Evolution of SIVmac239 following serial passaging in humanized mice

James Z. Curlin[1,2,*], **Kimberly Schmitt**[1,*], **Leila Remling-Mulder**[1], **Jared Morrison**[1], **John J. Baczenas**[3], **Corina Valencia Tibbits**[3], **Kelly Goff**[4], **Shelby O'Connor**[3], **Mark Stenglein**[1], **Preston Marx**[4,5], **Ramesh Akkina**[1,**]

[1]Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO, United States

[2]ADEAR Training Program, Department of Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO, United States

[3]Department of Pathology and Laboratory Medicine, University of Wisconsin School of Medicine and Public Health, Madison, WI, United States

[4]Department of Tropical Medicine, School of Public Health and Tropical Medicine, New Orleans, LA, United States

[5]Tulane National Primate Research Center, Covington, LA, United States

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

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

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

Correspondence to Ramesh Akkina at akkina@colostate.edu, **Telephone: 970 491 1009, Address: Colorado State University, 1619 Campus Delivery, Fort Collins, CO 80521.**
*The first two authors contributed equally

CONFLICT OF INTEREST STATEMENT

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

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 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.

MATERIALS AND METHODS

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]

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 ($p<0.0001$). CD4$^+$ T cell decline and Plasma viral loads were displayed as mean ± SD.

Illumina-based deep sequencing

Overlapping amplicon pools were generated from viral RNA collected from two separate infected mice at 3-, 11-, 19- and 25-weeks 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, Carlsbad, CA).

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 was deposited to the Sequence Read Archive and are publicly available (Accession Numbers: SRR17194610; SRR20736401-SRR2073606; SRR20736413-SRR2073614)

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 two weeks of infection, with a significant, but gradual decline continuing throughout the rest of the viremic phase relative to the uninfected control (*p < 0.0001; 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 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]

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 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.

ACKNOWLEDGEMENTS

REFERENCES

1. Apetrei C et al. Molecular epidemiology of simian immunodeficiency virus SIVsm in U.S. primate centers unravels the origin of SIVmac and SIVstm. J Virol 79, 8991–9005 (2005). 10.1128/JVI.79.14.8991-9005.2005 [PubMed: 15994793]

2. Kestler H et al. Induction of AIDS in rhesus monkeys by molecularly cloned simian immunodeficiency virus. Science 248, 1109–1112 (1990). 10.1126/science.2160735 [PubMed: 2160735]

3. Naidu YM et al. Characterization of infectious molecular clones of simian immunodeficiency virus (SIVmac) and human immunodeficiency virus type 2: persistent infection of rhesus monkeys with molecularly cloned SIVmac. J Virol 62, 4691–4696 (1988). [PubMed: 2846880]

4. Daniel MD et al. Isolation of T-cell tropic HTLV-III-like retrovirus from macaques. Science 228, 1201–1204 (1985). 10.1126/science.3159089 [PubMed: 3159089]

5. Curlin JZ et al. In vivo Infection Dynamics and Human Adaptive Changes of SIVsm-Derived Viral Siblings SIVmac239, SIVB670, and SIVhu in Humanized Mice as a Paralog of HIV-2 Genesis. Frontiers in Virology 1 (2021). 10.3389/fviro.2021.813606

6. Curlin J et al. Evolution of SIVsm in humanized mice towards HIV-2. J Med Primatol (2020). 10.1111/jmp.12486

7. Curlin J et al. SIVcpz cross-species transmission and viral evolution toward HIV-1 in a humanized mouse model. J Med Primatol 49, 40–43 (2020). 10.1111/jmp.12440 [PubMed: 31576587]

8. Schmitt K et al. SIV progenitor evolution toward HIV: A humanized mouse surrogate model for SIVsm adaptation toward HIV-2. J Med Primatol 47, 298–301 (2018). 10.1111/jmp.12380 [PubMed: 30255956]

9. Schmitt K et al. Mimicking SIV chimpanzee viral evolution toward HIV-1 during cross-species transmission. J Med Primatol 49, 284–287 (2020). 10.1111/jmp.12485 [PubMed: 33460210]

10. Schmitt K et al. Cross-Species Transmission and Evolution of SIV Chimpanzee Progenitor Viruses Toward HIV-1 in Humanized Mice. Frontiers in Microbiology 11 (2020). 10.3389/fmicb.2020.01889

11. Schmitt K et al. Modeling the evolution of SIV sooty mangabey progenitor virus towards HIV-2 using humanized mice. Virology 510, 175–184 (2017). 10.1016/j.virol.2017.07.005 [PubMed: 28750321]

12. Murphey-Corb M et al. Isolation of an HTLV-III-related retrovirus from macaques with simian AIDS and its possible origin in asymptomatic mangabeys. Nature 321, 435–437 (1986). 10.1038/321435a0 [PubMed: 3012358]

13. Akkina R. New generation humanized mice for virus research: comparative aspects and future prospects. Virology 435, 14–28 (2013). 10.1016/j.virol.2012.10.007 [PubMed: 23217612]

14. Akkina RK, Rosenblatt JD, Campbell AG, Chen IS & Zack JA. Modeling human lymphoid precursor cell gene therapy in the SCID-hu mouse. Blood 84, 1393–1398 (1994). [PubMed: 7520766]

15. Bai J et al. Characterization of anti-CCR5 ribozyme-transduced CD34+ hematopoietic progenitor cells in vitro and in a SCID-hu mouse model in vivo. Mol Ther 1, 244–254 (2000). 10.1006/mthe.2000.0038 [PubMed: 10933940]

16. Berges BK, Akkina SR, Folkvord JM, Connick E & Akkina R. Mucosal transmission of R5 and X4 tropic HIV-1 via vaginal and rectal routes in humanized Rag2−/− gammac −/− (RAG-hu) mice. Virology 373, 342–351 (2008). 10.1016/j.virol.2007.11.020 [PubMed: 18207484]

17. Veselinovic M, Charlins P & Akkina R. Modeling HIV-1 Mucosal Transmission and Prevention in Humanized Mice. Methods Mol Biol 1354, 203–220 (2016). 10.1007/978-1-4939-3046-3_14 [PubMed: 26714714]

18. Quick J et al. Multiplex PCR method for MinION and Illumina sequencing of Zika and other virus genomes directly from clinical samples. Nat Protoc 12, 1261–1276 (2017). 10.1038/nprot.2017.066 [PubMed: 28538739]

19. Bushnell B, Rood J & Singer E. BBMerge - Accurate paired shotgun read merging via overlap. PLoS One 12, e0185056 (2017). 10.1371/journal.pone.0185056

20. Langmead B & Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat Methods 9, 357–359 (2012). 10.1038/nmeth.1923 [PubMed: 22388286]

21. Doring M et al. geno2pheno[ngs-freq]: a genotypic interpretation system for identifying viral drug resistance using next-generation sequencing data. Nucleic Acids Res 46, W271–W277 (2018). 10.1093/nar/gky349 [PubMed: 29718426]
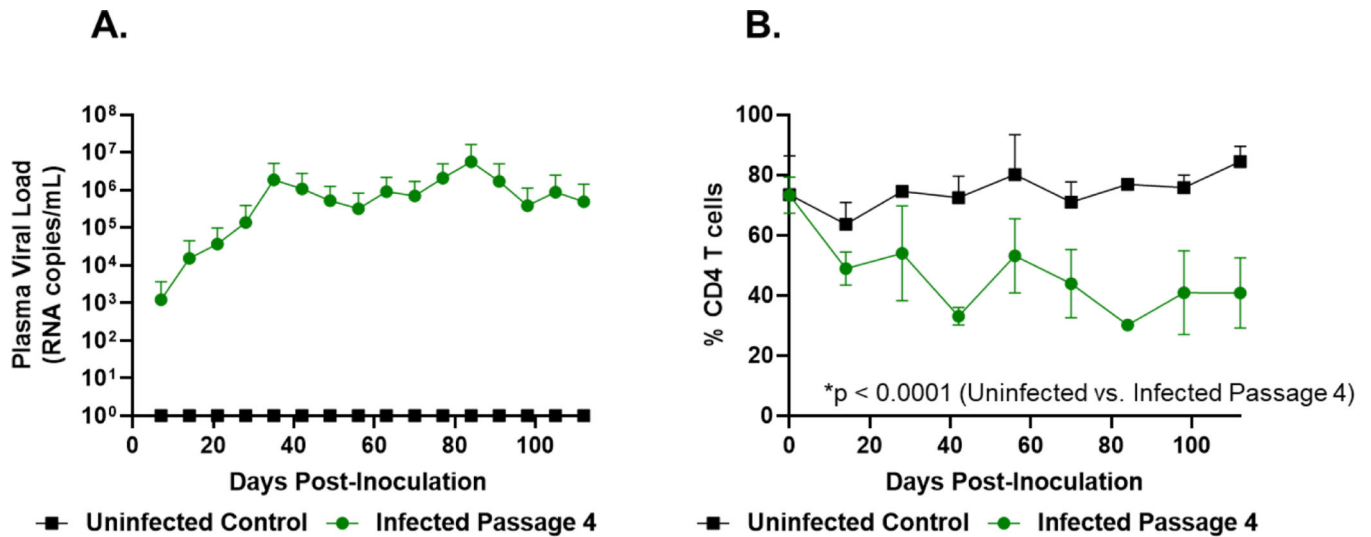
Figure 1. SIVmac239 Plasma Viral Loads and CD4$^+$ T cell decline after four serial passages.
(A) SIVmac239 fourth passage plasma viral loads. Plasma viral loads peaked around 84 days post-inoculation. (B) SIVmac239 fourth passage CD4$^+$ T cell decline. Both data sets are represented as mean ± SD. CD4$^+$ depletion was significant by the end of the serial passage (two-tailed Student's *t*-test, p<0.0001).
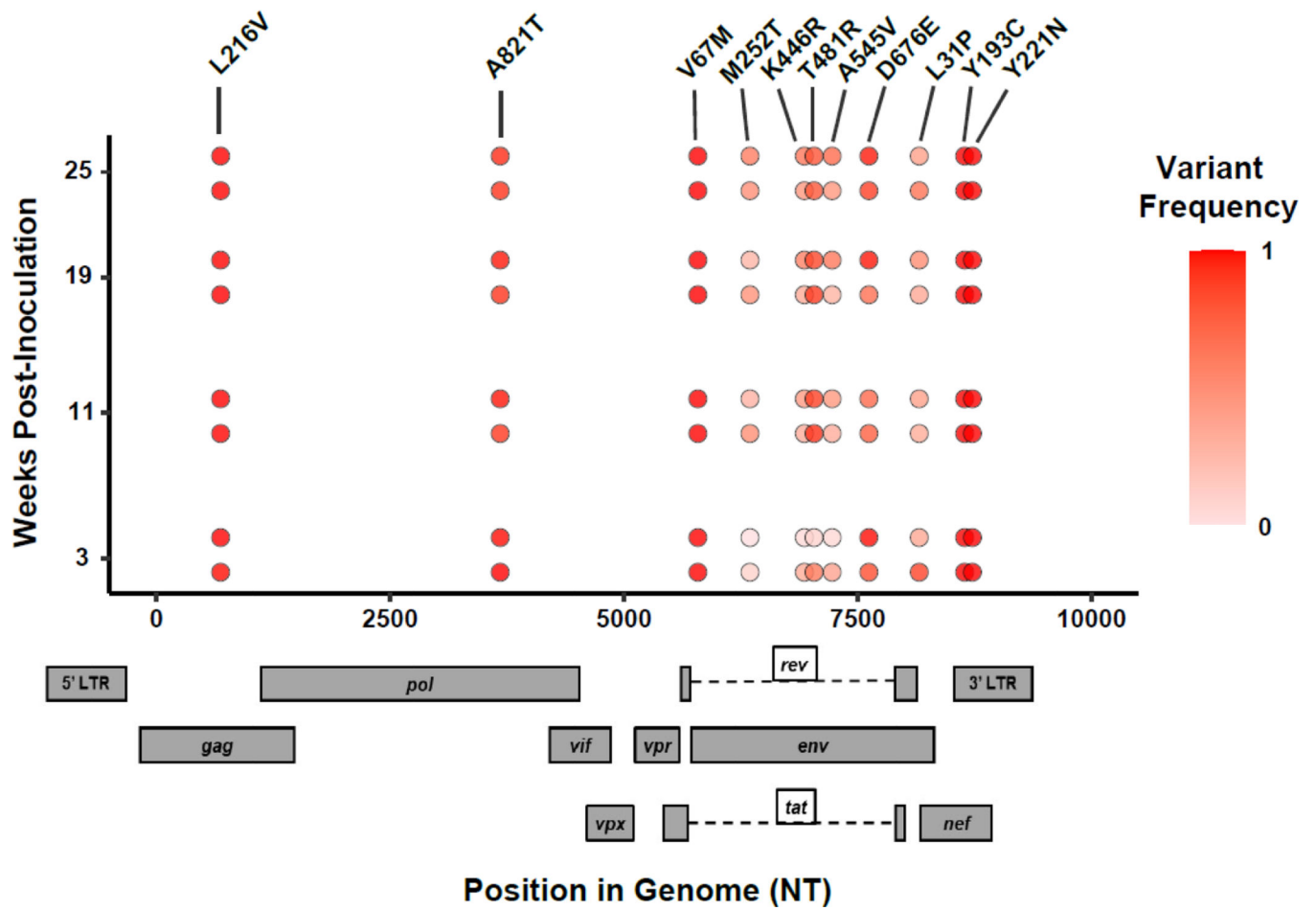
Figure 2. Nonsynonymous mutations occurring at >50% frequency following four serial passages of SIVmac239.

SNPs identified had a minimum variant frequency of   50% and required   100 read depth of coverage.