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SIVcpz cross-species transmission and viral evolution towards HIV-1 in a humanized mouse model

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Abstract

HIV-1 evolved from its progenitor SIV strains, but details are lacking on its adaptation to the human host. We followed the evolution of SIVcpz in humanized mice to mimic cross-species transmission. Increasing viral loads, CD4⁺ T-cell decline, and non-synonymous mutations were seen in the entire genome reflecting viral adaptation.

INTRODUCTION

How HIV-1 viral strains originating from SIV progenitor viruses SIVcpz and SIVgor evolved to become highly pathogenic to humans is still unclear. The dominant theory posits that serial transmissions in the human population led to increased viral fitness by accumulating adaptive mutations.¹ Humanized mice (hu-mice) present an ideal model to mimic the initial transmission and subsequent viral evolution. Hu-mice harbor a transplanted human immune system which includes human CD4⁺ T-cells, macrophages and dendritic cells permissive for viral infection.^{2–12} While three previous studies utilized hu-mice to evaluate this question, the data obtained was limited: in one case, only parts of the viral genomes were subjected to sequence analysis and in each study, only variants from a single terminal timepoint after 14–16 weeks of infection were analyzed.^{13–15} To overcome these limitations, we infected hu-mice with several SIVcpz strains, including SIVcpzEK505 which is closely related to HIV-1 group N. Viremia and CD4⁺ T-cell levels were monitored for 120 days. To understand viral adaptation and the associated genetic changes, full genome sequence analysis was performed at multiple time points during viral infection (Figure 1A).

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RESULTS

Successful infection resulting in viremia was seen in hu-mice challenged with a number of SIVcpz isolates, with the SIVcpzEK505 results presented here. Virus was detected in plasma within one week of inoculation. Viral loads increased gradually by approximately 2 orders of magnitude, peaking around 80 days post-challenge before declining slightly (Figure 1B). There was slow CD4⁺ T-cell decline from the starting point in the infected mice versus those of the uninfected controls (Figure 1C). Viral genomic RNA obtained from plasma samples from viremic mice at 3, 11, 19 and 22 weeks was subjected to next-generation sequencing (NGS). Numerous synonymous and non-synonymous single-nucleotide changes (SNPs) were seen throughout the genome. Non-synonymous SNPs that increased in frequency within the viral population by at least 20% are presented in Table 1.

DISCUSSION

Here we utilized the humanized mouse model to mimic the initial cross-species transmission of SIVcpz and understand the viral phenotypic and genetic adaptation in an *in vivo* context. SIVcpz readily infected hu-mice and was viremic in a week confirming successful transmission to humans. Gradual viral load increases suggest improved viral fitness in human cells. CD4⁺ T-cell decline indicates viral predilection to these cells comparable to observed immunosuppression in humans. In addition to other shortcomings, previous studies did not provide enough time to adequately determine the variants needed for cross-species adaptation.^{13–15} We analyzed the viral variants at different time points spanning 22 weeks. Multiple SNPs were identified throughout the viral genome in contrast to only a few variants identified in the envelope gene previously. While many mutations were seen at individual early time points, some disappeared subsequently, others persisted across multiple time points, with some even increasing in frequency (Table 1). SNPs displaying a 20% increase in occurrence are presented. These encompass Gag, Pol, Vif, Env, and Nef genes suggesting SIV adaptation to humans requires many allelic changes. We are currently investigating the genomes of later passages of the virus in an effort to identify the major changes that confer improved fitness to overcome human restriction factors.

MATERIALS AND METHODS

Preparation of humanized mice

Hu-HSC mice were prepared as described previously (Figure 1A).^{16–20} All animals were cared for according to the CSU IACUC approved protocols.

EK505 Viral propagation and *in vivo* infection

HEK 293T cells were transfected with the SIVcpzEK505 plasmid, kindly provided by Dr. Preston Marx. After 48 hours, the supernatant was ultracentrifuged using a 20% sucrose cushion and 200 μ L (TCID₅₀ 3.2×10^5) of the concentrated virus was injected intraperitoneally into 5 well-engrafted (>60% CD45⁺, >50% CD4⁺) hu-HSC mice (Figure 1A). Viral Titer was determined using TZM-bl reporter cells as previously described.^{21–25}

Plasma viral load (PVL) and CD4⁺ T-cell level evaluation

To determine the PVLs and CD4⁺ T-cell engraftment, peripheral blood was obtained on a weekly and bimonthly basis respectively by tail vein puncture (Figure 1). The E.Z.N.A Viral RNA kit (Omega bio-tek, Norcross, CA) was used to extract viral RNA from the plasma, which was quantified as previously described using the iScript One-Step RT-PCR kit with SYBR Green per the manufacturer's guidelines (Bio Rad, Hercules, CA).¹⁶ Mouse anti-human CD45-FITC (eBioscience), CD3-PE (eBioscience), and CD4-PE/Cy5 (BD Pharmingen, San Jose, CA) antibodies were used to stain whole blood samples. CD4⁺ T-cell levels were determined within the CD45⁺CD3⁺ population using the BD Accuri C6 cytometer as described previously.¹⁶ CD4⁺ T-cell decline between the infected and uninfected mice was assessed using a two-tailed Student's *t*-test. ($p < 0.05$).

NGS and genomic analysis

Amplicons for sequencing were generated using RNA samples from timepoints 3-, 11-, 19- and 22-weeks post-inoculation. Multiplexed whole-genome spanning primer pools were designed using the Primal Scheme software.²⁶ Samples were prepared using the Nextera XT DNA Library preparation kit and sequenced at the University of Wisconsin, Madison sequencing core facility using a MiSeq Illumina desktop sequencer (Invitrogen, Carlsbad, CA).

Reads were mapped to the SIVcpzEK505 stock virus (GenBank accession Number: DQ373065.1) using bowtie2 software v2.2.5.²⁷ These alignments were combined with lofreq software v2.1.2 to call variants.²⁸ Each variant required >20 reads depth of coverage and $>20\%$ frequency increase relative to the stock virus.

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Figure 1.

Schematic representation of the hu-HSC mouse generation, viral infection, SIVcpzEK505 plasma viral loads and CD4⁺ T-cell decline following inoculation. (A.) Neonatal mouse pups were intrahepatically injected with human CD34⁺ hematopoietic stem cells following sublethal irradiation. Mice were screened 8–12 weeks post-reconstitution to determine engraftment levels of human immune cells. (B.) Plasma viral loads and (C.) CD4⁺ T-cell depletion of SIVcpzEK505-infected and uninfected hu-HSC mice. Statistically significant

CD4⁺ T-cell depletion was seen in the infected mice relative to the uninfected mice (two-tailed Student's t-test, $p < 0.05$).

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Table 1.

Nonsynonymous single-nucleotide polymorphisms (SNPs) that showcase a >20% increase in viral population frequency by 22 weeks post inoculation.

Gene	Codon Number ^a	AA Change ^b	Highest Endpoint Frequency ^c
Gag	35	V→I	0.88
Gag	105	R→K	0.23
Pol	175	P→Q	0.34
Pol	364	E→K	0.22
Vif	13	V→A	0.41
Vif	55	I→T	0.61
Vif	118	S→A	0.92
Env	402	V→G	0.56
Env	403	G→E	0.99
Env	414	P→T	0.6
Env	601	Q→K	0.77
Env	630	K→R	0.74
Env	668	R→K	0.3
Env	703	E→K	0.6
Env	773	I→K	0.58
Env	829	G→S	0.2
Nef	79	M→L	0.35

^aCodon number based on the beginning of the respective gene

^bThe amino acid change resulting from the nonsynonymous mutation

^cThe highest detected frequency of the change between samples at the last time point collected