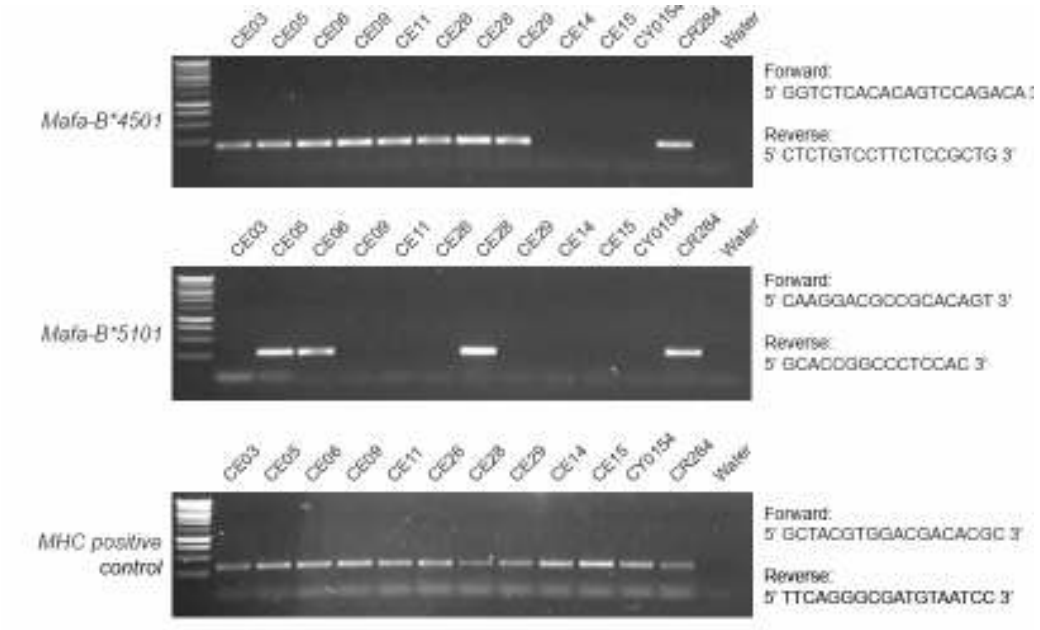

Figure 1 PCR-SSP results with illustrating allele sharing for *Mafa-B*4501* and *Mafa-B*5101* among Indonesian and Mauritian cynomolgus macaques. According to STR data (not shown), CR284 was included as a positive control Mauritian cynomolgus macaque having both Mafa-B*4501 and Mafa-B*5101. Similarly, CY0154 was included as a negative control Mauritian cynomolgus macaque lacking both alleles. A set of positive control primers designed to bind to a conserved area of the MHC region were included to confirm the validity of the cDNA.

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