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## Exploring the anatomical HIV reservoirs: role of the testicular tissue

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Interruption of combination antiretroviral therapy (cART) almost invariably leads to the re-emergence of detectable HIV replication, typically in about two weeks [1]. The source of persistent viremia or viral rebound following cART interruption is likely multifactorial. It could arise from residual ongoing viral replication during cART and/or from reactivation of HIV expression from viral compartments and reservoirs.

In vivo virologic compartments are cell types or tissues between which there is a restriction of virus *exchange*, while virologic reservoirs are cell types or tissues in which there is a relative restriction of *replication* [2]. The distinction between compartments and reservoirs is important for understanding the testes as a sanctuary for HIV-1 [2]. The HIV-1 reservoirs are considered as the major source of viral recrudescence after stopping cART and consequently the most important hurdle to HIV-1 eradication.

Advances in the last years have indicated that resting memory CD4<sup>+</sup> T cells harboring a replication-competent form of HIV constitute the main cellular reservoir [3]. Most scientific efforts to reduce viral persistence have largely focused on eradication of HIV-1-infected cells within this reservoir. However, recent studies have challenged this dogma and suggested that follicular T-helper (TFH) cells also could be an important cell type for HIV-1 persistence [4]. There is definitely an urgent need to better identify the cell types or anatomical sites of HIV-1 persistence since major knowledge gaps exist in this area.

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The brain and genital tracts have been postulated as reservoirs for latent infection, but their roles in HIV-1 persistence remain unclear. Indeed, characterizing the HIV-1 anatomical compartments and reservoirs is challenging because the virus resides in difficult-to-access tissues. This limitation explains why supporting data on the role of the testes as both a viral compartment and reservoir derive from rare studies on animals. That the testis is a privileged, immune-tolerant site has been known since 1767 when a cock testis was transplanted into the abdomen of a hen from which a testis of normal structure was later recovered [5, 6]. A small proportion of HIV-1 infected men (<10%) achieve viral suppression in their blood but continue to shed HIV-1 episodically in their semen [7]. As such, the situation of the testis as an immunologically privileged site and as a potential antiretroviral drug sanctuary for HIV-1 offers insight into the complexities of HIV-1 infection in the male genital tract.

In this issue, Jenabian *et al.* [8] used an original and creative way to assess the immune privilege properties of human testes and to study the ability of the testicular tissue to be an anatomical site for HIV-1 persistence. Indeed, the authors had the unique opportunity to obtain bilateral testes from six HIV-1-infected but virally suppressed and 10 HIV-1-uninfected volunteers desiring orchiectomy in the context of gender reassignment surgery.

Jenabian and colleagues present new findings describing the immunological milieu of the testis. They convincingly demonstrated that testes may represent a preferential target for HIV persistence due to higher frequencies of activation markers, to a markedly higher expression of CCR5 co-receptor or to immune tolerance properties. In particular, they observed a higher frequency of CD39<sup>+</sup> T regs. This finding is of particular interest since these cells are associated with suppression of anti-HIV specific CD8<sup>+</sup>-mediated immune responses [9].

The testes consequently appear as a tissue with features that favor persistence of viral replication. This is especially true given the additional presence of a physical barrier between the blood vessels and the seminiferous tubules. This blood-testis barrier may indeed significantly decrease the penetration of antiretroviral drug into the testes. For example, tissue penetration of cART through the blood-testes-barrier can be blocked by efflux transporters P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP), which are both known to limit penetration of atazanavir and darunavir into semen [10, 11]. However, the testes as a drug-impermeable compartment for HIV-1 replication is attractive but simplistic given that there are multiple genital tract sources of HIV-1 [11-13].

The accompanying manuscript by Jenabian *et al.* provides further insight into the role of the testes as an anatomical compartment/reservoir for HIV-1. Indeed, the authors detected HIV-1 genomic DNA in the majority of the testicular tissue samples examined. However, HIV-1 DNA was not detected in all testes from these virally suppressed patients. The frequency of cells carrying HIV-1 DNA was also extremely low (range 1-6 copies per millions cells). Considering that most of the integrated proviruses are defective [14], the relevance of these few detected copies in the testes is not known and could represent blood-associated HIV-1 infected mononuclear cells transiting through the testes tissue at the time of the orchiectomies. In situ hybridization localization of HIV-1 DNA and RNA in the tissues

would have been helpful. The scarcity of detected proviruses may be explained by the use of total cell lysates from testicular tissue; as such, HIV-1 target cells represent only a minority of the testicular cells sampled. From our perspective, this technical sampling issue stands as a limitation of the work presented by Jenabian and colleagues [8]. When considering the testes as a distinctive anatomical reservoir for HIV persistence during cART, the contribution of testicular-associated HIV-1 to semen is generally considered to be minimal since vasectomy has no effect on the HIV-1 RNA level in seminal plasma and other sources of virus from the more distal genital tract glandular structures are more important sources of semen virus [12, 15, 16]. As a consequence, although the work is certainly of interest, it remains to be seen whether a replication-competent HIV can be found in testes from cART treated patients.

A number of additional questions remain unanswered regarding the testes role as an anatomical reservoir for HIV-1 replication and persistence: What is the distribution of virus across CD4<sup>+</sup> T-cell sub-populations in the testes? Do macrophages contribute to HIV-1 persistence in the specific immune privileged testicular tissue? And importantly, is there HIV-1 phylogenetic support to define and differentiate between the testes as a site of restricted viral flow or exchange (compartment) and restricted viral replication (reservoir)?

In conclusion, although the ability of the testicular HIV-1 reservoir to effectively give rise to replication-competent virus is not known, the further characterization by Jenabian and colleagues of the testis as an immunologically-privileged compartment adds important insight into the diversity of this difficult-to-access HIV-1 anatomical reservoir and paves the way for other immunological and virological studies aimed at exploring and identifying other HIV-1 tissue reservoirs.

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