

SHORT REPORT

Evolution of SIVmac239 following serial passaging in humanized mice

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Abstract

Serial passage of SIVmac239 allows for greater understanding of the genetic changes necessary for cross-species transmission of primate lentiviruses into humans. Using humanized mice, we show that adaptive mutations continue to accumulate in SIVmac239 during four serial passages, with persistent CD4⁺ T cell decline and increases in plasma viral loads.

KEYWORDS

cross-species SIV transmission, HIV evolution surrogate model, humanized mice, rhesus macaques, SIVmac239 cross-species transmission, SIVmac239 evolution, SIVmac239 infection of humanized mice

1 | INTRODUCTION

SIVmac and its derivatives are widely used as models for HIV infection due to their ability to mimic AIDS-like pathogenesis in rhesus macaques.¹⁻⁴ Previously, we showed successful infection and serial passaging of SIVmac239 as well as other SIVs in the humanized

mouse model, which harbors a fully functional human immune system.²⁻¹⁵ Here, we characterized SIVmac239 following four serial passages in humanized mice and identified the genetic adaptations that arose following in vivo adaptation to the human immune cell environment. Viral pathogenesis was determined by monitoring plasma viral loads weekly using qRT-PCR and CD4⁺ T cell decline biweekly

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using flow cytometry. The resulting virus was subjected to Illumina-based deep sequencing to identify mutations that arose at increasing frequencies within the viral population.

2 | MATERIALS AND METHODS

2.1 | Generation of hu-HSC mice, infection with SIVmac239 and serial passaging

All animal studies have been approved and reviewed by the Colorado State University Institutional Animal Center and Use Committee (Protocol #1202), and animals were maintained by the CSU Painter Animal Center. The preparation of hu-HSC mice was performed according to the previously described procedures.^{5,10,11,14-17} A total of 8 mice (3 female; 5 male) were used for these experiments.

A human-cell adapted SIVmac239 strain that was serially passaged three times in humanized mice was used to infect hu-HSC mice as previously described.⁵ Briefly, mice displaying high plasma viral loads 24-weeks post-inoculation were euthanized and cells were collected to propagate and passage the virus for future serial passages of hu-mice.^{5,10,11}

2.2 | Assessment of viral pathogenesis

Pathogenesis of the virus was determined through plasma viral load detection and assessment of CD4⁺ T cell decline as described previously.⁵ Briefly, the E.Z.N.A. Viral RNA kit (Omega bio-tek, Norcross, CA) was used to extract viral RNA from plasma isolated weekly from peripheral blood. Quantification of viral loads was performed using qRT-PCR and SYBR Green with the iScript One-Step RT-PCR kit (BioRad, Hercules, CA) based on the manufacturer's instructions.⁵

CD4⁺ T cell levels were assessed as a fraction of CD45⁺/CD3⁺ cells following staining of whole blood using mouse anti-human antibodies CD45-APC (eBioscience), CD3-FITC (eBioscience), and CD4-PE (BD Pharmingen, San Jose, CA) and the BD Accuri C6 cytometer as previously described.^{5,6} CD4⁺ T cell decline was assessed relative to uninfected controls using a two-tailed Student's *t*-test in

GraphPad Prism 8.1.0 ($p < .0001$). CD4⁺ T cell decline and plasma viral loads were displayed as mean \pm SD.

2.3 | Illumina-based deep sequencing

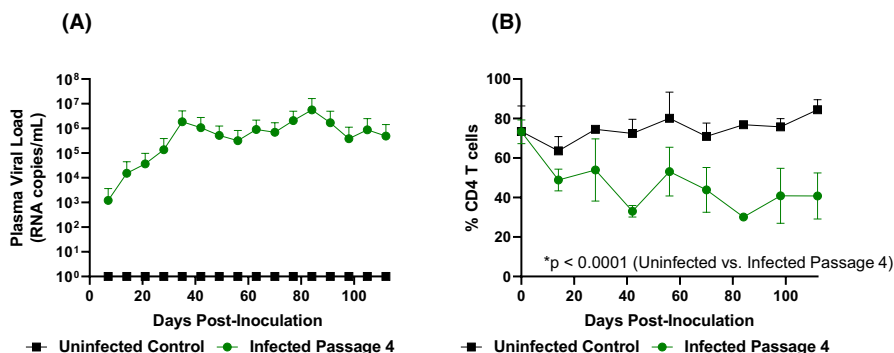
Overlapping amplicon pools were generated from viral RNA collected from two separate infected mice at 3-, 11-, 19-, and 25-week post-inoculation using Primal Scheme designed primers as described previously.^{5,18} Amplicons were prepared for sequencing using the TruSeq Nano DNA Library Preparation Kit and run on the MiSeq Illumina desktop sequencer (Invitrogen).

Sequence read processing and variant identification was performed using Geneious Prime v2022.1.1. Paired-end reads were merged using BBMerge version 38.84, and read ends were trimmed with a 0.05 error probability rate.¹⁹ Reads were mapped against our previously sequenced SIVmac239 reference consensus sequence using Bowtie2 v2.3.0.^{5,20} SNPs were identified based on a minimum variant frequency of 0.5 and minimum depth of coverage of 100 reads. R and ggplot2 (ISBN: 0387981403) scripts were used to create the genome plot and can be found at https://github.com/stenglein-lab/viral_variant_explorer. Raw sequencing data were deposited to the Sequence Read Archive and are publicly available (Accession Numbers: SRR17194610; SRR20736401-SRR2073606; and SRR20736413-SRR2073614).

3 | RESULTS

The hu-mouse adapted SIVmac239 fourth passage virus was able to cause viremia with a rapid increase in viral loads reaching a titer of over 1×10^6 RNA copies/mL within 35 days before peaking around 84 days post-inoculation and gradually declining (Figure 1A). CD4⁺ T cell decline was observed within 2 weeks of infection, with a significant, but gradual decline continuing throughout the rest of the viremic phase relative to the uninfected control ($*p < .0001$; Figure 1B). In addition to mutations identified in previous serial passages, we found that the frequency of nonsynonymous SNPs producing M252T, K446R, and A545V in *env* and L31P in *nef* had increased to >50% of the viral population by the end of the fourth serial passage (Figure 2).⁵ Additionally, we determined using Geno2pheno

FIGURE 1 SIVmac239 Plasma Viral Loads and CD4⁺ T cell decline after four serial passages. (A) SIVmac239 fourth passage plasma viral loads. Plasma viral loads peaked around 84 days post-inoculation. (B) SIVmac239 fourth passage CD4⁺ T cell decline. Both data sets are represented as mean \pm SD. CD4⁺ depletion was significant by the end of the serial passage (two-tailed Student's *t*-test, $p < .0001$)



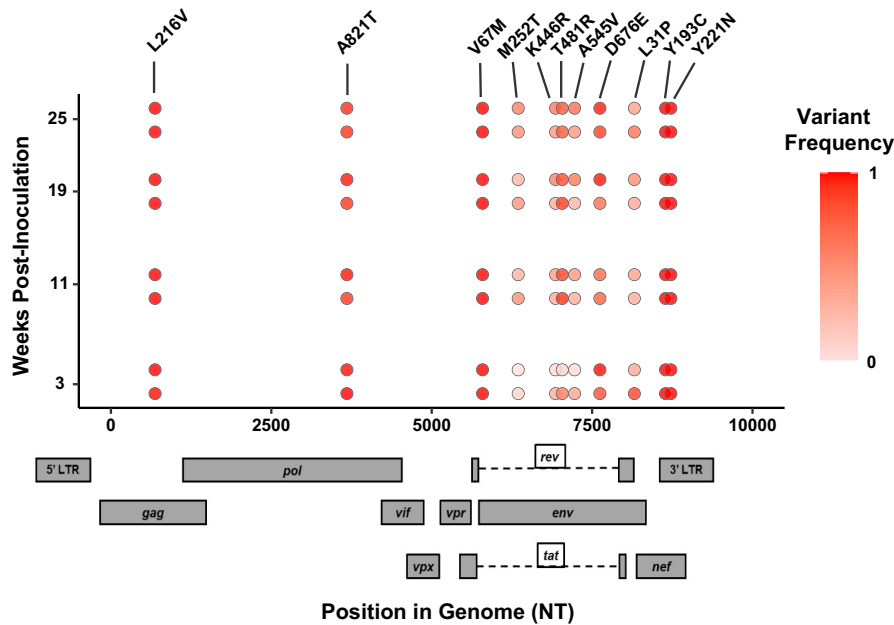


FIGURE 2 Nonsynonymous mutations occurring at >50% frequency following four serial passages of SIVmac239. SNPs identified had a minimum variant frequency of $\geq 50\%$ and required ≥ 100 read depth of coverage

that the adapted virus is still primarily a CCR5-tropic virus and has not yet shifted to either an X4 or dual-tropic phenotype by the end of the fourth generation of serial passaging.²¹

4 | DISCUSSION

The fourth serial passage of SIVmac239 contrasts the earlier passages in both pathogenesis and genetic changes.⁵ The first three passages showed moderate increases in viral loads, while the fourth passage starting viral loads were greater than 1×10^3 RNA copies/mL, and continued to rise over 3 logs relatively quickly. This suggests that the fourth serial passage virus is more adapted to human cells than earlier passages.⁵ Additionally, while CD4⁺ T cell decline was significant relative to the uninfected controls, there was not as large of a difference between the previous passages and the fourth serial passage relative to the differences in viral loads.⁵ This indicates that genetic changes that arise in this passage had a greater impact on plasma viral loads than on CD4⁺ T cell decline.

While the majority of the previously identified mutations were maintained at high frequencies within the viral population, several mutations in *env* and *nef* rose above 50% frequency that were not previously seen.⁵ Furthermore, the retention of previously identified high-frequency mutations indicates the importance for countering increased fitness for viral replication in the human. Future functional studies on these mutations are likely to shed more light on their specific roles in human host adaptation.

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CONFLICT OF INTEREST

The authors confirm that there are no conflicts of interest involved with these studies.

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