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SIV progenitor evolution towards HIV: A humanized mouse surrogate model for SIVsm adaptation towards HIV-2

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Abstract

How SIV progenitors evolved into deadly HIV-1 and HIV-2 following initial cross-species transmission still remains a mystery. Here we used humanized mice as a human surrogate system to evaluate SIVsm evolution into HIV-2. Increased viral virulence to human $CD4^+$ T cells and adaptive genetic changes were observed during serial passages.

INTRODUCTION

Thirteen independent SIV cross-species transmission events have led to epidemic HIV strains through the accumulation of adaptive mutations in SIV resulting in global pandemics/epidemics by HIV-1 and HIV-2.[1] However, data is lacking on how the adaptive changes occurred in these progenitor SIVs due to the lack of suitable experimental system.

In order to evaluate this, we used a human surrogate model of hu-HSC humanized mice transplanted with human hematopoietic stem cells to elucidate the adaptation of SIVsm into HIV-2 *in* vivo.[2–6] These mice harbor human T cells, B cells, monocytes/macrophages and DC cells[3, 6–8] and are highly susceptible to HIV infection.[2, 7, 9–12] To determine if SIVsm could cross the species barrier, we used primary SIVsm isolate E041 to infect hu-HSC mice followed by its sequential passage for seven generations. Mice were assessed for plasma viral loads and $CD4^+$ T cell decline. Viral isolates from different passages were assessed by next-generation sequencing to determine genetic changes.

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RESULTS

Following the initial challenge with SIVsmE041, hu-mice showed detectable viremia within 2 weeks. The viremia levels gradually increased by three logs peaking at 6.69×10^4 copies/ml at 10 weeks post-inoculation (Figure 2A). Virus from this first passage was then subsequently passaged six more times in hu-HSC mice to mimic the evolution of SIVsm into HIV-2. By the seventh generation, viral loads were found to be 2.5-logs higher at 2 weeks post-inoculation compared to that of initial first passage indicating an increase in viral fitness (Figure 2B) to human cell infection.

One of the hallmarks of HIV infection is the depletion of $CD4^+$ T cells. There was no significant human $CD4^+$ T cell decline during the initial few weeks of infection with SIVsmE041. However, a gradual decline is noted during subsequent weeks relative to the uninfected controls (Figure 2A). By comparison, during the seventh passage, SIVsm displayed a more rapid, statistically significant ($p < 0.05$) $CD4^+$ T cell decline by day 10 (Figure 2B), suggesting an increased pathogenicity of the serially passaged seventh generation viral strain.

DISCUSSION

Many theories exist regarding the origin and transmission of HIV pathogens in the human population. Compelling genomic evidence suggests that HIV-2 arose through cross-species transmission events from sooty mangabeys.[1] Viral adaptive changes needed for this successful cross-species evolution are not well understood due to the lack of an ideal *in vivo* system for experimental evaluation. In this study, we used a hu-HSC mouse model to test this hypothesis. Our results showed that SIVsm can readily infect hu-mice and give rise to chronic viremia consistent with HIV-2 infection in humans. Increased viral fitness to human cells as evidenced by the higher viral loads observed by the seventh generation resulting in a more rapid $CD4^+$ T cell decline suggests selection and evolution of the progenitor SIVsm *in vivo*. This is consistent with the hypothesis that SIVsm evolved gradually during sequential infections in the human host to become more pathogenic.[1] At the genomic level, we previously identified 14 nonsynonymous substitutions in *gag*, *vif*, *vpr*, *rev*, *env* and *nef* that became fixed in the human adapted viral population. This was observed in multiple mice across five passages/generations.[13] These substitutions are likely indicative of increased viral fitness to the human host. Studies are currently underway to elucidate if these nonsynonymous substitutions indeed determine cross-species transmissibility.

MATERIALS AND METHODS

Generation of humanized mice

Humanized mice were generated as previously described (Figure 1A).[13–17] Mice were maintained at the Colorado State University Painter Animal Center. The studies conducted in this publication have been reviewed and approved by the CSU Institutional Animal Center and Use Committee.

SIVsmE041 Infection of humanized mice

The SIVsmE041 (Genbank accession HM059825.1) used in this study was obtained from a 21 year old sooty mangabey euthanized for displaying clinical signs of AIDS[18–22] and then propagated in sooty mangabey PBMC creating a true primary isolate comprised of a population of closely related viruses.

Plasma viral load and $CD4^+$ T cell level determination

Five hu-HSC mice were injected intraperitoneally (i/p) with 200 μl of SIVsmE041 ($TCID_{50}$ 811). Peripheral blood was collected by tail vein puncture weekly for viral loads and bimonthly for engraftment (Figure 1B). Viral RNA was extracted using the E.Z.N.A. Viral RNA kit (Omega bio-tek) and viral loads determined using the iScript One-Step RT-PCR kit with SYBR Green and the manufacturer's instructions (Bio Rad) as described previously.[13] Whole blood was stained using fluorophores conjugated to mouse anti-human CD45-FitC (eBioscience), CD3-PE (eBioscience) and CD4-PE/Cy5 (BD Pharmingen) and assessed using the BD Accuri C65 FACS Analyzer as described previously.[13] $CD4^+$ T cell levels were calculated within the $CD45^+CD3^+$ double positive population and decline was assessed using a two-tailed Student's *t*-test ($p<0.05$).

Preparation of SIVsmE041 for sequential passaging

At the end of each generation (Figure 1C), SIVsm infected mice with the highest titer were euthanized and various tissues such as the spleen, bone marrow, lymph nodes and whole blood were harvested and used to propagate virus as previously described.[13] For each subsequent generation, five hu-HSC mice were injected with 200 μl of viral supernatant.

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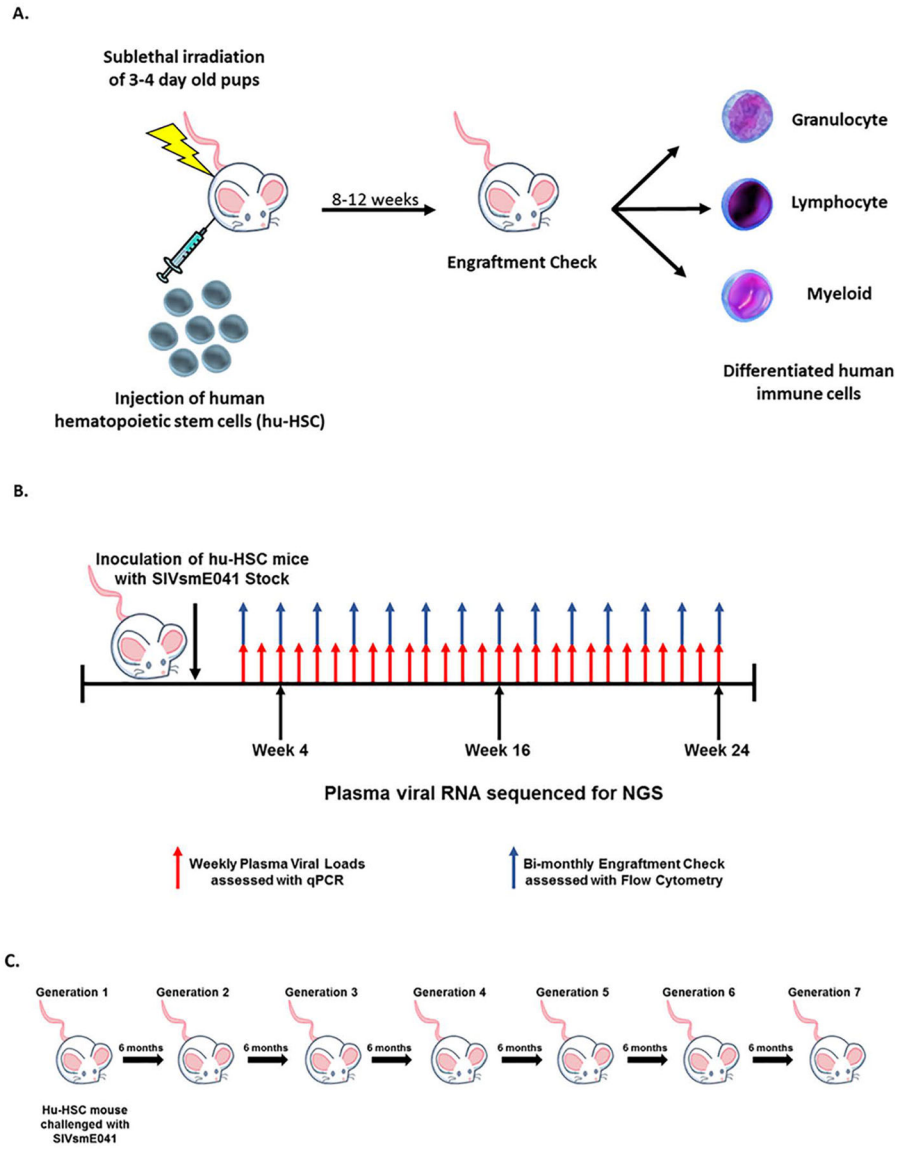

Figure 1.

Hu-HSC mice generation and experimental scheme with SIVsmE041 progenitor virus. A, Neonatal mice were sublethally irradiated and injected intrahepatically with human CD34+ hematopoietic stem cells. Mice are screened 8-12 wk post-reconstitution for human immune cell engraftment. B, Schematic representation of SIVsmE041 infection. C, Serial passaging methodology in hu-HSC mice

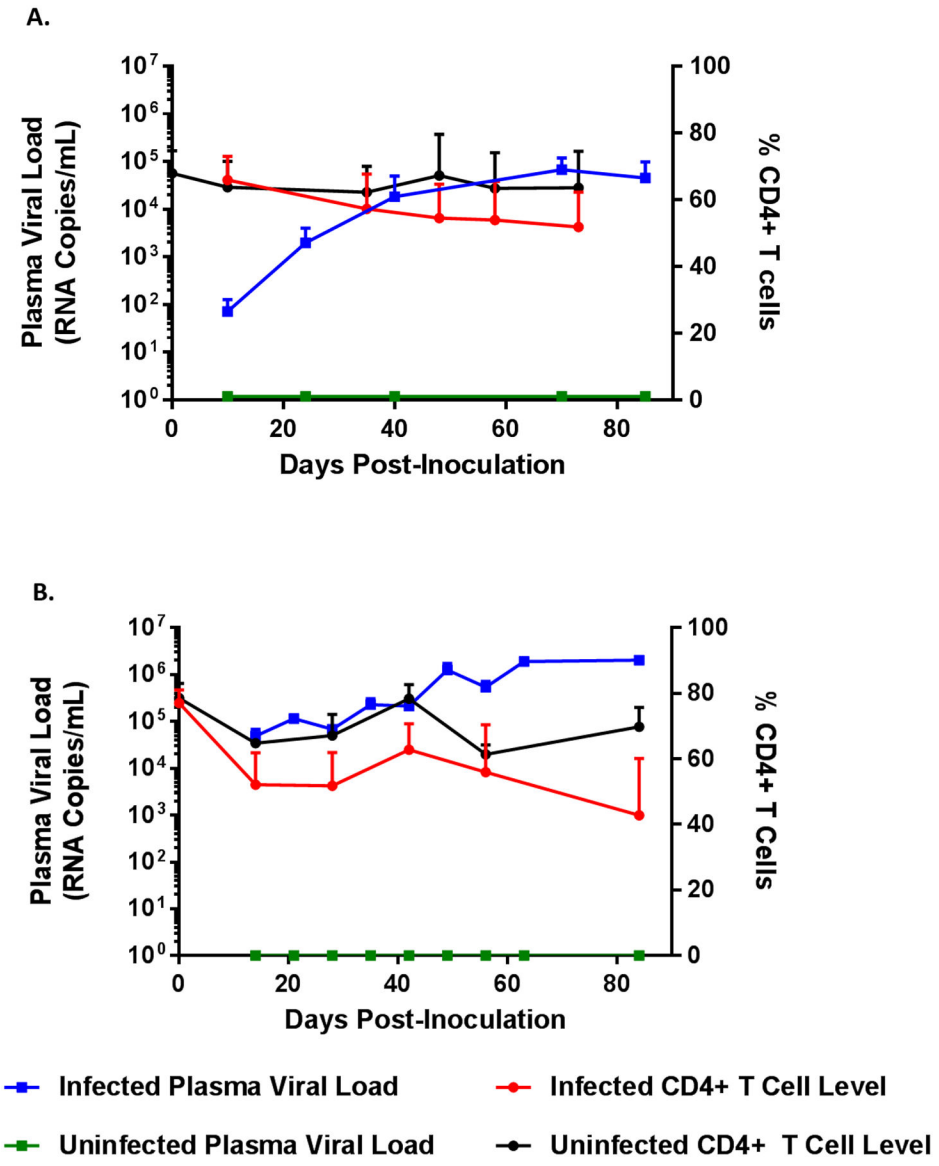

Figure 2.

Kinetics of SIVsmE041 infection and CD4+ T-cell decline during in vivo passages in hu-HSC mice. A, First- and (B) seventh-generation plasma viral loads and CD4+ T-cell decline seen in SIVsmE041-infected and SIVsmE041-uninfected hu-HSC mice. Statistically significant CD4+ T-cell depletion was seen in both the first- and seventh-generation infected mice relative to the uninfected mice (two-tailed Student's t test, $P < 0.05$)