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.

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 Use Committee (IACUC). The studies were conducted in accordance with the standards recommended by the IACUC. All procedures were performed in accordance with the guidelines established by the IACUC.

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
SCHMITT et al. Use Committee (Protocol Review No. 1202). Humanized (hu-HSC) mice were prepared as previously described. 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. 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. 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. 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. Bimonthly, whole blood was stained with fluorophore conjugated antihuman CD45-FITC (eBioscience), CD3-PE (eBioscience), and CD4-PE/Cy5 (BD Pharmigen, San Jose, CA) to determine CD4+ T-cell decline as previously described. 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 < 0.001) to compare infected and uninfected mice.

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 < 0.0001; Figure 1A). 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 < 0.001; Figure 1B). Taken together, these data show that the pathogenicity and viral fitness continue to increase with
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. 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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