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REVIEWS

Spotlight

For Viral Reservoir Studies, Timing Matters

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New strategies to eliminate persistent HIV-1 during therapy will benefit from animal models, such as non-human primates infected with simian immunodeficiency viruses (SIV) or chimeras (SHIV). Understanding the genetic composition of SIV and SHIV reservoirs during therapy is therefore crucial for the future application of this model.

Despite advances in antiretroviral therapy (ART) a cure for HIV-1 remains elusive due to the persistence of HIV-infected cells. During active infection, and in the initial stages of ART, the majority of HIV-1-infected $CD4^+$ T cells die during the course of productive infection. However, some cells, including long-lived memory $CD4^+$ T cells, may not express the integrated HIV-1 provirus without stimulation. If this integrated provirus is genetically intact, these latently infected cells contain the genetic material that could reinitiate HIV infection at any time, even after long-term ART [1,2]. This replication-competent HIV-1 reservoir must be targeted when developing any HIV-1 curative therapy.

There are two approaches to HIV-1 cure: the 'kick-and-kill' method, where latency-reversing agents stimulate productive infection to purge infected cells from the reservoir, and the 'lock-and-block' method, which aims to indefinitely silence the integrated provirus [3]. When developing these HIV-1 interventions for use in humans, the preclinical trial stage typically tests the potential therapeutic agent in a non-human primate model; most commonly this is the rhesus macaque infected with either simian immunodeficiency virus (SIV), or the SIV–HIV chimera, SHIV. To be of benefit, these models must be a good reflection of the human disease; for HIV-1 curative therapies, this means that the non-human primate SIV or SHIV reservoir must approximate the HIV-1 reservoir. In a recent study published in *Cell Host and Microbe* [4], Bender *et al*. aimed to characterize these latent reservoirs in SIV- or SHIV-infected macaques to determine how well the non-human primate model used in HIV-1 curative studies reflects clinically relevant HIV-1 reservoirs. Bender *et al*. also investigated the HIV-2 reservoir in persons infected with this viral strain [4].

Bender *et al*. describe the SIV reservoir in seven rhesus macaques who were treated with suppressive ART during the chronic stage of infection using full-length sequencing methods. Over 250 SIV sequences were assessed, and similar to previous studies of HIV-1 [5–8], the majority of these viral sequences were defective, with the most common defect being a large internal deletion. However, a far greater proportion of SIV sequences (28%) were genetically intact compared with the HIV-1 reservoir (1–10%) [5–8]. Sequences that were hypermutated or containing a large number of G-to-A mutations resulting in early stop codons, were also observed to a higher extent in SIV-infected macaques. Two pairs of identical SIV sequences could be isolated from only a single macaque.

SIV infection was then compared with SHIV infection in similarly treated macaques; 131 SHIV sequences were isolated from four animals, with the majority being defective. Only 15% were considered intact, which is lower than the proportion of intact sequences observed for SIV but again higher than the 1–10% observed in HIV-1 infection [5–8]. The proportions of specific defective mutations within the SHIV and SIV reservoirs were similar. In addition, only three pairs of identical SHIV sequences could be isolated from a single macaque.

In contrast, the reservoir in three HIV-2-infected participants was very similar to that of HIV-1, despite the fact that only 41 sequences (approximately 15 per participant) were able to be isolated. Only a single sequence was considered genetically intact. A smaller fraction of sequences was considered hypermutated when compared with the macaque reservoirs. As such, Bender *et al*. [4] highlighted discrepancies in the retroviral reservoirs between macaques and humans. These may reflect host-related differences rather than any virological differences between the viral strains.

The macaque reservoir observed by Bender *et al*. [4] is comparable to what is found in the peripheral blood mononuclear cells (PBMCs) of untreated HIV-1-infected participants, or early after ART-initiation [6,8] (Figure 1). In these participants, the high number of genetically intact proviruses results from short-lived HIV-infected cells that are not part of the reservoir but have not undergone decay as of yet. Moreover, as clonal expansion of HIV-infected cells is strongly linked to time on therapy, identical sequences are rarely observed in untreated participants or early after ART initiation [6,8,9]. However, for the majority of HIV-1 reservoir studies, proviruses are analysed after 3 years of ART [5–7], as previous studies have indicated that viral decay continues until therapy has been continued for at least 3 years [2]. The SIV- and SHIV-infected macaques were sampled at only 36 weeks post initial ART treatment. While all SIV-infected macaques were virally suppressed (<50 copies per ml) by 20 weeks post ART initiation (the majority by 16 weeks) and no viral evolution was demonstrated after 8 weeks of therapy, we note that the authors used only one assay for viral evolution, root-to-tip analysis of a neighbour-joining tree, and other phylogenetic methods of evolution should have

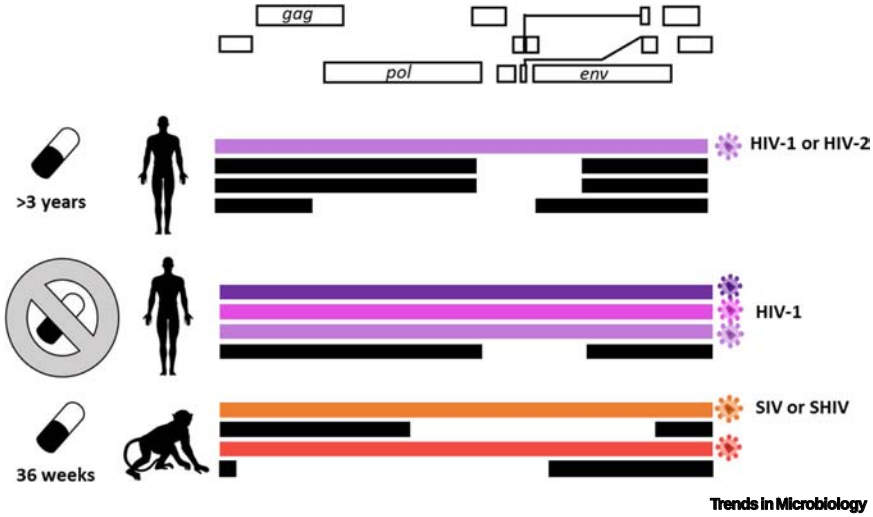

Figure 1. Comparison of Retroviral Reservoirs in Different Hosts and Treatment Contexts. The retroviral reservoirs in rhesus macaques infected with either simian immunodeficiency virus (SIV) or a chimera (SHIV) [4] as compared to humans infected with either HIV-1 [5–8] or HIV-2 [4]. The proportion of intact genomes found in the reservoir is similar between macaques infected with either SIV or SHIV [4], and is much higher in macaques than in humans after 3 years of antiretroviral therapy (ART) [5–8]. The macaque reservoir approximates the untreated or early post-treatment-initiation human HIV-1 CD4$^+$ T cell reservoir [6,8].

been employed to confirm this finding [10]. As such, the major differences in the intact reservoir found between macaques and humans delineated in the Bender study may be due to the fact that the reservoir in each host was observed at a different stage of decay. Follow-up analysis of the reservoir within macaques at >3 years post ART initiation is warranted.

While there seems to be striking differences between retroviral reservoirs in macaques and humans, the results of Bender *et al*. do not imply that the non-human primate model is inadequate for HIV-1 curative research. While a higher proportion of intact provirus was observed in macaques infected with either SHIV or SIV, the intact proviruses did not make up the majority of sequences observed in the reservoir. If the discrepancies in the proportions of intact virus between humans and macaques are indeed due to differences in the time at which the reservoirs were analysed, then this knowledge can be used when designing new non-primate studies. The value of an animal model is largely influenced by its ability to mimic human disease. Analysing the SIV or SHIV reservoir in macaques after they have been suppressed for 3 or more years would increase the likelihood that the reservoir in the non-human primate model is more reflective of the HIV-1 reservoir in humans on prolonged effective ART. While financially challenging, this would increase the usability and translatability of this model in preclinical trials of HIV-1 curative strategies.

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Forum

Imaging the Hepatitis B Virus: Broadcasting Live

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Although important breakthroughs in our understanding of the hepatitis B virus (HBV) life cycle have been made since the discovery of its main entry factor, the spatiotemporal dynamics of HBV–host interactions remains understudied. Here, we discuss recent advances and continuing challenges to image the HBV life cycle in live cells.

Context

Over 250 million people worldwide are chronically infected with HBV, significantly increasing risks of fibrosis, cirrhosis, and ultimately hepatocellular carcinoma. While an efficient vaccine