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An SIV Macaque Model of HAART: Viral Latency in the Periphery and the Central Nervous System

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Abstract

Purpose of Review—Here, SIV macaque models are examined for their strengths in identifying *in vivo* sites of HIV latency and persistent virus replication during HAART. The best-characterized HIV reservoir in HAART-treated persons is resting CD4+ T cells in blood, although residual virus also comes from other reservoirs. Nonhuman primate/SIV models of HAART have been developed to characterize potential HIV reservoirs, in particular the central nervous system (CNS) and stem cells in bone marrow, known and potential reservoirs of latent virus that are difficult to study in humans.

Recent Findings—Few SIV macaque models of HAART have examined plasma and CSF virus decay, the number of resting CD4+ T cells harboring replication-competent latent SIV, HAART-treatment effect on the CNS, or residual viral replication or viral DNA levels in that tissue. Using a consistent, accelerated SIV macaque model, we characterized peripheral viral reservoirs, including those in the CNS, among HAART-treated macaques. The SIV model reproduces latency in memory CD4+ T cells throughout the body and indicates that the CNS contains a stable SIV DNA reservoir.

Summary—An SIV macaque model of HAART recapitulating viral latency, particularly in the CNS, is required to study therapeutic approaches for a functional HIV cure.

Keywords

SIV; macaque; HAART; reservoirs; CNS

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Introduction

The SIV macaque model of HIV/AIDS and CNS disease has been used for both pathogenesis and vaccine research. It is now crucial to refine and apply these models to the next stage of HIV research and treatment involving eradication of HIV latent reservoirs or “functional cures” that reduce viral reservoirs and boost immune responses to prevent virus reactivation.

The first step is to comprehensively characterize potential latent reservoirs of HIV in the SIV model in which there is virus control with HAART. A well-characterized SIV macaque model is needed to serve this role and several models have recently been reported that use either SIV or SHIV macaque models.[1–5] Recent studies have used antiretroviral therapy in SIV-infected macaques to study aspects of immune recovery[1, 2, 4–6] and a recent study used a combined antiretroviral regimen to examine SHIV RNA and DNA in selected tissues.[5] Another SIV study used three antiretroviral drugs in a short treatment regimen to examine the impact on inflammation in the brain.[6]

A Consistent, Accelerated Macaque Model of SIV

While consensus is yet to be reached regarding the use of SIV versus SHIV infection in these models, a major challenge is the development of an antiretroviral regimen that more closely mimics treatment in humans. Over the past 12 years we have studied viral pathogenesis in peripheral blood, immune tissues, lung, gut, heart, peripheral nervous system and brain in our consistent, accelerated model SIV macaque model.[7–15] These studies provide a comprehensive understanding of acute, chronic and late stage disease, as well as the impact of the innate and adaptive immune responses in the brain. We have developed the only model that uses a HAART-like regimen of antiretroviral drugs that includes a reverse transcriptase inhibitor, two protease inhibitors and an integrase inhibitor, thereby targeting 3 different steps in the viral life cycle. Using this model, we rigorously characterized peripheral virus replication and quantitated the number of resting CD4+ T cells in blood and lymphoid tissues.[3] Further, we used this SIV HAART model to demonstrate that, although virus replication is controlled in the CNS, SIV DNA levels are comparable to those seen in untreated SIV-infected macaques and animals have persistent CNS inflammatory responses.[16] Thus, this model has the potential to characterize additional latent reservoirs of HIV and can be used to determine the circumstances and mechanisms of activation of latent reservoirs. The model will be valuable to test if treatment or immune manipulation would be sufficient to maintain latent HIV without concurrent HAART. This review provides an overview of this model in comparison to other SIV macaque models that use three or more antiretroviral drugs.

Virus Decay in Plasma and Latently CD4+ T cells in Blood and Tissues in the SIV Macaque HAART model

SIV-infected macaques were treated with a four antiretrovirals: saquinavir (Hoffman-LaRoche), integrase inhibitor L-870812 (Merck), atazanavir (Bristol-Myers Squibb) and 9-R-(2-phosphonomethoxypropyl)adenine (tenofovir; Gilead) beginning at 12 days post-inoculation. Treatment was initiated immediately after acute infection, when viral load in both plasma and CSF were declining but had not reached a set point. After initiating HAART, we measured the decay of virus in plasma and CSF and the number of latently infected resting CD4+lymphocytes in the peripheral blood and immune tissues (lymph nodes and spleen) (Figure 1 A–C). The treated animals experienced at least a five log₁₀ decrease in plasma viremia relative to untreated animals, consistent with what has been reported in humans.[17–19] There was a dramatic biphasic decline in viremia in the plasma of all the

treated macaques. Each phase exhibited a decay constant similar to that reported for HIV-1 infected humans on HAART and reported recently in a SHIV model by North and colleagues.[5, 17]

In addition to the rapid viral decline in plasma, the number of CD4⁺ T cells in peripheral blood increased following the initiation of HAART, similar to that observed following treatment in HIV-infected humans.[18, 19] Thus, both viral and immune responses to HAART in this model parallel those in HIV-infected individuals.

In patients on HAART, HIV-1 persists in resting CD4⁺ T cells in a latent state. We used an SIV coculture assay to measure frequencies of resting CD4⁺ T cells harboring replication competent virus.[20, 21] In our HAART-treated, SIV-infected macaques the frequency of resting CD4⁺ T cells in peripheral blood harboring replication-competent virus showed an initial decay similar to that observed in HIV-1-infected humans on HAART.[3] The initial decay in these cells reflected turnover of labile preintegration complexes that cannot integrate into the host genome due to the resting state of the infected cell. However, it has been postulated that before decay of these resting cells is complete, release of virus occurs in resting CD4⁺ T cells that have become activated either a) when preintegration complexes integrate into the host genome upon activation of the cell and produce virus or b) when the resting cell carries a stably integrated but latent viral genome and becomes activated to produce virus. Our results suggest that the frequencies measured at necropsy are representative of cells in the stable post-integration state of latency. This had not been previously shown in any animal model of HIV-1 infection and HAART. Thus, this model recapitulates the level of replication-competent virus present in resting CD4⁺ T cells in peripheral blood and provides a realistic model of HIV-1 infection and HAART as defined by previous work in HIV-1-infected humans.[22]

Latent HIV is present not only in resting CD4⁺ T cells in the peripheral blood, but also in immune tissues. We therefore determined the frequency of resting CD4⁺ T cells harboring replication-competent virus in pooled head (cervical, retropharyngeal, and submandibular) lymph nodes, pooled gut (mesenteric and colonic) lymph nodes, spleen, and peripheral blood mononuclear cells (PBMC) isolated at necropsy. In all HAART-treated animals, frequencies were substantially lower than the untreated animals. Geometric mean frequencies in HAART-treated animals were 1.3 IUPM for head lymph nodes, 2.0 IUPM for gut lymph nodes, 1.9 IUPM for spleen, and 1.4 IUPM for PBMC.[3]

The frequencies of latently infected resting CD4⁺ T cells were not significantly different in the HAART-treated macaques between different tissues, [3] a finding that is consistent with observations in untreated HIV-1-infected humans.[23] Taken together, these results show that the model of HAART closely mimics the virologic and immunologic states achieved by HIV-1-infected humans on HAART with respect to the degree of suppression of viremia, the rebound in circulating and lymphoid tissue-associated CD4⁺ T cell counts, and persistence of replication competent virus in resting CD4⁺ T cells. These results confirm the persistence of latently infected resting CD4⁺ T cells in the lymphoid tissue and raise the possibility that there is an even distribution of this latent viral reservoir in the lymphoid tissues throughout the body of an infected individual on HAART.

Virus Decay in the CSF and Decreased Viral RNA but Not Viral DNA in Brain

The CNS is a viral reservoir in HIV, and most antiretroviral drugs do not reach significant levels in the brain. Therefore, we used our SIV macaque model to determine the extent to which non-CNS penetrant antiretrovirals would affect virus replication in the brain and alter innate and adaptive immune responses that ultimately lead to HIV encephalitis and neurocognitive deficits. The HAART regimen used in our model was chosen because the

combination of drugs did not reach effective levels in the brain.[24] CSF viral loads declined rapidly after initiating HAART in parallel with the virus decline in plasma (Figure 1B), which had similar viral decay kinetics to HIV-infected individuals treated with HAART (Figure 1C).[3, 16] Viral RNA in brain measured by quantitative RT-PCR was undetectable in treated animals but interestingly viral DNA levels were not different from those seen in untreated SIV-infected macaques. CNS inflammation was significantly reduced, with decreased expression of MHC Class II and GFAP in brain and reduced CSF CCL2 and IL-6 (Figure 2A). In treated macaques there was significantly lower expression of IFN β , MxA, and IDO mRNA in brain suggesting suppressed immune hyperactivation, and fewer CD4+ and CD8+ T cells, suggesting reduced trafficking of T cells from peripheral blood (Figure 2B). Nonetheless, expression of CD68 on macrophages and TNF α and IFN γ mRNA levels in the brain of HAART-treated macaques, while reduced, were not significantly different than in the brains of untreated SIV-infected macaques. This continued CNS inflammation in some macaques suggested that there might be low level residual SIV RNA expression in the absence of effective antiretroviral drug levels in the brain. Chronic activation of Type I IFNs is observed in the last stage of infection in brain in our consistent, accelerated SIV macaque model and correlates with inflammation and CNS lesions.[8] Type I IFNs also have been proposed as a pathogenic mechanism in HIV systemic disease since they upregulate immunosuppressive molecules including programmed death ligand-1 (PD-1) and PDL-1. [25] IFN γ expression is produced by the adaptive immune response and is a strong inducer of the immunosuppressive enzyme IDO.[16] Thus, low-level persistent virus replication in brain may drive expression of innate and adaptive immune responses that contribute to continued immunosuppression and local inflammation that may underlie the neurocognitive changes that persist even with successful treatment with HAART.[8, 26, 27]

Our studies of HAART therapy in a rigorous, high viral load, accelerated SIV macaque model showed significant benefits in reducing CNS virus replication and inflammation when treatment was initiated early in infection, even when the drugs did not cross the blood-brain barrier.[16] This model is now established and is well suited to address many important questions that remain in the treatment of HIV and presents a unique opportunity to study the CNS as measuring HIV DNA levels in the brains of patients undergoing therapy is impossible.

A number of questions remain to be answered by this model: When is the ideal stage of infection to initiate HAART therapy to preserve the immune system as well as to protect the central and peripheral nervous systems? Is it possible to purge latent virus from tissue reservoirs or decrease the level of residual viremia to allow immune control of virus replication? Are there combinations of antiretroviral therapy and neuroprotective drugs that will prevent cognitive decline and other neurological symptoms in HIV-infected individuals?

A recent study using short-term combined antiretroviral therapy with non-CNS penetrant drugs described results similar to those in our studies.[6] Despite a lack of decrease in virus in the CSF, and modest reduction in viral RNA in brain there was a reduction in some CNS inflammatory parameters but not in the expression of CD68 or TNF α mRNA.[6] Similar results have been observed in another SIV macaque model in which animals were treated with a regimen of tenofovir with or without the protease inhibitor nelfinavir, both of which are poorly CNS penetrant. Tenofovir monotherapy was shown to reverse neurophysiological abnormalities, which returned upon treatment cessation.[28] Movement abnormalities were not affected by tenofovir treatment. In another study, early treatment with tenofovir and nelfinavir prevented development of characteristic neurophysiological and motor alterations following SIV infection, and significantly decreased viral RNA in brain.[29] Treatment also altered immune responses in the brain as indicated by decreased IFN α and the IFN-

responsive gene G1P1 RNA and increased CCL5 RNA.[29] Together, these studies showed decreased viral replication in the CNS following antiretroviral treatment in SIV-infected macaques, even when using a poorly CNS penetrant HAART regimen. However, there also appear to be residual immune responses that may contribute to persistent inflammation and CNS disease in HIV-infected individuals on HAART.

Conclusion

To study the potential eradication of HIV, well-characterized, rigorous animal models will be necessary to first exhaustively identify reservoirs of latent virus and then to insure that these reservoirs are either purged of replication competent virus or that the levels of latent virus are lowered sufficiently to allow for virus control by the immune response, resulting in a functional cure. This review has compared the SIV macaque HAART models that have been developed to our SIV HAART model based on a consistent, accelerated SIV macaque model, characterized by high viral load in the plasma and CSF and the development of AIDS and CNS disease in three months. Non-CNS penetrant HAART controlled virus in both the periphery and the brain and prevented the development of AIDS and CNS lesions. In addition, this SIV HAART model closely mirrors the decay in viral load in the blood of HIV individuals on HAART, and the level of resting CD4⁺ T cells that harbor latent replication competent SIV in blood and immune tissue was very similar to HIV individuals on HAART. Thus, this model is ideally suited to examine the efficacy of adjunctive therapy to prevent ongoing CNS inflammation that may be the underlying cause of the neurocognitive changes observed in 50% of individuals on long-term HAART, as well as to test whether it is possible to purge or decrease existing latent viral reservoirs to a level that will prevent virus reactivation with the cessation of HAART.

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Figure 1. Virus replication and decay in SIV-infected macaques treated with HAART
(A) Viral RNA levels in plasma increase rapidly during the first 7 to 10 days after virus inoculation, decline by approximately 1 log, then increase again and remain at 10^7 to 10^8 for the remainder of the infection period. Viral RNA levels in CSF increase during acute infection, decline by several logs during the next 2 weeks, then increase again only in macaques that will develop moderate to severe encephalitis. CD4+ cell counts in peripheral blood decline during acute infection, recover briefly, then begin to decline again during the asymptomatic stage of infection. **(B)** Viral RNA levels in plasma and CSF increase rapidly during the first 7 to 10 days prior to HAART treatment, which was initiated at 12 days p.i. Within a few days after initiation of HAART, plasma and CSF viral load begin to decline. By approximately 60 days p.i., plasma and CSF viral load have declined to below the level of detection (~ 100 copy eq./mL) and viral loads remained low throughout the remainder of the experiment. **(B & C)** The decline in plasma and CSF viral RNA occurred in two phases—an initial short-term rapid decline followed by a longer term slower decline similar to the two-phase decline seen in the plasma of HIV-infected individuals on HAART. At 80 days p.i., there were 8 to 10 latently infected resting CD4+ T cells per million resting CD4+ T cells in the blood. These numbers declined gradually to approximately one latently infected resting CD4+ T cells per million by 175 days p.i. p.i. = post-inoculation; CSF = cerebrospinal fluid

Figure 2. Viral, inflammatory, and immune parameters in the accelerated, consistent SIV/ macaque model of HIV-associated neurological disease

(A) CSF:Plasma CCL2 ratio, an estimate of the gradient of CCL2 in the brain as compared to the periphery, rose acutely, but declined significantly after initiation of HAART therapy. Macrophage surface markers MHC Class II and CD68, astrocyte marker GFAP, and immune markers IFN β RNA and Mx RNA all were expressed at very low levels in brain at necropsy as compared to untreated macaques (Figure 1). In addition, viral RNA in brain was undetectable at necropsy. (B) CCL2 production in the CNS, expressed as the ratio of CCL2 in CSF:plasma rises during acute infection, declines by day 21 p.i., then increases again in macaques that will develop moderate to severe encephalitis. Markers of macrophage infiltration and activation (CD68 and MHC class II) are elevated in brain tissue during acute and terminal infection. In contrast, activation of astrocytes as measured by expression of GFAP in brain tissue increases gradually throughout infection. p.i. = post-inoculation