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MHC haplotype frequencies in a UK breeding colony of Mauritian cynomolgus macaques mirror those found in a distinct population from the same geographic origin

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Abstract

Background—Mauritian cynomolgus macaques have greatly restricted genetic diversity in the MHC region compared to other nonhuman primates; however the frequency of common MHC haplotypes among captive-bred populations has not been reported.

Methods—Microsatellite PCR was used to determine MHC haplotype frequencies among captive macaques at a UK breeding facility. Allele-specific PCR and Reference Strand Conformational Analysis were used to determine the allele expression profile of a subset of animals.

Results—Haplotypes H3 (21%) and H1 (19%) were most common in the captive population of Mauritian cynomolgus macaques. Predicted alleles were detected by allele-specific PCR-SSP in 99% of animals. Allele expression profiles were similar in animals with identical haplotypes.

Conclusion—Mauritian cynomolgus macaques in the UK breeding facility have restricted MHC diversity comparable to a previously described population. Microsatellite-derived haplotypes are highly predictive of allele expression. A selective breeding program has been established to produce MHC-identical animals for biomedical research.

Keywords

immunogenetics; selective breeding; haploidentical animals; *Macaca fascicularis*

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Introduction

Cynomolgus macaques are a valid and more readily available alternative to Indian rhesus macaques for use in non-human primate models of HIV infection. They have been used for many years (predominantly in Europe) in studies of pathogenesis [5, 6, 8, 17, 20, 29], evaluation of antiretroviral therapy [3, 9, 15, 32] and vaccine development [7, 18, 19, 23, 28, 31]. Until recently however, thorough characterization of cellular immune responses induced by infection or immunization with candidate vaccines has been limited due to a lack of information about MHC class I gene sequences in cynomolgus macaques.

The MHC alleles expressed by macaques can dramatically influence cellular immune responses and infection dynamics. For example certain rhesus macaques MHC class I alleles, e.g. *Mamu-A*01* and *Mamu-B*17*, present SIV-derived peptides that elicit protective cellular immune responses, leading to lower viral loads [21, 22, 24, 38, 39]. While such effects are of interest in determining the role of cellular immunity in SIV infection, atypical infection dynamics (which are frequently observed, regardless of the species used) in just one animal can confound the interpretation of experimental data, since experiments are ethically and financially limited in size.

Only recently have comprehensive studies of MHC diversity at the genetic level in cynomolgus macaques been carried out [1, 12, 16, 25, 33–36]. The population of cynomolgus macaques on the island of Mauritius has been shown to have a remarkably restricted degree of MHC diversity, with six common haplotypes (designated H1 – H6) accounting for almost all MHC class I and II diversity seen in these animals [25, 36]. In contrast, the extensive diversity of MHC genes in rhesus macaques [2, 4, 13, 26] means that only limited matching of specific MHC class I alleles among experimental animals is possible and completely shared MHC haplotypes have been reported only in closely related animals [27, 37].

Mauritian cynomolgus macaques thus represent a very valuable resource for biomedical research, since their restricted MHC diversity makes it feasible to identify animals with identical MHC class I and II genes. Furthermore, a pilot study has shown that Mauritian cynomolgus macaques carrying identical MHC haplotypes mount comparable cellular immune responses following infection with the widely-used virus isolate SIVmac239 [36]. The use of MHC-matched animals would contribute to reduced experimental variation, facilitate more detailed characterization of cellular immune responses and increase the power of the cynomolgus macaque model in SIV/HIV pathogenesis and vaccine research.

Cynomolgus macaques derived from stock of Mauritian origin at a UK breeding facility are used for SIV/HIV and other infectious disease research. The self-sustaining colony, comprising approximately 355 animals, was established initially from animals imported from a breeding and export facility in Israel which sources the animals from Mauritius. We sought to determine the frequency of common MHC haplotypes among these animals and to determine whether the frequencies of individual haplotypes among captive animals were similar to those previously reported in animals obtained directly from Mauritius [36]. Furthermore, we sought to determine whether it would be feasible to establish a new

breeding group of Mauritian cynomolgus macaques comprised of animals carrying specific MHC haplotypes, to allow for selective breeding of MHC-identical animals for future studies. We employed microsatellite PCR with a panel of markers that span the MHC region to determine haplotype frequencies in our captive population. Using allele-specific PCR for selected alleles, we evaluated the correlation between predicted allele expression on the basis of microsatellite haplotypes and allele transcripts detected in mRNA. Using Reference Strand Conformation Analysis, we confirmed that animals carrying identical haplotypes had similar allele expression profiles, and selected 19 animals expressing the two most common haplotypes to form a new breeding group.

Materials and methods

Animals

Three hundred and fifty five Mauritian cynomolgus macaques housed at the breeding facility were included in the study. The colony was founded from five batches of first generation captive-bred animals imported into the UK from a distributor in Israel between 1996 and 2004 and 11 second generation captive-bred animals imported from Mauritius in 2003. The current colony is comprised of these founder animals and their first or second generation offspring. Animals were housed and maintained in accordance with UK Home Office guidelines for care and maintenance of nonhuman primates. Samples from 331 cynomolgus macaques imported into the US from Mauritius, including 117 previously described animals [36], were typed for comparison.

DNA extraction

EDTA-treated blood samples were obtained from all animals. DNA was extracted by standard phenol-chloroform method, resuspended in molecular-grade water, quantified using Hoescht dye (Sigma-Aldrich) and diluted to 10 ng/ μ l.

RNA extraction and cDNA synthesis

RNA was extracted from 1.5 ml fresh EDTA-treated blood using the QIAamp RNA blood mini kit (Qiagen) according to the method recommended by the manufacturer.

Approximately 1 μ g RNA was converted to cDNA using the Accuscript High Fidelity 1st Strand cDNA Synthesis Kit (Stratagene).

Microsatellite analysis

Microsatellite analysis was performed using one of two methods, incorporating modifications to the previously described assay [36] in order to improve sensitivity. Ten reference samples representing the six common haplotypes were typed by both method 1 and method 2 to assess consistency and to set bins for genotyping.

Method 1—Microsatellite analysis was performed on 207 samples using the 18 primer sets described in [36], with the complementary primer for D6S2972 replaced with 5'-GAT AAG GGG GAA CTA CTA CA-3'. A 10ng amount of DNA was amplified in 15 μ l multiplex or single reactions using either 0.3U Phusion Hot Start DNA polymerase in 1 $\times$ HF buffer (New England BioLabs) or 0.6U Fast Start Taq polymerase in 1 $\times$ Fast Start buffer containing

1.8mM MgCl₂ (Roche), 200 µM each dNTP and primer concentrations as detailed in Supplemental Table 1. Products were amplified using T3 thermocyclers (Biometra) in 0.2ml thin walled 8 tube strips (ABgene). Cycling conditions were: 95°C for 4 minutes; 3 cycles of 95°C for 30 seconds, 64°C for 30 seconds, 72°C for 30 seconds; 3 cycles of 95°C for 30 seconds, 62°C for 30 seconds, 72°C for 30 seconds; 3 cycles of 95°C for 30 seconds, 60°C for 30 seconds, 72°C for 30 seconds; 6 cycles of 95°C for 30 seconds, 58°C for 30 seconds, 72°C for 30 seconds; 25 cycles of 95°C for 30 seconds, 50°C for 30 seconds, 72°C for 30 seconds; 72°C for 30 minutes. One microliter of neat or 1:10 dilution of PCR product was mixed with 0.4 µl Genescan ROX-500 size standard and 8.6 µl HiDi formamide (Applied Biosystems). Samples were denatured for 5 minutes at 95°C and electrokinetically injected at 2.0 kV for 10 seconds into a 36cm capillary filled with POP7 polymer and separated at kV for 900 seconds on an ABI 3130xl genetic analyzer (Applied Biosystems). Analysis was carried out using GeneMapper software v4.0 (Applied Biosystems).

One hundred and forty eight samples were amplified with Method 2, which represents a refinement of Method 1, with incorporation of more informative markers in certain regions of the MHC [as described in 11]. Briefly, in panel 2, C4_2_25 was replaced with P03-193435 at a primer concentration of 0.3 µM and the concentrations of D6S2704 and MICA primers were reduced to 0.08 µM and 0.15 µM respectively. The two markers in panel 6 were replaced with D05-104184 and D05-144699, each at a primer concentration of 0.3 µM.

Haplotypes were inferred according to the previously described marker sizes [36]. Where data from parents or siblings were available, haplotypes were confirmed by segregation analysis. A haplotype was assigned if 16/18 markers had the expected allele sizes. In most cases, discrepancies between the expected and apparent allele sizes could be explained by the addition or subtraction of a single repeat unit from the marker. In several cases where some markers failed to amplify due to PCR failure, the haplotype was assigned if at least 10 unambiguous markers were present. Of the cohort of animals obtained directly from Mauritius, 117 were previously described [36], while the remainder were typed according to Method 2.

Detection of transcribed alleles by PCR using Sequence-Specific Primers (PCR-SSP)

Due to limited quantities of cDNA, full-length *M. fascicularis* class I B (*Mafa-B*) transcripts were first amplified non-specifically from the cDNA using 0.05µM degenerate primers 5'-MHC-FULL-D: 5'-GCG GCC GCA TGS SSR TCA TGG CKC CSS G-3' and 3'-MHC-FULL-D: 5'-GGT ACC TCA AGC YGT GAG AGA CWC ATC AGA GCC-3'. A 1.2µl volume of cDNA was amplified in a 20µl reaction containing 0.4U Phusion Hot Start DNA polymerase, High Fidelity Buffer, 2% DMSO (NEB) and 200µM each dNTP (Roche) using the following cycling conditions: 98°C for 60 seconds; 25 cycles of 98°C for 5 seconds, 65°C for 1 second, 72°C for 20 second; 72°C for 5 minutes. Four class I B alleles were amplified from cDNA using 0.5µM sequence specific primers: B*430101 was amplified with sense primer 5'-CGC ACT CCA TGA GGT ATT TCA C-3', and antisense primer 5'-CCC TCC TGC TCC ATC CAC T-3'; B*440101 was amplified with sense primer 5'-GAG CCC TGG TAT CTC GAA GTT-3' and antisense primer 5'-GGT GAG TCT GTG CGG

TGA-3'; B*460101 was amplified with sense primer 5'-GGG AGC CCC GCT TCA C-3' and antisense primer 5'-CCA TCT CCT GCT GGC CTA-3'; B*510101 was amplified with sense primer 5'-CAA GGA CGC CGC ACA GT-3' and antisense primer 5'-GAT ACC CGC GGA GGA GGT-3'. One microliter of amplified MHC from the first round reaction was amplified in a 20 μ l reaction using the same cycling conditions, but with the annealing temperature increased to 67°C (B*440101, B*460101 and B*510101) or 69°C (B*430101). Full-length *Mafa-B* was amplified from each sample as a positive control for the presence of intact *Mafa-B* cDNA. Five picograms of plasmid containing clones of the specific alleles were amplified as positive controls. Amplified fragments were separated on 1.7% (w/v) agarose gels and sized relative to a ϕ X174/HaeIII size ladder (Promega). Samples were scored positive for each allele if an appropriate sized allele specific band (B*430101, 163bp, B*440101, 169bp, B*460101, 664bp, B*510101, 140bp) and full-length *Mafa-B* (1098 bp) were present.

Reference Strand Conformational Analysis (RSCA)

RSCA was adapted from a previously described method [36]. A 305 bp fluorescent-labeled reference strand (FLR) was amplified from three plasmid clones (*Mafa-A**200101 (GenBank no. AY958088), *Mafa-B**370101 (GenBank no. AY958132) and *Mamu-B**07 (GenBank no. AY958132)) using the labeled primer 5'RSCA-Long-N: 5'-6-carboxyfluorescein-[FAM] GCT ACG TGG ACG ACA CGC-3' and the complementary primer 3'RSCA-Long-C: 5'-GTT CAG GGC GAT GTA ATC-3' (Sigma Genosys). Product was amplified in 20 μ l reactions using 0.5 μ M primers, 200 μ M each dNTP (Roche), 0.4 U Phusion Hot Start DNA polymerase and High Fidelity Buffer (NEB) and 1 ng plasmid containing the allele clone. Cycling conditions were: 98°C for 60 seconds; 25 cycles of 98°C for 5 seconds, 63°C for 1 second, 72°C for 20 seconds; 72°C for 5 minutes. FLRs were purified using the QIAquick PCR Purification Kit (Qiagen) and diluted 1:30 in water immediately prior to use. Comparable fragments were amplified from 18 *Mafa-A* and *Mafa-B* clones (A*200101, A*250201, A*290101, A*320101, A*470101, B*370101, B*430101, B*440101, B*460101, B*470101, B*480101, B*490101, B*510101, B*600101, B*630101, B*640101, B*650101, B*660101) to ensure that each allele displayed a unique mobility profiles, and from sample cDNA using unlabelled primers. A 1.5 μ l volume of standard or 3 μ l sample was mixed with 3 μ l diluted FLR and denatured at 95°C for 5 minutes. The reaction was then cooled at -1°C/s to 55°C, held for 5 minutes and cooled at -1°C/s to 15°C. Samples were refrigerated until use. Immediately prior to use the sample was diluted 1:5 and 2 μ l was added to 8 μ l water containing 0.08 μ l ROX-ET900 size ladder (GE Healthcare). Samples were electrokinetically injected at 7.5kV for 10 seconds into a 36cm capillary filled with 6.3% non-denaturing GeneScan polymer (Applied Biosystems) and separated at 7.5 kV for 20 minutes at a constant temperature of 30°C. Apparent mobility was defined in arbitrary units corresponding to the scan number at which the peaks of the ROX-ET900 ladder were detected. Standards were used to set mobility bins for each FLR/allele combination using GeneMapper software v4.0 (Applied Biosystems). Samples were scored positive for a given allele if they contained a peak falling into the appropriate bin for all three FLRs. All samples were re-examined manually to ensure correct calling of peaks.

Results

Microsatellite-based haplotyping of the MHC region

Three hundred and fifty five animals were typed for the six common MHC haplotypes (H1 – H6) previously described in Mauritian cynomolgus macaques [36] using either of two microsatellite-based PCR assays. Haplotypes were assigned based on previously described marker sizes, and confirmed by segregation analysis where data from parents and/or siblings was available. The assay used did not affect the haplotype assignment, as assessed by typing of ten samples with both assays (data not shown). H3 was most common among this group of animals (21%), followed by H1 (19%) and H2 (9%) (Fig. 1A). Thirty-five percent of haplotypes were recognized as being simple recombinants of two or three of the six major haplotypes. A total of 53 recombinant configurations were identified, with elements of all major haplotypes represented. With the exception of recombinant haplotypes comprising elements of H1, H2 or H3 (which share class I A alleles [36]), and having the breakpoint in the class I A region, or between class I A and class I B, all recombinant haplotypes are predicted to carry different configurations of class I alleles to the parent haplotypes.

The distribution of haplotypes was comparable to that reported previously [36] as well as an expanded cohort of 331 animals sourced directly from Mauritius (Fig. 1B). Haplotype H3 was somewhat more common among the captive-bred animals, while H4 appeared slightly less common (Fig. 1). Of the animals characterized, 37% carried at least one copy of H3 and 34% carried at least one copy of H1, with 22 animals homozygous for one of the six common haplotypes, predominantly H1 and H3 (Fig. 1C). The distribution of haplotypes was similar irrespective of the original origin of the animals, i.e. imported directly from Mauritius, imported from Israel or UK-born (data not shown). Haplotype H7 was detected in four animals (~1 % of total haplotypes) in this study. The microsatellite allele sizes for haplotype H7 are described in Table 1. For markers D6S2691 and D6S2793, two different allele sizes were associated with haplotype H7, likely representing expansion and contraction of the intrinsically unstable microsatellites. Although this haplotype was not detected in the initial study [36], it has since been detected in four animals in the expanded cohort of 331 animals from Mauritius as well as in six additional Mauritian cynomolgus macaques obtained from a facility in Israel (E.M., A.B., R.W., J.K., unpublished data).

Comparison of predicted and transcribed *Mafa-B* alleles

Presence of specific class I B allele transcripts was determined in cDNA from 70 animals in the current study by allele-specific PCR using sequence specific primers (PCR-SSP). In 57 of 58 (98%) animals with unambiguous predictions on the basis of microsatellite-derived MHC haplotypes, detected alleles were consistent with those predicted (Table 2). In the animal with discordant results, the unexpected SSP bands detected were faint relative to other samples (Fig. 2 B, C, lane 4 and Table 2, animal M899). In 12 animals (17%), microsatellite-based predictions were ambiguous due to recombination. In these cases, PCR-SSP resolved the MHC class IB genotypes. For example, PCR-SSP demonstrated that three animals (I142, M582A, and M693) had inherited the class IB alleles of the H3 haplotype (Mafa-B*510101-positive) rather than those of the H1 haplotype (Table 2).

Selection of animals for formation of MHC-defined breeding colony

From the 355 animals typed by microsatellite PCR, 19 animals homozygous for H1 or H3, or heterozygous for these two haplotypes were combined into a new breeding colony (Fig. 3). The new colony consists of one male, homozygous for H1, and 18 females: four homozygous for H1, two homozygous for H3 and 12 heterozygous for H1 and H3. The age range of these animals is 7 – 14 years.

Expression of predicted class I alleles as determined by RSCA

Among the 19 animals included in the haplotype-defined colony, RSCA revealed complete concordance between predicted and expressed alleles for B*440101 (17/17 animals) and B*510101 (14/14) (Table 3). The peaks corresponding to these alleles were of significantly greater intensity than other peaks in the sample (Fig. 4). Allele B*430101 was detected in 15 of 17 animals in which expression was predicted. In the remaining two animals, a peak of appropriate mobility was detected using two FLR, but not with the third FLR. Alleles A*250201/A*250301 were detected in 14 of 19 animals and in a further two animals peaks of appropriate mobility were detected with two FLR. Allele A*290101 was detected in 3 of 19 animals. In those animals in which it was detected, peak intensity was typically less than 100 fluorescence units (threshold value for a positive peak was set at 75 units). Allele B*450101 was detected in 7 of 14 animals in which expression was predicted, and in a further two animals peaks of appropriate mobility were detected with two FLR. Allele B*460101 was detected in 6 of 17 animals in which it was predicted. In a further 8 animals a peak of appropriate mobility was detected with two FLR. Animals homozygous for H1 or H3 had comparable profiles of expressed alleles, as did the animals heterozygous for the two haplotypes (Fig. 4 A-F). Furthermore, the RSCA profiles of the heterozygote animals were additive for the RSCA profiles of the homozygous animals (Fig. 4 E, F).

Discussion

The identification and characterization of the restricted MHC diversity of Mauritian cynomolgus macaques [25, 36] has established the exceptional potential of this population for biomedical research that can benefit from the use of MHC-defined nonhuman primates. We have shown that the distribution of MHC haplotypes among Mauritian cynomolgus macaques housed at the UK-based breeding facility is comparable to that previously reported in cynomolgus macaques obtained directly from Mauritius [36], with minor differences observed in the frequencies of haplotypes H3 and H4. The frequency of recombinant haplotypes is also comparable between the two populations. The identification of the novel haplotype H7 in the two populations of macaques described in this study, as well as in an expanded cohort of animals derived from Mauritius [E.M., A.B., J.K., R.W., unpublished data], suggests that it is a genuine low-frequency Mauritian haplotype as opposed to genetic contamination of our captive-bred population due to mixing with non-Mauritian cynomolgus macaques.

The high frequency of haplotypes H1, H2 and H3 will enable MHC-identical animals to be selected from this facility for use in biomedical research, reducing variation due to unequal distribution of MHC haplotypes among experimental groups. If the data obtained for this

study are reproduced at other breeding facilities around the world then it is highly likely that similar frequencies of each haplotype will be available to all researchers using Mauritian cynomolgus macaques, regardless of the origin of the animals.

In order to confirm that the MHC alleles predicted by microsatellite analysis are actually expressed, we used PCR-SSP to type four common Mauritian class IB alleles (B*430101, B*440101, B*460101 and B*510101) in cDNA from approximately one fifth of the animals in the current study. These alleles were previously reported to be the highest frequency class IB alleles among MCM [12] and are therefore most likely to reveal discrepancies between microsatellite-based predictions and allele expression. Class IA alleles A*250201/A*250301 and A*290101 are predicted at higher frequency (greater than 90% [36]) but their expression on multiple high-frequency haplotypes may mask discordant predictions. In animals carrying two A*250201/A*250301/A*290101-containing haplotypes it would be unclear whether the detected allele was transcribed from one or both haplotypes. In the 58 animals with unambiguous microsatellite-based predictions, there was complete concordance between predicted allele expression and detected messenger RNA transcript in all but one animal where weak PCR-SSP signals were observed for two of three class IB alleles associated with the H1 haplotype. Thus the predictive value of microsatellite-derived haplotypes is very high in the absence of recombination. Discrepancies may arise through non-specific amplification of certain alleles, resulting in a band of the expected size being detected, but these can be excluded by sequence analysis of the PCR-SSP product. It is also possible that localized recombination between two microsatellite markers has led to the generation of a haplotype that expresses alleles other than those predicted in the original study [36]. Nevertheless, our allele-specific PCR data would suggest that such events do not occur at high frequency in the class IB region of cynomolgus macaques. Twelve animals (17%) had ambiguous allele predictions due to recombinant haplotypes. In these cases, PCR-SSP successfully resolved the expressed alleles.

Given the high frequency of H1 and H3 haplotypes in the population of animals studied, we sought to establish a selective breeding program to produce MHC-identical animals carrying haplotypes H1 and H3. This will facilitate immunogenetic analysis of these haplotypes and their influence on animal models of infectious disease. The aim is to enrich certain alleles (or combinations of alleles) amongst the breeding population. From the 355 animals studied, 19 were selected based on H1 or H3 homozygosity or heterozygosity. Through the application of RSCA, it was demonstrated that the subsets of animals with identical MHC haplotypes expressed similar class I alleles. Not all of the alleles predicted by microsatellite analysis were detected by RSCA for every animal however. Discordance between predicted and expressed alleles were noted for certain alleles, particularly A*290101, B*450101 and B*460101. It should be noted that the peaks corresponding to these alleles were close to the threshold of detection of the assay while peaks corresponding to alleles B*440101 and B*510101 were of significantly greater intensity. In the case of B*450101, the high level of concordance was actually somewhat surprising given that this allele has a mismatched base pair with the forward primer which may result in suboptimal amplification. Failure to detect the expression of certain predicted alleles was noted most commonly with the Mafa-B*370101 FLR which appeared to be the least sensitive of the three FLR employed. In the case of B*460101, PCR-SSP demonstrated that the allele was expressed in all animals in

which it was predicted, hence transcript was present in the cDNA sample but was below the detection limit of our RSCA protocol. It has been reported that MHC class I alleles display differential levels of expression in macaques [14, 26]. Thus, it is likely that alleles expressed at low levels may fall below the detection limit of our RSCA protocol, particularly in heterozygous animals where an increased number of alleles are competing for amplification and hybridization with the FLR. The use of locus-specific primers to selectively amplify the class I A and B alleles separately would be expected to improve the sensitivity of detection for such alleles.

Despite discordance between the predicted and detected expression for certain alleles, the expression profiles for animals with identical haplotypes were similar, and the profiles of H1 and H3 homozygous animals were additive in animals heterozygous for these two haplotypes. These 19 animals were grouped into a new breeding colony, with a sire that is homozygous for the H1 haplotype. All offspring from this colony will therefore carry at least one copy of the H1 haplotype. The relevance of haplotypes H1 and H3 to cellular immune responses against intracellular pathogens is not yet clear. Observations in a cohort of SIV-infected cynomolgus macaques suggest that neither haplotype is associated with improved control of SIVmac251 infection [E.M, N.A., N.R. unpublished data], and two animals expressing class I alleles from haplotype H3 displayed a typical course of infection when challenged with SIVmac239 [36]. The selection of haplotypes associated with improved control of viraemia would be useful for characterisation of protective immune responses against SIV, but would not be appropriate for vaccine evaluation, as subtle vaccine effects could not be easily distinguished from naturally occurring protective immune responses. Haplotypes H1 and H3 are therefore relevant to SIV pathogenesis and vaccine evaluation. We are also planning to establish additional breeding groups comprised of animals with other high-frequency MHC haplotypes in order to study these in more detail. In addition, the high-throughput nature of microsatellite-based typing means that all offspring from any breeding facility, whether selectively bred or not, can be readily typed in order to select the most appropriate animals and control for MHC haplotypes between experimental groups. In the first instance, selectively bred animals will be used for investigation of the role of cellular immunity in SIV infection of cynomolgus macaques, but will also be made available to researchers using cynomolgus macaques in models of other human infectious diseases such as tuberculosis.

While MHC-identical groups of animals will not be representative of the outbred human population, the use of such animals will allow for detailed study of vaccine- or infection-induced cellular immune responses against intracellular pathogens without the confounding effects of MHC variation common to non-Mauritian macaques. The use of a well-defined model with low genetic diversity will provide fundamental information about cellular immunity in macaques which can then be extended to more outbred populations, such as Indonesian cynomolgus, or Chinese or Indian rhesus, macaques.

The historical reproductive rate across the entire captive cynomolgus macaque UK breeding colony over the past 10 years has been about 50% of females having a live birth in a given year. It would therefore be predicted that the new breeding group would give rise to approximately 9 offspring per year, 56% of which would be homozygous for H1 and 44%

heterozygous for H1/H3. It will be of interest to determine whether the high degree of haplotype similarity among the animals in this new colony will have an effect on reproductive success. It has been reported that captive MHC class II-identical rhesus macaques display a lower degree of reproductive success than MHC-discordant animals [30]. This may reflect a biological mechanism to promote diversity of MHC alleles in order to ensure resistance to a wide variety of pathogens at the species level. We will monitor the reproductive success of the newly formed breeding groups relative to the whole colony over the next five years to determine whether MHC homozygosity is depressing reproductive success. Furthermore, the availability of detailed breeding and pedigree records for the facility since its establishment will permit retrospective analysis of breeding fitness in relation to MHC haplotype diversity.

To maximize the benefit of the newly established breeding groups, we are developing reagents for use in studies of cellular immunity, such that the immune responses elicited by vaccination or infection of MHC-identical animals can be characterized. In the first instance, we will determine whether certain MHC haplotypes are associated with improved cellular immune responses and control of viral loads following vaccination and infection with SIVmac251. Such studies will provide valuable information about the role of cellular immunity in SIV infection of cynomolgus macaques and will aid HIV vaccine development efforts.

In conclusion, we have demonstrated that Mauritian cynomolgus macaques in the primary UK breeding and supply facility have a comparable low level of genetic diversity in the MHC region to that in a previously described cohort sourced directly from Mauritius [36]. We have also found microsatellite-inferred haplotypes to be strongly predictive of MHC class IB allele expression. Furthermore we have established a new breeding colony of animals carrying only the two most common haplotypes, such that all offspring from this colony will share at least one MHC haplotype, with almost two thirds predicted to be homozygous. We have demonstrated that the pattern of class I allele expression in these animals is generally consistent with the predictions based on microsatellite analysis. While certain rhesus macaque breeding colonies have been genotyped for selected *Mamu-A* and *-B* alleles to facilitate matching of experimental animals for those alleles [10], to our knowledge this is the first report of a selective breeding program designed to generate a supply of macaques with completely characterized and matched MHC class I and II haplotypes for biomedical research. These animals will prove to be a valuable resource for studies to investigate the role of immunogenetics on the outcome of pathogenesis and/or vaccination in nonhuman primate models of infectious disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

MHC haplotype frequencies in UK populations of Mauritian cynomolgus macaques. (A) Frequency of MHC haplotypes in 355 Mauritian cynomolgus macaques (710 chromosomes) housed at the UK breeding facility, determined by microsatellite analysis. (B) Frequency of MHC haplotypes in an expanded cohort of 331 Mauritian cynomolgus macaques obtained directly from Mauritius. This cohort includes 117 animals described previously [36]. (C) Percentage of animals in UK breeding facility carrying at least one copy of intact and recombinant haplotypes. Haplotype H7 is a rare Mauritian haplotype (frequency ~1%) that was found in four animals in the current study as well as 4 of 331 animals obtained directly from Mauritius.

Fig. 2. Allele-specific PCR for 4 common alleles. Alleles (A) B*430101, (B) B*440101, (C) B*460101 and (D) B*510101 were amplified from cDNA using sequence specific primers. (E) Full-length MHC was amplified as a positive control. Lane M: Φ X HaeIII digest size marker, 1–20: cDNA samples, lane 21: B*430101 positive control, lane 22: B*440101 positive control, lane 23: B*460101 positive control, lane 24: B*510101 positive control, lane 25: no template control. Indicated size markers are (A) 194 bp, (B) 194 bp, (C) 603 bp, (D) 118 bp, (E) 1078 bp.

	I074 ♀	I315C ♀	I331 ♀	M572 ♀	M883 ♂	M010 ♀	M069 ♀
D6S2972	126 126	126 126	126 126	126 126	126 126	120 120	120 120
D6S2970	301 301	301 301	301 301	301 301	301 301	293 293	293 293
D6S2854	191 191	191 191	191 191	191 191	191 191	191 191	191 191
D6S2704	163 163	163 163	163 163	163 163	163 163	143 143	143 143
D6S2847	318 318	318 318	318 318	318 318	318 318	318 318	318 318
C4_2_25	233 233	233 233	233 233	233 233	233 233	233 233	233 233
D6S2691	255 255	255 255	255 255	na na	255 255	290 294	294 294
9268	235 235	235 235	235 235	235 235	235 235	235 235	235 235
MICA	204 204	204 204	204 204	204 204	204 204	201 201	201 201
D6S2793	274 274	274 274	274 274	274 274	274 274	243 243	243 243
D6S2782	321 321	321 321	321 321	321 321	321 321	334 334	334 334
D6S2669	129 129	129 129	129 129	129 129	129 129	141 141	141 141
D6S2892	206 206	206 206	206 206	206 206	206 206	204 204	204 204
DRA_CA	234 234	234 234	234 234	234 234	234 234	269 269	269 269
D6S2876	214 214	214 214	214 214	214 214	214 214	216 216	216 216
D6S2747	206 206	206 206	206 206	206 206	206 206	null null	null null
D6S2745	297 297	297 297	297 297	297 297	297 297	301 301	301 301
D6S2771	396 396	396 396	396 396	396 396	396 396	397 397	397 397

	I007B ♀	I007C ♀	I087B ♀	I142B ♀	I321B ♀	M206 ♀	M224 ♀
D6S2972	126 120	126 120	126 120	126 120	126 120	126 120	126 120
D6S2970	301 293	301 293	301 293	301 293	301 293	301 293	301 293
D6S2854	191 191	191 191	191 191	191 191	191 191	191 191	191 191
D6S2704	163 143	163 143	163 143	163 143	163 143	163 143	163 143
D6S2847	318 318	318 318	318 318	318 318	318 318	318 318	318 318
C4_2_25	233 233	233 233	233 233	233 233	233 233	233 233	233 233
D6S2691	285 294	255 294	250 300	255 294	255 298	255 294	255 294
9268	235 null	235 null	235 null	235 null	235 null	235 null	235 null
MICA	204 201	204 201	204 201	204 201	204 201	204 201	204 201
D6S2793	274 243	274 243	274 243	274 243	274 243	274 243	274 243
D6S2782	321 334	321 334	321 334	321 334	321 334	321 334	321 334
D6S2669	129 141	129 141	129 141	129 141	129 141	129 141	129 141
D6S2892	206 204	206 204	206 204	206 204	206 204	206 204	206 204
DRA_CA	234 269	234 269	234 269	234 269	234 269	234 269	234 269
D6S2876	214 216	214 216	214 216	214 216	214 216	214 216	214 216
D6S2747	206 null	206 null	206 null	206 null	206 null	206 null	206 null
D6S2745	297 301	297 301	297 301	297 301	297 301	297 301	297 301
D6S2771	396 397	396 397	396 397	396 397	396 397	396 397	397 397

	M652 ♀	M855 ♀	M891 ♀	M934 ♀	M1655 ♀
D6S2972	126 120	126 120	126 120	126 120	126 120
D6S2970	301 293	301 293	301 293	301 293	301 293
D6S2854	191 191	191 191	191 191	191 191	191 191
D6S2704	163 143	163 143	163 143	163 143	163 143
D6S2847	318 318	318 318	318 318	318 318	318 318
C4_2_25	233 233	233 233	233 233	233 233	233 233
D6S2691	255 290	255 290	255 298	255 294	255 285
9268	235 null	235 null	235 null	235 null	235 null
MICA	204 201	204 201	204 201	204 201	204 201
D6S2793	274 243	274 243	274 243	274 243	274 243
D6S2782	321 334	321 334	321 334	321 334	321 334
D6S2669	129 141	129 141	129 141	129 141	129 141
D6S2892	206 204	206 204	206 204	206 204	206 204
DRA_CA	234 269	234 269	234 269	234 269	234 269
D6S2876	214 216	214 216	214 216	214 216	214 216
D6S2747	206 null	206 null	206 null	206 null	206 null
D6S2745	297 301	297 301	297 301	297 301	297 301
D6S2771	396 397	396 397	396 397	396 397	396 397

Fig. 3.
MHC haplotypes of 19 animals carrying H1 (black) and H3 (blue) haplotypes included in haplotype-defined breeding colony. Haplotypes were established on the basis of 18 microsatellite markers distributed across the 5 Mb macaque MHC region. Alleles are identified on the basis of apparent size in basepairs relative to an internal size standard. Discrepant microsatellite allele sizes are shaded in white. na: no amplification.

Fig. 4.

Comparison of RSCA profiles in selected animals included in haplotype-defined breeding colony using *Mamu-B*07* FLR. (A) animal I074, (B) M572, both H1 homozygous. (C) M010, (D) M069, both H3 homozygous. (E) I007C, (F) M934, both H1-H3 heterozygous. Bins corresponding to alleles carried by haplotypes H1 (A*250301, A*290101, B*430101, B*440101, B*460101) and H3 (A*250201, A*290101, B*450101, B*510101) are indicated. The amplified RSCA fragment does not distinguish between alleles A*250201 and A*250301. Residual FLR homoduplex is present in all samples at an apparent mobility of approximately 360.

Table 1

Rare H7 MHC haplotype of Mauritian cynomolgus macaques defined by panel of 18 microsatellite markers.

Marker	Allele size (bp)
D6S2972	120
D6S2970	309
D6S2854	191
D6S2704	160
D6S2847	320
C4_2_25	233
D6S2691	245, 249
9268	null
MICA	204
D6S2793	274, 276
D6S2782	334
D6S2669	108
D6S2892	200
DRA_CA	267
D6S2876	209
D6S2747	190
D6S2745	null
D6S2771	392

Table 2

Comparison of predicted and expressed class I B alleles in 70 Mauritian cynomolgus macaques. The expression of four *Maf/a-B* alleles was predicted on the basis of MHC haplotypes derived from microsatellite analysis (MS). Black shaded box: expression of allele is predicted; white shaded box: expression of allele is not predicted. Grey shaded box: ambiguous prediction due to recombination within the class I B region. Transcribed alleles were detected in cDNA by allele-specific PCR using sequence specific primers (SSP). Shaded box: allele transcript detected; W: weak positive by SSP.

Sample	Animal ID	Haplotype H1						H3	
		B*430101		B*440101		B*460101		B*510101	
		MS	SSP	MS	SSP	MS	SSP	MS	SSP
1	I050								
2	I132								
3	I082								
4	M899				W		W		
5	I001								
6	I150								
7	I147								
8	I133								
9	I017								
10	M891								
11	I142								
12	I074								
13	I012								
14	I320								
15	I149C								
16	I015								
17	I446								
18	I331								
19	I320F								
20	I331E								
21	I1531								
22	M636F								

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Sample	Animal ID	Haplotype H1								H3	
		B*430101		B*440101		B*460101		B*510101		MS	SSP
		MS	SSP	MS	SSP	MS	SSP	MS	SSP		
23	M136										
24	M561										
25	M561E										
26	M567										
27	M572										
28	M582A										
29	M582B										
30	M596										
31	M598										
32	M633										
33	M636F										
34	M648										
35	M652										
36	M652D										
37	M666										
38	M669										
39	M671										
40	M675										
41	M677										
42	M689										
43	M693										
44	M694										
45	M1464										
46	M1464E										
47	M1559										
48	I458A										
49	M738										

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Sample	Animal ID	Haplotype H1								H3	
		B*430101		B*440101		B*460101		B*510101		MS	SSP
		MS	SSP	MS	SSP	MS	SSP	MS	SSP		
50	I007B										
51	I089										
52	I089I										
53	I130										
54	I130I										
55	I319A										
56	I326A										
57	I326AD										
58	I430										
59	I430H										
60	M005										
61	M093										
62	M119										
63	M150										
64	M234										
65	M290										
66	M733										
67	M855										
68	M862										
69	M957										
70	M957C										

Table 3

Comparison of predicted and detected class I allele expression for animals included in haplotype-defined breeding colony. Expression of H1 (A*250301, A*290101, B*430101, B*440101, B*460101) and H3 (A*250201, A*290101, B*450101, B*510101) class I alleles was predicted on the basis of microsatellite analysis. Presence of transcript in cDNA was detected by RSCA using three class I FLR. RSCA does not discriminate between alleles A*250201 and A*250301 which differ by only a single amino acid in the signal peptide. Shaded box: concordance between predicted and expressed allele; 0/3: predicted alleles not detected with any FLR; 1/3: allele detected with 1 FLR; 2/3: allele detected with 2 FLR; np: expression of allele not predicted.

Animal	Haplotype	A*25	A*29	B*43	B*44	B*45	B*46	B*51
I074	H1-H1					np		np
I315C	H1-H1		1/3			np		np
I331	H1-H1		0/3			np	2/3	np
M572	H1-H1		1/3			np		np
M883	H1-H1	2/3	1/3			np	2/3	np
M010	H3-H3		1/3	np	np		np	
M069	H3-H3			np	np		np	
I007B	H1-H3		1/3				2/3	
I007C	H1-H3			2/3				
I087B	H1-H3		1/3				2/3	
I142B	H1-H3		1/3	2/3		0/3	2/3	
I321B	H1-H3		0/3			2/3	1/3	
M206	H1-H3	2/3	0/3			0/3	2/3	
M224	H1-H3		1/3					
M652	H1-H3	0/3	1/3			0/3	1/3	
M855	H1-H3	0/3	1/3			0/3	1/3	
M891	H1-H3		2/3			2/3	2/3	
M934	H1-H3		2/3					
M1655	H1-H3	0/3	1/3			0/3	2/3	

Table 4

Predicted MHC class I and II allele expression for animals in selective breeding group. Adapted from [25, 36].

	Haplotype	
	H1	H3
Class I A	A*250301	A*250201
	A*290101	A*290101
Class I B	B*430101	B*450101
	B*440101	B*510101
	B*460101	I*100101
Class II DR	DRA*0201	DRA*0102
	DRB*w501	DRB1*1002
	DRB*w2101	
Class II DQ	DQA1*2403	DQA1*0503
	DQB1*1801	DQB1*1601
Class II DP	DPA1*0702	DPA1*0202
	DPB1*42	DPB1*29