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Mimicking SIV chimpanzee viral evolution towards HIV-1 during cross-species transmission

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Abstract

HIV-1 evolved from SIV during cross-species transmission events, though viral genetic changes are not well understood. Here we studied the evolution of SIVcpzLB715 into HIV-1 Group M using humanized mice. High viral loads, rapid $CD4^+$ T-cell decline, and non-synonymous substitutions were identified throughout the viral genome suggesting viral adaptation.

Keywords

HIV-1 viral evolution from SIV; SIV chimpanzee evolution towards HIV-1; Cross-species SIV transmission; SIVcpz infection in humanized mice; hu-HSC mice for cross-species viral transmission

INTRODUCTION

Many genetic changes across the viral genome were necessary for the successful transformation of SIVs into pathogenic HIVs due to differences in host T-cell receptor structure, restriction factors, and immune system barriers.[1–7] Here, we experimentally simulated the human exposures of SIVcpzLB715 and serial propagation in multiple human immune environments to determine the mutations that led to the generation of the pathogenic HIV-1 Group M virus. We used humanized hematopoietic stem cell (hu-HSC) mice harboring a complete repertoire of human immune cells susceptible to SIV and HIV infection to model these genetic adaptions.[8–15] SIVcpzLB715 was used to infect a cohort of hu-HSC mice and passaged into a second generation of mice (Figure 1A). Plasma viral loads

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CONFLICT OF INTEREST STATEMENT

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

(PVL), $CD4^+$ T-cell decline and Illumina-based deep sequencing at multiple time points were used to determine the adaptation of this virus over time.

MATERIALS & METHODS

Generation of humanized mice and ethics

Humanized mice were generated according to methods previously described (Figure 1A).[16–20] All mice were maintained at the Colorado State University Painter Animal Center. The studies conducted here have all been reviewed and approved by the CSU Institutional Animal Care and Use Committee.

LB715 viral propagation, *in vivo* infection, and serial passage

The SIVcpzLB715 plasmid was obtained from Dr. Preston Marx and used in cell transfections to generate stock virus as described previously.[10] For the initial infection, 200 μl ($TCID_{50}$ 2.0×10^5) virus was injected intraperitoneally into five (>75% $CD45^+$, >60% $CD4^+$) hu-HSC mice. SIVcpzLB715 infected mice with the highest viral titer after 6 months were euthanized and infected tissues were cultured to harvest the first passage stock virus as described previously.[13,14] For the next generation, a new cohort of five hu-HSC mice were injected with 200 μl of first passage virus.

Determination of PVL and $CD4^+$ T-cell decline

To evaluate PVL and $CD4^+$ T-cell decline, peripheral blood was obtained on a weekly and bimonthly basis, respectively. Plasma RNA was extracted utilizing the E.Z.N.A. Viral RNA kit (Omega bio-tek, Norcross, CA) and the manufacturer's instructions. Viral load was quantified using the iScript One-Step RT-PCR kit with SYBR Green (BioRad, Hercules, CA) per the manufacturer's instructions as described previously.[10,14] $CD4^+$ T-cell decline was determined by staining whole blood with fluorophore conjugated anti-human CD45-FitC (eBioscience), CD3-PE (eBioscience), and CD4-PE/Cy5 (BD Pharmigen, San Jose, CA) antibodies. BD Accuri C6 FACS Analyzer was used to determine cell counts as described previously.[13,14] The $CD4^+$ T-cell decline between the infected and uninfected mice was assessed using a two-tailed Student's *t*-test ($p<0.001$).

Illumina-based deep sequencing and analysis

Viral RNA samples from timepoints 3, 11, 19- and 24-weeks post-inoculation were used to generate amplicons for sequencing. Whole-genome primer pools were made with Primal Scheme.[21] Amplicons were prepared using the TruSeq Nano DNA HT Library Preparation Kit and sequenced utilizing the MiSeq Illumina sequencer (Invitrogen, Carlsbad, CA). Reads were mapped to the SIVcpzLB715 stock virus (GenBank accession number: KP861923.1) using bowtie2 software v2.2.5.[22] This output was used as input for lofreq software v2.1.2 to call variants.[23] Each variant required >20 reads depth of coverage, >20% last timepoint frequency, a change of at least 30% frequency over time and were present in at least 5 datasets.

RESULTS

Virus from the first hu-mice passage was used to infect a second cohort of five hu-mice to evaluate the potential evolution of SIVcpzLB715 into HIV-1 Group M (Figure 1A). Viral loads showed an approximate 4.5-log increase throughout the duration of infection (Figure 1B). $CD4^+$ T-cell depletion occurred within 14 days and significantly declined ($p<0.001$) throughout the duration of the second-generation infection (Figure 1B). These data show SIVcpzLB715 can establish chronic viremia upon serial passage that produces significant $CD4^+$ T-cell decline by the end of the second generation.

Viral RNA was extracted from plasma of viremic mice at weeks 3, 11, 19 and 24 and subjected to deep sequencing. Detected amino acid substitutions were scored using a BLOSUM62 matrix to determine the likelihood of any given residue substitution based on known protein alignments (Figure 2). Mutations in *gag*, *pol, nef* and *env* were identified as more favorable for viral protein structure.

DISCUSSION

We assessed the phenotypic and genetic changes that facilitated the evolution of SIVcpzLB715 towards HIV-1 Group M utilizing a humanized mouse model to recapitulate cross-species transmission. Hu-mice became infected with SIVcpzLB715 within 2 weeks after initial viral challenge. Enhanced $CD4^+$ T-cell loss was observed during the second serial passage suggesting increased pathogenicity and viral fitness. Additionally, one of the mutations in Env gene (E523Q) located in the CD4 binding site appears to have a favorable BLOSUM62 score (Figure 2).[24,25] This implies that the CD4 binding site is adapting to improve virus-receptor binding. With increased knowledge of how SIVcpzLB715 first adapted to the human host, we may be able to gain better insight on how SIVs cross over into the human population.

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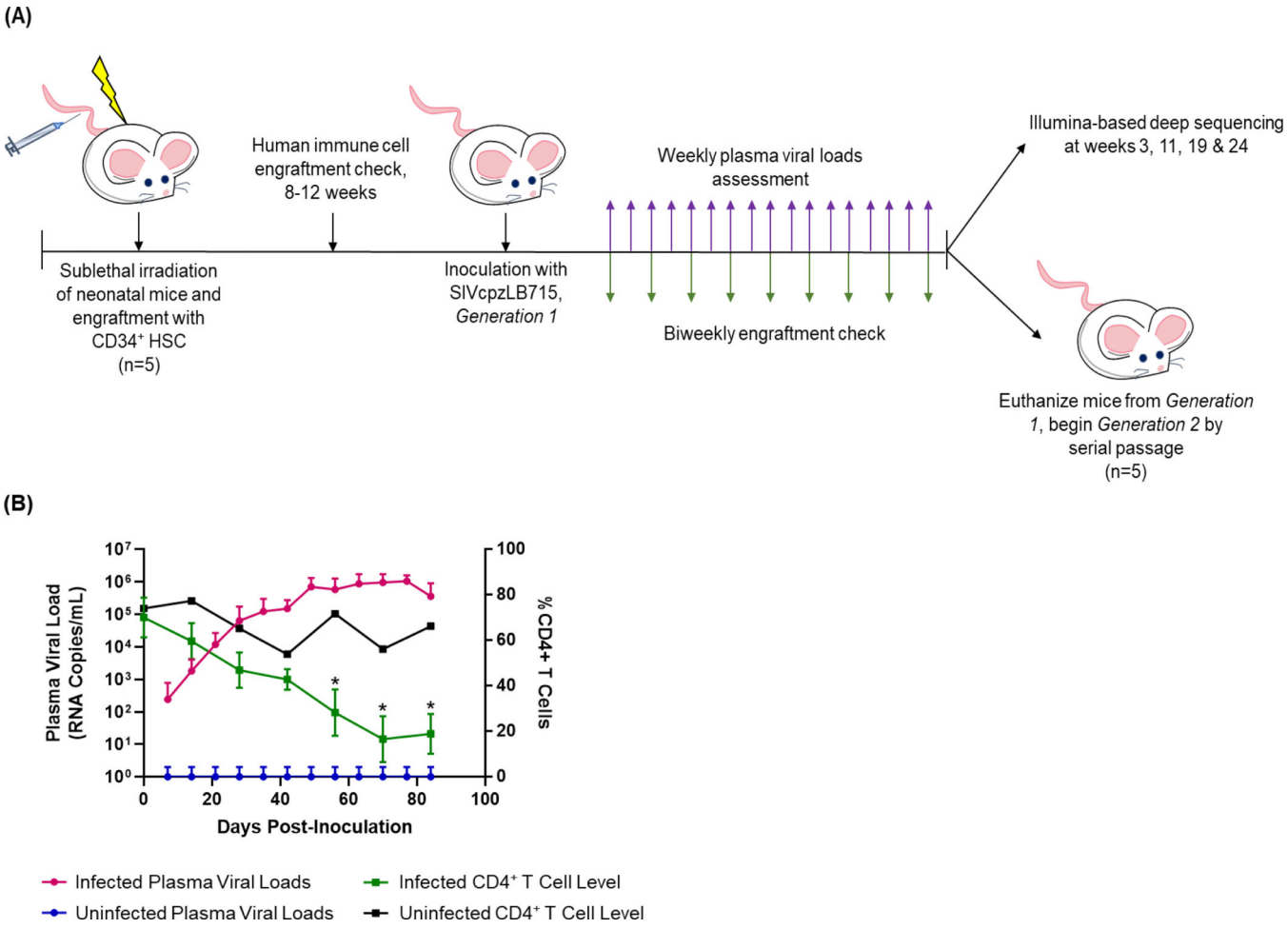

Figure 1. SIVcpzLB715 infection kinetics during the second generation in hu-HSC mice.

(A) Experimental scheme for SIVcpzLB715 infection of hu-HSC mice. Neonatal mice are sublethally irradiated and inoculated intrahepatically with $CD34^+$ hematopoietic stem cells. Following SIVcpzLB715 inoculation, PVL were assessed weekly and $CD4^+$ T-cell decline bimonthly. **(B)** Second generation PVL and $CD4^+$ T-cell decline seen in SIVcpzLB715 infected hu-HSC mice. Statistically significant $CD4^+$ T-cell depletion occurred by the end of the second generation in infected mice relative to the uninfected mice (two-tailed Student's *t*-test, *$p<0.001$).

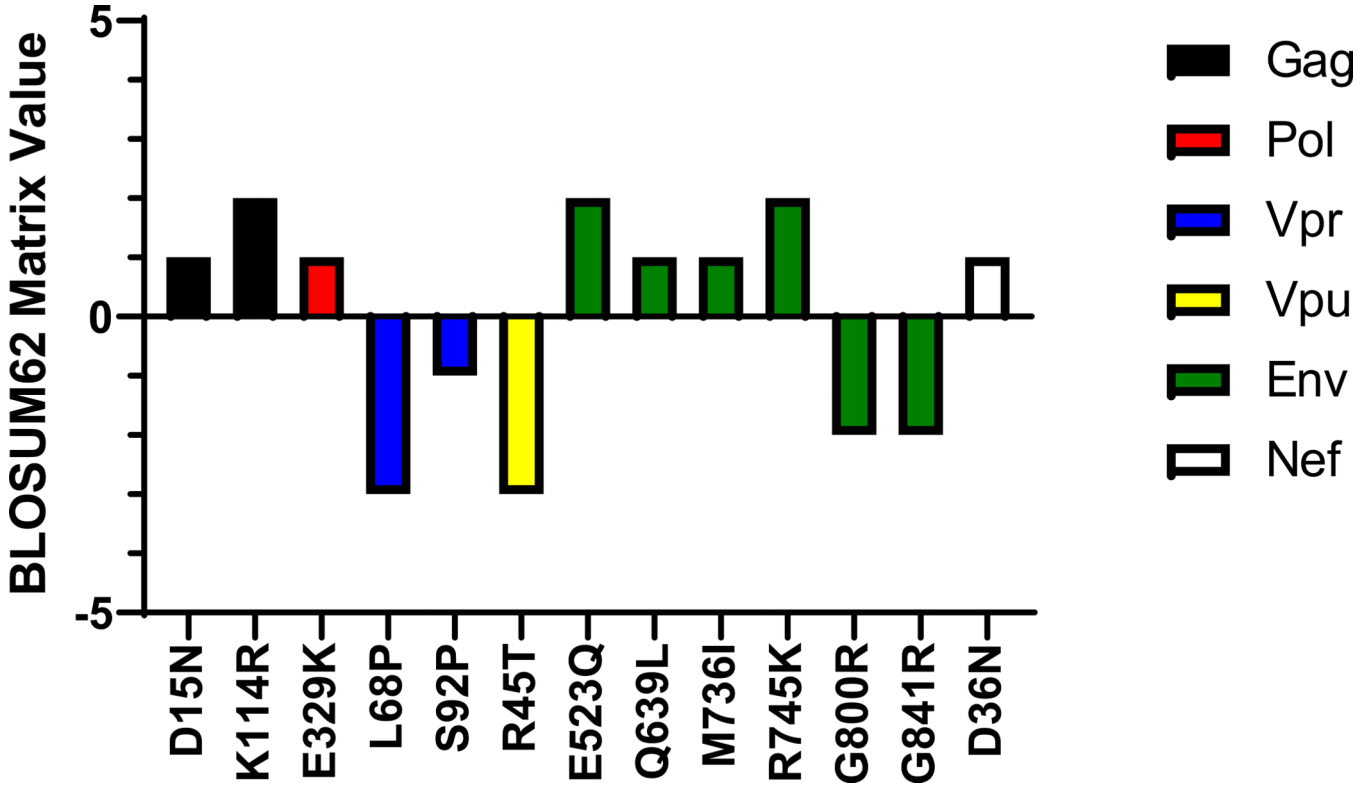

Figure 2. BLOSUM62 matrix scores of identified residue changes.

Variant residues were identified as described in the results and methods. Some variants in Vpr, Vpu and Env appear to be disfavored, yet still became more frequent in the viral population.