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SHORT REPORT

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Long-term evolutionary adaptation of SIVcpz toward HIV-1 using a humanized mouse model

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Abstract

Critical genetic adaptations needed for SIV chimpanzee to evolve into HIV-1 are not well understood. Using humanized mice, we mimicked the evolution of SIVcpzLB715 into HIV-1 Group M over the course of four generations. Higher initial viral load, increased $CD4^+$ T-cell decline, and nonsynonymous substitutions arose suggesting viral evolution.

KEYWORDS
HIV-1 viral evolution, modeling SIV evolution using humanized mice, SIV cross-species transmission, SIVcpz evolution into HIV-1, viral evolution in humanized mice

1 | INTRODUCTION

The genetic adaptations required for SIV progenitor viruses to become pathogenic and established as HIVs in the human population are still unclear. Chimpanzee-derived SIVs (SIVcpz) are believed to have evolved into the highly pathogenic HIV-1 Group M.[1–3] An ideal model to recapitulate the genetic adaptations for the cross-species transmission of SIVcpzLB715 into HIV-1 Group M is the humanized mouse (hu-HSC).[4,5] These hu-HSC mice harbor a complete functional human immune system permissive for viral infection.[4,6–21] In this study, we used hu-HSC mice to mimic the selective immune pressures of natural infection by serially passaging SIVcpzLB715 to reproduce the nonsynonymous mutations that resulted in the evolution of HIV-1. Hu-HSC mice were inoculated with SIVcpzLB715 and sequentially passaged for four generations cumulatively for 2 years. Mice were monitored weekly for plasma viral loads and biweekly for $CD4^+$ T-cell decline to assess viral fitness over time. Illumina-based deep sequencing was used to identify potential nonsynonymous mutations throughout the viral genome likely necessary for adaptation in human immune cells.

2 | MATERIALS AND METHODS

2.1 | Ethics and the preparation of humanized mice

All animals were maintained in the Painter Animal Center at Colorado State University, and the studies conducted in this publication have been approved by the CSU Institutional Animal Care and

Kimberly Schmitt and James Curlin contributed equally to this article.

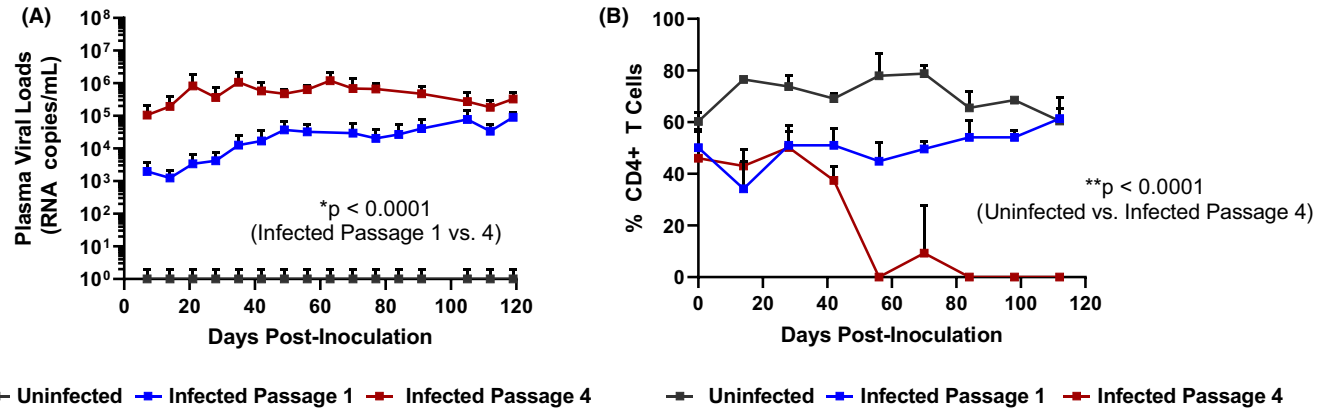

FIGURE 1 SIVcpzLB715 infection leads to chronic viremia and rapid $CD4^+$ T-cell decline. (A) Plasma viral loads and (B) $CD4^+$ T-cell depletion in SIVcpzLB715-infected and SIVcpzLB71-uninfected hu-HSC mice. Plasma viral load and $CD4^+$ T-cell decline data are represented as the mean ± SD. The plasma viral loads from the first to the fourth passage showed a statistically significant increase from the first to the fourth passage (two-tailed Student's *t*-test, $^*p < .0001$). Statistically significant $CD4^+$ T-cell depletion was also seen in the infected hu-HSC mice relative to the uninfected controls (two-tailed Student's *t*-test, $^{**}p < .0001$).

Use Committee (Protocol Review No. 1202). Humanized (hu-HSC) mice were prepared as previously described.[22–25] A total number of 15 humanized mice (seven female and eight male) were used in this study.

2.2 | SIVcpzLB715 infection and serial passage

SIVcpzLB715 was propagated, concentrated, and inoculated into five well-engrafted (>75% $CD45^+$ and >60% $CD4^+$) hu-HSC mice as previously described to begin the first generation.[4,5] After approximately 6 months, the mice were euthanized, and the virus was propagated from mice with the highest plasma viral titer to begin the next generation as previously described.[4,5,10,17] This was repeated for four sequential passages.

2.3 | Plasma viral loads and $CD4^+$ T-cell assessment

Plasma viral loads were assessed on a weekly basis as previously described.[4,5] Briefly, the E.Z.N.A. Viral RNA kit (Omega bio-tek, Norcross, CA) was used to extract plasma RNA from peripheral blood per the manufacturer's instructions. Viral loads were quantified using the iScript One-Step RT-PCR kit with SYBR green (BioRad, Hercules, CA) according to the manufacturer's instructions. Bimonthly, whole blood was stained with fluorophore conjugated antihuman CD45-FITC (eBiosceince), CD3-PE (eBioscience), and CD4-PE/Cy5 (BD Pharmigen, San Jose, CA) to determine $CD4^+$ T-cell decline as previously described.[4,5] Data were analyzed using GraphPad Prism 8.1.0. Both the plasma viral loads and $CD4^+$ T-cell decline are presented as mean ± SD. Statistical significance in $CD4^+$ T-cell decline was determined using a two-tailed Student's *t*-test ($p < .001$) to compare infected and uninfected mice.

2.4 | Illumina-based deep sequencing and sequence analysis

Overlapping 400-bp amplicons were generated at 3, 11, 19, and 25 weeks postinoculation from viral RNA using whole-genome spanning primer pools created using the Primal Scheme software described previously.[26] Amplicons were further prepared for the MiSeq Illumina desktop sequencer (Invitrogen, Carlsbad, CA) using the TruSeq Nano DNA HT Library Preparation kit (Illumina, San Diego, CA) according to the manufacturer's instructions. Geneious Prime v2022.1.1 was used to process sequence reads and identify variants. BBMerge v38.84 was used to merge paired-end reads that were trimmed with a 0.05 error rate probability.[27] Bowtie2 v2.3.0 was used to map the reads to the previously sequenced SIVcpzLB715 stock virus.[4,28] The variants identified had ≥100 read depth and ≥50% viral population frequency. The genome plots were created using R and ggplot2 (ISBN:0387981403) scripts, which can be found at https://github.com/stenglein-lab/viral_variant_explorer. The raw data supporting the conclusions of this article can be found on the sequence read archive (SRA; Accession Numbers: SRR12081901; SRR12081911-SRR12081919; SRR20736399-SRR20736400; and SRR20736407-SRR20736412).

3 | RESULTS

The fourth serial passage of SIVcpzLB715 in hu-HSC mice resulted in viral loads 2-logs higher (1.05×10^5 RNA copies/ml) within 1 week of inoculation compared with the first viral passage ($^*p < .0001$; Figure 1A).[4,5] Rapid, statistically significant, $CD4^+$ T-cell decline occurred by Day 56 and continued throughout the duration of the fourth generation of infection when compared to the uninfected controls ($^{**}p < .0001$; Figure 1B). Taken together, these data show that the pathogenicity and viral fitness continue to increase with

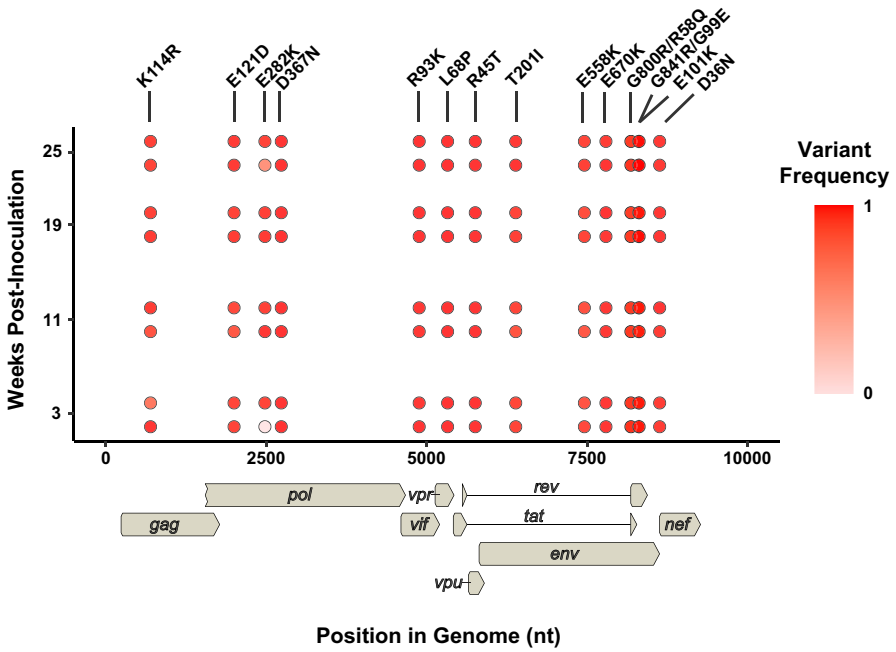

FIGURE 2 Viral variants increasing in frequency after four serial passages of SIVcpzLB715 in hu-HSC mice. Nonsynonymous variant frequencies that reached ≥50% of the viral population with ≥100 read depth of coverage. The viral variant frequency is indicated by the red scale, and the amino acid residue changes for each position are listed above their respective locations.

each serial passage of SIVcpzLB715 in hu-HSC mice. Illumina-based deep sequencing of viral RNA identified numerous adaptive nonsynonymous variants within the viral population with at least 50% frequency toward the end of the fourth serial passage with the majority of these variants becoming fixed (Figure 2).

4 | DISCUSSION

Humanized mice constitute an ideal model to assess the genetic adaptations required for SIVcpz to evolve into HIV-1 through serial passaging. At the end of four sequential passages in hu-HSC mice, SIVcpzLB715 was able to achieve a high viral set point that was maintained throughout the duration of the passage. Furthermore, significant $CD4^+$ T-cell decline was more pronounced during the fourth passage relative to previous passages.[4] Sixteen nonsynonymous mutations resulting in amino acid substitutions that may be critical for cross-species adaptation were identified throughout the viral genome in genes such as *gag*, *pol*, *vif*, *vpr*, *vpu*, *env*, *rev*, and *nef* with the majority of these variants detected in *env* (Figure 2). Overall, these data showed increased viral fitness and pathogenicity of the fourth generation serially passaged virus. Our data also demonstrated the utility of humanized mice in recreating the adaptive pressures necessary for the evolution of SIVcpz into HIV-1.

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CONFLICT OF INTEREST

The authors confirm that there were no conflicts of interest during the preparation of this manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Sequence Read Archive (SRA) at https://www.ncbi.nlm.nih.gov/sra/.

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