SUPPLEMENT ARTICLE

In Vivo Models of Human Immunodeficiency Virus Persistence and Cure Strategies

Christopher C. Nixon,[1] Maud Mavigner,[2] Guido Silvestri,[3] and J. Victor Garcia[1]

[1]Division of Infectious Diseases, Center for AIDS Research, University of North Carolina at Chapel Hill School of Medicine; [2]Department of Pediatrics, Emory University School of Medicine, and [3]Emory Vaccine Center and Yerkes National Primate Research Center, Emory University, Atlanta, Georgia

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 eradiation 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

DOI: 10.1093/infdis/jiw637

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, spleen, 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.

Table 1. Comparison of Humanized Mice and Nonhuman Primates for the In Vivo Evaluation of Cure Strategies

Parameter	huMice	NHPs
Demonstrated HIV latency in T cells during ART	Yes	Yes
Tissue reservoirs similar to humans	Yes	Yes
Platform for testing treatment interruption and viral rebound	Yes	Yes
Platform for testing novel HIV induction (kick) strategies	Yes	Yes
Platform for testing novel HIV kill strategies	Yes	Yes
Anatomy similar to humans	No	Yes
Susceptibility to HIV infection	Yes	No
Susceptibility to SIV infection	No	Yes
T-cell responses to HIV	Yes	Yes
Modes of transmission routes similar to humans	Yes	Yes
Responsive to anti-HIV drugs used in humans	Yes	Yes /No
Responsive to anti-HIV antibodies	Yes	Yes

Abbreviations: ART, antiretroviral therapy; HIV, human immunodeficiency virus; huMice, humanized mice; NHP, nonhuman primates; SIV, simian immunodeficiency virus.

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

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 latently infected cells carrying replication-competent SIV [67, 68].

NONHUMAN PRIMATE MODELS TO CHARACTERIZE THE DYNAMICS OF ESTABLISHMENT AND MAINTENANCE OF HUMAN IMMUNODEFICIENCY VIRUS RESERVOIR

Early Seeding of Simian Immunodeficiency Virus Reservoir

One of the main barriers to HIV eradication is the rapid seeding of the viral reservoir very early following infection. In 2014, a study provided definitive evidence of the early establishment of SIV reservoir using RM initiated on suppressive ART on days 3, 7, 10, or 14 after intrarectal SIVmac251 infection [65]. Although treatment on day 3 abrogated the induction of SIV-specific humoral and cellular immune responses and prevented the emergence of viral RNA in the plasma and proviral DNA in the peripheral blood mononuclear cells, detectable levels of SIV DNA were found in the lymph nodes (LNs) 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 histocompatability 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

Siman 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_{CM}) and transitional memory (T_{TM}) 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_{SCM}), T cells that are uniquely able to both self-renew and differentiate into all other memory T cell subsets [87–90], disproportionally 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 showed high levels of SIV DNA in the CD4+ T_{SCM} of untreated monkeys [92]. Moreover, we showed that, although the frequency of infection of T_{TM} and T_{EM} declined approximately 100-fold following ART initiation in both blood and lymph nodes, it remained stable in the T_{SCM} and T_{CM} compartments [93]. The observed stable level of virus in CD4+ T_{SCM} 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_{FH}) are the major site of viral infection and replication [94–96]. Several factors might contribute to this high frequency of infection of the T_{FH}, 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_{FH} in the germinal centers during infection [96, 106]. In the RM model, the first description of T_{FH} 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_{FR}) in chronic SIV infection might contribute to this accumulation of T_{FH} in the LNs [108]. Interestingly, productive SIV infection of elite controller (EC) RMs have been shown to be restricted to CD4+ T_{FH} [109]. On ART, normalization of T_{FH} 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_{FH} 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 Siman 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 LRAs 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

Coinhibitory 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 leukophoresis 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

References

1. Chun TW, Carruth L, Finzi D, et al. Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. Nature **1997**; 387:183–8.
2. Finzi D, Hermankova M, Pierson T, et al. Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. Science **1997**; 278:1295–300.
3. Chun TW, Finzi D, Margolick J, Chadwick K, Schwartz D, Siliciano RF. In vivo fate of HIV-1-infected T cells: quantitative analysis of the transition to stable latency. Nat Med **1995**; 1:1284–90.
4. Chun TW, Davey RT Jr, Engel D, Lane HC, Fauci AS. Re-emergence of HIV after stopping therapy. Nature **1999**; 401:874–5.
5. Chun TW, Stuyver L, Mizell SB, et al. Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy. Proc Natl Acad Sci U S A **1997**; 94:13193–7.
6. Davey RT Jr, Bhat N, Yoder C, et al. HIV-1 and T cell dynamics after interruption of highly active antiretroviral therapy (HAART) in patients with a history of sustained viral suppression. Proc Natl Acad Sci U S A **1999**; 96:15109–14.
7. Le Douce V, Herbein G, Rohr O, Schwartz C. Molecular mechanisms of HIV-1 persistence in the monocyte-macrophage lineage. Retrovirology **2010**; 7:32.
8. Sonza S, Mutimer HP, Oelrichs R, et al. Monocytes harbour replication-competent, non-latent HIV-1 in patients on highly active antiretroviral therapy. AIDS **2001**; 15:17–22.
9. Eisele E, Siliciano RF. Redefining the viral reservoirs that prevent HIV-1 eradication. Immunity **2012**; 37:377–88.
10. Tucker JD, Rennie S; Social and Ethical Working Group on HIV Cure. Social and ethical implications of HIV cure research. AIDS **2014**; 28:1247–50.
11. Eyal N, Kuritzkes DR. Challenges in clinical trial design for HIV-1 cure research. Lancet **2013**; 382:1464–5.
12. Shultz LD, Brehm MA, Garcia-Martinez JV, Greiner DL. Humanized mice for immune system investigation: progress, promise and challenges. Nat Rev Immunol **2012**; 12:786–98.
13. Traggiai E, Chicha L, Mazzucchelli L, et al. Development of a human adaptive immune system in cord blood cell-transplanted mice. Science **2004**; 304:104–7.
14. Ishikawa F, Yasukawa M, Lyons B, et al. Development of functional human blood and immune systems in NOD/SCID/IL2 receptor {gamma} chain(null) mice. Blood **2005**; 106:1565–73.
15. Shultz LD, Lyons BL, Burzenski LM, et al. Human lymphoid and myeloid cell development in NOD/LtSz-scid IL2R gamma null mice engrafted with mobilized human hemopoietic stem cells. J Immunol **2005**; 174:6477–89.
16. Olesen R, Wahl A, Denton PW, Garcia JV. Immune reconstitution of the female reproductive tract of humanized BLT mice and their susceptibility to human immunodeficiency virus infection. J Reprod Immunol **2011**; 88:195–203.
17. Wege AK, Melkus MW, Denton PW, Estes JD, Garcia JV. Functional and phenotypic characterization of the humanized BLT mouse model. Curr Top Microbiol Immunol **2008**; 324:149–65.
18. Lan P, Tonomura N, Shimizu A, Wang S, Yang YG. Reconstitution of a functional human immune system in immunodeficient mice through combined human fetal thymus/liver and CD34+ cell transplantation. Blood **2006**; 108:487–92.
19. Melkus MW, Estes JD, Padgett-Thomas A, et al. Humanized mice mount specific adaptive and innate immune responses to EBV and TSST-1. Nat Med **2006**; 12:1316–22.
20. Rongvaux A, Takizawa H, Strowig T, et al. Human hemato-lymphoid system mice: current use and future potential for medicine. Annu Rev Immunol **2013**; 31:635–74.
21. Brainard DM, Seung E, Frahm N, et al. Induction of robust cellular and humoral virus-specific adaptive immune responses in human immunodeficiency virus-infected humanized BLT mice. J Virol **2009**; 83:7305–21.
22. Dudek TE, No DC, Seung E, et al. Rapid evolution of HIV-1 to functional CD8+ T cell responses in humanized BLT mice. Sci Transl Med **2012**; 4:143ra98.
23. Sun Z, Denton PW, Estes JD, et al. Intrarectal transmission, systemic infection, and CD4+ T cell depletion in humanized mice infected with HIV-1. J Exp Med **2007**; 204:705–14.
24. Denton PW, Estes JD, Sun Z, et al. Antiretroviral pre-exposure prophylaxis prevents vaginal transmission of HIV-1 in humanized BLT mice. PLoS Med **2008**; 5:e16.
25. Denton PW, Krisko JF, Powell DA, et al. Systemic administration of antiretrovirals prior to exposure prevents rectal and intravenous HIV-1 transmission in humanized BLT mice. PLoS One **2010**; 5:e8829.
26. Denton PW, Olesen R, Choudhary SK, et al. Generation of HIV latency in humanized BLT mice. J Virol **2012**; 86:630–4.
27. Denton PW, Othieno F, Martinez-Torres F, et al. One percent tenofovir applied topically to humanized BLT mice and used according to the CAPRISA 004 experimental design demonstrates partial protection from vaginal HIV infection, validating the BLT model for evaluation of new microbicide candidates. J Virol **2011**; 85:7582–93.
28. Nischang M, Sutmuller R, Gers-Huber G, et al. Humanized mice recapitulate key features of HIV-1 infection: a novel concept using long-acting anti-retroviral drugs for treating HIV-1. PLoS One **2012**; 7:e38853.
29. Horwitz JA, Halper-Stromberg A, Mouquet H, et al. HIV-1 suppression and durable control by combining single broadly neutralizing antibodies and antiretroviral drugs in humanized mice. Proc Natl Acad Sci U S A **2013**; 110:16538–43.
30. Kovarova M, Council OD, Date AA, et al. Nanoformulations of rilpivirine for topical pericoital and systemic coitus-independent administration efficiently prevent HIV transmission. PLoS Pathog **2015**; 11:e1005075.
31. Greenblatt MB, Vrbanac V, Vbranac V, et al. Graft versus host disease in the bone marrow, liver and thymus humanized mouse model. PLoS One **2012**; 7:e44664.
32. Lavender KJ, Pang WW, Messer RJ, et al. BLT-humanized C57BL/6 Rag2-/-γc-/-CD47-/- mice are resistant to GVHD and develop B- and T-cell immunity to HIV infection. Blood **2013**; 122:4013–20.
33. Martinez-Torres F, Nochi T, Wahl A, Garcia JV, Denton PW. Hypogammaglobulinemia in BLT humanized mice–an animal model of primary antibody deficiency. PLoS One **2014**; 9:e108663.

34. Karpel ME, Boutwell CL, Allen TM. BLT humanized mice as a small animal model of HIV infection. Curr Opin Virol **2015**; 13:75–80.

35. Halper-Stromberg A, Lu CL, Klein F, et al. Broadly neutralizing antibodies and viral inducers decrease rebound from HIV-1 latent reservoirs in humanized mice. Cell **2014**; 158:989–99.

36. Klein F, Halper-Stromberg A, Horwitz JA, et al. HIV therapy by a combination of broadly neutralizing antibodies in humanized mice. Nature **2012**; 492:118–22.

37. Denton PW, Long JM, Wietgrefe SW, et al. Targeted cytotoxic therapy kills persisting HIV infected cells during ART. PLoS Pathog **2014**; 10:e1003872.

38. McCune JM, Namikawa R, Kaneshima H, Shultz LD, Lieberman M, Weissman IL. The SCID-hu mouse: murine model for the analysis of human hematolymphoid differentiation and function. Science **1988**; 241:1632–9.

39. Brooks DG, Kitchen SG, Kitchen CM, Scripture-Adams DD, Zack JA. Generation of HIV latency during thymopoiesis. Nat Med **2001**; 7:459–64.

40. Scripture-Adams DD, Brooks DG, Korin YD, Zack JA. Interleukin-7 induces expression of latent human immunodeficiency virus type 1 with minimal effects on T-cell phenotype. J Virol **2002**; 76:13077–82.

41. Korin YD, Brooks DG, Brown S, Korotzer A, Zack JA. Effects of prostratin on T-cell activation and human immunodeficiency virus latency. J Virol **2002**; 76:8118–23.

42. Brooks DG, Hamer DH, Arlen PA, et al. Molecular characterization, reactivation, and depletion of latent HIV. Immunity **2003**; 19:413–23.

43. Honeycutt JB, Wahl A, Archin N, Choudhary S, Margolis D, Garcia JV. HIV-1 infection, response to treatment and establishment of viral latency in a novel humanized T cell-only mouse (TOM) model. Retrovirology **2013**; 10:121.

44. Baenziger S, Tussiwand R, Schlaepfer E, et al. Disseminated and sustained HIV infection in CD34+ cord blood cell-transplanted Rag2-/-gamma c-/- mice. Proc Natl Acad Sci U S A **2006**; 103:15951–6.

45. Akkina R, Berges BK, Palmer BE, et al. Humanized Rag1-/- γc-/- mice support multilineage hematopoiesis and are susceptible to HIV-1 infection via systemic and vaginal routes. PLoS One **2011**; 6:e20169.

46. Berges BK, Akkina SR, Folkvord JM, Connick E, Akkina R. Mucosal transmission of R5 and X4 tropic HIV-1 via vaginal and rectal routes in humanized Rag2-/-gammac -/- (RAG-hu) mice. Virology **2008**; 373:342–51.

47. Berges BK, Akkina SR, Remling L, Akkina R. Humanized Rag2(-/-)gammac(-/-) (RAG-hu) mice can sustain long-term chronic HIV-1 infection lasting more than a year. Virology **2010**; 397:100–3.

48. Choudhary SK, Rezk NL, Ince WL, et al. Suppression of human immunodeficiency virus type 1 (HIV-1) viremia with reverse transcriptase and integrase inhibitors, CD4+ T-cell recovery, and viral rebound upon interruption of therapy in a new model for HIV treatment in the humanized Rag2-/-{gamma}c-/- mouse. J Virol **2009**; 83:8254–8.

49. Choudhary SK, Archin NM, Cheema M, Dahl NP, Garcia JV, Margolis DM. Latent HIV-1 infection of resting CD4+ T cells in the humanized Rag2−/− γc−/− mouse. J Virol **2012**; 86:114–20.

50. Nochi T, Denton PW, Wahl A, Garcia JV. Cryptopatches are essential for the development of human GALT. Cell Rep **2013**; 3:1874–84.

51. Wahl A, Swanson MD, Nochi T, et al. Human breast milk and antiretrovirals dramatically reduce oral HIV-1 transmission in BLT humanized mice. PLoS Pathog **2012**; 8:e1002732.

52. Chateau ML, Denton PW, Swanson MD, McGowan I, Garcia JV. Rectal transmission of transmitted/founder HIV-1 is efficiently prevented by topical 1% tenofovir in BLT humanized mice. PLoS One **2013**; 8:e60024.

53. Balazs AB, Chen J, Hong CM, Rao DS, Yang L, Baltimore D. Antibody-based protection against HIV infection by vectored immunoprophylaxis. Nature **2011**; 481:81–4.

54. Balazs AB, Ouyang Y, Hong CM, et al. Vectored immunoprophylaxis protects humanized mice from mucosal HIV transmission. Nat Med **2014**; 20:296–300.

55. Balazs AB, West AP Jr. Antibody gene transfer for HIV immunoprophylaxis. Nat Immunol **2013**; 14:1–5.

56. Blankson JN, Finzi D, Pierson TC, et al. Biphasic decay of latently infected CD4+ T cells in acute human immunodeficiency virus type 1 infection. J Infect Dis **2000**; 182:1636–42.

57. Archin NM, Vaidya NK, Kuruc JD, et al. Immediate antiviral therapy appears to restrict resting CD4+ cell HIV-1 infection without accelerating the decay of latent infection. Proc Natl Acad Sci U S A **2012**; 109:9523–8.

58. Chun TW, Nickle DC, Justement JS, et al. HIV-infected individuals receiving effective antiviral therapy for extended periods of time continually replenish their viral reservoir. J Clin Invest **2005**; 115:3250–5.

59. Sattentau QJ, Stevenson M. Macrophages and HIV-1: an unhealthy constellation. Cell Host Microbe **2016**; 19:304–10.

60. Honeycutt JB, Wahl A, Baker C, et al. Macrophages sustain HIV replication in vivo independently of T cells. J Clin Invest **2016**; 126:1353–66.

61. Evans DT, Silvestri G. Nonhuman primate models in AIDS research. Curr Opin HIV AIDS **2013**; 8:255–61.

62. Del Prete GQ, Smedley J, Macallister R, et al. Short communication: comparative evaluation of coformulated injectable combination antiretroviral therapy regimens in simian immunodeficiency virus-infected rhesus macaques. AIDS Res Hum Retroviruses **2016**; 32:163–8.

63. Micci L, Ryan ES, Fromentin R, et al. Interleukin-21 combined with ART reduces inflammation and viral reservoir in SIV-infected macaques. J Clin Invest **2015**; 125:4497–513.

64. Shytaj IL, Norelli S, Chirullo B, et al. A highly intensified ART regimen induces long-term viral suppression and restriction of the viral reservoir in a simian AIDS model. PLoS Pathog **2012**; 8:e1002774.

65. Whitney JB, Hill AL, Sanisetty S, et al. Rapid seeding of the viral reservoir prior to SIV viraemia in rhesus monkeys. Nature **2014**; 512:74–7.

66. Mavigner M, Lee ST, Habib J. Quantifying integrated SIV-DNA by repetitive-sampling Alu-gag PCR. J Virus Erad **2016**; 2:219–26.

67. Avalos CR, Price SL, Forsyth ER, et al. Quantitation of productively infected monocytes and macrophages of simian immunodeficiency virus-infected macaques. J Virol **2016**; 90:5643–56.

68. Shen A, Yang HC, Zhou Y, et al. Novel pathway for induction of latent virus from resting CD4(+) T cells in the simian immunodeficiency virus/macaque model of human immunodeficiency virus type 1 latency. J Virol **2007**; 81:1660–70.

69. Ananworanich J, Chomont N, Eller LA, et al. HIV DNA set point is rapidly established in acute HIV infection and dramatically reduced by early ART. EBioMedicine **2016**; 11:68–72.

70. Ananworanich J, Schuetz A, Vandergeeten C, et al. Impact of multi-targeted antiretroviral treatment on gut T cell depletion and HIV reservoir seeding during acute HIV infection. PLoS One **2012**; 7:e33948.

71. Hocqueloux L, Avettand-Fènoël V, Jacquot S, et al. Long-term antiretroviral therapy initiated during primary HIV-1 infection is key to achieving both low HIV reservoirs and normal T cell counts. J Antimicrob Chemother **2013**; 68:1169–78.

72. Sáez-Cirión A, Bacchus C, Hocqueloux L, et al. Post-treatment HIV-1 controllers with a long-term virological remission after the interruption of early initiated antiretroviral therapy ANRS VISCONTI Study. PLoS Pathog **2013**; 9:e1003211.

73. Steingrover R, Pogány K, Fernandez Garcia E, et al. HIV-1 viral rebound dynamics after a single treatment interruption depends on time of initiation of highly active antiretroviral therapy. AIDS **2008**; 22:1583–8.

74. Luzuriaga K, Gay H, Ziemniak C, et al. Viremic relapse after HIV-1 remission in a perinatally infected child. N Engl J Med **2015**; 372:786–8.

75. Borrow P, Lewicki H, Hahn BH, Shaw GM, Oldstone MB. Virus-specific CD8+ cytotoxic T-lymphocyte activity associated with control of viremia in primary human immunodeficiency virus type 1 infection. J Virol **1994**; 68:6103–10.

76. Koup RA, Safrit JT, Cao Y, et al. Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome. J Virol **1994**; 68:4650–5.

77. Altfeld M, Addo MM, Rosenberg ES, et al. Influence of HLA-B57 on clinical presentation and viral control during acute HIV-1 infection. AIDS **2003**; 17:2581–91.

78. Costello C, Tang J, Rivers C, et al. HLA-B*5703 independently associated with slower HIV-1 disease progression in Rwandan women. AIDS **1999**; 13:1990–1.

79. Evans DT, Jing P, Allen TM, et al. Definition of five new simian immunodeficiency virus cytotoxic T-lymphocyte epitopes and their restricting major histocompatibility complex class I molecules: evidence for an influence on disease progression. J Virol **2000**; 74:7400–10.

80. Evans DT, Knapp LA, Jing P, et al. Rapid and slow progressors differ by a single MHC class I haplotype in a family of MHC-defined rhesus macaques infected with SIV. Immunol Lett **1999**; 66:53–9.

81. Haynes BF, Pantaleo G, Fauci AS. Toward an understanding of the correlates of protective immunity to HIV infection. Science **1996**; 271:324–8.

82. Kaslow RA, Carrington M, Apple R, et al. Influence of combinations of human major histocompatibility complex genes on the course of HIV-1 infection. Nat Med **1996**; 2:405–11.

83. Klein MR, van der Burg SH, Hovenkamp E, et al. Characterization of HLA-B57-restricted human immunodeficiency virus type 1 Gag- and RT-specific cytotoxic T lymphocyte responses. J Gen Virol **1998**; 79 (Pt 9):2191–201.

84. Chowdhury A, Hayes TL, Bosinger SE, et al. Differential impact of in vivo CD8+ T lymphocyte depletion in controller versus progressor simian immunodeficiency virus-infected macaques. J Virol **2015**; 89:8677–86.

85. Metzner KJ, Jin X, Lee FV, et al. Effects of in vivo CD8(+) T cell depletion on virus replication in rhesus macaques immunized with a live, attenuated simian immunodeficiency virus vaccine. J Exp Med **2000**; 191:1921–31.

86. Cartwright EK, Spicer L, Smith SA, et al. CD8(+) lymphocytes are required for maintaining viral suppression in SIV-infected macaques treated with short-term antiretroviral therapy. Immunity **2016**; 45:656–68.

87. Gattinoni L, Lugli E, Ji Y, et al. A human memory T cell subset with stem cell-like properties. Nat Med **2011**; 17:1290–7.

88. Gattinoni L, Zhong XS, Palmer DC, et al. Wnt signaling arrests effector T cell differentiation and generates CD8+ memory stem cells. Nat Med **2009**; 15:808–13.

89. Lugli E, Dominguez MH, Gattinoni L, et al. Superior T memory stem cell persistence supports long-lived T cell memory. J Clin Invest **2013**; 123:594–9.

90. Muranski P, Borman ZA, Kerkar SP, et al. Th17 cells are long lived and retain a stem cell-like molecular signature. Immunity **2011**; 35:972–85.

91. Buzon MJ, Martin-Gayo E, Pereyra F, et al. Long-term antiretroviral treatment initiated at primary HIV-1 infection affects the size, composition, and decay kinetics of the reservoir of HIV-1-infected CD4 T cells. J Virol **2014**; 88:10056–65.

92. Cartwright EK, McGary CS, Cervasi B, et al. Divergent CD4+ T memory stem cell dynamics in pathogenic and nonpathogenic simian immunodeficiency virus infections. J Immunol **2014**; 192:4666–73.

93. Cartwright EK, Palesch D, Mavigner M, Paiardini M, Chahroudi A, Silvestri G. Initiation of antiretroviral therapy restores CD4+ T memory stem cell homeostasis in simian immunodeficiency virus-infected macaques. J Virol **2016**; 90:6699–708.

94. Folkvord JM, Armon C, Connick E. Lymphoid follicles are sites of heightened human immunodeficiency virus type 1 (HIV-1) replication and reduced antiretroviral effector mechanisms. AIDS Res Hum Retroviruses **2005**; 21:363–70.

95. Hufert FT, van Lunzen J, Janossy G, et al. Germinal centre CD4+ T cells are an important site of HIV replication in vivo. AIDS **1997**; 11:849–57.

96. Perreau M, Savoye AL, De Crignis E, et al. Follicular helper T cells serve as the major CD4 T cell compartment for HIV-1 infection, replication, and production. J Exp Med **2013**; 210:143–56.

97. Allam A, Majji S, Peachman K, et al. TFH cells accumulate in mucosal tissues of humanized-DRAG mice and are highly permissive to HIV-1. Sci Rep **2015**; 5:10443.

98. Kohler SL, Pham MN, Folkvord JM, et al. Germinal center T follicular helper cells are highly permissive to HIV-1 and alter their phenotype during virus replication. J Immunol **2016**; 196:2711–22.

99. Haase AT, Henry K, Zupancic M, et al. Quantitative image analysis of HIV-1 infection in lymphoid tissue. Science **1996**; 274:985–9.

100. Heath SL, Tew JG, Tew JG, Szakal AK, Burton GF. Follicular dendritic cells and human immunodeficiency virus infectivity. Nature **1995**; 377:740–4.

101. Schacker T, Little S, Connick E, et al. Rapid accumulation of human immunodeficiency virus (HIV) in lymphatic tissue reservoirs during acute and early HIV infection: implications for timing of antiretroviral therapy. J Infect Dis **2000**; 181:354–7.

102. Spiegel H, Herbst H, Niedobitek G, Foss HD, Stein H. Follicular dendritic cells are a major reservoir for human immunodeficiency virus type 1 in lymphoid tissues facilitating infection of CD4+ T-helper cells. Am J Pathol **1992**; 140:15–22.

103. Connick E, Folkvord JM, Lind KT, et al. Compartmentalization of simian immunodeficiency virus replication within secondary lymphoid tissues of rhesus macaques is linked to disease stage and inversely related to localization of virus-specific CTL. J Immunol **2014**; 193:5613–25.

104. Connick E, Mattila T, Folkvord JM, et al. CTL fail to accumulate at sites of HIV-1 replication in lymphoid tissue. J Immunol **2007**; 178:6975–83.

105. Tjernlund A, Zhu J, Laing K, et al. In situ detection of Gag-specific CD8+ cells in the GI tract of SIV infected rhesus macaques. Retrovirology **2010**; 7:12.

106. Lindqvist M, van Lunzen J, Soghoian DZ, et al. Expansion of HIV-specific T follicular helper cells in chronic HIV infection. J Clin Invest **2012**; 122:3271–80.

107. Petrovas C, Yamamoto T, Gerner MY, et al. CD4 T follicular helper cell dynamics during SIV infection. J Clin Invest **2012**; 122:3281–94.

108. Chowdhury A, Del Rio Estrada PM, Del Rio PM, et al. Decreased T follicular regulatory cell/T follicular helper cell (TFH) in simian immunodeficiency virus-infected rhesus macaques may contribute to accumulation of TFH in chronic infection. J Immunol **2015**; 195:3237–47.

109. Fukazawa Y, Lum R, Okoye AA, et al. B cell follicle sanctuary permits persistent productive simian immunodeficiency virus infection in elite controllers. Nat Med **2015**; 21:132–9.

110. Horiike M, Iwami S, Kodama M, et al. Lymph nodes harbor viral reservoirs that cause rebound of plasma viremia in SIV-infected macaques upon cessation of combined antiretroviral therapy. Virology **2012**; 423:107–18.

111. Banga R, Procopio FA, Noto A, et al. PD-1(+) and follicular helper T cells are responsible for persistent HIV-1 transcription in treated aviremic individuals. Nat Med **2016**; 22:754–61.

112. Fletcher CV, Staskus K, Wietgrefe SW, et al. Persistent HIV-1 replication is associated with lower antiretroviral drug concentrations in lymphatic tissues. Proc Natl Acad Sci U S A **2014**; 111:2307–12.

113. Brenchley JM, Price DA, Schacker TW, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nat Med **2006**; 12:1365–71.

114. Cecchinato V, Trindade CJ, Laurence A, et al. Altered balance between Th17 and Th1 cells at mucosal sites predicts AIDS progression in simian immunodeficiency virus-infected macaques. Mucosal Immunol **2008**; 1:279–88.

115. Favre D, Lederer S, Kanwar B, et al. Critical loss of the balance between Th17 and T regulatory cell populations in pathogenic SIV infection. PLoS Pathog **2009**; 5:e1000295.

116. Raffatellu M, Santos RL, Verhoeven DE, et al. Simian immunodeficiency virus-induced mucosal interleukin-17 deficiency promotes Salmonella dissemination from the gut. Nat Med **2008**; 14:421–8.

117. Xu H, Wang X, Veazey RS. Th17 cells coordinate with Th22 cells in maintaining homeostasis of intestinal tissues and both are depleted in SIV-infected macaques. J AIDS Clin Res **2014**; 5:pii: 302.

118. Chun TW, Nickle DC, Justement JS, et al. Persistence of HIV in gut-associated lymphoid tissue despite long-term antiretroviral therapy. J Infect Dis **2008**; 197:714–20.

119. Guadalupe M, Reay E, Sankaran S, et al. Severe CD4+ T-cell depletion in gut lymphoid tissue during primary human immunodeficiency virus type 1 infection and substantial delay in restoration following highly active antiretroviral therapy. J Virol **2003**; 77:11708–17.

120. Mehandru S, Poles MA, Tenner-Racz K, et al. Lack of mucosal immune reconstitution during prolonged treatment of acute and early HIV-1 infection. PLoS Med **2006**; 3:e484.

121. Yukl SA, Gianella S, Sinclair E, et al. Differences in HIV burden and immune activation within the gut of HIV-positive patients receiving suppressive antiretroviral therapy. J Infect Dis **2010**; 202:1553–61.

122. Ryan ES, Micci L, Fromentin R, et al. Loss of function of intestinal IL-17 and IL-22 producing cells contributes to inflammation and viral persistence in SIV-infected rhesus macaques. PLoS Pathog **2016**; 12:e1005412.

123. Cysique LA, Brew BJ. Prevalence of non-confounded HIV-associated neurocognitive impairment in the context of plasma HIV RNA suppression. J Neurovirol **2011**; 17:176–83.

124. Heaton RK, Clifford DB, Franklin DR Jr, et al. HIV-associated neurocognitive disorders persist in the era of potent antiretroviral therapy: CHARTER Study. Neurology **2010**; 75:2087–96.

125. Robertson KR, Smurzynski M, Parsons TD, et al. The prevalence and incidence of neurocognitive impairment in the HAART era. AIDS **2007**; 21:1915–21.

126. Clements JE, Li M, Gama L, et al. The central nervous system is a viral reservoir in simian immunodeficiency virus–infected macaques on combined antiretroviral therapy: a model for human immunodeficiency virus patients on highly active antiretroviral therapy. J Neurovirol **2005**; 11:180–9.

127. Dinoso JB, Rabi SA, Blankson JN, et al. A simian immunodeficiency virus-infected macaque model to study viral reservoirs that persist during highly active antiretroviral therapy. J Virol **2009**; 83:9247–57.

128. Marcondes MC, Morsey B, Emanuel K, Lamberty BG, Flynn CT, Fox HS. CD8+ T cells maintain suppression of simian immunodeficiency virus in the central nervous system. J Infect Dis **2015**; 211:40–4.

129. North TW, Higgins J, Deere JD, et al. Viral sanctuaries during highly active antiretroviral therapy in a nonhuman primate model for AIDS. J Virol **2010**; 84:2913–22.

130. Zink MC, Brice AK, Kelly KM, et al. Simian immunodeficiency virus-infected macaques treated with highly active antiretroviral therapy have reduced central nervous system viral replication and inflammation but persistence of viral DNA. J Infect Dis **2010**; 202:161–70.

131. Queen SE, Mears BM, Kelly KM, et al. Replication-competent simian immunodeficiency virus (SIV) Gag escape mutations archived in latent reservoirs during antiretroviral treatment of SIV-infected macaques. J Virol **2011**; 85:9167–75.

132. Allers K, Hütter G, Hofmann J, et al. Evidence for the cure of HIV infection by CCR5Δ32/Δ32 stem cell transplantation. Blood **2011**; 117:2791–9.

133. Hütter G, Nowak D, Mossner M, et al. Long-term control of HIV by CCR5 Delta32/Delta32 stem-cell transplantation. N Engl J Med **2009**; 360:692–8.

134. Mavigner M, Watkins B, Lawson B, et al. Persistence of virus reservoirs in ART-treated SHIV-infected rhesus macaques after autologous hematopoietic stem cell transplant. PLoS Pathog **2014**; 10:e1004406.

135. Archin NM, Liberty AL, Kashuba AD, et al. Administration of vorinostat disrupts HIV-1 latency in patients on antiretroviral therapy. Nature **2012**; 487:482–5.

136. Elliott JH, Wightman F, Solomon A, et al. Activation of HIV transcription with short-course vorinostat in HIV-infected patients on suppressive antiretroviral therapy. PLoS Pathog **2014**; 10:e1004473.

137. Rasmussen TA, Tolstrup M, Brinkmann CR, et al. Panobinostat, a histone deacetylase inhibitor, for latent-virus reactivation in HIV-infected patients on suppressive antiretroviral therapy: a phase ½, single group, clinical trial. Lancet HIV **2014**; 1:e13–21.

138. Søgaard OS, Graversen ME, Leth S, et al. The depsipeptide romidepsin reverses HIV-1 latency in vivo. PLoS Pathog **2015**; 11:e1005142.

139. Wei DG, Chiang V, Fyne E, et al. Histone deacetylase inhibitor romidepsin induces HIV expression in CD4 T cells from patients on suppressive antiretroviral therapy at concentrations achieved by clinical dosing. PLoS Pathog **2014**; 10:e1004071.

140. Elliott JH, McMahon JH, Chang CC, et al. Short-term administration of disulfiram for reversal of latent HIV infection: a phase 2 dose-escalation study. Lancet HIV **2015**; 2:e520–9.

141. Del Prete GQ, Oswald K, Lara A, et al. Elevated plasma viral loads in romidepsin-treated simian immunodeficiency virus-infected rhesus macaques on suppressive combination antiretroviral therapy. Antimicrob Agents Chemother **2015**; 60:1560–72.

142. Del Prete GQ, Shoemaker R, Oswald K, et al. Effect of suberoylanilide hydroxamic acid (SAHA) administration on the residual virus pool in a model of combination antiretroviral therapy-mediated suppression in SIVmac239-infected indian rhesus macaques. Antimicrob Agents Chemother **2014**; 58: 6790–806.

143. Klatt NR, Canary LA, Sun X, et al. Probiotic/prebiotic supplementation of antiretrovirals improves gastrointestinal immunity in SIV-infected macaques. J Clin Invest **2013**; 123:903–7.

144. Beq S, Nugeyre MT, Ho Tsong Fang R, et al. IL-7 induces immunological improvement in SIV-infected rhesus macaques under antiviral therapy. J Immunol **2006**; 176:914–22.

145. Velu V, Titanji K, Zhu B, et al. Enhancing SIV-specific immunity in vivo by PD-1 blockade. Nature **2009**; 458:206–10.

146. Amancha PK, Hong JJ, Rogers K, Ansari AA, Villinger F. In vivo blockade of the programmed cell death-1 pathway using soluble recombinant PD-1-Fc enhances CD4+ and CD8+ T cell responses but has limited clinical benefit. J Immunol **2013**; 191:6060–70.