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Eradication of HIV from Tissue Reservoirs: Challenges for the Cure

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Abstract

The persistence of HIV infection, even after lengthy and successful combined antiretroviral therapy (cART), has precluded an effective cure. The anatomical locations and biological mechanisms through which the viral population is maintained remain unknown. Much research has focused nearly exclusively on circulating resting T cells as the predominant source of persistent HIV, a strategy with limited success in developing an effective cure strategy. In this study, we review research supporting the importance of anatomical tissues and other immune cells for HIV maintenance and expansion, including the central nervous system, lymph nodes, and macrophages. We present accumulated research that clearly demonstrates the limitations of using blood-derived cells as a proxy for tissue reservoirs and sanctuaries throughout the body. We cite recent studies that have successfully used deep-sequencing strategies to uncover the complexity of HIV infection and the ability of the virus to evolve despite undetectable plasma viral loads. Finally, we suggest new strategies and highlight the importance of tissue banks for future research.

Keywords: cure, cART, persistence, evolution, replication, compartment

Introduction

Despite long-term treatments with combined antiretroviral therapy (cART) that effectively reduce HIV plasma viral loads to undetectable levels, viral rebound is inevitable when treatment is interrupted. The inability to entirely eradicate the virus has precluded an effective cure, as described in several recent reviews.[1,2] In this report, we focus on the challenges to a cure that are specific to targeting the nonblood tissue(s) that harbor virus throughout infection. From this perspective, the three preeminent challenges of HIV cure research are: (1) identify the location of the anatomical reservoir/sanctuary from which virus repopulates blood upon cessation of cART; (2) define the mechanism by which virus is maintained at low or undetectable levels in such locations; (3) develop a treatment that will eradicate or silence the virus without damaging nearby sensitive or irreplaceable tissues [e.g., central nervous system (CNS)].

In this study, we will use the term ''reservoir'' as defining a tissue or cell in which latent virus is archived without replication/infection cycles and is instead perpetuated through cellular expansion and/or cellular longevity (as per Ref.[3]), and ''sanctuary'' as describing a tissue or cell in which virus is shielded from the effects of cART and which permits some low level of ongoing and complete virus replication cycles (e.g., an ''active reservoir''[4]). The expectation is that virus harbored in reservoirs will remain genetically similar to their earlier precursor, whereas virus in sanctuaries will accumulate diversity over time as the infection cycle continues and maintains the potential to migrate among compartments.[3]

The two viral persistence mechanisms are not mutually exclusive, and given the high viral mutability and widespread infection, both are likely and have been shown to work synergistically in HIV controllers not on cART.[5] However, they are too often presented in opposition, with an undue emphasis on memory T cells as the dominant, if not only,

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means by which HIV infection is maintained.[4] As a result, much cure research, including clinical trials and mathematical modeling,[6,7] has focused intensively on the reactivation of virus from T cell reservoirs to provide a method of viral detection and eradication. In these models, viral reservoir T cells are drug induced to express HIV, which in turn kills them through a program known as ''shock and kill''; however, this strategy has been largely ineffective at eliminating the reservoirs completely and preventing rebound after cART cessation.[2] In this study, we review evidence supporting the importance of sanctuaries for HIV maintenance and expansion, allowing another understudied target for further research in advancing strategies to cure HIV infection.

The mechanism of HIV persistence is largely dependent upon the infected cell type (e.g., T cells, macrophages, microglia, astrocytes) and its anatomical location (e.g., lymph node, gut, blood, CNS, cancer tissues, etc.). There is no debate that various subsets of T cells are major cellular reservoirs of HIV during cART[8–14] and maintain virus through clonal proliferation without interference from cART.[15,16] As expected under this scenario, virus obtained from circulating T cells during suppressive therapy showed a lack of genetic evolution compared with pretherapy virus, consistent with the model of maintenance through latency rather than replication.[17–19] However, an expanding body of evidence now demonstrates that circulating T cells do not adequately represent the totality of infection, as they only represent a small fraction of all T cells in the body, and ignore the importance of HIV infection in other cell types.[20] In a recent study, deep sequencing techniques, which were not employed in the above-cited studies, revealed evolution within HIV provirus-harboring peripheral blood mononuclear cells (PBMC) during cART therapy.[21] Another study found that while PBMC proviral DNA sequences were similar to residual plasma viremia, episomal DNA sequences in PBMCs were not, suggesting an alternative tissue-based source of infectious virus independent of residual plasma virus.[22] Furthermore, the inclusion of an integrase inhibitor along with the standard cART protease inhibitor resulted in an increase in unintegrated 2-long terminal repeat (LTR) circles in some subjects. 2-LTR circles are the byproduct of an unsuccessful integration attempt, which occurs after viral reverse transcription, thus blocking successful provirus integration, a step required for production of new viral RNA species. This observation suggests that in some cases, the virus successfully completed the replication cycle and theoretically could have integrated into cellular DNA without the integrase inhibitor treatment.[2]

Many studies of HIV persistence have focused on the identity of HIV within T cell subsets from blood-derived lymphocyte subsets. Equally plausible sites of HIV persistence are within tissue-resident macrophages, which are long-lived and capable of harboring replicating HIV.[23–25] Macrophages may provide conditions consistent with a sanctuary, as they are more resistant to the cytopathic effects of the virus[24,26] and contain lower intracellular concentrations of cART than T cells,[27–29] both of which decrease the effectiveness of cART[30] and may result in ongoing replication. The potential for transmission of HIV between cells within anatomical tissue sites may be variable.[31] For example, the dynamics of infection are different in macrophages than in T cells: macrophages efficiently promote cell-to-cell transfer of virus through virological synapses[32] and contain virions in cytoplasmic channels that are not immediately released. Cell-to-cell spread reduces sensitivity to drug therapy due to the higher number of virions per cell compared with cell-free infection,[33,34] although this effect is perhaps attenuated with combination therapy.[35] Cell-to-cell spread was shown to induce viral gene expression more quickly than cell-free infection *ex vivo*, independent of the higher number of virions transmitted through this route.[36] Additionally, macrophages can selectively uptake infected T cells, thereby becoming productively infected.[37] Other cell types involved in cell-to-cell spread through virological synapses include dendritic cells and keratinocytes, which are abundant at sites relevant to sexual transmission.[38,39] Although not likely reservoirs, these cell types could nonetheless play an important role in maintaining virus and/or increasing cellular activation which enhances infection.[40]

Considerable evidence suggests that lymphoid tissue may act as a sanctuary for virus during cART. Lower concentration of drugs were found in lymphatic tissues than in blood,[41] which may allow for continued HIV replication. Our group sequenced multiple HIV RNA and DNA species isolated from lymph nodes, brain, and other postmortem tissues from subjects with undetectable viral loads at the time of death.[42,43] The evolutionary rate of these HIV sequences was similar to the previously estimated rate of pre-cART and wild-type virus,[44–46] suggesting an important role for these tissues as viral sanctuaries. Another recent study, using cART-treated subjects, similar analytical techniques, and deep sequencing, found evidence of ongoing replication of wild-type virus in lymph nodes and continual seeding of the blood from lymph node sanctuary sites.[47] Interestingly, one group reported evidence for HIV RNA+ cells in lymph nodes before cART therapy interruption, suggesting ongoing tissue-based HIV replication. Moreover, during viral rebound after cessation of cART, the presence of diverse populations of virus suggested that the rebounding viral population originated from multiple sources, including lymph nodes.[48] A recent study of HIV controllers who were not on cART showed that blood virus was largely the result of clonally expanded archival provirus, whereas lymph node virus was actively replicating.[5] In this study, a small number of circulating T cells showed evidence of recent infection, suggesting a model in which active infection in lymph nodes could become clonally expanded provirus in blood. This supports the findings that the lymph node is an important site of ongoing replication despite undetectable viral load (VL).

The CNS represents another potential reservoir/sanctuary for virus. Resident brain cells, including astrocytes, perivascular macrophages, and microglial cells, are long-lived and capable of inducing latency.[49] Interestingly, astrocytes and neurons, actively express HIV nef and rev proteins.[50–52] The blood/brain barrier may protect the brain to some degree from viral infiltration into the CNS, as not all HIV-infected patients have detectable virus in brain tissues and/or cerebrospinal fluid (CSF). Viral populations in the CNS are established early in infection, and may remain compartmentalized with respect to plasma virus throughout the course of infection, and even among different brain compartments.[53,54] This pattern has occurred even when cART was begun in the first few months of infection, with the accumulation of genetic diversity pre-cART.[55] A recent study found that, upon cessation of cART, rebound virus in the

CSF was distinct from that in plasma and was detected at multiple time points, suggesting independent sources of replication-competent virus between the CNS and the periphery.[56] We recently reported the presence of viral DNA using digital droplet polymerase chain reaction (ddPCR) from 48/87 autopsy brain tissues from 20 subjects with undetectable viral loads at death.[43] Furthermore, viral RNA and DNA from cerebellum and lymph nodes were evolutionarily related. The similarity of the brain and lymph node virus is especially interesting in light of a recent study, which suggested that the meninges may be part of the lymphatic system,[57] which could provide the virus an alternative entry to the brain than through the blood–brain barrier.[53,58]

Adipose tissue is another potential reservoir/sanctuary. One study found HIV DNA in the adipose tissue of all studied subjects who were on ART with undetectable VL. Additional experiments showed that the concentration of virus in the CD4+ fraction of the adipose tissue was higher than that of PBMC. Furthermore, HIV RNA was also detected using *in situ* hybridization.[59] Another study found HIV provirus in subcutaneous, abdominal visceral, and deep neck fat deposits in all subjects studied and was associated with cellular activation.[60]

New methods are needed that can accurately detect and measure tissue-based HIV-infected cells *in vivo* without the need for invasive procedures. A recent study described a method for quantifying virus in gut biopsies using ddPCR[61]; however, quantitative PCR methods that probe for small regions of conserved HIV likely overestimate the size of the reservoir. Thus, an additional challenge is determining the viability of virus populations detected in sanctuaries. Deep sequencing techniques have already been useful in more comprehensively evaluating the viral population[47,62] and will be an informative technique going forward. Recent studies show that after cART, viral RNA may be present, but is largely nonfunctional and evolutionarily inert due to deleterious mutations and/or truncated virus[63–65]; therefore, incompletely sequenced proviral DNA does not necessarily imply the presence of functional virus.[66] On the other hand, at least some proviruses, including those in clonally expanded cells are replication competent [67] and can continue to proliferation after activation,[68] indicating that these proviruses cannot be entirely dismissed as irrelevant. Furthermore, the identification of replication-competent fusion proteins in patients on cART that generate HIV-1 chimeric proteins needs further investigation.[63] The current gold standard assay to determine virus functionally is the quantitative viral outgrowth assay (QVOA), which is expensive and only provides the minimum estimate of the potential reservoir.[69] A recently described approach uses a reporter cell-based assay, which improves upon the QVOA in terms of expense, time, and blood volume, and showed that the reservoir was 70-fold greater than previously reported.[69] Quantitative imaging (e.g., positron emission tomography [PET] scan) is a promising alternative as are indirect biological markers.[70] More complex *in vitro* systems that realistically replicate the microenvironment may also present a novel methodology to study in-depth viral dynamics in tissues.[70]

Toward eradication of the virus from the body, the most current and high-profile ''kick and kill'' cure strategy aims to activate latent CD4+ T cells with latency-reversing agents that target host cell mechanisms (reviewed recently in Ref.[71]). This approach has thus far been targeted to CD4+ cells, and may not effectively eradicate virus from other cell types, nor necessarily from the heterogeneous set of CD4+ cells themselves.[20] Improving drug delivery to tissues is an important facet of any cure strategy, such as using nanoparticles[72] and targeting endosomes, where viral replication takes place.[73] Small interfering RNAs are another potential mechanism for silencing virus,[74] which could provide a functional cure (i.e., suppressed replication without eradication of virus) for pantropic targets.

These studies all suggest that the dynamics of post-cART infection are more complicated than initially perceived from the study of PBMCs. Eradicating virus from anatomical tissues is clearly an important goal in the development of new strategies aimed at curing HIV infection and its consequences. However, several challenges presently limit the research into this area. Obtaining tissues from living patients is difficult and often unethical, which has resulted in a relative paucity of research on tissues as compared with easily obtained blood samples. HIV tissue banks and their donors will therefore serve as an important resource going forward in the fight for an HIV cure.[75] For example, the National NeuroAIDS Tissue Consortium (NNTC) is funded by NIMH and NINDS to store and facilitate distribution of tissues (brain and nonbrain) collected from both HIV+ and HIV− donors.[76] At the time of publication, the NNTC contains tissues from 2,097 individuals and is actively following 587 HIV+ individuals at four clinical sites across the United States, who were identified as probable late-stage cases. These subjects have extensive information available regarding treatment, comorbidities, and donor demographics. Postmortem tissues are also donated to the bank, although the information about these subjects may be less complete. Another tissue resource is the AIDS and Cancer Specimen Resource (ACSR), which contains more than 300,000 specimens from individuals with a range of HIV-associated comorbidities from multiple countries and clinical trials.[77] ACSR specimens are available from multisite autopsies and both nontumor and tumor sites, along with some donor clinical information.

In summary, the central role of tissues as an important viral reservoir/sanctuary is becoming clear. Vital research must be directed toward understanding the dynamics of the virus in anatomical sanctuaries to develop a fully effective method of virus suppression and eradication.

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