In Vivo Models of Human Immunodeficiency Virus Persistence and Cure Strategies

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Current HIV therapy is not curative regardless of how soon after infection it is initiated or how long it is administered, and therapy interruption almost invariably results in robust viral rebound. Human immunodeficiency virus persistence is therefore the major obstacle to a cure for AIDS. The testing and implementation of novel yet unproven approaches to HIV eradication that could compromise the health status of HIV-infected individuals might not be ethically warranted. Therefore, adequate in vitro and in vivo evidence of efficacy is needed to facilitate the clinical implementation of promising strategies for an HIV cure. Animal models of HIV infection have a strong and well-documented history of bridging the gap between laboratory discoveries and eventual clinical implementation. More recently, animal models have been developed and implemented for the in vivo evaluation of novel HIV cure strategies. In this article, we review the recent progress in this rapidly moving area of research, focusing on the two most promising model systems: humanized mice and nonhuman primates.

Keywords. HIV latency; AIDS; humanized mice; animal models; non-human primates.

Implementation of combination antiretroviral therapy (ART) for the treatment of human immunodeficiency virus (HIV) infection in the 1990s dramatically changed the course of disease. Human immunodeficiency virus type 1 (HIV-1) infection changed from an almost immediately terminal diagnosis to one of a chronic illness that could be managed relatively well. Although ART is extremely effective at impeding new infection, there is no mechanism by which current treatment regimens will eliminate latently infected cells. Therefore, current ART regimens cannot cure HIV-1 infection. It has been clearly demonstrated that ART interruption results in rapid rebound of plasma viremia, presumably due to reactivation of a latent reservoir [1–6]. The persistent HIV reservoir represents long-lived, latently infected, resting, inducible cells present in tissues and blood of an infected individual.

Peripheral blood has been well characterized as an ample and readily accessible reservoir of latently infected cells that can persist for decades with an extremely long half-life [7–9]. However, latently infected cells are also present in tissues. Ethical and practical considerations limit studying the HIV tissue reservoirs in humans. Ethical considerations also prevent the evaluation of novel approaches to viral eradication that may present unnecessary risks to otherwise healthy individuals [10, 11]. Therefore, investigating the mechanisms of viral persistence in vivo and the in vivo evaluation of novel approaches to HIV eradication require animal models that recapitulate critical aspects of infection in humans.

In this review, we discuss two different but highly complementary models for the investigation of virtually all aspects of HIV persistence and eradication: humanized mice and nonhuman primates (NHPs). As illustrated here, both offer strong benefits that can be used to shed light on the efficacy and safety of novel approaches to HIV eradication. Likewise, both models have challenges that have to be overcome to more efficiently translate observations made into clinical practice.

Animal models that recapitulate key aspects of HIV infection are critical to HIV cure research because most new cure strategies cannot be directly tested in humans without major risks. Animal models allow for in-depth in vivo study of multiple anatomic reservoirs and allow for manipulation of various immune cell populations to understand their role in infection and persistence. The most commonly used animal models for cure research are humanized mice and NHPs. Although neither model perfectly recapitulates HIV infection in humans, each can be tailored to address questions critical to cure research.

OVERVIEW OF HUMANIZED MOUSE MODELS

Most modern humanized mouse models are produced by transplantation of human CD34+ hematopoietic stem-progenitor cells (HSPCs) and/or human tissues into one of several different strains of immunodeficient mice [12]. Depending on the specific model, systemic or local reconstitution with human hematopoietic cells can include human B cells, natural killer cells, T cells, monocytes, macrophages, and dendritic...
cells. Only specific strains of mice support human T-cell development when transplanted with human CD34+ HSPCs. In these strains, human T cells develop in the mouse thymus. It is unclear how or if these human T cells are educated in the mouse thymus [13–15]. When humanized mice are engineered by implanting human thymus and liver tissue, a functional human thymus results, and developing T cells are educated on human thymic epithelial cells, allowing for restriction by human leukocyte antigens (HLAs) I and II [16, 17]. Bone marrow–liver–thymus (BLT) mice are unique in that they receive an autologous bone marrow transplant in addition to human thymic tissue where T-cell progenitors can be educated in the context of HLAs [18, 19].

STRENGTHS AND LIMITATIONS OF CURRENT HUMANIZED MOUSE MODELS

All currently available humanized mouse models are capable of replicating HIV, and replication takes place in human cells present in peripheral blood and tissues. Both innate and adaptive immune responses to HIV have been demonstrated in these models [12, 19–23], and HIV infection responds to the same drugs that are used when treating human patients [24–30]. As with any animal model used for biomedical research, there are limitations to their use in HIV studies. Some limitations are intrinsic to the size and biology of the animal, and these include the relatively small volume of blood plasma that can be obtained for viral load analysis, the limited amount of peripheral blood cells that can be used for in vitro functional analysis, and the relatively short lifespan of the animal. However, humanized mice can be considered a useful accelerated model for the rapid evaluation of interventional therapies. Other limitations include those related to the nature of the xenografts between humans and mice. The structure of the lymph node tissues in humanized mice is not identical to those in humans, and some animals develop a wasting disease [13, 31]. Such concerns have been largely addressed by using new immunodeficient strains of mice [32]. Despite the fact that most humanized mouse models have demonstrated highly effective adaptive T-cell immune responses, their B cell functions are not optimal and are currently being improved [12, 13, 33, 34]. Still, the current models have been used extensively to test the efficacy of multiple immune-based approaches to control viral replication and to eliminate HIV-infected cells in vivo [29, 35–37].

HIV PERSISTENCE IN SEVERE COMBINED IMMUNODEFICIENCY-HUMAN THYMUS/LIVER IMPLANTED MICE

McCune et al first described SCID-hu thy/liv mice as a model for the study of human hematolymphoid differentiation and function [38]. Namikawa et al then showed these mice to be susceptible to HIV-1 infection [38], and Brooks et al followed with the demonstration of HIV latency during thymopoiesis [39]. In the latter study it was shown that CD4/CD8 double-positive thymocytes are targets of HIV-1 infection and that latently-infected, single-positive CD4+ T cells result from transcriptional silencing of the HIV promoter that occurs during T-cell development. Ex vivo induction experiments resulted in virus production, which led the authors to conclude that HIV latency could be established in thymocytes and that this could contribute to systemic viral persistence. Moreover, in this model, latently HIV-infected cells were shown to be reactivated ex vivo with prostratin and interleukin 7 (IL-7) [40, 41] and were used to demonstrate the ability of immunotoxins to eliminate latently infected cells that had been reactivated [42].

HUMAN IMMUNODEFICIENCY VIRUS PERSISTENCE IN T CELL–ONLY MICE

Two limitations of the SCID-hu thy/liv model are that these mice possess very few human T cells outside the human thymic organoid and that infection of the thymic organoid results in very low levels of plasma viremia. The introduction of a new strain of immunodeficient mouse has expanded the utility of the tissue implant model by allowing for increased levels of peripheral and systemic reconstitution with human T cells. Implantation of thy/liv tissue into NOD/SCID Common Gamma Chain Knockout (NSG) mice results in the development of a thymic organoid that is similar to that in SCID-hu thy/live mice [43]. NSG thy/liv mice, however, have significant levels of human T cells in all tissues analyzed, including peripheral blood, spleen, thymus, lymph nodes, bone marrow, liver, and lung. This model is distinguished from NSG mice transplanted with human CD34+ hematopoietic stem/progenitor cells by the complete absence of human antigen-presenting cells. NSG thy/liv mice lack human B cells, monocytes, macrophages, or dendritic cells, thereby receiving the functional designation of T cell–only mice (ToM). T cell–only mice are not only susceptible to HIV infection but also support high levels of viral replication and lifelong viremia if untreated. Consistent with the systemic distribution of human CD4+ T cells, HIV-infected cells can be found in all tissues examined. As in patients, ART suppresses viremia in these mice, and treatment interruption results in viral rebound [43]. In an experiment to determine if HIV latency is established in this model, cells obtained from infected and suppressed mice were harvested from multiple tissues, and resting CD4+ T cells were isolated for ex vivo induction assays. Indeed, it was shown that ToM establish HIV latency with a similar frequency to that seen in patients [43]. ToM represent a significant advance over the original thy/liv implant model that permits studies of T cells in the complete absence of human antigen-presenting cells.

HUMAN IMMUNODEFICIENCY VIRUS PERSISTENCE IN RAγ2/IL2RY DOUBLE KNOCKOUT MICE

Double knockout (DKO) mice are humanized by a transplant of human CD34+ HSPCs, and they efficiently replicate HIV following intravenous or vaginal exposure [28, 44–47]. It has been
demonstrated that ART regimens can suppress viral replication in these mice, and that when there is breakthrough, drug-resistant viruses can be identified [48]. Furthermore, when ART is interrupted, rapid viral rebound and loss of peripheral CD4+ T cells result. Together, these data demonstrate that DKO mice recapitulate critical aspects of HIV infection.

Chaudhary et al have also investigated the establishment of HIV latency in these mice [49]. By isolating resting CD4+ T cells from tissues of both untreated and ART-suppressed infected mice and stimulating them in the culture, HIV production was consistently detected. Furthermore, their analysis showed that latency was established at a frequency between 2 and 12 infectious units per million (IUPM) resting CD4+ T cells.

**HUMAN IMMUNODEFICIENCY VIRUS PERSISTENCE IN BONE MARROW–LIVER–THYMUS MICE**

In addition to implantation of human thymus and liver tissue, BLT mice receive a human bone marrow transplant of autologous hematopoietic stem cells [19]. Along with T-cell development described in the thy/liv implant models previously, due to bone marrow engraftment, BLT mice develop virtually all other human hematopoietic cell types, including lymphocytes, NK cells, monocytes, macrophages, and dendritic cells. Moreover, human cells in BLT mice are distributed throughout all organs, including bone marrow, lymph nodes, thymus, liver, lung, digestive tract, and male and female reproductive tracts [19, 23, 26, 50, 51]. The distribution of human HIV target cells in mucosal sites renders BLT mice susceptible to rectal, vaginal, and oral HIV infection. Mucosal or parenteral exposure to HIV results in systemic infection [23–25, 27, 32, 51, 52]. Human immunodeficiency virus infection is readily detected in plasma using standard viral load assays. Systemic viremia is followed by T-cell activation and CD4+ T cell depletion, hallmarks of HIV infection in humans.

Pharmacokinetic studies of plasma drug levels in BLT mice [26, 37] have established ART drug combinations consisting of tenofovir, emtricitabine, and raltegravir that suppress virus in plasma to levels that are below detectable limits. As in humans, therapy interruption results in rebound viremia to levels that match those before treatment. Furthermore, the study of suppressed BLT mice demonstrates the presence of latently infected cells at a frequency of approximately 8 infectious units per million resting CD4+ T cells [26]. When cell-associated viral RNA levels in the tissues of BLT mice are analyzed during ART treatment, there is a rapid initial decrease that plateaus 28 days after the initiation of ART. Moreover, administration of immunotoxin-conjugated antibodies targeting HIV env results in an additional decrease in tissue viral RNA levels and infected cells [37]. BLT humanized mice serve as an excellent platform to investigate HIV persistence in vivo with well-established and fully validated methodology for the accurate measurement of RNA and DNA reservoirs, as well as the quantitative analysis of latently infected resting CD4+ T cells. BLT mice are therefore poised to serve as a valuable model to evaluate novel approaches to eliminate HIV-infected cells in tissues and to test new eradication approaches.

**IN VIVO EVALUATION OF HUMAN IMMUNODEFICIENCY VIRUS CURE STRATEGIES IN HUMANIZED MICE**

A tremendous amount of work has been performed in humanized mice evaluating the utility of antibodies to combat HIV infection. Recently discovered antibodies have broad in vitro neutralization activity, but when evaluated in vivo in humanized mice for their ability to control infection, they were shown to provide only short and variable reduction in peripheral blood plasma viral loads [36]. It was shown that sustained viral replication in the face of anti-HIV antibodies was associated with mutations mapped to the respective antibody specificity [36]. When combinations of three different antibodies were used to treat infected humanized mice similar results followed, but it was subsequently found that a combination of 5 antibodies resulted in a dramatic drop in plasma viral load to undetectable limits. These results were obtained in all animals treated, and the suppression was maintained for up to 60 days [29]. Alternative approaches to deliver antibodies have also been evaluated in humanized mice. Using adeno-associated virus (AAV) vectors, sustained levels of antibody delivery have been demonstrated in vivo in mice [53–55]. It is of note that administration of single antibodies by AAV delivery in vivo results in more robust suppression in humanized mice than does intermittent dosing [29].

Because latently infected cells do not express viral antigens, they are not expected to be recognized by the immune system or any engineered immunotherapy [56–58]. Engagement of the immune system or immune-based approaches to eliminate infected cells requires the induction of expression of HIV

<table>
<thead>
<tr>
<th>Parameter</th>
<th>huMice</th>
<th>NHPs</th>
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<tbody>
<tr>
<td>Demonstrated HIV latency in T cells during ART</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>Tissue reservoirs similar to humans</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>Platform for testing treatment interruption and viral rebound</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>Platform for testing novel HIV induction (kick) strategies</td>
<td>Yes</td>
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<tr>
<td>Platform for testing novel HIV kill strategies</td>
<td>Yes</td>
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<tr>
<td>Anatomy similar to humans</td>
<td>No</td>
<td>Yes</td>
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<tr>
<td>Susceptibility to HIV infection</td>
<td>Yes</td>
<td>No</td>
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<td>Susceptibility to SIV infection</td>
<td>No</td>
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<tr>
<td>T-cell responses to HIV</td>
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<td>Modes of transmission routes similar to humans</td>
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<td>Responsive to anti-HIV drugs used in humans</td>
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<td>Responsive to anti-HIV antibodies</td>
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Abbreviations: ART, antiretroviral therapy; HIV, human immunodeficiency virus; huMice, humanized mice; NHP, nonhuman primates; SIV, simian immunodeficiency virus.
antigens, a process known as latency reversal. Three latency reversing agents (LRAs) were evaluated in humanized mice for their ability to prevent or delay viral rebound: vorinostat, I-BET151, and CTLA-4 [35]. The LRAs were administered individually or in combination to infected humanized mice that had been suppressed with a combination of antibodies (3BNC117, 10–1074, and PG16). Individual inducers had no effect on viral rebound as 31 of 33 mice rebounded. On the other hand, 57% of animals that received a combination of all 3 inducers failed to rebound, suggesting a possible reduction in the HIV reservoir [35].

HUMAN IMMUNODEFICIENCY VIRUS REPLICATION IN MYELOID-ONLY MICE

Most latency and cure studies have focused on T cells as the major HIV reservoir in vivo. Macrophages have long been considered to be significant targets of HIV infection, but their contribution to persistence has not been fully established [59]. Studies on the role of macrophages during the course of HIV infection are confounded by the abundance of human T cells that can be engulfed by macrophages. Honeycutt et al developed a novel humanized mouse model designated myeloid-only mice (MoM) in which the only targets of HIV infection are human myeloid cells [60]. With this model, it was demonstrated that, in the absence of human T cells, macrophages can sustain robust HIV infection. Human immunodeficiency virus replication in vivo in macrophages was confirmed by detection of viral RNA in the plasma and in all tissues analyzed, and HIV antigen was detected in tissue macrophages. In addition, electron microscopy showed HIV virion budding from bone marrow macrophages. Notably, HIV DNA and RNA were readily detected in the brains of infected MoM, and p24-expressing macrophages were detected throughout the brain. Moreover, it was demonstrated that infected macrophages isolated from MoM can establish de novo infection in uninfected animals [60].

NONHUMAN PRIMATE MODELS OF SIMIAN IMMUNODEFICIENCY VIRUS PERSISTENCE

Nonhuman primate models have been used extensively for studies of HIV/AIDS pathogenesis and vaccines, but have to date been relatively underutilized in the area of HIV cure. The model of simian immunodeficiency virus (SIV) infection of macaques is well-established, robust, and possesses many similarities with HIV infection in terms of transmission, acute/early infection events, viral and CD4+ T-cell dynamics, establishment of reservoirs, and disease progression and is widely used as an excellent animal model for HIV infection [61]. Historically, a major limitation of the SIV/macaque model to study virus reservoirs has been the lack of optimized ART regimens consistently suppressing virus replication below detectable limits. However, new ART combinations and formulations can now be used in SIV-infected rhesus macaques (RMs, Macaca mulatta), cynomolgus macaques (CMs, Macaca fascicularis), and pigtail macaques (PTMs, Macaca nemestrina). Several groups have demonstrated consistent suppression of plasma viremia with viral dynamics that replicate those of HIV-infected patients started on ART [62–65], thus allowing the NHP to be used as a translational animal model for HIV cure studies. In addition, our labs and others have worked at developing and adapting assays measuring the SIV reservoir, including polymerase chain reaction–based assays measuring total, or integrated, SIV DNA [66] and cell culture assays estimating the frequency of latent SIV DNA were found in the lymph nodes (LN)s and colorectal mucosa of these monkeys. Moreover, following ART cessation after 24 weeks of fully suppressive therapy, virus rebounded in all animals, although animals treated on day 3 exhibited a delayed viral rebound compared with the RMs treated at later time points. Consistent with human studies, this experiment suggests that, although early ART initiation can reduce the reservoir size, delay/reduce the viral rebound following ART interruption, or slow disease progression [69–73], ART alone, even initiated early after infection, is not sufficient to prevent the establishment of HIV latency or eliminate latently infected cells [74].

Role of CD8+ T Cells in Maintaining Virus Suppression on Antiretroviral Therapy

Several lines of evidence indicate that CD8+ T cells control virus replication during untreated HIV/SIV infection, including the postpeak decline of viremia when the antigen-specific CD8+ T cells expand [75, 76], the association between certain major histocompatibility complex class I alleles and disease progression [77–83], or the increased viremia following experimentally induced CD8+ T-cell depletion in SIV-infected RMs [84, 85]. However, little is known on the role of CD8+ T cells during ART. We recently showed in 13 ART-treated, SIV-infected RMs that experimental depletion of CD8+ T cells resulted in increased virus levels in both plasma and lymphoid tissues in all animal studied and that repopulation of CD8+ T cells (but not CD8+ NK...
cells) was associated with reestablishment of virus control [86]. These results demonstrate a role for CD8+ T cells in controlling virus production during ART and confirm the importance of exploring immunotherapeutic approaches in ART-treated, HIV-infected individuals.

**NONHUMAN PRIMATE MODELS TO CHARACTERIZE THE CELLULAR AND ANATOMICAL DISTRIBUTION OF HUMAN IMMUNODEFICIENCY VIRUS RESERVOIR**

**Simian Immunodeficiency Virus Persistence in Long-lived CD4+ T Memory Stem Cells**
The best-characterized HIV-1 reservoir and main barrier to a cure consists of a small population of latently infected resting memory CD4+ T cells carrying integrated, transcriptionally silent, but replication-competent HIV. This latent reservoir involves several subsets of memory CD4+ T cells at distinct differentiation stages with different half-lives and phenotypic and functional properties that contribute differentially to the HIV reservoir. On long-term ART, central memory (T<sub>CM</sub>) and transitional memory (T<sub>TM</sub>) CD4+ T cells have been shown to be the main contributors to the HIV reservoir. More recently, it has been shown that the CD4+ T memory stem cells (T<sub>SCM</sub>), T cells that are uniquely able to both self-renew and differentiate into all other memory T cell subsets [87–90], disproportionately contribute to the total HIV reservoir in patients on long-term ART despite their small contribution to the overall CD4+ T-cell pool [91]. In the RM model of SIV infection, our group found high levels of SIV DNA in the CD4+ T<sub>SCM</sub> of untreated monkeys [92]. Moreover, we showed that, although the frequency of infection of T<sub>TM</sub> and T<sub>EM</sub> declined approximately 100-fold following ART initiation in both blood and lymph nodes, it remained stable in the T<sub>SCM</sub> and T<sub>CM</sub> compartments [93]. The observed stable level of virus in CD4+ T<sub>SCM</sub> following ART initiation supports the hypothesis that these cells are a critical contributor to SIV persistence.

**Critical Role of Germinal Center CD4+ T Follicular Helper Cells in Simian Immunodeficiency Virus Persistence**
In untreated HIV and SIV pathogenic infections, CD4+ T follicular helper cells (T<sub>FH</sub>) are the major site of viral infection and replication [94–96]. Several factors might contribute to this high frequency of infection of the T<sub>FH</sub> including (1) a high permissiveness to infection [97, 98], (2) the presence of virions bound to the surface of the adjacent follicular dendritic cells [99–102], (3) low frequencies of virus-specific cytotoxic T lymphocytes in the B follicles [103–105], and (4) expansion/accumulation of the T<sub>FH</sub> in the germinal centers during infection [96, 106]. In the RM model, the first description of T<sub>FH</sub> showed an accumulation of these cells in the LNs during chronic SIV infection, sustained by a constant flow of activated CD4+ T cells entering the B-cell follicles [107]. Consistent with other studies, our work on SIV-infected RMs has suggested that a decrease in follicular regulatory T cells (T<sub>FR</sub>) in chronic SIV infection might contribute to this accumulation of T<sub>FH</sub> in the LNs [108]. Interestingly, productive SIV infection of elite controller (EC) RMs have been shown to be restricted to CD4+ T<sub>FH</sub> [109]. On ART, normalization of T<sub>FH</sub> functions and numbers is incomplete, and high levels of SIV RNA have been shown to persist in the LNs of ART-suppressed, SIV-infected RMs [110]. Moreover, a recent study showed that PD-1+ T<sub>FH</sub> are the major source of replication-competent and infectious HIV-1 in treated aviremic individuals [111]. Additional to the factors listed herein, reduced penetration and suboptimal concentration of ART in the LNs have also been suggested as potential causes of HIV/SIV persistence in the LNs [112].

**Simian Immunodeficiency Virus Persistence in the Gastrointestinal Tract**
The NHP models have played a critical role in the description of HIV pathogenesis in the gastrointestinal tract by demonstrating a massive loss of mucosal CD4+ T cells early in SIV infection, with specific depletion of intestinal Th17 and Th22 CD4+ T cells, damages to the gut barrier integrity, and development of microbial translocation [113–117]. Antiretroviral therapy fails to fully restore intestinal immunity and integrity, and the gut mucosa represents a major site of HIV/SIV persistence on ART and a source of persistent chronic activation [118–121]. A recent study in RMs showed that SIV infection induced profound frequency changes and functional impairments of the colorectal Th17 and Th22 cells that ART failed to restore [122]. Additionally, the study showed that interleukin 17 (IL-17)– and interleukin 22 (IL-22)–producing T cell numbers and function were predictive of residual immune activation and SIV persistence.

**Simian Immunodeficiency Virus Persistence in the Central Nervous System**
Significant rates of HIV-associated neurocognitive disorders persist in ART-suppressed patients [123–125]. Given the difficulty in obtaining tissues from the human central nervous system (CNS), SIV infection of macaques provides a good model to study neurological HIV infection and has demonstrated the persistence of SIV DNA in brain tissues and cerebrospinal fluid [126–130]. Additionally, data obtained in PTMs suggest that immune escape variants might be archived in the brain and reemerge after ART interruption [131]. The NHP models afford the best opportunity to further characterize SIV persistence in the CNS to design therapeutic approaches specifically targeting the CNS reservoir.

**IN VIVO EVALUATION OF HUMAN IMMUNODEFICIENCY VIRUS CURE STRATEGY IN NONHUMAN PRIMATES**

**Hematopoietic Stem Cell Transplant in Antiretroviral Therapy–Treated Simian Immunodeficiency Virus–Infected Rhesus Macaques**
The single case of functional HIV cure reported to date is an HIV-infected individual (the “Berlin patient”) who developed leukemia and received myeloablative chemotherapy and an allogeneic
hematopoietic stem cell transplant (HSCT) from a Δ32ccr5 homozygous donor [132, 133]. To better understand the factors that have contributed to this apparent cure, we conducted, in ART-suppressed, SIV-infected RMs, a controlled test of the contribution of myeloablative total body irradiation and autologous HSCT to the viral reservoir clearance [134]. Myeloablative total body irradiation followed by infusion of autologous hematopoietic stem cells collected before infection was performed in 3 RMs infected with a chimeric simian-human immunodeficiency virus (SHIV) and treated with suppressive ART for 5–8 weeks. The irradiation eliminated 94%–99% of the circulating CD4+ T cells, and a successful engraftment of the HSCT was observed in all animals. However, a rapid rebound of plasma viremia was observed in 2 of the 3 transplanted RMs following ART interruption, and the third animal was sacrificed at 2 weeks after ART interruption with undetectable viremia but detectable virus in lymphoid tissues. This study indicates that the massive reset of the hematopoietic compartment is not sufficient to eliminate the virus reservoir in the setting of short-term ART but provides a new platform to investigate HIV eradication strategies in RMs.

Reversing Simian Immunodeficiency Virus Latency
A key approach to cure HIV infection is to purge the reservoir through a “shock and kill” strategy, where HIV transcription is reactivated, allowing the immune system to clear the cells expressing reactivated virus. Recent clinical trials that focused on reactivating latent gene expression with histone deacetylase inhibitors (HDACi) such as Vorinostat, Panobinostat, and Romidepsin [135–139] or with disulfiram [140] demonstrated increased levels of plasma and/or cell-associated HIV RNA but failed to reduce the latent reservoir. The possibility to achieve sustained suppression of plasma viremia in ART-treated, SIV-infected macaques allows the testing of different LRA in this model. Results similar to those obtained in clinical trials were seen using Vorinostat or Romidepsin in ART-suppressed, SIV-infected RMs, with increase in viral production but detectable levels of SIV RNA and DNA in blood and tissues on ART following Vorinostat treatment or absence of difference in plasma viral rebound following ART cessation in the Romidepsin-treated animals compared with untreated controls [141, 142]. Combinatorial strategies will likely be required to effectively and comprehensively purge the HIV reservoir. These approaches could include HDACis, histone methylation inhibitors, protein kinase C agonists, bromodomain inhibitors, or toll-like receptor agonists. Given the incomplete knowledge about cellular and anatomical distribution of the reservoir and toxicity of new LRAs, the use of NHP models will be critical to screen compounds aimed at reactivating the latent reservoir.

Targeting Intestinal Mucosa Immunity
Due to the persistence of HIV/SIV infection and immune dysfunction in the gut mucosa under ART, therapeutic interventions aimed at restoring intestinal integrity and immunity have been proposed as part of HIV cure research. To that extent, a combination of probiotics and prebiotics were administered to ART-treated, SIV-infected PTMs, which resulted in an improved reconstitution and functionality of CD4+ T cells in the colon and a reduced fibrosis [143]. Several immune-based interventions have also been evaluated. Administration of IL-7 to ART-treated, SIV-infected RM has been shown to improve peripheral CD4+ T-cell restoration [144]. In a recent study in ART-treated, SIV-infected RMs, administration of IL-21 led to a better restoration of intestinal Th17 and Th22 cells and to a reduction of intestinal and systemic immune activation [63]. Interestingly, a sustained reduction in plasma viremia and in the frequency of CD4+ T cells harboring replication-competent SIV was observed in IL-21–treated animals. These results suggest that IL-21 treatment may represent a promising immune-based intervention in the HIV cure armamentarium.

Blocking Immune Inhibitory Pathway
Ccoinhibitory receptors, also called immune checkpoint molecules, are overexpressed by T cells during HIV/SIV infection and are associated with immune exhaustion. Among these molecules, PD-1 has been of central interest, and its blockade has been shown to enhance cellular and humoral immune responses and reduce SIV production in RMs [145]. A study in ART-treated, SIV-infected RMs suggests that blockade of PD-1 can enhance T-cell responses and slow plasma viremia rebound following ART interruption [146]. The model of ART-suppressed NHPs will permit the exploration of other inhibitory pathway blockades targeting notably CTLA-4, LAG-3, TIM-3, or TIGIT.

SUMMARY
Although neither humanized mouse nor primate models perfectly mirror HIV disease in humans, there are a multitude of ways in which these 2 models complement each other and synergize to generate a robust and multifaceted research platform for in vivo research in HIV cure strategies. Currently, neither model allows for the in-depth type of analysis that is performed on human leukopheresis products due to their sheer volume. It is important to note the differences in sample size availability between the 2 models. The NHP model certainly allows for larger samples to be obtained (ie, blood, plasma, and tissue biopsy) over a longer time course than is currently possible with humanized mice. However, humanized mice allow for more frequent sampling of entire animals with a full complement of individual tissues.

Perhaps the most apparent distinction between these models is the presence in humanized mice of the human primary cell targets of HIV infection. Specifically, although humanized mice do not recapitulate the entirety of the human being, they certainly possess human cells that represent the natural targets of HIV infection in vivo. Nonhuman primates do not possess human cells but
are a natural in vivo systemic model of viral immunodeficiency. It follows that all studies in NHPs must be performed with SIV and its derivatives (including HIV/SIV chimeras or SHIVs), whereas studies in humanized mice can be performed with highly relevant HIV and its natural targets. Another significant distinction between humanized mice and NHPs is their different lifespan. The lifespan of humanized mice is shorter than that of NHPs. Although this allows for fast initial modelling in mice, it falls to the NHP models to generate data regarding long time-course experiments. It is also important to be cognizant of the fact that reagents designed for use in humans in most cases can be directly applied to humanized mouse models, whereas they might require modification for use in NHPs, even more so when targeting SIV. These therapeutic reagents include, but are not limited to, monoclonal antibodies, engineered bispecific antibodies, and other immunomodulatory protein constructs. Importantly, there is a significant push to implement cell- and gene therapy–based HIV cure strategies. These 2 models compliment each other and synergize for in vivo testing of such approaches to treat HIV infection.

Finding a cure for HIV/AIDS is a daunting enterprise that will require the use of informative animal models in which novel advances can be evaluated for safety and efficacy. Nonhuman primate and humanized mice represent the 2 best in vivo platforms currently available to carry out this important work. The natural complementarity of both systems will benefit from careful coordination and harmonization of approaches and reagents to obtain maximum progress and fast translation into clinical practice.

Notes

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