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Evolution of SIVsm in humanized mice towards HIV-2

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Abstract

Through the accumulation of adaptive mutations, HIV-2 originated from SIVsm. To identify these evolutionary changes, a humanized mouse model recapitulated the process that likely enabled this cross-species transmission event. Various adaptive mutations arose, as well as increased virulence and $CD4^+$ T-cell decline as the virus was passaged in humanized mice.

Keywords

HIV-2 viral evolution; SIVsm evolution towards HIV-2; Sooty Mangabeys; cross-species SIV transmission; SIVsm infection in humanized mice; hu-HSC mice for viral cross-species jump

2 INTRODUCTION

SIV sooty mangabey (SIVsm) is believed to have accumulated a number of genetic changes during cross-species transmission events between humans and sooty mangabeys that eventually resulted in HIV-2.[1–3] Humanized hematopoietic stem cell (Hu-HSC) mice are an effective model to study this question, as they provide an *in vivo* human immune environment that mimics the selective pressures of natural human infections.[4–11] Here, we used serial passaging to simulate the repeated cross-species exposures of SIVsm and reproduce the mutations that likely facilitated the transition of SIVsm into HIV-2.[12–14] Following the inoculation of hu-mice, SIVsmE041 was sequentially passaged for eight generations. Mice were assessed weekly for plasma viral loads and $CD4^+$ T-cell decline was monitored biweekly to determine viral pathogenicity over time. Additionally, the adapted

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Conflict of Interest Statement

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

virus was sequenced using Illumina-based deep sequencing to identify mutations that potentially played role in SIVsmE041 adaption to human cells for higher viral fitness.

3 MATERIALS AND METHODS

3.1 Hu-HSC mouse preparation and Ethics

Humanized hu-HSC mice were prepared as previously described.[15–19] Experimental animals were maintained at the Colorado State University Painter Animal Center. All studies were approved and reviewed by the CSU Institutional Animal Center and Use Committee.

3.2 SIVsmE041 infection of hu-HSC mice and serial passaging

SIVsmE041 (GenBank accession HM059825.1), isolated from sooty mangabey PBMC, was used to infect hu-HSC mice as previously described.[9,10] Approximately 24 weeks after inoculation, mice with the highest plasma viral loads were euthanized to propagate the virus as previously described.[6,9,10] This process was repeated for 8 sequential passages.

3.3 $CD4^+$ T-cell decline and plasma viral load detection

Peripheral blood was collected weekly by tail vein puncture and viral RNA was isolated from plasma using the E.Z.N.A Viral RNA kit (Omega bio-tek, Norcross, CA). RNA was quantified using qRT-PCR and SYBR Green with the iScript One-Step RT-PCR kit (BioRad, Hercules, CA) according to the manufacturer's instructions to determine plasma viral loads. Bimonthly, whole blood collected from the mice was stained with mouse anti-human CD45-APC (eBioscience), CD3-FITC (eBioscience), and CD4-PE (BD Pharmingen, San Jose, CA) antibodies. $CD4^+$ T-cell levels were assessed as a percentage of $CD45^+/CD3^+$ cells with the BD Accuri C6 cytometer as described previously.[6,9,10] Comparison of $CD4^+$ T-cell decline between control and infected mice was assessed with a two-tailed Student's *t*-test. ($p<0.001$)

3.4 SNP detection and Illumina-based deep sequencing

Viral RNA from two infected mice at weeks 3, 11, 19, and 23 post-infection in the eighth passage were used to generate amplicons with primers designed using Primal Scheme software.[20] Amplicons were prepared using the TruSeq Nano DNA Library Preparation Kit and the MiSeq Illumina desktop sequencer (Invitrogen, Carlsbad, CA). Nonsynonymous SNPs were identified by aligning reads to the SIVsm stock virus consensus sequence using bowtie2 software v2.2.5, and then calling variants at >100 coverage depth and >1% frequency using lofreq software v2.1.2.[21,22] Genome plots were generated using R and ggplot2 (ISBN: 0387981403). R scripts can be found at https://github.com/stenglein-lab/viral_variant_explorer

4 RESULTS

Eight serial passages in hu-mice resulted in a human adapted SIVsmE041 capable of rapid infection within one week of inoculation that was sustained for over 100 days and maintained above 10^5 RNA copies/ml (Figure 1A). $CD4^+$ T-cell levels showed significant ($p<0.001$) decline that commenced around 60 days post-inoculation and persisted through the remainder of the passage (Figure 1B). This is a markedly drastic decline compared to

earlier passages with SIVsm in humanized mice. Numerous nonsynonymous single nucleotide polymorphisms (SNPs) were identified using Illumina-based deep sequencing throughout the viral genome at high frequencies (Figure 2).

5 DISCUSSION

Serial passaging in humanized mice provided an effective model for recapitulating the genetic changes that allowed SIVsm to adapt to human immune cells similarly to HIV-2. After eight sequential passages, SIVsm is consistently capable of readily infecting humanized mice lasting for the duration of the passage. Additionally, the $CD4^+$ T-cell decline displayed after eight passages is drastically more pronounced than that seen in earlier passages of SIVsmE041.[9,10] This indicates that the virus has continued to develop a greater affinity for $CD4^+$ T-cells than in earlier passages.

Many previously characterized SNPs could still be seen even after the eighth passage, suggesting that these mutations became fixed.[9,10] Furthermore, several additional substitutions such as Pol I172M and Nef F65I that were only present at less than 30% frequency early in the passage and/or previously unidentified began to increase dramatically to over 70% frequency. Further studies are needed to ascertain their function and determine why these mutations with potential advantage have begun to arise during later passages of SIVsmE041.

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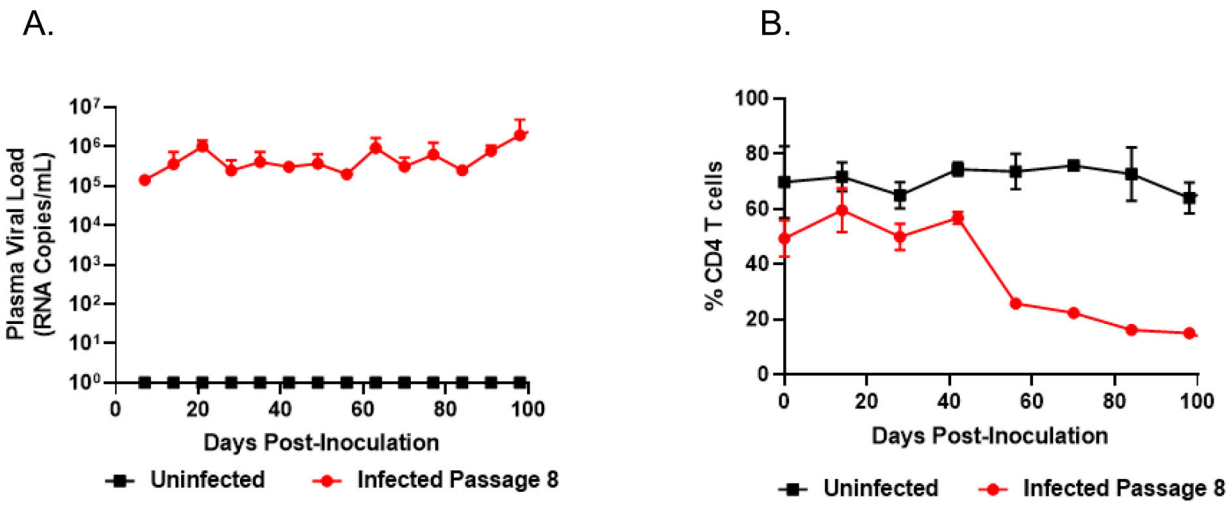

Figure 1. Plasma Viral Loads and CD4$^+$ T-cell decline following eight serial passages of SIVsmE041 in hu-HSC mice.

(A) Plasma viral loads collected from the eighth passage of SIVsmE041 infected mice (B) CD4$^+$ T-cell depletion following SIVsmE041 infection. CD4$^+$ T cell depletion was rapid and significant within 60 days post-inoculation (two-tailed Student's *t*-test, $p<0.001$).

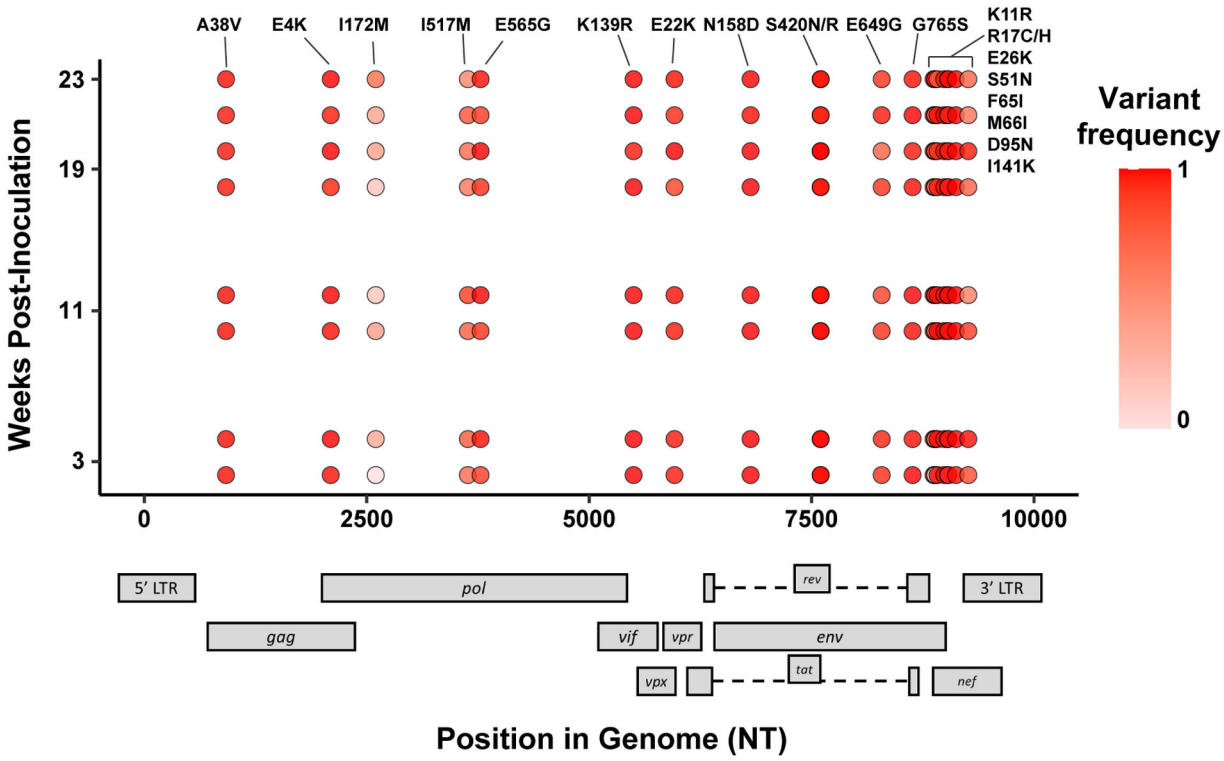

Figure 2. SNP frequencies identified after eight serial passages in hu-HSC mice that reached >50% of the viral population at the last timepoint.

Lofreq v2.1.2 was used to identify SNP frequencies following read alignment by bowtie2 v2.2.5. Mutations were present throughout the genome, with the highest concentration in *env* and *nef*.