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Review: HIV-1 Phylogeny during Suppressive Antiretroviral Therapy

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Abstract

PURPOSE OF REVIEW: Studies of HIV-1 genetic diversity can provide clues on the effect of antiretroviral therapy (ART) on viral replication, the mechanisms for viral persistence, and the efficacy of new interventions. This article reviews methods for interrogating intra-host HIV-1 diversity, addresses the ongoing debate regarding HIV-1 compartmentalization and replication during ART, and summarizes recent findings on the effects of curative strategies on HIV-1 populations.

RECENT FINDINGS: HIV-1 replication in the blood is virtually halted upon the initiation of ART. However, proliferation of cells infected prior to ART provide a self-renewing reservoir for infection during ART. Current evidence supports that proliferation of infected cells is a mechanism for HIV-1 persistence in both the blood and the tissues. However, more studies are required to determine if tissue sanctuaries exist that may also allow viral replication during ART. Recent studies investigating potential curative interventions show little effect on the genetic landscape of HIV-1 infection and highlight the need to develop strategies targeting the proliferation of infected cells.

SUMMARY: Using phylogeny to characterize HIV-1 genetic diversity and evolution during ART has demonstrated a lack of viral replication, the proliferation of infected cells, and provides one metric to measure the effect of new interventions aimed at achieving a functional cure for HIV-1.

Keywords

antiretroviral therapy; ART; HIV genetics; HIV diversity; HIV replication; HIV phylogenetics

Introduction

Characteristic of HIV-1 infection is the vast genetic diversity that accumulates within and between hosts. High HIV-1 genetic diversity is largely due to the lack of a proofreading mechanism in the reverse transcriptase (RT) enzyme by which the virus converts its sense-RNA genome into double-stranded DNA for integration into the host genome where it either

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co-opts host machinery and is expressed or enters a latent (transcriptionally-silent) state. Other factors that drive the accumulation of HIV-1 diversity within an infected individual include a high rate of viral recombination [1], large population size [2], host APOBEC-mediated hypermutation [3], changes in infected cell populations including clonal expansion [4*, 5, 6*, 7, 8], differential microenvironments of anatomical compartments [9, 10, 11, 12] and immune selection for antibody and CD8+ T cell escape variants [13**, 14]. It is the interplay of all these factors that define the scope of HIV-1 genetic diversity and should be considered in analyses of intra-patient HIV-1 phylogenetics. The study of within-host HIV-1 populations, especially of those that persist during long-term antiretroviral therapy (ART), gives clues as to the mechanisms that sustain the HIV-1 reservoir and result in viral rebound when ART is interrupted.

The interrogation of intra-host HIV-1 genetics is typically done via the inference of a phylogenetic tree from sequence data generated by single-genome sequencing [15]. Most studies employ a neighbor joining and/or maximum likelihood approach to estimate the phylogenetic distance between viral RNA or proviral variants. While neighbor joining trees are the most prevalent and the quickest way to visualize the sequence diversity within a dataset, the application of substitution models to generate maximum likelihood trees provide more robust inferences of evolutionary relationships between variants in the population. The generated branch lengths and the inferred tree topology, in addition to the sequence distances, can be used to answer biological questions related to viral evolution [16, 17*, 18, 19], compartmentalization [12, 20, 21, 22], T-cell expansion [6, 23], and the effects of curative interventions [24*, 25*, 26*].

HIV-1 genetics on ART

Characterizing the HIV-1 genetics on ART allows us to investigate mechanisms by which the reservoir is established prior to ART and maintained during ART. Upon ART initiation, the majority of infected cells will die [5, 27], the cell-free virus load will drop precipitously to clinically undetectable levels [28], and the vast majority, if not all, of new viral replication rounds will be prevented [16, 17, 18, 19, 20]. It is these events that underscore genetic analyses of intra-host populations during ART and must be considered during interpretations of such analyses. For example, HIV-1 sequences gained by single-proviral sequencing of cells obtained from individuals on ART are not likely the result of new rounds of viral replication and, therefore, any shift in the proviral population over time probably reflect the waxing and waning of infected T cell clones [23]. Such clonal populations are a proverbial fossil record of HIV-1 infection dating back to the time of transmission [7, 8, 29*, 30]. A recent study by Jones, et al. utilized the placement of a fossil record to estimate the dates of integration of the observed proviral sequences that persisted on ART when trained on serial pre-ART data to calibrate a molecular clock [30**]. This study utilized nef sequences from two individuals with serial plasma virus sampled over many years of uncontrolled infection to generate a phylogenetic tree wherein the diversity was tracked over the duration of uncontrolled infection that could be used for linear regression of root-to-tip distances of those sequences obtained on ART and, thereby, provide an estimated date for the original integration event. The authors found that the on-ART sequences were seeded in both early infection and near time of initiation of ART. They further provided phylogenetic evidence

that viral “blips” were stochastic reactivations of cells infected before ART initiation due to the estimated dates of integration [30]. However, Jones and colleagues note that the use of a small, sub-genomic region underestimates the diversity of the population due to unseen differences elsewhere in the genome. In fact, such analyses performed on sub-genomic regions of HIV-1 must be carefully interpreted as a recent study by Laskey and colleagues showed that they often result in an overestimation of clonality [31].

Because low-level viremia persists despite ART, the question of reservoir maintenance by ongoing rounds of viral replication remains debatable. Residual viremia is not necessarily indicative of ongoing rounds of viral replication since there is no evidence that such particles go on to infect new cells in the presence of ART. A study published on a cohort of individuals with persistently detectable viremia on ART showed no genetic shift in the HIV populations over time indicating that persistent, clinically-detectable, low-level viremia does not, by itself, signal ongoing replication in the blood [16]. Furthermore, because HIV-1 requires some time to acquire a substantial amount of genetic diversity detectable by single-genome sequencing, a study by Van Zyl and colleagues looked for a phylogenetic shift in the HIV-1 populations of perinatally-infected children put on ART shortly after birth and sustained on ART for 6–9 years [17]. This study provided a sensitive measure to probe for an increase in proviral diversity and/or a phylogenetic shift in the proviral population during ART because of the limited initial diversity in samples. The authors found no detectable increase in the HIV-1 diversity and no phylogenetic shift in the proviral population in the children for 6–9 years on ART, suggesting a lack of ongoing viral replication in the blood [17]. In another study, the addition of maraviroc to intensify ART regimens also failed to show evidence for ongoing cycles of viral replication, a result consistent with previous ART intensification studies [19]. The authors of this study utilized a Bayesian phylogenetic framework with a tip-date randomization procedure to look for evidence of a population shift during ART and, consistent with previous studies, found none that suggested ongoing viral replication on ART [19]. Perhaps the clearest evidence for a lack of viral replication on ART, however, comes from two studies showing no accumulation of genetic diversity over time in variants known to be replication competent [18, 32]. Taken together, these results suggest that virtually all ongoing cycles of viral replication in the blood are halted by ART. Further studies are needed to determine if the same is true for tissues, such as the lymph nodes and the gut.

Studies attempting to directly characterize the reservoir via quantitative viral outgrowth assays (QVOA) or by more recent methods, such as the Intact Proviral Detection Assay [33**] or Matched Integration Site and Proviral Sequencing Assay [34**], have provided insights into HIV-1 genetics on ART. Using QVOA, Bui et al., Hosmane et al, and Lorenzi et al. each showed that clonal expansion of cells containing replication-competent proviruses are common among HIV-1 infected individuals with viremia fully suppressed on ART [4, 35, 36*]. Such clones have also been shown to be a source of persistent and rebound viremia [32, 37], to be transcriptionally active both before and during therapy [37, 38*], and to wax and wane in size over the course of treatment [23]. Furthermore, a recent study by Cohn and colleagues described a method by which singly-reactivated cells from a QVOA can be analyzed from the culture well to describe the sub-population of infected cells containing intact proviruses that are most easily reactivated. The authors again describe the presence of

cellular clones arising from both outgrowth recovery as well as the single cells captured by their assay termed LURE -- latent cell capture [39**]. They further describe the whole-cell transcriptional profile of reactivated cells and found that the pattern of expressed genes included those involved in HIV-1 repression, implicating a mechanism that allows for cellular division upon reactivation without pathways that end in cell death [39]. Although these studies contribute to our understanding of what, potentially, maintains the latent state and the persistence of some infected T cell clones, further studies are needed to fully elucidate the mechanisms by which some cells are transcriptionally silent while others are not and the relative ease with which intact proviruses may be reactivated. More recently developed methods mentioned above [33, 34], can more accurately measure of the size or characterize the genetics of the HIV-1 reservoir and will be useful tools in the future to evaluate the efficacy of potential curative interventions.

HIV-1 compartmentalization

HIV-1 compartmentalization describes the state of having distinct viral populations across different tissues or across different cell types within an infected individual, suggesting that a biological barrier restricts the flow of virus particles or of infected cells. Such compartmentalization can be detected phylogenetically or using statistical tests for panmixia. Compartmentalization between the central nervous system (CNS) and the peripheral blood has been demonstrated phylogenetically [11, 21, 40, 41] and shown to be associated with HIV-associated neurodegenerative disorder (HAND) [42, 43]. Compartmentalization of HIV-1 in the CNS has been attributed to viral tropism, especially differences in macrophage-tropic (CD4 low) vs. T-cell tropic (CD4 high) in the two compartments [44]. Furthermore, a recent study showed that the CNS reservoir is seeded and exhibits a genetically distinct population structure shortly after infection, although early administration of ART limits the overall diversity [11]. However, conflicting studies exist regarding compartmentalization of HIV-1 across other anatomical sites such as lymph nodes, gut, and genital secretions.

The majority of studies investigating HIV-1 genetics in the vaginal tract suggest viral compartmentalization [45, 46]. However, Bull, et al. claim that such conclusions may be influenced by shallow sampling [47, 48, 49]. Similar studies on seminal fluid show a lack of compartmentalization in both untreated and treated individuals [10, 50], although some suggest trends towards compartmentalization [22]. Compartmentalization in lymphoid tissues still remains an open question with some studies suggesting the presence of distinct genetic compartments found in the spleen as well as the lymph nodes [9, 12], although the lymph nodes are a current point of controversy within the field [5, 6, 20]. More studies will need to be done on the lymphoid tissues to fully explore the HIV-1 populations within. Understanding the contributions of various tissues to the HIV-1 reservoir on ART is paramount to furthering progress towards functional cures as curative interventions will require activity in all tissues wherein HIV-1 persists and can rebound.

Recent compartmentalization analyses have gravitated towards understanding which CD4+ T cell subsets harbors the HIV-1 reservoir on ART. Hiener and colleagues showed that intact, identical proviruses were largely found in the effector memory subset [51*]. In

addition, Lee *et al.* demonstrated a preferential bias for intact proviruses within Th1-polarized CD4⁺ T cells [52*]. Although largely disputed [53, 54, 55, 56, 57], CD32a was proposed as a marker for enrichment of HIV-infected cells [58]. Taken together, these data present a new view in measuring the latent reservoir in HIV-infected individuals. Further research is needed to fully elucidate the contribution of various cell subsets to HIV-1 persistence during ART.

HIV-1 genetics to investigate the effect of potential curative interventions

Current ART is highly effective at blocking viral replication. However, ART has no effect on latently-infected cells or on proliferating infected cell populations. Consequently, HIV-1 infected cells persist indefinitely *in vivo* despite ART. The persistence of HIV-1 during ART has shifted the focus of recent research efforts from blocking viral replication to targeting the viral reservoir. One strategy for targeting HIV-1 infected cells that persist on ART is infusion with anti-HIV broadly neutralizing antibodies (bNAbs) [59]. To investigate the efficacy of such bNAbs, one study of 15 HIV-1 infected individuals undergoing infusions of the bNAb, 3BNC117, found that the reservoir size and clonal composition were not altered [26]. Other interventions, such as kick and kill approaches that attempt to activate latent proviruses then target and kill the activated cells that produce viral proteins with, for example, CAR T-cells, have also had limited success thus far [60, 61]. Most recently, Winckelmann, et al. showed that a large fraction of proviruses that were activated after exposure to romidepsin, a histone deacetylase inhibitor, were clonal in nature [24], suggesting that romidepsin either induced the proliferation of infected cells or induced the expression of proviruses that were already in clonally expanded populations of infected cells. A follow-up study by Winckelmann, et al. in a cohort of individuals infused with both Vacc-4x, a peptide-based therapeutic vaccine, and romidepsin showed that neither Vacc-4x nor romidepsin affected the diversity of the proviral or cell-associated RNA populations and that rebound virus during an analytic treatment interruption did not match proviruses or cell-associated RNA sampled during ART [25], highlighting the depth of the latent reservoir and the challenges to the development of potent curative interventions. Future efforts towards a cure will investigate the effect of combinations of latency reversal agents, bNAbs, and other strategies on the size of the reservoir, on the clonal landscape of infected cells, and on the transcriptional activity of the HIV-1 proviruses that were present prior to the intervention.

Conclusion

Much of our understanding of the HIV-1 reservoir has come from the genetic characterization of the viral population in donors before, during, and after treatment with combination ART. It appears that we are approaching a scientific consensus that ART virtually halts ongoing viral replication in the blood of infected individuals [16, 17, 18, 19]. However, significant debate on whether or not residual replication is largely halted in lymphoid tissue still remains [5, 12, 20]. This controversy may be fueled, in part, due to publication biases towards positive results [62] showing evidence for compartmentalization and replication vs. those showing a lack for such evidence. It has been well-established that only a small fraction of HIV-infected cells survives on ART and that they persist through clonal expansion during ART [6, 23, 31, 32, 51]. Furthermore, it has been shown that those

cells are largely transcriptionally silent, perhaps allowing for their survival by evading immune sensors [38]. These clonally expanded HIV proviruses can and, in one case, have been shown to be the source of persistent and rebound viremia [32]. Understanding the mechanisms that maintain the HIV-1 reservoir will inform the design of curative strategies to achieve a functional cure. Determining the efficacy of potential curative interventions will depend, in part, on understanding their effects on intra-host viral genetics, on the size of the HIV-1 reservoir, and on the dynamics of clonal expansion.

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Key Points:

- Phylogenetic analyses have shown that optimal ART virtually halts all ongoing rounds of HIV-1 replication in the blood, preventing further diversification of the proviral population.
- Some infected cells clonally proliferate and can have proviruses that are transcriptionally active, producing virions that are the source of residual viremia and rebound upon ART cessation.
- Anatomical compartmentalization of HIV-1 within lymphoid tissues remains an ongoing focus of research to understand the mechanisms of persistence therein.
- Studies investigating the contributions of various T-cell subsets to HIV-1 persistence on ART have provided key insights into cells that harbor replication-competent proviruses.
- Studies of HIV-1 populations in individuals undergoing potential curative interventions have shown limited effects on the latent reservoir.