

# Influence of MHC class I and II haplotypes on the experimental infection of Mauritian cynomolgus macaques with SHIV<sub>SF162P4cy</sub>

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## Key words

acquired immunodeficiency syndrome; major histocompatibility complex; mauritian cynomolgus macaques; simian/human immunodeficiency virus

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## Abstract

Mauritian cynomolgus macaques (MCM) are widely used in human immunodeficiency virus research because of their restricted major histocompatibility complex (MHC) diversity which provides the opportunity to address the influence of host factors on vaccine studies. We herein report the impact of MHC haplotype on the outcome of 21 MCM infections with the CCR5-tropic simian/human immunodeficiency virus (SHIV)<sub>SF162P4cy</sub>. MCM were susceptible to SHIV<sub>SF162P4cy</sub> infection as shown by viremia and loss of CD4<sup>+</sup> T cells. A significant association between haplotype M7 (class IA, IB, II) and persistent viremia was observed in chronic phase, whereas recombinant class IA haplotype was associated with a reduction of viral RNA during acute infection. Class IB M4 haplotype displayed significantly lower acute phase provirus copy numbers. In addition, statistical analysis indicated a detrimental effect of haplotype M4 (class IA, IB) on the course of infection as indicated by lower CD4<sup>+</sup> T-cell levels during chronic infection. A decrease in post-acute phase CD4<sup>+</sup> T-cell numbers was also observed in haplotype M2 animals. This is the first report that documents the effects of host MHC class I and II molecules on the SHIV<sub>SF162P4cy</sub> infection in MCM, particularly with regard to the association between recombinant class IA, M4, and M7 haplotypes and the dynamic of viral replication and level of CD4<sup>+</sup> T cells.

## Introduction

The simian/human immunodeficiency virus (SHIV)-macaque model of infection has proven invaluable in providing insights into human immunodeficiency virus-1 (HIV-1) pathogenesis and for HIV-1/acquired immunodeficiency syndrome (AIDS) vaccine development (1, 2). Asian macaques have been extensively used for the preclinical evaluation of vaccine candidates, evidencing a different susceptibility to primate lentivirus-induced diseases among different species of monkeys (3). In fact, many factors such as the origin of the virus stocks prepared and titrated from different laboratories and the genetic diversity of the animals, could contribute to determining the susceptibility of macaques to infection, making difficult the interpretation of vaccine studies. SHIV<sub>SF162</sub> is a

CCR5-tropic virus capable of establishing persistent infection and causing simian AIDS with a varying disease progression that is characteristic of the human HIV disease (4–6). The differential pathogenicity of SHIV<sub>SF162</sub> in monkeys of different origin (7) highlights the importance of considering host-related genetic background and immunological factors in the evaluation of vaccine efficacy in the different monkey species used for these studies.

The major histocompatibility complex (MHC) is involved in both innate and adaptive immunity and plays a primary role in the immune response. In the rhesus macaque/simian immunodeficiency virus (SIV) model disease progression to AIDS is clearly influenced by MHC class I and class II allelic polymorphism (8–13). Recently, many studies have reported an association between the MHC class I haplotype and the control of lentiviral infection in Mauritian cynomolgus macaques (MCM) (14–20). MCM exhibit an unexpected

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degree of allele sharing caused by a strong founder effect on the geographically isolated island of Mauritius (14, 21–26). In fact, just seven common MHC haplotypes, termed M1–M7, have been characterized in feral MCM, in contrast to the extensive diversity of MHC genes in rhesus macaques (27, 28). A genetically defined population of non-human primates with such a limited MHC diversity is an extraordinarily valuable resource to study the effect of MHC haplotypes on the progression of SIV/SHIV infection. Indeed, the restricted MHC polymorphism increases the potential for powerful genetic-immunological studies, especially when working with experimental groups of limited size like MCM.

While the impact of MHC haplotype on viral outcome in MCM infected with a CXCR4-tropic SHIV, SIVmac239 or SIVmac251 has already been described (14, 15, 17–20, 25, 29), to date no study has reported the effect of MHC haplotypes on CCR5-tropic SHIV replication. It would be very useful to know the relative susceptibility of MCM to different viruses in light of their remarkably restricted MHC genetics, in particular to characterize the effects of MHC haplotypes on the replication kinetics of each commonly used challenge virus.

This is of particular importance in light of the fact that previous reports suggested that the influence of MHC genetics may vary depending on the virus isolate used.

In this article, we describe the consequences of intrarectal infection of 21 MCM with different doses of SHIV<sub>SF162P4cy</sub>, a virus obtained by the infection of a cynomolgus monkey with the rhesus-derived SHIV<sub>SF162P4</sub>, and the effect of MHC class I and II haplotypes on control of SHIV<sub>SF162P4cy</sub> replication in genetically defined MCM. Appreciation of the role played by the MHC haplotypes in the complex virus–host interaction will contribute to a better design of vaccine protocols and to a clearer interpretation of results, in the effort to generate adequate interventions against HIV/AIDS.

## Materials and methods

### Animals and infections

Adult cynomolgus macaques (*Macaca fascicularis*) imported from Mauritius and tested seronegative for simian T-lymphotropic virus-1 (STLV-1), SIV, simian type D retrovirus, and simian herpes B virus infections were housed in single cages within level 3 biosafety facilities according to the European guidelines for non-human primate care (EEC, Directive No. 86-609, November 24, 1986). Before the start of the experiment, the animal protocol was approved by the ethics committee of the Istituto Superiore di Sanità. Animals were clinically examined, and weight and rectal temperature were measured while they were under ketamine hydrochloride anaesthesia (10 mg/kg). Macaques used in this study were part of different experimental protocols as naive or control animals. Control animals were treated with different adjuvants such as aluminum phosphate (Alum), 250 µl administered subcutaneously or non-toxic mutant of the heat-labile

enterotoxin (LTK63), administered 30 µl intranasally. LTK63 is an *Escherichia Coli* heat-labile enterotoxin mutant, which proved generally safe and effective as an intranasal adjuvant both in animals and in humans (30). Monkeys AH694, AK407, AK484, AK803, AK952, AL639 received LTK63 at weeks 0, 4, 8 and Alum at weeks 24 and 36 before challenge performed at week 44; monkeys AQ271, AP511, AQ882 received alum at weeks 23 and 36 before challenge performed at week 48; monkey AF318 received Alum at weeks 0, 4, 12, 23, 43, 87, and LTK63 at weeks 57 and 67 before challenge performed at week 96. SHIV<sub>SF162P4cy</sub> was obtained by infecting intravenously a cynomolgus macaque (monkey AH595) with 78 tissue culture infectious dose<sub>50</sub> (TCID<sub>50</sub>) of the SHIV<sub>SF162P4</sub> obtained from the National Institutes of Health (NIH). Monkey AH595 was killed at day 12 after infection and blood, lymph nodes, and spleen mononuclear cells were collected and CD8+-depleted cells were stimulated with phytohemagglutinin (PHA) and interleukin-2 to induce massive virus replication. At the peak of reverse transcriptase (RT) activity, the cell-free supernatant was aliquoted and frozen. The new viral stock termed SHIV<sub>SF162P4cy</sub> was titrated *in vitro* on human cell lines (TZM-bl) resulting in titers of  $3.2 \times 10^3$  TCID<sub>50</sub>/ml. To titer the virus *in vivo*, 10 cynomolgus monkeys were rectally exposed to 10-fold serial dilutions of SHIV<sub>SF162P4cy</sub> (undiluted virus one monkey, 1:10 two monkeys, 1:100 three monkeys, 1:1000 two monkeys, 1:10,000 two monkeys). According to this titration, the SHIV<sub>SF162P4cy</sub> viral stock contained  $1.79 \times 10^2$  MID<sub>50</sub>/ml. Animals were inoculated mucosally with different doses of the same SHIV<sub>SF162P4cy</sub> virus stock. All animals used in these studies were males.

### Plasma viral RNA measurements and proviral DNA detection

Plasma levels of SHIV<sub>SF162P4cy</sub> were determined using a 'one-step' real-time RT-PCR (RNA-polymerase chain reaction assay), with a threshold limit for detection of 50 RNA eq/ml (31). To quantitate the cell-associated viral load, DNA was extracted from 400 µl of whole citrated blood by using the QIAmp DNA Blood Mini Kit (QIAGEN, Milan, Italy) according to the manufacturer's instructions. SHIV proviral copies were determined using Taqman real-time PCR. Probe and primers to specifically amplify a region of 71 bp within the gag gene of SIVmac251 (gi:334657) were designed using Primer Express software (Applied Biosystems, Foster City, CA) as described (32). Samples were analyzed in triplicate by PCR in a total volume of 25 µl of a mixture containing 400 ng DNA, 12.5 µl PCR master mix (Applied Biosystems), 900 nM each primer, and 180 nM probe. Thermal cycling conditions were 2 min at 50°C (to allow uracil *N*-glycosylase digestion which prevents cross-over contamination), 10 min at 95°C (to allow thermal activation of the Amplitaq Gold), and 45 cycles of 15 s at 95°C and 1 min at 60°C. Amplification,

data acquisition, and analysis were performed using the ABI Prism 7700 sequence detection system (Applied Biosystems). The lower limit of detection of this assay was 1 SHIV proviral copy/ $\mu\text{g}$  of DNA.

### Lymphocyte subset determination

Citrated peripheral blood mononuclear cells (PBMC) were stained with FITC-conjugated anti-CD3, PE-conjugated anti-CD4, and PERCP-conjugated anti-CD8 mAb (Becton-Dickinson, Mountain View, CA), and analyzed with a FAC-Scan cytometer and software (Becton-Dickinson) as described previously (33). Absolute cell numbers were calculated from the blood cell counts, performed by an automatic cell counter (Coulter Onyx, Beckman Coulter, Milan, Italy).

### Microsatellite analysis and allele-specific PCR

MHC class IA and IB and class II haplotypes were determined by microsatellite PCR with resolution of recombinant class IB haplotypes by allele-specific PCR as previously described (16).

### Statistical methods

Viral load and proviral DNA as well as CD4 T-cell counts were evaluated for different phases of infection: acute (2–4 weeks), post-acute (8–16 weeks), and chronic (24–46 weeks). Acute, post-acute, and chronic phases included all determinations among 2–4 weeks, 8–16 weeks, and 24–46 weeks, respectively. CD4+ T-cell decline was obtained using the last CD4+ T-cell count prior to challenge as baseline. For viral load and proviral DNA, all data were expressed as  $\log_{10}$  copies/ml; a value of  $\log_{10}(50)/2$  was assigned to viral loads below the cut-off level (50 copies/ml).

Analysis of variance (ANOVA), was applied to the plasma viral load, proviral DNA, and CD4+ T cells in the acute, post-acute, and chronic phase of infection, after controlling the normality assumption underlying the model through the residual analysis. In the ANOVA, the previous continuous response variables were considered, separately, as a dependent variable, and were evaluated in relationship with classification variables, known as independent variables. The variation in the response was assumed to be due to effects in the classification (presence or absence of haplotype effect), with random error accounting for the remaining variation. M1, M2, M3, and M4 haplotypes (the most represented) were included in the multivariate model, separately for IA, IB, and II MHC classes, and all factors were adjusted for each other. For the class IA, the recombinant effect was also included in the model.

Statistical analyses were carried out at two-sided with a 0.05 significance level, using SAS<sup>®</sup> software, version 9.2 (SAS Institute, Cary, NC). Viral load, proviral load, and CD4+ T-cell count of naive, control alum, and control LTK monkeys were assessed at different time points (acute 2–4 weeks,

post-acute 8–16 weeks, and chronic 24–46 weeks) with Kruskal–Wallis nonparametric ANOVA test with Dunn's adjustment for multiple comparisons (Graphpad InStat 3.05, San Diego, CA).

## Results

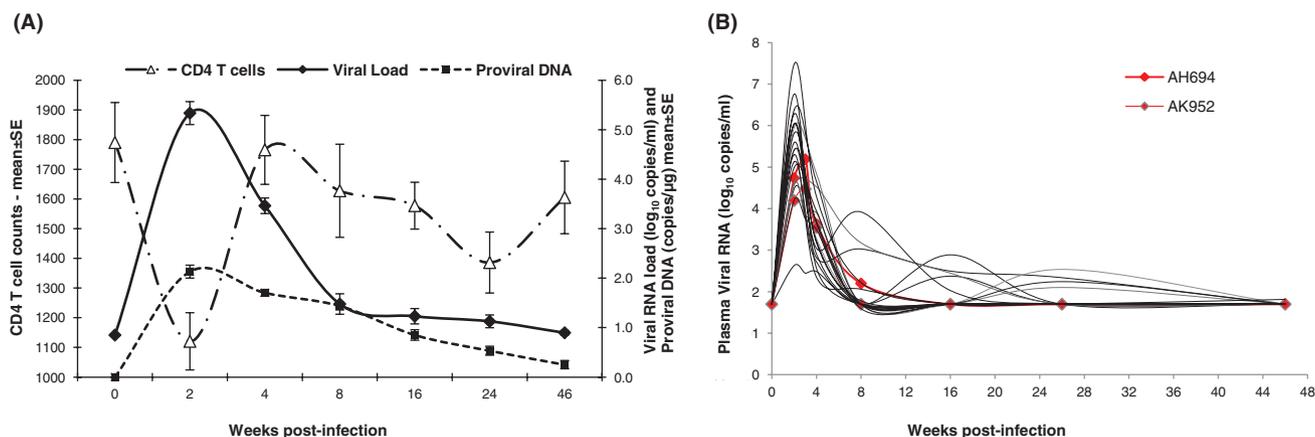
### Study profile and infection with SHIV<sub>SF162P4cy</sub>

By using SHIV<sub>SF162P4cy</sub>, a virus obtained by passaging SHIV<sub>SF162P4</sub> (5) in a cynomolgus macaque of Mauritian origin (31), we investigated the dynamics of viral replication in MCM. Twenty-one naive or control MCM, which were part of different experimental protocols, were analyzed. The identification number, treatment, challenge dose, age, and MHC haplotype of each of the animals are listed in Table 1. Monkeys were challenged rectally with an identical stock of SHIV<sub>SF162P4cy</sub>: 14 were infected with 70 MID<sub>50</sub>, 2 with 35 MID<sub>50</sub>, 2 with 17.9 MID<sub>50</sub>, 2 with 1.79 MID<sub>50</sub>, and 1 with 179 MID<sub>50</sub> of SHIV<sub>SF162P4cy</sub>, respectively. The dynamics of infection were followed by monitoring plasma viral load, peripheral blood CD4+ T-cell counts, proviral DNA levels, and disease progression for up to 46 weeks post-infection (p.i.) (Figure 1A). Viral RNA levels assessed over three different time periods p.i. were analyzed and compared among animals. Time periods were defined as follows: acute phase (mean of 2–4 weeks p.i.: when plasma viremia peaks occur), post-acute phase (mean of weeks 8–16), and chronic phase of infection (mean of weeks 24–46), respectively. As shown in Figure 1B, high levels of viral RNA were detected in all animals at 2 weeks p.i., except for two monkeys that showed a delayed peak at 3 weeks p.i. The levels of plasma viremia ranged from  $4 \times 10^2$  to  $3 \times 10^7$  RNA copies/ml during the acute phase of infection, decreased from  $1 \times 10^2$  to  $8 \times 10^3$  RNA copies/ml at 8–16 weeks, and varied from 65 to 400 RNA copies/ml at 24–46 weeks (Figure 1A, B). Statistical analysis did not show any significant correlation between viral dynamics and size of virus inoculum. The CD4+ T-cell levels of all monkeys were also monitored during the acute, post-acute, and chronic phases of infection (Figure 1A). Concomitant with peak viral loads there was a moderate drop in the absolute CD4+ T-cell counts. However, this cell population rebounded to near baseline values 4–8 weeks p.i., even though pre-infection levels were never restored throughout the 46 weeks of follow-up. Provirus copy number was also assessed in PBMC of infected monkeys at various time points after SHIV challenge and they ranged from 5 to  $1 \times 10^3$  copies/ml during the acute phase of infection (2–4 weeks), declined to 1–100 copies/ml at 8–16 weeks p.i., and stabilized at about 10–25 copies/ml at 24–46 weeks p.i. (Figure 1A). None of the infected monkeys showed any other clinical signs of disease throughout the 46 weeks study period. As some of the animals enrolled in this study had received adjuvant controls, to exclude a bias in the results due to prior treatment, analysis of viral load, proviral load and CD4+ T-cell count of naive, control Alum, and

**Table 1** Summary of monkeys used in this study. Naive or control Mauritian cynomolgus macaques were infected with the same stock of SHIV<sub>SF162P4cy</sub> in different vaccine evaluation studies. Where there is evidence of recombination between markers each haplotype contribution is listed, separated by commas. All animals used in these studies were males

Animal ID	Treatment	Challenge dose	Age (months)	MHC class IA genotype	MHC class IB genotype	MHC class II genotype
AH960	Naive	179 MID50	48	M4/M6	M4/M6	M4/M6
AH694	LTK63/Alum	70 MID50	54	M1/M1	M1/rec (M1, M3)	M1/M3
AK407	LTK63/Alum	70 MID50	44	M3/rec (M2, M4)	M3/M4	M3/M1
AK484	LTK63/Alum	70 MID50	44	M3/rec (M3, M1)	M3/M4	M3/M4
AK803	LTK63/Alum	70 MID50	42	M3/M1	M3/M1	M3/M6
AK952	LTK63/Alum	70 MID50	42	M4/rec (M4, M1)	M4/M1	M4/M1
AL639	LTK63/Alum	70 MID50	42	M1/rec (M1, M4)	M1/M1	M3/M1
AQ271	Alum	70 MID50	41	M1/M1	M1/M3	M1/M3
AP511	Alum	70 MID50	44	M7/rec (M1, M3)	M7/M3	M7/M3
AQ882	Alum	70 MID50	45	M7/M2	M7/M6	M7/M6
AS377	Naive	70 MID50	43	M5/M3	M5/M1	M5/M1
AU676	Naive	70 MID50	36	M7/M3	M7/M3	M7/rec (M3, M2)
AS167	Naive	70 MID50	41	M2/M3	M2/M3	M2/M3
AQ498	Naive	70 MID50	48	M6/M4	M6/M4	M6/M2
AU427	Naive	70 MID50	36	M1/M4	M1/M4	M1/M4
AF318	LTK/Alum	35 MID50	41	M3/M4	M3/M1	M3/M1
AG172	Naive	35 MID50	45	M2/M1	M2/M3	rec (M2, M3)/M3
AG981	Naive	17.9 MID50	50	M3/rec (M3, M2)	M3/M2	M3/M2
AH522	Naive	17.9 MID50	48	M1/M4	M1/M4	M1/M4
AF238	Naive	1.79 MID50	48	M2/M7	M2/M7	M4/M3
AE624	Naive	1.79 MID50	48	M2/M3	M2/M3	M2/M3

MHC, major histocompatibility complex; SHIV, simian/human immunodeficiency virus.



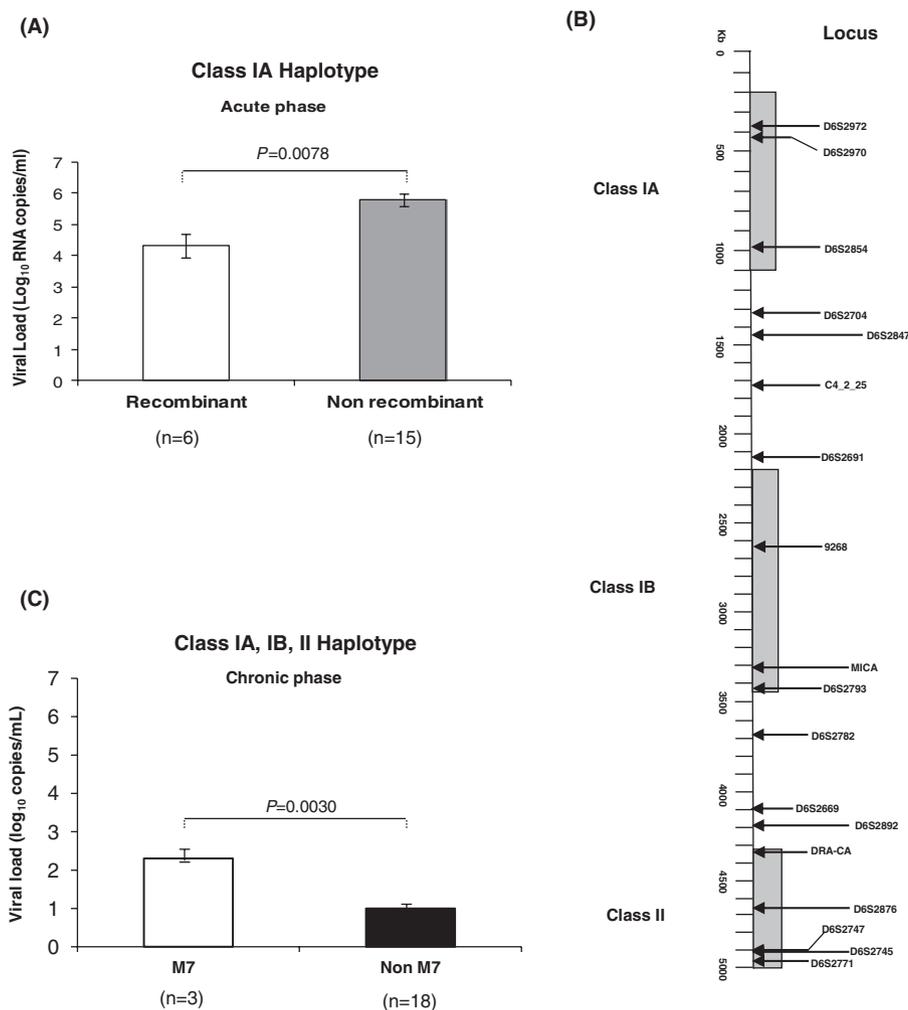
**Figure 1** Dynamics of viral infection in 21 cynomolgus monkeys inoculated with SHIV<sub>SF162P4cy</sub> during acute [2–4 weeks post-infection (p.i.)], post-acute (8–16 weeks p.i.), and chronic phase (24–46 weeks p.i.) of infection. (A) Data represent mean values with standard error of log plasma RNA load, log proviral DNA, and CD4+ T-cell counts from 0 to 46 weeks p.i. (B) Viral RNA levels in plasma of all infected animals over time. The red curves denote peaks of viral load at 3 weeks in two animals AH694 and AK952.

control LTK monkeys were assessed during acute, post-acute, and chronic phase of infection and no significant associations were found.

#### Effects of MHC haplotype combinations on plasma viral RNA levels in SHIV<sub>SF162P4cy</sub>-infected cynomolgus monkeys

To investigate whether the MHC haplotype could influence the outcome of infection, MHC class I and II haplotypes were

defined by microsatellite analysis with the alleles for class I and class II regions being inferred on the basis of established haplotype allele associations (14, 24). Seven haplotypes (M1–M7) or simple recombinants thereof, were detected in the monkeys infected with SHIV<sub>SF162P4cy</sub>. M3 was the most commonly represented haplotype in the cohort, whereas M5 was found in only one animal (Table 1). When analyzed on the basis of MHC class I and II haplotypes or recombinants thereof, we found a significant reduction of the mean



**Figure 2** Effect of major histocompatibility complex (MHC) class IA, IB, and II haplotypes on viral load following infection with SHIV<sub>SF162P4cy</sub>. (A) Mean values with standard error of plasma viremia level for recombinant ( $n = 6$ ) and non-recombinant ( $n = 15$ ) class IA haplotypes are shown. (B) Localization of microsatellite markers in the MHC region of cynomolgus macaque. The class IA gene clusters are shown as shaded boxes. The markers are positioned according to Wiseman *et al.* (14) and Watanabe *et al.* (34). (C) Mean viral load of animals carrying M7 class IA, IB, II ( $n = 3$ ) and non-M7 class IA, IB, II ( $n = 18$ ) MHC haplotypes. A value of  $\log_{10} (50)/2$  assigned to viral loads below the cut-off level (50 copies/ml).  $n$ , number of animals in each group.

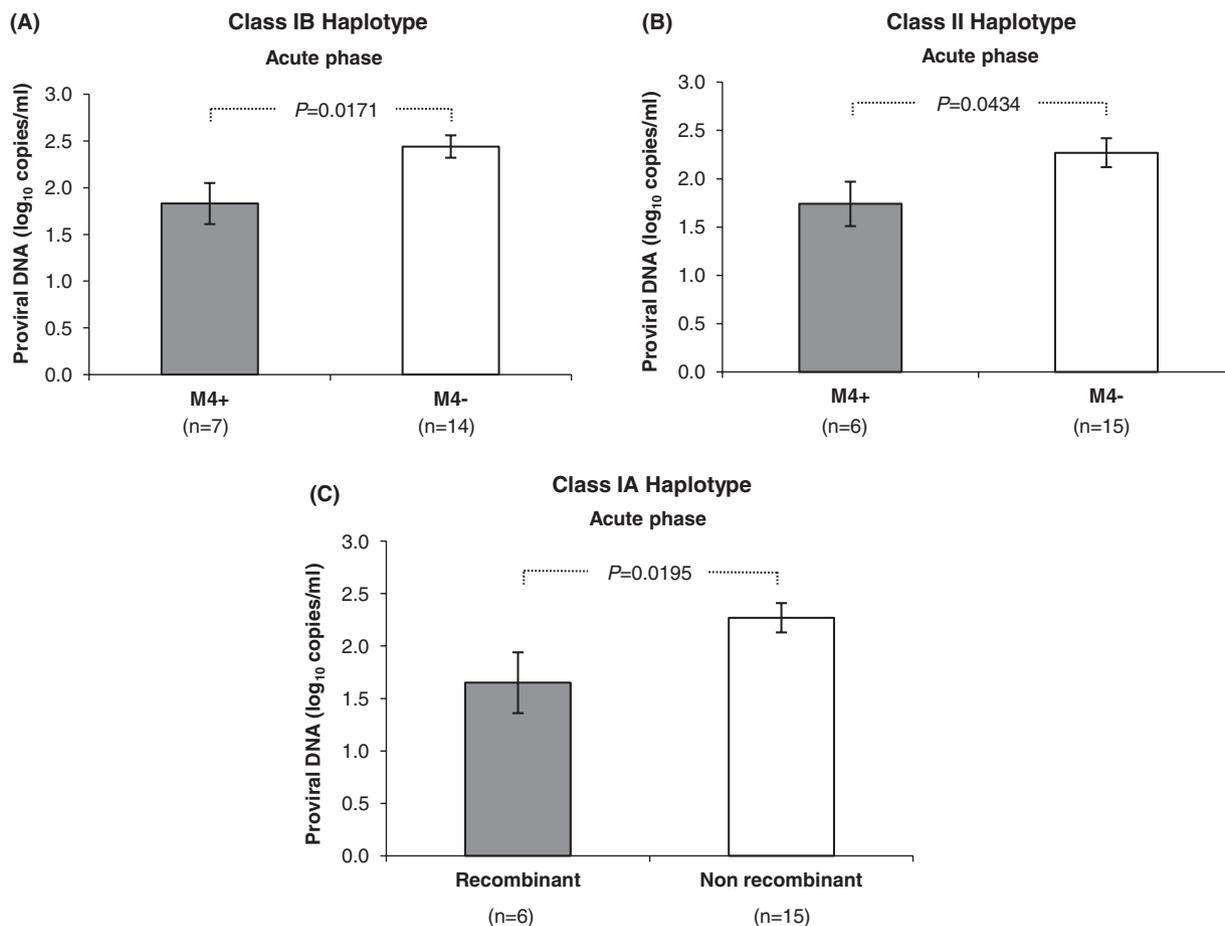
viral load (1.47  $\log_{10}$  RNA copies/ml) during the acute phase of infection associated with the recombinant MHC class IA haplotype ( $n = 6$ ), as compared with macaques with a non-recombinant MHC class IA haplotype ( $n = 15$ ,  $P = 0.0078$ , Figure 2A). Class IA recombinants were defined as falling between the first four markers, D6S2972, D6S2970, D6S2854, and D6S2704 described by Wiseman *et al.* (14). The relative locations of the four markers D6S2972, D6S2970, D6S2854, and D6S2704, adapted from Watanabe *et al.* and Wiseman *et al.* (14, 34), are shown in Figure 2B. Because several alleles are shared among the three most frequent class IA haplotypes, M1/M3 and M2/M3 recombinations should not alter the class IA allele composition. However, at 2–4 weeks p.i., the reduction of the peak viral load observed in the animals with M1/M3 or M2/M3 recombinant IA haplotypes ( $n = 3$ ) maintained statistical significance even when animals with M1/M4 or M2/M4 recombinant IA haplotypes ( $n = 3$ ) were excluded from the analysis ( $P = 0.0186$ ; data not shown). Moreover, when the opposite was performed and the three monkeys carrying the M1/M4 or M2/M4 recombinant IA haplotypes were compared with animals with non-recombinant haplotypes,

the former group still showed improved control of viremia ( $P = 0.0213$ ).

During the chronic phase of infection a persistent viremia was observed in four monkeys. Three out of four monkeys (AQ882, AU676, and AP511) carried the M7 (class IA, IB, and II)/rec haplotype, whereas the fourth (AQ271) carried M1 (class IA, IB, and II)/rec haplotype (Figure S1, *Supporting Information*, violet boxed haplotypes). At week 24, statistical analysis showed higher viral load in the M7 (class IA, IB, and II)/rec haplotype animals (Fisher's Exact test,  $P = 0.0030$ ) than in the other animals (Figure 2C), included monkey AF238 carrying the M7 haplotype only in class IA and IB regions, which was plasma viremia negative in the chronic phase of infection.

#### Effects of MHC haplotype combinations on proviral DNA levels in SHIV<sub>SF162P4cy</sub>-infected cynomolgus monkeys

Next we found that M4 and recombinant IA haplotypes also impacted on proviral copy number. In particular, M4 monkeys had lower proviral DNA values than non-M4 macaques in the



**Figure 3** Effects of major histocompatibility complex class IA, IB, and II haplotypes on proviral DNA following infection with SHIV<sub>SF162P4cy</sub>. Mean values with standard error are depicted for all animals positive and negative for the indicated haplotype: M4 ( $n = 7$ ), non-M4 ( $n = 14$ ) class IB; M4 ( $n = 6$ ), non-M4 ( $n = 15$ ) class II; recombinant ( $n = 6$ ) and non-recombinant ( $n = 15$ ) class IA.  $n$ , number of animals in each group.

acute phase of infection (Figure 3A, B). The association was statistically significant for the class IB ( $P = 0.0171$ ) and II regions ( $P = 0.0434$ ), but not for class IA. Recombinants in the class IA haplotype were also associated with lower proviral DNA copy numbers, which reached statistical significance at 2–4 weeks p.i. ( $P = 0.0195$ ) (Figure 3C). Such a reduction was observed in the animals with M1/M3 or M2/M3 recombinant IA haplotypes ( $n = 3$ ) at 2–4 weeks p.i. also when animals with M1/M4 or M2/M4 recombinant IA haplotypes ( $n = 3$ ) were excluded from the analysis ( $P = 0.0086$ ). In contrast, the three monkeys carrying the M1/M4 or M2/M4 recombinant IA haplotypes did not show control of provirus levels, when compared with animals with non-recombinant haplotypes.

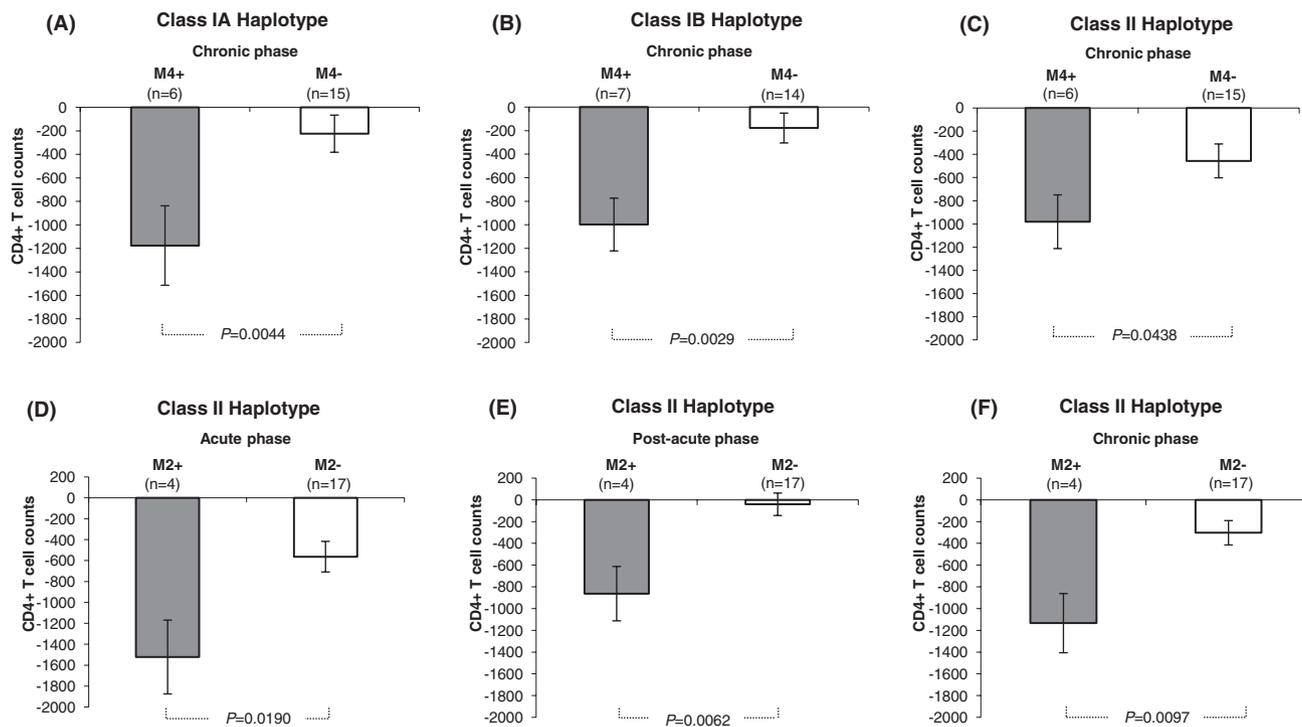
#### Effects of MHC haplotype combinations on CD4 T-cell counts in SHIV<sub>SF162P4cy</sub>-infected cynomolgus monkeys

When differences in post-infection CD4<sup>+</sup> T levels were considered, a significant association was noted between lower

CD4<sup>+</sup> T-cell counts and haplotype M4 for MHC class IA (chronic phase  $P = 0.0044$ , Figure 4A), class IB (chronic phase  $P = 0.0029$ , Figure 4B), class II (chronic phase  $P = 0.0438$ , Figure 4C). A significant detrimental effect of the MHC class II haplotype M2 was also observed on CD4<sup>+</sup> T-cell loss throughout the entire follow-up (acute phase  $P = 0.0190$ , Figure 4D; post-acute phase  $P = 0.0062$ , Figure 4E; chronic phase  $P = 0.0097$ , Figure 4F). The influence of MHC class II polymorphisms on CD4<sup>+</sup> T-cell counts in naive cynomolgus macaques was recently reported (35). However, in our monkey cohort no correlation could be established between MHC class IA haplotype M4, MHC class IB haplotype M4, or MHC class II haplotype M2 and pre-infection CD4<sup>+</sup> T-cell counts (data not shown).

#### Discussion

In this study, the infectivity of the SHIV<sub>SF162P4cy</sub> was assessed by evaluating levels of plasma viremia, CD4<sup>+</sup> T-cell counts, and disease progression (36). Similar to what has been



**Figure 4** Effects of major histocompatibility complex class IA, IB, and II haplotypes on CD4+ T-cell numbers in the acute (2–4 weeks p.i.), post-acute (8–16 weeks p.i.), and chronic phase (24–46 weeks p.i.) of infection. Mean values with standard error are depicted for all animals positive and negative for the indicated haplotype: M4 ( $n = 6$ ) non-M4 ( $n = 15$ ) class IA; M2 ( $n = 4$ ) non-M2 ( $n = 17$ ) class II.  $n$ , number of animals in each group.

reported in the rhesus monkey model (4, 7), SHIV<sub>SF162P4cy</sub> was mildly pathogenic in cynomolgus macaques as compared with more aggressive X4 SHIVs, indicating that in this model the virus more closely mimics the natural course of HIV infection observed in humans, thus showing a comparable susceptibility of the two monkey species with these viruses (7). We and others have previously observed an advantage of M3 or M6 haplotypes in the control of viral load in cynomolgus monkeys infected with SIV or CXCR4-tropic SHIV (15, 17, 25, 29). In this study, MHC genetics showed a significant effect of the M7 haplotype on viral persistence in macaques infected with the CCR5-tropic SHIV<sub>SF162P4cy</sub>. In particular, the detrimental effect of the M7 haplotype was associated with class IA, IB, and II regions, though with three of four animals possessing an intact M7 haplotype, localization of the effect to a specific region was not possible. Despite an effect on plasma viremia during chronic infection, no association was observed between MHC M7 haplotype and proviral DNA and CD4+ T-cell counts. Because only 1% of MCM carry the less frequent MHC class I M7 haplotype (25, 26), identifying large numbers of SHIV<sub>SF162P4cy</sub>-infected, M7 haplotype-carrying animals will be challenging. However, identification of those haplotypes associated with resolving/persistent plasma viremia will improve the validity of the MCM model. A further observation was that animals with recombinant class IA haplotypes controlled plasma viremia early after infection. In particular,

of the six animals with a recombinant class IA region, three recombinations occurred either between M1 and M3 (M1/M3;  $n = 2$ ) or M2 and M3 (M2/M3;  $n = 1$ ). When recombinant IA haplotypes were compared with the non-recombinants, animals carrying the M1/M3 or M2/M3 haplotypes showed significantly better control of infection. Recombinations among M1, M2, and M3 class IA haplotypes are not expected to change the class I alleles expressed, because these three haplotypes transcribe an identical *Mafa-A4\*01:01* allele and highly similar *Mafa-A1\*063* alleles. Moreover, the M1 and M2 haplotypes encode an identical allele, *Mafa-A2\*05:01*, while the M3 haplotype encodes an allelic variant *Mafa-A2\*05:11* that differs by seven nucleotides (26). Hence, the class I alleles expressed by the recombinant IA haplotypes are very similar, yet not identical. A possible explanation for the observed result is that the recombination event resulted in altered transcriptional activity in the class IA region, such that certain alleles are up or downregulated (37) though full analysis of the recombination junction regions by single-nucleotide polymorphism (SNP)/direct sequence analysis in our animals would be required to confirm this hypothesis. Changes in the expression pattern of alleles might not be the sole reason for the lower viremia detected in monkeys with a recombinant IA haplotype. To explain the results obtained from the recombinant animals both MHC haplotypes should be considered, because these animals are 'recombinant' for half of their MHC

genes. In fact, as all recombinant IA haplotype animals in this study inherited a non-recombinant haplotype from the other parent, it is possible that the protective effect displayed early in infection by the recombinant haplotype resulted from the combinatorial complexity of sequences located in the class IA gene region and their relative transcripts. This phenomenon has been already suggested by Wojcechowskyj *et al.* (38) in *Mamu-B\*17*-containing haplotypes in rhesus macaques.

In order to formally investigate this hypothesis, one could study SHIV infection of full siblings who inherit the identical recombinant-containing haplotype from one parent and an identical haplotype from their other parent. On a practical level, this is possible but challenging given the long generation time and high cost of selectively breeding sufficient macaques. Due to the limited number of animals, to strengthen the results obtained from the recombinant class IA haplotypes, it would be very interesting to expand the case study to include a large number of animals infected with the SHIV<sub>SF162</sub> lineage viruses. The lower CD4<sup>+</sup> T-cell levels observed during the chronic phases of infection in class IA, IB, and II M4 haplotype animals were concomitant with the lower proviral DNA values associated with class IB and II M4 haplotype in acute infection. However, the mechanism underlying these apparently contradictory associations is unclear. One possibility is that M4 animals are poorly able to control the virus; hence the pronounced drop in CD4<sup>+</sup> T cells and therefore a reduction in virus target cells leading to lower proviral loads. It is also possible that a redistribution of CD4<sup>+</sup> T cells could occur, due to the pro-inflammatory processes of the acute infection which may be influenced by stronger innate responses linked to specific haplotypes. Another explanation stems from a recent report showing the influence of the MHC on the blood CD4<sup>+</sup> counts in an outbred uninfected population of Filipino macaques (35). However, in our cohort of animals, counts of circulating CD4<sup>+</sup> T cells before infection were not influenced by the MHC haplotype.

A further observation was that class II-M2 was associated with lower CD4<sup>+</sup> T-cell levels throughout the entire p.i. follow-up. The above result is unusual, because very few associations between class II haplotypes and effects on the course of HIV/SIV infection, especially as early as 2 weeks p.i., have been reported (12, 16), in contrast with the many effects described for the MHC class I haplotypes on the dynamics of infection. However, the results obtained in M2 haplotype animals are consistent with other studies suggesting a negative effect of this haplotype in SHIV infection (15, 17). In the case of SIV infection, however, M2 was associated with a beneficial effect (19). It should be noted that in our study, bias in the results due to prior treatment of the animals with Alum or LTK adjuvant or differences in size of virus inoculum can be excluded because no significant differences in viral load, proviral load and CD4<sup>+</sup> T-cell counts were observed between groups of animals during the observation

period. While several of the associations we identified are consistent with those reported previously, a number were novel or contrasted with previous studies. However, it is to be stressed that the interplay between MHC host genetic background and the outcome of MCM infection is likely dependent on the strains of virus used, which possess distinct envelope sequences and different coreceptor usage that may influence viral pathogenesis. Therefore a direct comparison of studies using different monkey species/virus combinations is difficult to make at the present; as previous reports have noted, the effect of MHC haplotypes on infection should be determined for each virus isolate.

In summary, we conclude that SHIV<sub>SF162P4cy</sub> is a suitable virus for assessing vaccine efficacy in cynomolgus macaques and the SHIV-cynomolgus macaque system is a relevant non-human primate model for studying lentivirus pathogenesis. Here we have presented for the first-time evidence of the effects of MHC class I polymorphism on the replication of a cynomolgus macaque-derived CCR5-tropic SHIV by comparing the combination of three different parameters of infection, namely viral load, proviral load and CD4<sup>+</sup> T-cell counts. The most relevant and unexpected results reported are (1) the association among the M2, M4, and M7 haplotypes and the less favorable course of infection and (2) the effect of recombinant class IA haplotype on the control of infection. Although MCM represent an extraordinarily valuable model for understanding HIV disease, the source and the tropism of the challenging virus as well as the prevalence of the MHC haplotypes should be considered before randomization of monkeys among experimental groups in future studies in order to ensure a reliable interpretation of the data.

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## Conflict of interest

The authors have declared no conflicting interests.

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### Supporting Information

The following supporting information is available for this article:

Figure S1. Major histocompatibility complex haplotypes of 21 Mauritian cynomolgus macaques infected with simian/human immunodeficiency virus<sub>SF162P4cy</sub>. Violet boxes indicate haplotypes of the four monkeys with detectable viremia during the chronic infection.

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