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HIV-1 Induced AIDS in Monkeys

Theodora Hatzioannou^{1,*}, Gregory Del Prete², Brandon F. Keele², Jacob D. Estes², Matthew W. McNatt^{1,3}, Julia Bitzegeio^{1,3}, Alice Raymond¹, Anthony Rodriguez¹, Fabian Schmidt^{1,3}, C. Mac Trubey², Jeremy Smedley⁴, Michael Piatak Jr.², Vineet N. KewalRamani^{5,*}, Jeffrey D. Lifson^{2,*}, and Paul D. Bieniasz^{1,3,6,*}

¹Aaron Diamond AIDS Research Center, 455 First Avenue, New York, NY 10016, USA

²AIDS and Cancer Virus Program, Leidos Biomedical Research, Frederick National Laboratory, Frederick, MD 21702, USA

³Laboratory of Retrovirology, The Rockefeller University, 455 First Avenue, New York, NY 10016, USA

⁴Laboratory Animal Sciences Program, Leidos Biomedical Research, Frederick National Laboratory, Frederick, MD 21702, USA

⁵HIV Drug Resistance Program, National Cancer Institute, Frederick, MD 21702, USA

⁶Howard Hughes Medical Institute, 455 First Avenue, New York, NY 10016, USA

Abstract

Primate lentiviruses exhibit narrow host tropism, reducing the occurrence of zoonoses but also impairing the development of optimal animal models of AIDS. To delineate the factors limiting cross-species HIV-1 transmission, we passaged a modified HIV-1 in pigtailed macaques that were transiently depleted of CD8⁺ cells during acute infection. During adaptation over four passages in macaques, HIV-1 acquired the ability to antagonize the macaque restriction factor tetherin, replicated at progressively higher levels and ultimately caused marked CD4⁺ T-cell depletion and AIDS-defining conditions. Transient treatment with anti-CD8 during acute HIV-1 infection caused rapid progression to AIDS, while untreated animals exhibited an elite controller phenotype. Thus, an adapted HIV-1 can cause AIDS in macaques, and stark differences in outcome can be determined by immunological perturbations during early infection.

HIV-1 replicates well in humans, but like other primate lentiviruses, it encounters impediments to replication in atypical host species (1–3). This fact has limited the development of optimal animal models of AIDS (4, 5). To delineate the requirements for primate lentivirus to colonize a divergent host and to aid development of better animal

*Corresponding author. thatzio@adarc.org (T.H.); vineet.kewalramani@nih.gov (V.N.K.); lifsonj@mail.nih.gov (J.D.L.); pbienias@adarc.org (P.D.B.).

The data presented in this paper are tabulated in the main paper and in the supplementary materials. HIV-1 Env sequences used in Fig. 3 have been deposited in Genbank (accession numbers KJ201545 through KJ201764) Sequences encompassing the full-length viral genome for P5 and P6 have also been deposited in GenBank (accession numbers KJ767573 through KJ767592).

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models of AIDS, we adapted HIV-1 to replicate efficiently and cause AIDS in a monkey species.

We inoculated pigtailed macaques (*Maccaca nemestrina*), which lack an HIV-1 restricting TRIM5 protein (6–9), with HIV-1 clones encoding SIV_{MAC} Vif, which antagonizes several macaque APOBEC3 proteins that would otherwise restrict HIV-1 replication (10). The inoculum contained four clonal HIV-1_{NL4-3}-derived viruses, each encoding a gp120 envelope protein from a prototype HIV-1 strain that utilizes the CCR5 co-receptor (Fig. 1A, fig. S1). In two passage-1 (P1) animals, acute plasma viremia reached $\sim 10^5$ viral RNA (vRNA) copies/ml and declined thereafter (Fig. 1B). This outcome was similar to our previous findings using an HIV-1 derivative encoding a primarily CXCR4-utilizing envelope protein (KB9) (11). One P1 animal (P1-A), in which viremia of $\sim 10^3$ vRNA copies/ml was maintained for 32 weeks (Fig. 1B), was treated with a monoclonal antibody to deplete CD8⁺ T cells to relieve immune pressure and thereby increase the viral population size. Its blood was used to initiate P2 infection (Fig. 1A, B, fig. S2). For P2, and each passage thereafter, recipient animals were treated with anti-CD8, at the time of inoculation and one week later, to augment early viremia (12). This treatment caused transient CD8⁺ T-cell depletion in blood and lymph nodes, without major impact on CD8⁺ T-cells in gut-associated lymphoid tissue (GALT) (Fig. S2, S3). In P2-A, acute viremia reached 10^6 vRNA copies/ml, declining to $\sim 10^4$ vRNA copies/ml at 5 to 6 weeks after infection (Fig. 1B). In P1 and P2 animals, the levels of blood CD4⁺ T-cells appeared unperturbed (Fig. 2A).

Animal P3-A was inoculated with blood taken from P2-A at 23 weeks post infection whereas animal P3-B was inoculated with blood taken 1 week later, after CD8⁺ cell depletion of P2-A (Fig. 1A, B). P3 recipients exhibited acute viremia at $\sim 10^6$ to 10^7 vRNA copies/ml. Blood CD4⁺ T-cells numbers and the proportion of CD4⁺ T-cells in GALT decreased following infection (Fig. 2A, B) but these perturbations were transient or modest in magnitude. Moreover, viremia declined over the ensuing 50–60 weeks (Fig. 1B). We depleted CD8⁺ cells in P3 macaques on three subsequent occasions (fig. S2). Although transient increases in plasma viremia followed antibody administration (Fig. 1B), neither animal exhibited disease.

P4 animals were inoculated with blood taken from either or both P3 macaques at 50 weeks post infection (Fig. 1A, B). Each developed acute viremia at $\sim 10^6$ – 10^7 vRNA copies/ml (Fig. 1B). Notably, marked and sustained CD4⁺ T-cell depletion occurred in the blood of 2 out of 3, and in the GALT of all P4 animals (Fig. 2A, B, C). Additional indications of HIV-1-induced pathogenesis included immune activation, as evidenced by more frequent Ki67 expression in GALT CD4⁺ cells (fig. S4A), as well as fibrotic disruption and frequent Ki67 expression in T-cell zones of lymph nodes (fig. S4 B, C, D). In P4-C, pathology was especially evident, and viremia continued to increase after the acute phase, approaching $\sim 10^8$ vRNA copies/ml (Fig 1B). Loss of blood and GALT CD4⁺ T-cells in P4-C was virtually complete at 28 weeks (Fig 2A, B, C). At this point, P4-C succumbed to an AIDS-defining, multifocal extranodal B-cell lymphoma with large retro-orbital and paraspinal tumors, and extensive epicardial and renal involvement (Fig. 2D, fig. S5A, B, C). Such tumors, associated with SIV-induced AIDS in macaques have been linked to opportunistic

lymphocryptoviruses or rhadinoviruses and are analogous to B-cell neoplasms in humans co-infected with HIV-1 and Kaposi's sarcoma-associated herpesvirus (KSHV) (13). AIDS-defining *Pneumocystis pneumonia* was also detected in P4-C lung biopsies (Fig. 2E) but not in controls (fig S6A).

To confirm that HIV-1 had adapted to replicate efficiently and cause AIDS in macaques, 4 animals (P5-A-D, Fig 1A) were inoculated with blood from P4-C. Two macaques that were transiently depleted of CD8⁺ cells at inoculation (P5-C and P5-D, Fig. 1A, fig. S2) developed persistently high viremia ($\sim 10^7$ vRNA copies/ml) with blood and GALT CD4⁺ T-cell depletion (Fig. 1B, Fig 2A). P5-C was euthanized at week 30 after infection, with *Pneumocystis pneumonia* (fig. S6B), whereas P5-D was euthanized at week 7 following onset of severe bilateral hind limb weakness with wasting. In P5-A and P5-B, which were not CD8 depleted, acute viremia reached $\sim 10^6$ vRNA copies/ml, but was controlled thereafter (Fig. 1B). Blood and GALT CD4⁺ T-cells were depleted but recovered thereafter (Fig. 2 A, B). Subsequent CD8⁺ cell depletion in P5-A and P5-B at week 23 (fig. S2), caused transient increases (10^3 -fold) in viremia, and blood CD4⁺ cell depletion in P5-A, but did not recapitulate the effect of CD8⁺ cell depletion during acute infection (Fig. 1B, Fig. 2A). Rather, as CD8⁺ cells recovered in P5-A and P5-B, viremia and CD4⁺ T-cells returned to pre-depletion levels (Fig. 1B, Fig. 2A).

This dichotomous outcome of HIV-1 infection was reproduced in animals that received blood from P5-C. Specifically, P6-A, (no CD8 depletion), showed acute viremia that was subsequently controlled with modest and transient GALT CD4⁺ T-cell depletion (Fig. 1A, B, Fig 2A, B). Conversely, in P6-B (CD8 depleted at infection, fig. S2), viremia persisted at 10^6 – 10^7 vRNA copies/ml while blood and GALT CD4⁺ cells were nearly completely depleted (Fig. 1B, Fig. 2A, B). P6-B was euthanized at 9 weeks with symptoms of nephropathy or glomerulonephritis (azotemia, hyperphosphatemia, hypoproteinemia and anemia). Necropsy revealed myocardial fibrosis, lymphoid depletion with histiocytosis, and diffuse expansion of the glomerular mesangial matrix. Although not AIDS defining, these conditions have frequently been described in human AIDS patients (14) and occasionally in SIV-infected macaques (15).

Single viral genome amplification and sequencing (SGA) (16–18) at various points during adaptation revealed that one envelope gene in the initial inoculum (AD8) dominated the viral population in P1-A and was the only strain detected in P2 to P6 (Fig. 3A). Indeed, Env sequences in P2-P6 macaques sampled over ~ 3.5 years exhibited only $\sim 2\%$ divergence from AD8. Additionally, although T-cells constituted the dominant infected cell type in lymph nodes, the propensity of AD8 to infect macrophages in GALT was preserved throughout P3-P6 (fig. S7A, B, C). In P5-C, that succumbed following a precipitous decline in blood CD4⁺ T-cells (Fig. 2A), about half of the envelope sequences had a four amino-acid deletion in the V3 loop (Fig. 3B), a determinant of coreceptor utilization (19). This deletion was associated with a change in coreceptor utilization, as evidenced by viral sensitivity to Maraviroc (CCR5 antagonist) or AMD3100 (CXCR4 antagonist) (Fig. 3C, D). Thus, HIV-1 infection in P5-C recapitulated a common feature of HIV-1 disease, namely coreceptor switching, that rarely occurs in non-human primate lentivirus infections (20). Interestingly, the V3 loop

deletion associated with CXCR4 utilization was not detected in P6-A or P6-B, both of which received blood from P5-C.

Although the initial virus was not restricted by TRIM5 α and APOBEC3, HIV-1 Vpu does not antagonize macaque tetherin, rendering the initial inoculum sensitive to this potential host-range restriction (21, 22). Notably, an insertion of an amino-acid at position 15 in the Vpu transmembrane (TM) domain (Fig. 4A) was detected in P1-A and was found in 100% of P2-A sequences at 52 weeks, and all sequences thereafter. Another Vpu TM mutation, V21G, (Fig. 4A) was detected in P3 animals and in all sequences from P4 onwards. TM domains govern Vpu:tetherin interaction (21) and each of the TM domain mutations contributed to the ability of Vpu 15/21 to antagonize pigtailed macaque tetherin (Fig. 4B). We previously showed that Vpu gained the ability to antagonize human tetherin during the emergence of HIV-1(23), and the passage of HIV-1 in pigtailed macaques apparently resulted in an analogous adaptation (Fig. 4B).

Antibodies to HIV-1 proteins were apparent in P4-A beginning at week 4, but were not detected until week 16 after infection in P4-B, which had higher viremia and some degree of blood CD4⁺ T-cell depletion (fig. S8, Fig. 1B, Fig. 2A). Animal P4-C, the first to succumb to AIDS, did not seroconvert (fig. S8). In fact, each macaque that progressed to AIDS following transient CD8 depletion did not seroconvert, whereas those that were not CD8 depleted and did not progress to AIDS mounted robust antibody responses (fig. S8). Previously, failure to seroconvert has been associated with rapid progression in SIV infection (24). HIV-1-induced loss of CD4⁺ helper T-cells in CD8-depleted animals may play a role in the failure to mount an antibody response in macaques that developed AIDS (12).

The approaches employed herein have enabled HIV-1 infection of macaques to recapitulate salient features of HIV-1 infection in humans. Our findings suggest that evasion or antagonism of antiretroviral proteins is key for the colonization of new hosts by primate lentiviruses. Because transient depletion of CD8⁺ cells during acute infection determined whether macaques became rapid progressors or elite controllers, our findings suggest that the immunological insult during acute HIV-1 infection can determine subsequent clinical course. It is also possible that adaptation to the host major histocompatibility complexes, and evasion of adaptive immune responses may be required for HIV-1 pathogenesis in immunologically intact hosts. Finally, because the viral strain used herein contains nearly all the targets of HIV-1 vaccines and drugs, further development of a pigtailed macaque-based model could facilitate the evaluation of novel clinical HIV-1 therapies, prevention strategies and interventions for virus eradication.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1. Adaptation of HIV-1 to pigtail macaques

(A) Outline of serial animal-to-animal HIV-1 passages. Animals colored black were transiently depleted of CD8+ cells at the time of inoculation. Inverted animals with colored asterisks indicate animals that were euthanized for clinical cause, including AIDS defining conditions. (B) Viremia in the plasma of HIV-1 infected macaques. Color-coded arrowheads indicate anti-CD8 treatment and asterisks indicate deaths (color code is the same for panel (A)). Grey arrows indicate times at which blood was taken to initiate the subsequent passage.

Fig. 2. HIV-1 pathogenesis in pigtail macaques

(A) CD4⁺ T-cell counts in blood of HIV-1 infected macaques. Color codes, arrowheads and asterisks are as described in Fig. 1. (B) CD4⁺ T-cell subset frequencies in GALT at the indicated weeks after infection. 'Pre' = 0 to 1 week before infection. (C) Immunohistochemical staining of CD4⁺ T-cells (brown) and myeloid cells (CD68⁺/CD163⁺; red) in the GALT of P4-C. Scale bar = 100µm. (D) Immunohistochemical staining (brown) of CD3⁺ T-cells (top) CD20⁺ B-cells (bottom) in tumor (marked T) effacing normal kidney tissue (marked N) in P4-C. Scale bar = 200µm. (E) Grocott's methenamine silver stain revealing *Pneumocystis* organisms (black spots) in P4-C lung tissue. Scale bar = 50µm.

Fig. 3. Envelope evolution and coreceptor switching in HIV-1 infected macaques

(A) Phylogenetic analysis of Env sequences (using SGA and neighbor joining method) found in plasma of HIV-1 infected macaques. (B) V3 loop amino acid sequences from the AD8 inoculum and representative viral genomes in P5-C plasma at week 27 after infection. (C, D) Infectivity of HIV-1 Env-GFP pseudotyped with control X4 (NL4-3) or R5 (AD8) or P5-C-derived Env proteins in the presence of Maraviroc (8nM) (C) or AMD3100 (1μM) (D) relative to no inhibitor. Values are mean $\pm$ S.D. of three independent experiments.

Fig. 4. Adaptation of HIV-1 Vpu to antagonize Tetherin in macaques

(A) Amino acid alignment of the Vpu transmembrane domain from the initial viral clone, and after adaptation in pigtailed macaques. (B) Infectious virion yield from 293T cells transfected with HIV-1 proviral plasmids encoding the indicated Vpu proteins and plasmids expressing human or three naturally occurring variants (v1-v3) of pigtailed macaque Tetherin.