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MHC heterozygote advantage in simian immunodeficiency virus-infected Mauritian cynomolgus macaques

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Abstract

The importance of a broad CD8-T lymphocyte (CD8-TL) immune response to HIV is unknown. Ex vivo measurements of immunological activity directed at a limited number of defined epitopes provide an incomplete portrait of the actual immune response. Here we examined viral loads in SIV-infected MHC homozygous and heterozygous Mauritian cynomolgus macaques (MCM). Chronic viremia in MHC homozygous macaques was 80-fold greater than in MHC heterozygous macaques. Virus from MHC homozygous macaques accumulated 11 to 14 variants consistent with escape from CD8-TL responses after one year of SIV infection. The pattern of mutations detected in MHC heterozygous macaques suggests that their epitope-specific CD8-TL responses are a composite of

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This manuscript demonstrates unambiguous MHC heterozygote advantage in macaque monkeys infected with the same strain of simian immunodeficiency virus (SIV), suggesting that a prophylactic HIV vaccine should elicit a population of CD8+ T cells with broad specificity.

Author contributions: This project was coordinated by S.L.O. Blood was processed by J.J.L., S.L.O., B.J.B., and J.M.G. ELISPOT assays were performed by J.J.L. and S.L.O. Viral sequencing was performed by A.M.D., E.A.B, and S.L.O. MHC and KIR genotyping was performed by R.A.W., J.J.T., J.A.K., B.N.B., and S.M.L. Viral loads were measured by C.E.M and T.C.F. Statistical analysis of viral loads was performed by R.C.J. and M.C. Statistical analyses of viral sequences were performed by A.L.H. Data from SIVmac251 infected MCM was provided by E.T.M. and N.J.R. Neutralizing antibody titers were measured in the lab of R.C.D. This project was originally conceived by D.H.O. Data was collected and analyses were performed in the lab of D.H.O.

Competing interests: Dr. David O'Connor consults for the International AIDS Vaccine Initiative.

those present in their MHC homozygous counterparts. These results provide the clearest example of MHC heterozygote advantage among individuals infected with the same immunodeficiency virus strain, suggesting that broad recognition of multiple CD8-TL epitopes should be a key feature of HIV vaccines.

Keywords

MHC; heterozygosity; CD8; SIV

Introduction

Antiviral CD8 T lymphocytes (CD8-TL) play an important role in the containment of human and simian immunodeficiency virus (HIV/SIV). HIV vaccine trials designed to exclusively elicit antiviral CD8 T cells have failed to demonstrate effective protection (1,2). Although it has been suggested that limited CD8-TL responses elicited by these vaccines were insufficient to confer protection (1,2), nearly three decades of research has failed to define the breadth of cellular immune responses that should be elicited by vaccination.

HIV/SIV-specific CD8-TL responses measured by ex vivo immunological assays (e.g. IFN-γ ELISPOT, polyfunctional ICS) cannot provide a comprehensive picture of the cellular immune response. These assays rely on synthetic peptide stimulation and do not stimulate CD8-TL with the complete set of antigenic peptides seen in vivo (3,4). Defining the entire repertoire of immunodominant and subdominant CD8-TL epitopes with synthetic peptides is impossible, as the sequence of HIV in the host is always changing and presenting new antigens to CD8-TL (3,5). Accordingly, ex vivo quantified CD8-TL responses have not correlated with plasma viremia (3,6,7). Furthermore, CD8-TL responses measured in the periphery may not accurately represent the CD8-TL responses present at the sites of most intense viral replication (8-10). Lastly, CD8-TL responses fluctuate during the course of an infection, thus no single time point can be considered representative of the CD8-TL breadth within each individual (11,12). Given these challenges, it is not surprising that previous attempts to define the importance of the breadth of the entire CD8-TL response to HIV/SIV disease outcome have yielded mixed results (13,14).

The link between CD8-TL breadth and disease outcome has been studied in vivo in HIV+ individuals who are homozygous or heterozygous at Human Leukocyte Antigen (HLA) class I loci. HLA-heterozygous individuals express a greater number of MHC molecules than HLA-homozygous individuals, potentially broadening their HIV-specific CD8-TL response (15, 16). These studies indirectly link CD8-TL breadth with HIV disease progression, and they do not suffer from sampling biases associated with examining limited numbers of epitopes, certain tissue sites, or specific time points during infection. HLA-heterozygous HIV+ individuals progress to AIDS more slowly than patients who are homozygous at one or more HLA class I locus (17,18). One major drawback to these studies is that HLA genetics and viral strains are different for each HIV+ individual. These studies may also be influenced by frequency-dependent selection (19-22). To unambiguously define the relationship between MHC diversity, CD8-TL breadth, and disease outcome, it is essential to study individuals who have identical MHC genetics and are infected with the same viral strain. Unfortunately, such HIV + patients are exceedingly rare, so detailed studies of MHC heterozygote advantage can only be undertaken in animal models where both host and viral genetics can be controlled.

Mauritian cynomolgus macaques (MCM) exhibit limited MHC diversity, are susceptible to infection with the clonal simian immunodeficiency virus (SIV) strain SIVmac239, and mount CD8-TL responses that can select for SIV escape variants (23-25). Nearly all of the MHC

genetic diversity found in this population is distributed amongst 7 common haplotypes, referred to as M1 through M7 (25,26). Approximately 90% of feral MCM have at least one copy of one of the three most common haplotypes (24). MCM who are homozygous and heterozygous for these MHC haplotypes exist naturally and can be rapidly selected by genetic screening.

Here we infected eight MHC homozygous MCM and five MHC heterozygous MCM with SIVmac239. We found that the average chronic phase viral load set point in MHC homozygous MCM was 80-fold greater than the chronic phase viral load set point in MHC heterozygous MCM. SIV genome sequence data suggests that CD8-TL responses mounted in MHC heterozygous MCM were likely a composite of the responses mounted in each MHC homozygous counterpart. Our data suggests that this heterogeneous composition of CD8-TL responses likely contributed to the superior viral control exhibited in these animals.

Results

Selection of MHC homozygous MCM

The majority of MCM MHC genetic diversity can be explained by 7 haplotypes (M1-M7) (25,26). These seven haplotypes can be distinguished using a panel of microsatellite markers (25,27). MCM homozygous for all haplotypes, except for M7, were identified in a microsatellite screen of 425 MCM, and the frequency of MCM homozygous for a particular MHC haplotype ranged from <0.2% to 4.2% (Fig. 1). The homozygosity of animals was confirmed using a newly described deep sequencing method (28) (fig. S1), even though some low abundance transcripts were difficult to detect. Because M1, M2, and M3 homozygotes were most common, we focused on animals with these haplotypes. The M1, M2, and M3 haplotypes share MHC class I A alleles (24); however, we expect that the different MHC class I B alleles encoded on each haplotype will restrict a unique set of SIV-specific CD8-TL responses (25). Furthermore, since the number of macaque MHC class I B alleles encoded per haplotype is considerably greater than the number of MHC class I A alleles (29), MCM heterozygous for M1, M2, or M3 express nearly double the MHC alleles than an animal who is MHC homozygous for one of these three haplotypes (Table S1).

Association of MHC homozygosity with higher viremia during the chronic phase of SIV infection

Eight MHC homozygous MCM (two M1/M1, two M2/M2, and four M3/M3 MCM) were infected with SIVmac239 (Table 1) or were superinfected as part of an unrelated lymphocyte adoptive transfer study (fig. S2 and (30)). Viral loads were measured in these eight animals for one year post infection (Fig. 2A). The average chronic viral load set point was 1.5×10^5 copies/ml (Table 2A), approximately 10 to 100 times higher than the viral load set point observed in previous studies of cynomolgus macaques infected with the genetically complex biological swarm virus SIVmac251 (31,32).

Among the eight homozygous MCM, only CY0165 maintained low chronic viremia. We examined neutralizing antibody titers in CY0165 and the other three M3/M3 homozygous MCM at 24 weeks post infection (wpi). Antibody titers in CY0165 were lower than in the other three animals (Table S2), but this may be due to the lower viral loads present in this animal. We also examined killer immunoglobulin receptor genetics of the homozygous MCM (33), but did not identify any obvious differences in CY0165 (Table S3) that could account for this animal's superior viral control.

Significantly lower chronic phase SIV viremia in MHC heterozygous MCM than in MHC homozygous MCM

We retrospectively analyzed viral loads of five MHC heterozygous MCM used in other SIV studies to determine whether the high chronic viremia observed in MHC homozygous MCM was a consequence of their limited MHC diversity (Table 1). All five animals were either infected with SIVmac239 or were superinfected as part of unrelated lymphocyte adoptive transfer studies (Materials and Methods, fig. S2, and (30)). The chronic phase viral load set point in the MHC heterozygous cohort was 1.9×10^3 copies/ml (Fig. 2B and Table 2A). Remarkably, average chronic viremia in the MHC homozygous MCM was 80-times greater than average chronic viremia in the MHC heterozygous cohort, suggesting that MHC homozygosity is associated with a high viral load set point in animals infected with SIVmac239 (Fig. 2C, 2E and Table 2B). We did not observe differences in peak viral load, consistent with the hypothesis that viral load set point, rather than peak viral load, is affected by CD8 T cell responses (34). Additionally, we do not believe the adoptive transfers affected the outcome, as the lower viral load set point observed in MHC heterozygous recipients was comparable to the viral load set points observed in previous studies of SIVmac251-infected Mauritian cynomolgus macaques who were not selected for study based on whether they were MHC homo- or heterozygous (31,32).

Four out of five MHC heterozygous MCM used in this study contained a single copy of the M3 haplotype. The M3 MHC class I B haplotype appeared to be protective in a study of MCM infected with SHIV89.6p, but not SIVmac251 (31,35). Interestingly, average chronic phase viremia of M3 homozygous MCM was 283-times greater than average chronic phase viremia of M3 heterozygous MCM (Fig. 2D, 2E, and Table 2B). Taken together, these findings strongly suggest MHC heterozygote advantage in genetically matched macaques infected with the same strain of SIV.

In an effort to extend our observations, we retrospectively analyzed the MHC haplotypes of a previously described cohort of 19 MCM infected with SIVmac251 that included six MHC homozygous MCM (31,36,37). Unfortunately, the MHC heterozygote advantage did not appear to extend to this cohort of animals. It is possible that these animals were not studied long enough to observe a heterozygote advantage in animals infected with a swarm virus, as 11 of the 19 animals were monitored for 20 weeks or less. Alternatively, by using a swarm virus as the inoculum, rather than clonal SIVmac239, a greater number of animals may be necessary to reveal a heterozygote advantage. These ambiguous results, however, underscore the importance of studying the MHC heterozygote advantage in macaques where both the host and viral genetics are controlled.

Estimating the breadth of CD8-TL responses in MHC homozygous and heterozygous MCM

We examined SIV-specific CD8-TL responses mounted by M2/M2 and M3/M3 MCM using IFN-γ ELISPOT. We measured IFN-γ production following stimulation with 35 peptide pools spanning Gag, Nef, Tat, Rev, Vpr, Vpx, and Vif at 16 wpi. Five peptide pools elicited responses shared between both M2/M2 animals and five peptide pools elicited positive responses in at least three of the four M3/M3 animals (fig. S3). In contrast, we identified ten peptide pools that elicited responses in only one M2/M2 animal and 23 pools that induced positive responses in less than three M3/M3 animals. The inconsistent detection of certain CD8-TL responses in animals that are MHC-identical confounded our attempts to attribute a positive response by IFN-γ ELISPOT to the presence of a particular MHC genotype.

Previous studies of monozygotic twins infected with the same source of HIV (38,39) suggested that shared CD8-TL responses could be inferred from parallel patterns of viral evolution, as the majority of HIV/SIV sequence variation outside of Env is located in CD8-TL epitopes

(34,40,41). We sequenced bulk PCR amplicons spanning the entire SIV genome of viruses isolated from the eight MHC homozygous MCM at approximately 20 wpi (fig. S4-S6). We found five to eight sites consistent with CD8-TL selection in each of the three MHC homozygous groups (Fig. 3). These shared variants comprise 66% (range 46% to 86%) of total nonsynonymous amino acid changes detected outside of Env, in accord with previous estimates of CD8-TL contribution to HIV/SIV diversification (40,41). Three sites of variability are located within previously described CD8-TL epitopes restricted by MHC class I A alleles common to M1, M2, and M3 ($Gag_{385-394}$, $Nef_{103-112}$, and $Pol_{591-600}$) (24), while an additional site of variability shared among all three MHC homozygous groups ($Nef_{197-206}$) strongly suggests a heretofore unknown epitope restricted by one of these MHC class I A alleles. At least two additional sites consistent with CD8-TL selection were detected in each group of MHC homozygous MCM. This data implies that at least five CD8-TL responses selected for immunologic escape variants during the first 20 weeks of infection in the MHC homozygous animals, even though seven out of these eight animals failed to control viremia. It is likely that additional CD8-TL responses were present during acute infection, but failed to select for viral variants detectable by bulk sequencing at 20 wpi.

We additionally sequenced the entire SIV genome from viruses replicating in MHC homozygous MCM at approximately one year post infection (fig. S7-S9). Using the same criteria established to examine viral sequence variation at 20 wpi, we detected 11 to 14 shared mutations present within each MHC-identical group (Fig. 4, 5). These shared variants comprise an average of 62% (range 46% to 75%) of the total nonsynonymous changes outside of Env, suggesting that a minimum of six additional epitope-specific CD8-TL responses were lost to escape between 20 weeks and one year of infection. We found that d_N was significantly greater than d_S at these selected sites, consistent with the hypothesis that positive selection favored amino acid changes within these regions during the first year of infection (Table S4) (42). In the absence of knowing most SIV epitopes restricted by MCM MHC alleles, viral sequence variation provided us with the most comprehensive picture of the immune response currently available. Nevertheless, the actual magnitude of the shared CD8-TL response is greater than can be predicted by viral sequencing or IFN-γ ELISPOT alone.

As described above, MHC heterozygous MCM infected with SIVmac239 maintained low chronic phase viral loads, which could limit the emergence of variants selected by CD8-TL responses. Nonetheless, we sequenced viruses isolated from MHC heterozygous MCM at one year post infection to determine whether variation present in viruses isolated from these animals would contain the same sequence signatures observed in their MHC homozygous counterparts. We found 11 viral variants in viruses isolated from M1/M3 MCM that matched those observed in viruses from M1/M1 and M3/M3 animals (Fig. 4). We subsequently compared the sequences of SIV replicating in the M1/M2 heterozygote (CY0116) to the sequence of SIV in M1/M1 and M2/M2 MCM at approximately one year post infection. We found 15 variants in viruses isolated from CY0116 that matched the variant sequences of viruses isolated from M1/M1 and M2/M2 animals (Fig. 5). In both cases, we found that conservation of wild type SIV sequence in the heterozygotes occurred to a greater extent than expected by chance within these select regions (fig. S10), consistent with models suggesting that a lower viral load lengthens the time for escape variants to emerge (43), and may explain why some variants were not observed in the heterozygous animals. In sum, the variants emerging in the MHC-heterozygous MCM were a composite of variants that emerged in their MHC-homozygous counterparts, strongly suggesting that CD8-TL responses recognizing epitopes restricted by alleles from both MHC haplotypes were present in the MHC-heterozygous MCM.

Discussion

MHC heterozygote advantage has been previously described in HIV (17,18). Unfortunately, these studies were confounded by the diversity of HLA alleles and HIV sequences of participants. In contrast, we compared SIV viral loads in a unique population of non-human primates who were homozygous and heterozygous for the same MHC alleles, thus providing unambiguous evidence for MHC heterozygote advantage in MHC-identical nonhuman primates infected with the same strain of SIV. This provides the clearest evidence to date that maximizing the potential diversity of epitope-specific CD8-TL is important for containing viral replication, as the patterns of viral selection indicate that CD8-TL responses in heterozygous animals are a composite of the responses observed in homozygous animals. Until now, it has been impossible to determine whether the primary mechanism for MHC heterozygote advantage is the broad recognition of greater numbers of CD8-TL epitopes or the greater likelihood of mounting a single, particularly potent CD8-TL response in individuals with approximately twice as many MHC alleles. By studying M1/M1, M1/M3, and M3/M3 MCM infected with the same strain of SIV, it appears that the breadth of the CD8-TL response is principally responsible for controlling viral replication.

In addition to demonstrating the importance of CD8-TL breadth in SIV control, MHC homozygous macaques can be used to address several previously inaccessible questions about CD8-TL breadth in HIV/SIV. For example, attenuated strains of SIV offer outstanding protection against challenge with homologous strains of SIV and confer modest protection against heterologous challenge. Some have speculated that the effective CD8-TL breadth is diminished in animals challenged with heterologous virus; comparing the efficacy of attenuated vaccines in MHC homozygous and heterozygous macaques can now test this hypothesis. Additionally, in this study we estimate that at least eleven SIV-specific CD8-TL responses emerged during the first year of infection in MHC homozygous macaques yet failed to control SIV replication during chronic infection. MHC homozygous MCM may be a more rigorous animal model in which to test whether a vaccine candidate is effective when the potential diversity of CD8-TL responses is minimized, but consistent, among MHC-identical animals.

This study also highlights limitations of using ex vivo immunological assays to assess the importance of CD8-TL breadth. Results from IFN-γ ELISPOT assays performed with fresh PBMC from MHC-identical animals were inconsistent, even though sequences of viruses replicating in MHC-identical animals were highly concordant at two time points during infection. Since CD8-TL responses select for escape variants at different rates, however, simply replacing IFN-γ ELISPOT and other ex vivo immunological assays with viral sequencing is not appropriate. Some CD8-TL responses, particularly those in regions of the virus that are under structural or functional constraint, may not select for escape variants even after prolonged infection. It is therefore important to interpret the laboratory results from this study cautiously; at least eleven CD8-TL responses were likely mounted in MHC homozygous animals that failed to control SIV, however, the true breadth of the CD8-TL response in blood and tissue may have been considerably higher than could be measured with available laboratory assays.

Caution must also be taken when extrapolating these findings to other contexts. Our study broadly agrees with previous studies demonstrating HLA heterozygote advantage in HIV; however, HLA heterozygote advantage is not the same as exceptional HIV control: more than 75% of fully HLA heterozygous HIV+ patients have chronic phase viral loads in excess of 10,000 copies / mL blood plasma (75.3% of whites, N = 1351; 82.2% of blacks, N = 197). In contrast to the diversity of circulating HIV strains that may each contain CD8-TL epitopes with variable levels of antigenicity, clonal SIVmac239 may contain critical epitopes recognized by cynomolgus macaque CD8-TLs that could contribute to the superior rate of virologic control observed in many of the animals studied. Repeating these studies using SIVmac239 serially

passaged in MCM as the inoculum could increase the similarity of this model to HIV infection. Moreover, it is important to note that we were unable to exhaustively evaluate whether MHC heterozygote advantage applies to all combinations of MCM MHC haplotypes. Our cohort of MHC heterozygous animals was biased towards those containing the M3 haplotype. Although we infected one non-M3 containing MHC heterozygous MCM (CY0116), chronic phase viremia in this animal was markedly higher than in the M3-heterozygous MCM. It will also be important to verify that the observations described for SIVmac239 apply to other SIV strains. Prospective analysis of MHC homozygous and heterozygous MCM infected with viruses such as SIVsmE660 and SIVmac251 will be essential, as analyses of previously infected animals unlikely included enough MHC homozygous macaques and were not followed for a long enough time to draw generalized conclusions when using a swarm virus as the inoculum.

Despite these limitations, our study provides the most dramatic evidence to date of MHC heterozygote advantage in primate lentivirus infection. These results strongly suggest that a CD8-TL response directed at a maximally diverse set of epitopes is advantageous and provides a compelling experimental rationale for eliciting such responses by prophylactic vaccination.

Materials and Methods

Animals and SIVmac239 challenge

MCM were purchased from Charles River Laboratories (Charles River BRF, Houston, TX). Animals CY0111, CY0113, CY0152, and CY0161-166 were challenged intrarectally with a single dose of 50,000 TCID50 (tissue culture dose sufficient to infect 50% of cells) of molecularly cloned SIVmac239 Nef open virus (44). Animals CY0150, CY0153, and CY0154 were initially infected intravenously as recipients of purified CD8β T cells isolated from an SIVmac239-infected MCM whose bulk sequence matched SIVmac239, except within the previously described $Nef_{103\text{-}111}$RM9 epitope (fig. S2A and (30)). These three animals were then challenged intrarectally with 50,000 TCID50 SIVmac239 36 hours after transfer (30). CY0116 was a recipient of a lymphocyte adoptive transfer from an MHC-matched animal infected with SIVmac239Δnef and subsequently challenged with 50,000 TCID50 SIVmac239 12 hours after transfer. The sequence of *nef* replicating in CY0116 at one week post infection was wild type (fig. S2B), suggesting that he was not likely infected from the donor animal. All animals were monitored for approximately one year post infection. Animal studies were approved by the Wisconsin Institutional Animal Care and Use Committee (IACUC). All animals were maintained at the Wisconsin National Primate Research Center and cared for according to the guidelines of the IACUC.

MHC and KIR genotyping of MCM

Microsatellite analysis of genomic DNA was performed as previously described to identify MHC and KIR genotypes (25,27,45). Ultradeep-pyrosequencing was used to confirm the genotypes of the M3/M3 MCM and performed as previously described (28).

Plasma viral load analysis

SIVmac239 plasma viral loads were determined essentially as previously described (25,46, 47). Briefly, viral RNA was reverse transcribed and quantified with the SuperScript III Platinum One-Step Quantitative RT-PCR System (Invitrogen, Carlsbad, CA) on a Light Cycler 2.0 (Roche, Indianapolis, IN). Serial dilutions of an SIV gag in vitro transcript were used as an internal standard curve for each run. LightCycler software version 4.0 was used to interpolate samples onto the standard curve to determine the copy number. The detection limit of the assay is 30 vRNA copy equivalents per milliliter of plasma. The limit of detection was used as the data point when the viral load was at or below the limit of detection.

Statistical analyses

Average $\log_{10}$ chronic viral load, including all data after 14 weeks of infection, was modeled using linear mixed effects regression, with a random effects term for each animal. An MCMC sample of the data (100,000 iterations) was used to estimate 95% confidence intervals and p-values for all comparisons. Mean viral load trends were estimated on the $\log_{10}$ scale with a LOWESS smoother and plotted to visually compare trends in each group. Bootstrapped samples (10,000 iterations) were also used to approximate 95% confidence regions on the figures. All statistics were calculated in R, version 2.9.0 (R Foundation for Statistical Computing, Vienna, Austria), primarily using the lme4 package.

Measurement of neutralizing antibodies

Neutralizing activity against cloned SIVmac239 and against uncloned lab-adapted SIVmac251 was measured using a SEAP reporter cell assay as previously described (48,49).

IFN-γ enzyme linked immunospot (IFN-γ ELISPOT) assays

IFN-γ ELISPOT assays were performed, essentially, as previously described (24). Briefly, peripheral blood mononuclear cells (PBMC) were isolated from EDTA-anticoagulated blood by Ficoll-Paque Plus (GE Healthcare, Bioscience, Uppsala, Sweden) density gradient centrifugation. 10^5 PBMC were placed in each well of a precoated monkey IFNγ-ELISPOT^{PLUS} plate (Mabtech, inc., Mariemont, OH) with 10μM of each peptide pool. Each pool was tested in duplicate. Concavalin A was used at 10μM as a positive control. Experiments were performed according to the manufacturer's protocol. Wells were imaged with an AID ELISPOT reader, and spots were counted with consistent parameter settings. For each experiment, responses were considered positive if the mean number of spots per pool exceeded background plus two standard deviations. Spot forming cells (SFCs) per 10^6 PBMC were determined by calculating the average number of spots per pool, subtracting the average number of spots made by PBMC without peptide, and extrapolating to 10^6 PBMC. The minimum number of SFCs per 10^6 PBMC required to consider a response positive was determined by calculating two times the standard deviation and extrapolating to 10^6 PBMC.

Viral sequence analysis

Cell-free plasma was prepared by Ficoll density gradient centrifugation of EDTA anticoagulated whole blood. Viral RNA was then isolated from the plasma with the Qiagen MinElute vRNA isolation kit (Qiagen, Valencia, CA). The QIAGEN one-step reverse transcription PCR kit (Qiagen, Valencia, CA) was used to amplify viral sequences spanning the entire SIV genome using 16 overlapping PCR amplicons (Table S5). Thermocycling conditions for the RT-PCR reactions were as follows: 50°C for 30 minutes, 95°C for 15 minutes, 45 amplification cycles (98°C for 1 minute, 58°C for 1 minute, and 72°C for 2.5 minutes), and 68°C for 2 minutes. PCR products were gel purified with the Qiaquick gel extraction kit (Qiagen, Valencia, CA). Purified PCR products were bidirectionally sequenced (Table S5) with the DYEnamic ET Terminator cycle sequencing kit (GE Health Care, Piscataway, NJ), and then resolved on an ABI 3730xl (Applied Biosystems, Foster City, CA). Sequences were analyzed using CodonCode Aligner software (CodonCode Corporation, Dedham, MA). A custom assembly script was used to assemble high-quality sequences and identify nucleotide mutations to maintain consistency between each sample analysis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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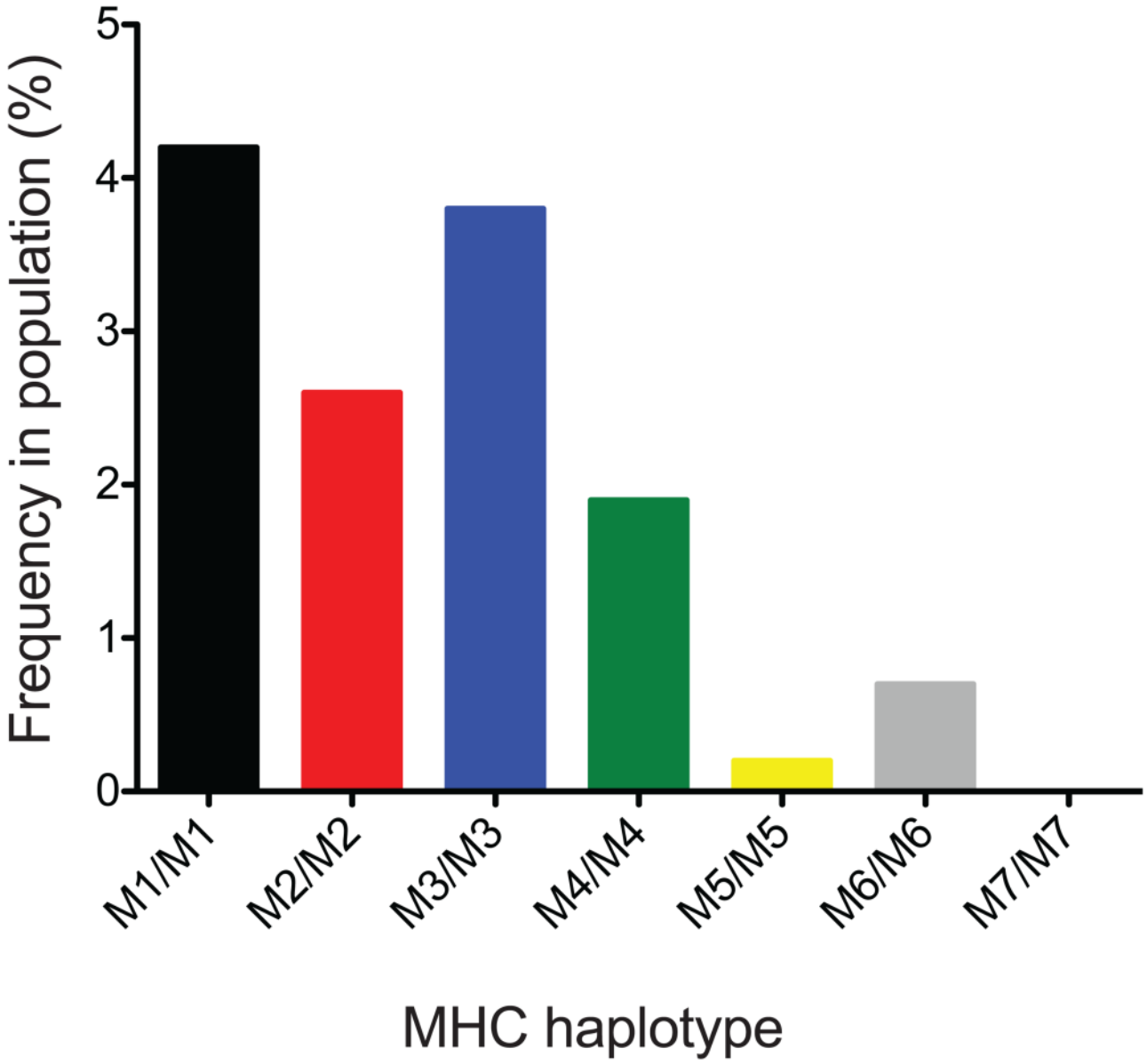

Figure 1. The frequency of MHC haplotypes among MHC-homozygous MCM was determined
Microsatellite typing was performed on 425 feral MCM. The frequency of MCM homozygous for the seven most common MHC haplotypes is shown.

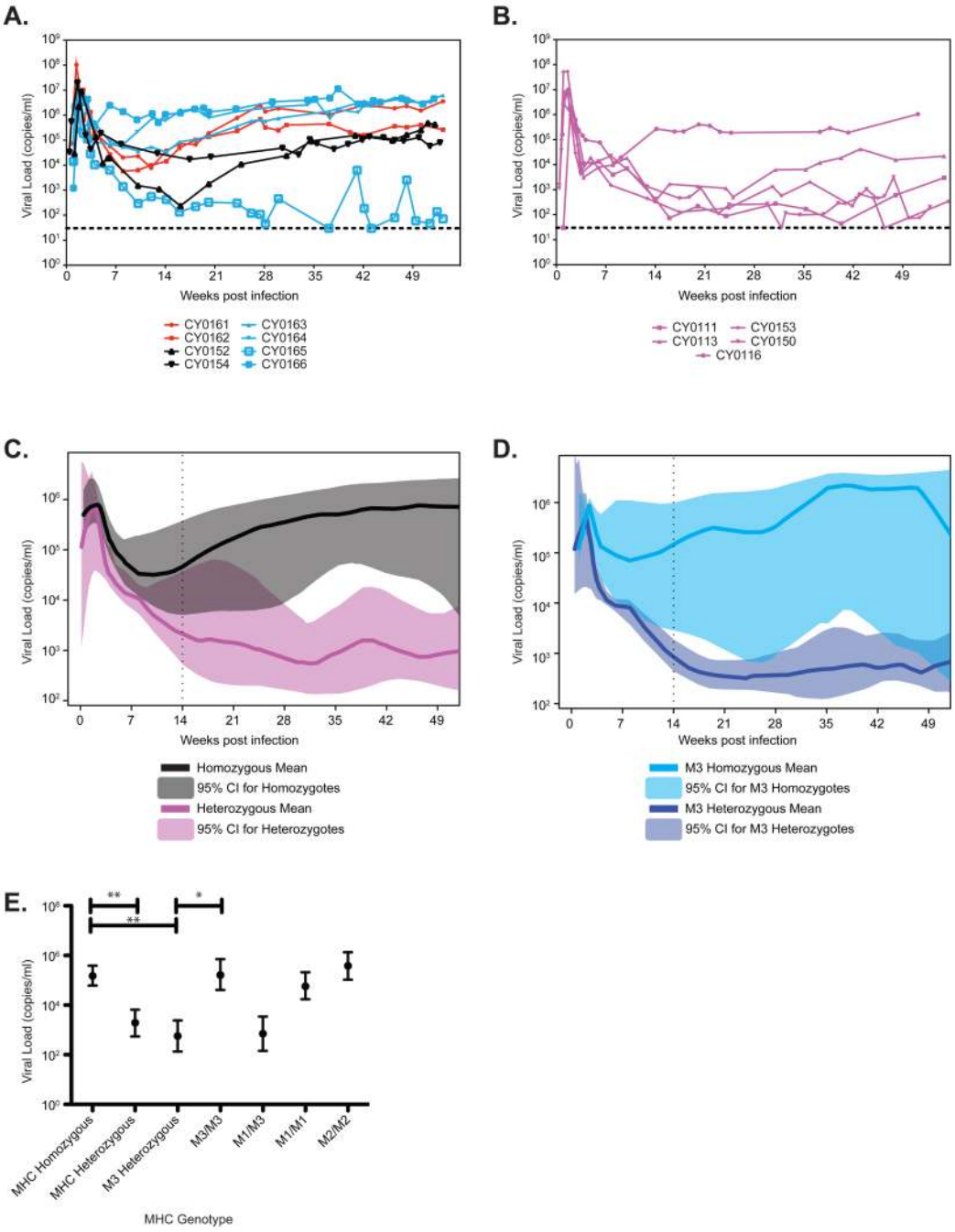

Figure 2. Plasma viral loads were determined in MHC homozygous and heterozygous MCM infected with SIVmac239 for one year

SIV viral loads were monitored for approximately one year post infection. The limit of detection is shown as a dotted line (30 copies/ml). (A) SIV viral loads in the MHC homozygous animals. The colors black, red, and blue represent individuals homozygous for the MHC haplotypes M1, M2, and M3, respectively. (B) SIV viral loads in the MHC heterozygous MCM. (C) Mean SIV viral loads for the MHC homozygous (n=8) and heterozygous MCM (n=5) cohorts were determined. The bootstrapped 95% confidence region is shown for each cohort as the shaded area. (D) Mean SIV viral loads for the M3 homozygous (n=4) and M3 heterozygous (n=4) cohorts were determined. The bootstrapped 95% confidence region is

shown for each cohort as the shaded area. (E) The chronic phase viral load set point was plotted for each of the indicated groups of MCM (Table 2A, Materials and Methods). Significant differences are indicated with asterisks.

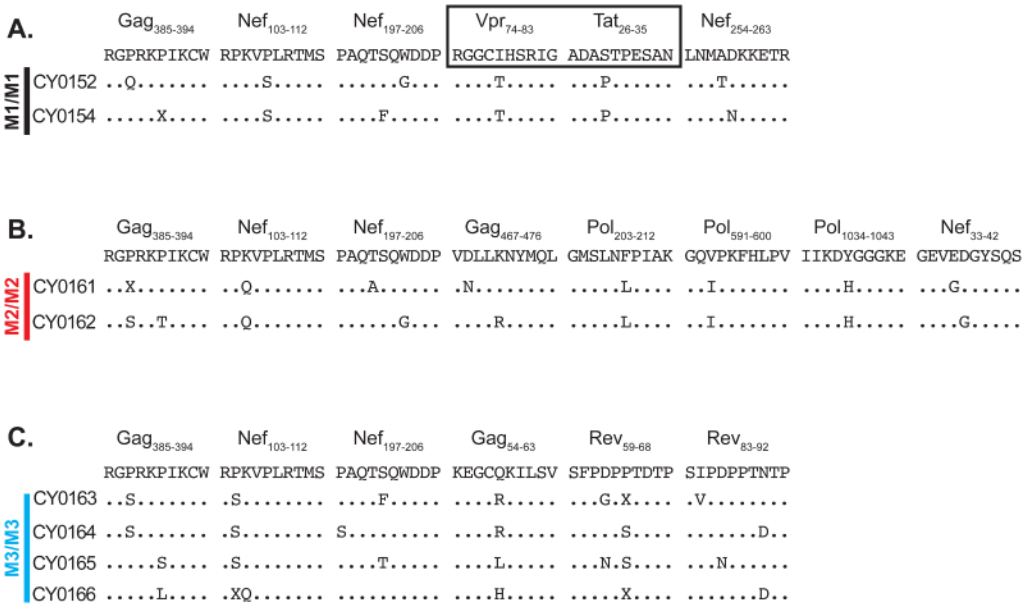

Figure 3. Patterns of viral sequence variation are shared in viruses isolated from MHC-identical and homozygous MCM at 20 wpi

(A) Viruses isolated from M1/M1 MCM were sequenced. Positions where high frequency variants were observed in viruses isolated from both animals are shown. The epitopes surrounded by a black box indicate that a single nucleotide mutation generated nonsynonymous mutations in both proteins. (B) Viruses isolated from M2/M2 MCM were sequenced. Positions where high frequency variants were observed in viruses isolated from both animals are shown. (C) Viruses isolated from M3/M3 MCM were sequenced. Positions where high frequency variants were observed in at least three out of four animals are shown. For A, B, and C, the SIVmac239 wild type sequence is shown on the top line, while the sequences isolated from each animal are shown below. Dots represent identity to the wild type sequence. A capital letter represents a complete amino acid replacement at that position. An 'X' represents a mixture of amino acids at that position.

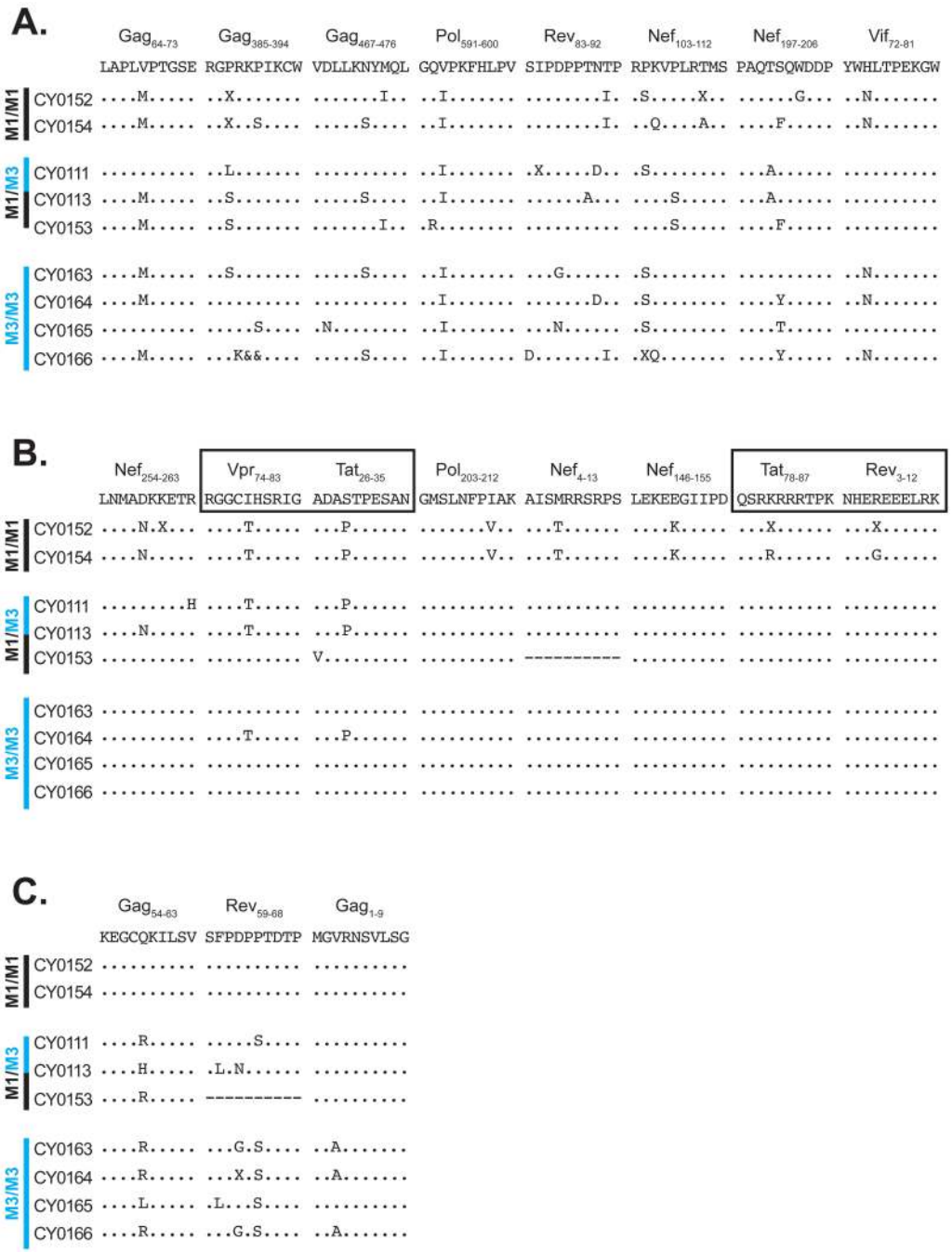

Figure 4. Viral sequence variants present in viruses isolated from M1/M3 MCM are a composite of those variants present in M1/M1 and M3/M3 MCM

Viruses were isolated and sequenced from all nine animals between 41 and 54 wpi. (A) Viral variants present in viruses replicating in both M1/M1 and three out of four M3/M3 animals are shown. The corresponding sequences observed in viruses replicating in the M1/M3 animals are shown. In animal CY0166 in $Gag_{385-394}$, the "&" represents a mixture of the amino acid sequences "KP," "KL," and "KSP" (B) Viral variants present in viruses replicating in both M1/M1 MCM, but in less than three M3/M3 MCM, are shown. The epitopes surrounded by a black box indicate that a single nucleotide mutation generated nonsynonymous mutations in both

proteins. (C) Viral variants present in viruses replicating in three out of four M3/M3 MCM, but not in both M1/M1 MCM, are shown. Dashes represent incomplete sequence.

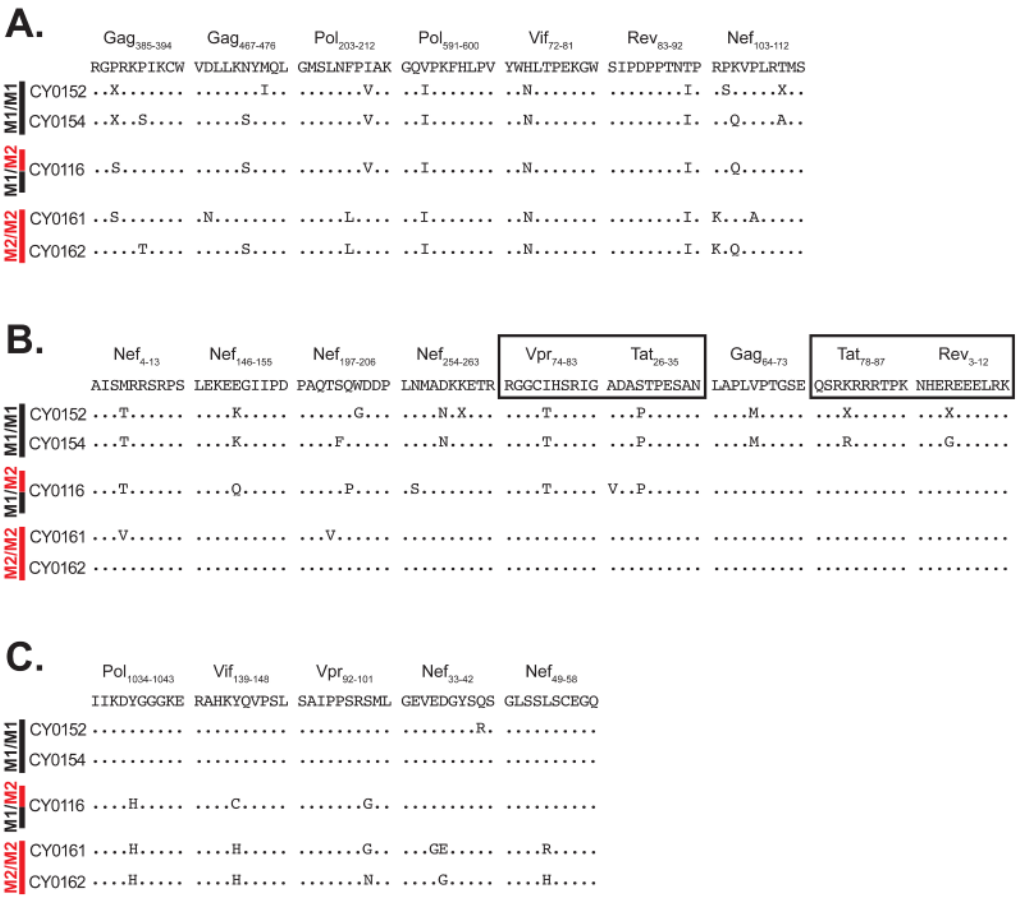

Figure 5. Viral sequence variants present in viruses isolated from an M1/M2 MCM are a composite of those variants present in M1/M1 and M2/M2 MCM

Viruses were isolated and sequenced from all five animals between 48 and 54 wpi. (A) Viral variants present in viruses replicating in both M1/M1 and M2/M2 animals are shown. Corresponding sequences observed in viruses replicating in the M1/M2 animal are shown. (B) Viral variants present in viruses replicating in both M1/M1 MCM, but not in both M2/M2 MCM, are shown. The epitopes surrounded by a black box indicate that a single nucleotide mutation generated nonsynonymous mutations in both proteins. (C) Viral variants present in viruses replicating in both M2/M2 MCM, but not in both M1/M1 MCM, are shown.

Table 1

MHC genotypes and method of infection for animals studied

Animal Number	MHC class I A genotype[1]	MHC class I B genotype[1]	MHC class II genotype[1]	Method of infection
CY0152	M1/M1	M1/M1	M1/M1	Intrarectal, 50,000 TCID50 SIVmac239
CY0154	M1/M1	M1/M1	M1/M1	Intravenous as adoptive transfer recipient[2]
CY0161	M2/M2	M2/M2	M2/M2	Intrarectal, 50,000 TCID50 SIVmac239
CY0162	M2/M2	M2/M2	M2/M2	Intrarectal, 50,000 TCID50 SIVmac239
CY0163	M3/M3	M3/M3	M3/M3	Intrarectal, 50,000 TCID50 SIVmac239
CY0164	M3/M3	M3/M3	M3/M3	Intrarectal, 50,000 TCID50 SIVmac239
Cy0165	M3/M3	M3/M3	M3/M3	Intrarectal, 50,000 TCID50 SIVmac239
Cy0166	M3/M3	M3/M3	M3/M3	Intrarectal, 50,000 TCID50 SIVmac239
CY0111	M1/M3	M1/M3	M1/M3	Intrarectal, 50,000 TCID50 SIVmac239
CY0150	M3/M4	M3/M4	M3/M4	Intravenous as adoptive transfer recipient[2]
CY0153	M1/M3	M1/M3	M1/M3	Intravenous as adoptive transfer recipient[2]
CY0113	M1/M2	M1/M3	M1/M3	Intrarectal, 50,000 TCID50 SIVmac239
CY0116	M2/M3	M1/M2	M1/M2	Intravenous as adoptive transfer recipient[3]

[1] The MHC genotypes refer to those previously described (25).

[2] These animals were challenged intrarectally with 50,000 TCID50 SIVmac239 after they received lymphocytes from an animal infected with SIV whose bulk sequence was identical to SIVmac239, except within the Nef$_{103\text{-}111}$RM9 epitope ((30) and fig. S2).

[3] CY0116 was challenged intrarectally with 50,000 TCID50 SIVmac239 after he received lymphocytes from an animal infected with SIVmac239Δnef. The bulk sequence of the initial virus replicating in CY0116 was identical to wild type SIVmac239 (fig. S2).

Table 2

Table 2A: Average chronic viremia present in each MCM cohort infected with SIVmac239

MCM cohort	Average viral load (log_{10})	Lower CI[1]	Upper CI[1]	Average viral load (copies/ml)
All MHC homozygous (n=8)	5.18	4.79	5.59	1.50×10^5
All MHC heterozygous (n=5)	3.29	2.74	3.82	1.96×10^3
M3 heterozygous (n=4)	2.76	2.13	3.39	5.71×10^2
M1/M3 heterozygous (n=3)	2.85	2.16	3.54	7.13×10^2
M1/M1 homozygous (n=2)	4.76	4.23	5.33	5.73×10^4
M2/M2 homozygous (n=2)	5.59	5.03	6.13	3.86×10^5
M3/M3 homozygous (n=4)	5.21	4.61	5.85	1.62×10^5

Table 2B: Difference in chronic viremia between MCM cohorts infected with SIVmac239

Cohort comparison	Difference in chronic viral loads (log_{10})	P
All heterozygous (n=5) vs. all homozygous (n=8)	-1.91	<0.001
M3 heterozygous (n=4) vs. all homozygous (n=8)	-2.45	<0.001
M3 heterozygous (n=4) vs. M3 homozygous (n=4)	-2.45	0.002
M2/M2 homozygous (n=2) vs. M3/M3 homozygous (n=4)	0.366	0.486
M2/M2 homozygous (n=2) vs. M1/M1 homozygous (n=2)	0.841	0.054
M3/M3 homozygous (n=4) vs. M1/M1 homozygous (n=2)	0.479	0.39

[1] 95% Confidence interval

Average chronic viral load set point for each animal grouping after 14 wpi was determined as described in the Materials and Methods.

The difference between the chronic viral load set point of the indicated animal groupings was determined as described in the Materials and Methods.