Mechanisms of HIV persistence in HIV reservoirs

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Keywords: HIV reservoirs, persistent viremia

Running head: HIV compartments and persistence
Summary

The establishment and maintenance of HIV reservoirs which lead to persistent viremia in patients on antiretroviral (ARV) drugs remains the greatest challenge of the highly active antiretroviral therapy (HAART) era. Cellular reservoirs include resting memory CD4+ T lymphocytes, these implicated as the major HIV reservoir, having a half-life of approximately 44 months while this is less than 6 hours for HIV in plasma. In some individuals persistent viremia consists of invariant HIV clones not detected in circulating resting CD4+ T lymphocytes suggesting other possible sources of residual viremia. Some anatomical reservoirs that may harbour such cells include the brain and the central nervous system (CNS), the gastrointestinal tract (GIT) and the gut-associated lymphoid tissue (GALT) and other lymphoid organs and the genital tract. The presence of immune cells and other HIV susceptible cells, occurring in differing compositions in anatomical reservoirs, coupled with variable and poor drug penetration which results in suboptimal drug concentrations in some sites, are all likely factors that fuel the continued low level replication and persistent viremia during treatment. Latently HIV infected CD4+ T cells harboring replication-competent virus, HIV cell-to-cell spread and HIV infected T cell homeostatic proliferation due to chronic immune activation represent further drivers of this persistent HIV viremia during HAART.
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>3TC</td>
<td>Lamivudine</td>
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<tr>
<td>ARV</td>
<td>Antiretroviral</td>
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<tr>
<td>ATV/r</td>
<td>Atazanavir</td>
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<tr>
<td>AZT</td>
<td>Zidovudine</td>
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<tr>
<td>CCR5</td>
<td>Chemokine receptor type 5</td>
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<tr>
<td>CD</td>
<td>Cluster of differentiation</td>
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<tr>
<td>CXCR4</td>
<td>Chemokine receptor type 4</td>
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<tr>
<td>CVF</td>
<td>Cervicovaginal fluid</td>
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<tr>
<td>D4T</td>
<td>Stavudine</td>
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<tr>
<td>DC</td>
<td>Dendritic cell</td>
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<tr>
<td>DC-SIGN</td>
<td>Cluster of differentiation - Specific intercellular adhesion molecule–grabbing integrin</td>
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<tr>
<td>DDI</td>
<td>Didanosine</td>
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<tr>
<td>DRV</td>
<td>Darunavir</td>
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<tr>
<td>GALT</td>
<td>Gut associated lymphoid tissue</td>
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<td>GIT</td>
<td>Gastrointestinal tract</td>
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<tr>
<td>HIVE</td>
<td>HIV encephalitis</td>
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<td>HPC</td>
<td>Hematopoietic progenitor cell</td>
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<tr>
<td>IDV</td>
<td>Indinavir</td>
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<td>LPV</td>
<td>Lopinavir</td>
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<td>NVP</td>
<td>Nevirapine</td>
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<td>RGV</td>
<td>Raltegravir</td>
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<tr>
<td>Tat</td>
<td>Transactivator of HIV gene expression</td>
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Remaining a serious challenge to health and development worldwide is ongoing infection with the human immunodeficiency virus (HIV), which if left untreated in the individual results in progressive depletion of CD4+ T cells and ultimately acquired immunodeficiency syndrome (AIDS). The use of highly active antiretroviral therapy (HAART) and the scale up of provision of antiretroviral therapy (ART) has shown enormous improvements in the well-being and survival of HIV infected persons. However, although HAART can reduce the viral load in plasma to levels below the limits of detection of clinical assays and can prevent transmission, the virus cannot be eradicated from the infected host. As current HAART regimens do not target latent proviral HIV, the virus continues to persist in anatomical and cellular reservoirs that are latent but have replication-competent HIV genomes. Anatomical compartments may be a source of viral rebound and circulating minority drug resistant variants due to continued low level independent replication and seeding from these sites. The virus spreads to different organs early in primary HIV infection\(^1\) and can form distinct viral populations suited to their environment and differences in selection pressures such as the local immune surveillance and the concentrations of antiviral drugs following HAART initiation. Although cellular reservoirs infected before HAART initiation are generally regarded as the sources of persistent viremia, the possibility of continued low level replication after HAART initiation in tissue anatomic sites where drug levels might be suboptimal cannot be excluded and warrants continued study. In this article we review the mechanisms of viral persistence in different anatomic and cellular compartments likely to harbor the virus during HIV therapy. These reservoirs together with the associated immune surveillance and HIV susceptible cells and drug penetration levels are summarized in Table 1. Determining the anatomic reservoirs responsible for persistent viremia and the types of cells involved is important in the current efforts for HIV eradication and identification of targets for new drugs.
Table 1: HIV anatomical reservoirs, associated HIV susceptible cells and penetrability of ARV drugs

<table>
<thead>
<tr>
<th>Compartment/Reservoir</th>
<th>Immune surveillance/susceptible cells</th>
<th>Drug penetration/concentrations</th>
<th>Comments</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>Genital tract</td>
<td>T lymphocytes, macrophages, dendritic cells (DCs), mucosal cells, epithelial cells, stromal cells, germ cells</td>
<td>Concentrations vary from optimal to suboptimal depending on class of drug. A barrier to penetration of some ARVs into the testes has been suggested</td>
<td>This site is important for the initial infection establishment and viral spread to different reservoirs</td>
<td>[2-4]</td>
</tr>
<tr>
<td>Lymphoid organs:</td>
<td>DCs, macrophages, T lymphocytes, hematopoietic progenitor cells, thymocytes</td>
<td>Concentrations of commonly used drugs tenofovir and emtricitabine may be suboptimal in lymph nodes</td>
<td>Multiple focal points of viral replication have been detected in lymph nodes during planned treatment interruption studies. Sub optimal drug levels in lymph nodes and other lymphatic tissues may result in low level or persistent viremia during treatment.</td>
<td>[5-8]</td>
</tr>
<tr>
<td>Gastro-intestinal tract</td>
<td>DCs, macrophages, T lymphocytes, mucosal cells, epithelial cells</td>
<td>Suboptimal penetration in areas such as the ileum and rectum</td>
<td>Site of the largest population of the body’s T cells and macrophages. Most of the T cells are activated and can be easily infected by HIV. Low level T cell homeostatic proliferation helps maintain the HIV reservoir during HAART</td>
<td>[9-11]</td>
</tr>
<tr>
<td>Brain/CNS</td>
<td>Microglia, T lymphocytes, macrophages, astrocytes, neurons</td>
<td>Suboptimal levels of some drugs. Protease inhibitors are pumped out by P-glycoproteins present in the blood brain barrier</td>
<td>There is restriction of viral and immune cells trafficking and blockage of drugs passage. Neurovirulent viruses and development of AIDS-dementia complex and HIV encephalitis</td>
<td>[12-15]</td>
</tr>
<tr>
<td>Respiratory tract</td>
<td>Bronchial, alveolar and interstitial macrophages, T lymphocytes, DCs, epithelial cells, fibroblasts</td>
<td>Drugs detectable at low levels in bronchoalveolar lavage</td>
<td>ARV treatment significantly lowers the HIV viral load in the broncho alveolar lavage fluid to undetectable levels although patients on HAART still have a high risk of pulmonary infections</td>
<td>[16-18]</td>
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<tr>
<td>Liver</td>
<td>DCs, macrophages, T lymphocytes, Kupffer cells, endothelial sinusoidal cells, stellate cells, hepatocytes</td>
<td>Liver fibrosis affects drug clearance by the liver leading to higher plasma drug concentrations in affected patients</td>
<td>HIV infection exacerbates liver damage in HBV and HCV co-infected patients. Most antiretroviral drugs are hepatotoxic and lower dosages may be required for patients with severe hepatic insufficiency</td>
<td>[19-22]</td>
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Genital tract

Subpopulations of HIV-1 from the male and female genital tracts have been shown to exhibit characteristics of being compartmentalized when compared to those of blood and lymphoid tissue. In the male genital tract, drug resistance has been shown to persist longer than in blood which may indicate local drug penetration barriers and independent selection pressures although antiretroviral therapy can effectively reduce HIV-1 levels in semen. Even with antiretroviral therapy, irregular seminal HIV shedding can occur. This may be due to the presence of both the HIV virus and CD4+ T lymphocytes in semen, which support and provide an environment for continued replication within the seminal tract, although some have suggested a viral source from outside the lumen which could include spill-over from...
blood and cells lining the lumen.\textsuperscript{12} However, since these external sources are fully exposed to drugs this does not explain the persistence of drug resistance in the genital tract.

In females, HIV has also been shown to be archived in the genital tract early in infection and has been detected in some women with undetectable levels in blood.\textsuperscript{13, 14} Mutation patterns from blood and genital tract specimens have been shown to differ.\textsuperscript{15} Even within the genital tract, viruses from the cervicovaginal lavage exhibited genetically distinct characteristics when compared to viruses from the endocervical secretions.\textsuperscript{15} Low level HIV replication in the male and female genital tract remains a possible cause for persistent viremia during HAART possibly due to poor drug penetration and suboptimal drug concentrations, and the presence of infected long-lived cells in these compartments.

Activities in the genital tract mucosa are very important for the initial establishment of infection and the spread of virus to different reservoirs (Figure 1). Dendritic cells (DCs) in the genital tract mucosa are the main HIV targets during sexual transmission.\textsuperscript{16, 17} DCs express the HIV receptors CD4, Chemokine receptors CCR5 and CXCR4 together with CD209 protein, also known as C-type lectin DC specific intercellular adhesion molecule–grabbing integrin (DC-SIGN).\textsuperscript{18} DC-SIGN enhances infection by having high affinity binding for the HIV glycoprotein gp120, aiding transmission of the virus to T lymphocytes in trans although in cis mechanisms have also been reported.\textsuperscript{19} Another DC protein known as Siglec-1 has also been identified and shown to contribute to HIV uptake by mature DCs.\textsuperscript{20} Replication within DCs is not necessary as DC-SIGN positive cells exposed to small amounts of HIV viruses can more efficiently infect activated T lymphocytes than unbound virus particles.\textsuperscript{21} HIV particles in DC-SIGN positive cells are not degraded and can remain stable and infectious for more
than 9 months which allows incoming virus to remain infective before target cells are infected. 21, 22

**Figure 1: HIV’s hiding places.** After exposure at mucosal surfaces (1), the virus is carried to the local lymph nodes (2) by dendritic cells. Fusion of dendritic cells with CD4+ T lymphocytes results in infection of the lymphocytes and viral replication in these cells. Infected CD4+ T lymphocytes are released into the blood stream (3) and disseminated to anatomical reservoirs in other organs (4) including the brain, CNS, spleen, bone marrow, thymus, lungs, kidneys, lymph nodes and gut associated lymphoid tissue (GALT) with infection of associated cellular reservoirs in these organs.

**Lymphoid organs**

After initial exposure and infection of antigen presenting DC-SIGN positive DCs at mucosal surfaces, the virus is internalized, processed and presented to T lymphocytes initiating an
adaptive immune response.\textsuperscript{26} HIV takes advantage of immature DCs in the genital tract which capture the HIV virus to gain access to lymphoid tissue compartmentalized CD4+ T cells.\textsuperscript{21} HIV then replicates in CD4+ T cells in the lymph nodes before infected T cells and free viruses enter the thoracic duct and spread into the bloodstream, other lymphoreticular tissues and immune associated organs such as the thymus, bone marrow, gut associated lymphoid tissue and spleen and to other possible reservoirs such as the CNS. Persistent replication in lymph nodes and lymphatic tissues despite HAART and lower concentrations of the antiretroviral drugs compared to levels in peripheral blood\textsuperscript{26-28} provides a "hiding place" for HIV in this compartment. Animal model studies have demonstrated that the lymph nodes may harbor viral reservoirs responsible for plasma virological failure if treatment is terminated,\textsuperscript{29} while some drugs such as non-nucleoside reverse transcriptase inhibitors tenofovir and emtricitabine, the nucleoside reverse transcriptase inhibitor drug efavirenz and atazanavir a protease inhibitor, have all been shown to have much lower levels in lymph nodes compared to peripheral blood.\textsuperscript{27}

Spleen and bone marrow may also be important HIV reservoirs with studies showing increased splenic inflammatory activity in HIV infected individuals compared to non-infected individuals.\textsuperscript{30} Abnormalities in hematopoietic progenitor cells due to bone marrow infection and the establishment of latent HIV infection have also been reported.\textsuperscript{31} Not much is known about the importance of the thymus as an HIV reservoir but \textit{in vitro} infection of thymocytes by HIV has been reported.\textsuperscript{32} \textit{In vivo} studies have also shown that HIV can infect the thymus and is accompanied by increased activation and depletion of CD4+ T cells.\textsuperscript{33} Animal model studies using bone marrow-liver-thymus humanized mice demonstrated that ARV drugs adequately penetrated the human thymic organoid but did not eliminate HIV replication in
this tissue or other tissues including the spleen and lymph nodes, further highlighting these tissues as possible reservoirs and sources of persistent viremia during HAART.

**Gastrointestinal tract**

Just as the genital tract mucosa is important in HIV infection establishment and spread during heterosexual transmission, the gastrointestinal tract (GIT) mucosa plays a major role in rectal HIV transmission as a portal of entry and viral spread, and in mother-to-child HIV transmission through the intestinal mucosa of the child during breast feeding or the swallowing of contaminated blood and fluids during birth. It is important to note that anal intercourse has a significantly higher risk of an individual contracting HIV per coitus act when compared to vaginal intercourse, demonstrating a high infection susceptibility of this mucosal membrane. The GIT contains a high proportion of the total lymphocytes in the body through the GALT which also harbors numerous innate immune cells such as macrophages and DCs, making this a favorable site for both HIV acquisition and replication. Prolonged immune activation results in depletion of GALT lymphocytes early in infection and levels remain depressed for the duration of the disease with incomplete restoration during HAART despite undetectable virus levels in blood. Early initiation of HAART also does not seem to completely restore and prevent CD4+ T cell activation and depletion in the GALT, although it may aid in maintaining homeostasis in the GALT mucosa.

HIV persistence in GALT even after 10 years of therapy has been reported with evidence of peripheral blood mononuclear cell infection from this reservoir. As a major site of HIV replication, a mechanism underlying HIV persistence in the GIT and GALT may be the persistent immune activation caused by antigenic stimulation of resting T and B cells.
resulting in their proliferation and increased turnover providing HIV with a constantly available source of susceptible cells.\textsuperscript{41} In addition to the CD4+ T lymphocytes, the GIT macrophages and follicular dendritic cells may also significantly contribute to the reservoir size of the GIT. Considering the large size of the GIT-associated mucosa these cells have been reported as a significant source of viral RNA in this reservoir.\textsuperscript{42}

Genotypic and phenotypic differences may exist between GIT and blood viral isolates,\textsuperscript{43, 44} which supports the idea of independent evolution in the GIT compartment. However more recent studies are reporting conflicting results with some finding less evidence of viral evolution and compartmentalization in the GALT over time in chronic HIV patients and in patients initiating HAART during acute HIV infection,\textsuperscript{45, 46} suggesting suppression of viral replication in the GALT during HAART therapy. Some however maintain that persistent HIV replication in the GIT may be occurring but there is dissemination of the virus to the peripheral blood resulting in equilibrium between these compartments.\textsuperscript{45} This is supported by a recent study which showed that viral quasispecies compartmentalization between the gut and peripheral blood exists early in infection, but that this compartmentalization is lost as the disease progresses.\textsuperscript{47} Interestingly, a study following the longitudinal course in patients administered HAART during primary infection, showed through viral sequencing that the gut mucosal reservoir was not the major source of rebound virus when treatment was interrupted and that these variants did not rapidly reseed existing GALT reservoirs.\textsuperscript{50}

**Central nervous system**

Different routes have been proposed by which HIV can invade the brain and these include access through the blood-brain barrier, the choroid plexus and the CSF.\textsuperscript{51, 52} On entering the CNS, HIV has access to susceptible microglial cells and macrophages which express the CD4
primary receptor and CCR5 chemokine co-receptor.\textsuperscript{53} Other CNS cells not bearing the primary CD4 receptor such as astrocytes can also be infected by HIV,\textsuperscript{54} but the mechanisms involved are still not well understood. Although post mortem samples have demonstrated the presence of viral DNA in microglia,\textsuperscript{55, 56} it is still not known if this proviral DNA can be activated to produce replication-competent virus and act as a viral reservoir.

Viral populations in the CSF and blood are nearly identical early in primary HIV infection,\textsuperscript{57} but are substantially different at later stages\textsuperscript{58, 59} probably due to differences in selective pressures and the restricted exchange of genetic information between the blood and the CNS. The virus may remain detectable in the CSF ten years after initiation of therapy and there is strong evidence of the CNS acting as an HIV reservoir and site that supports low level replication.\textsuperscript{59, 60} Suboptimal ARV drug levels in the CNS most likely result in continued replication in this compartment leading to the selection for neurotrophic variants and development of neurological symptoms in patients with undetectable plasma virus levels.\textsuperscript{61 - 63} However drugs with better CNS penetration seem to result in improved outcomes.\textsuperscript{64, 65} Continuous HIV replication in the CSF as the main mechanism of viral persistence has, as for the GIT, been disputed because of lack of evidence of viral evolution in the CSF.\textsuperscript{59} In contrast, a recent study reported independent replication detected exclusively four months after infection in 20% of analyzed samples that compared paired blood and CSF HIV populations.\textsuperscript{66} The identity of the CNS cells that are infected and harbor the virus early in infection and the cells which later act as a reservoir, and the effect of this reservoir after treatment interruption require further study to obtain conclusive answers. It has been established that infection of the CNS and the brain results in microglial and astrocyte activation and is associated with various neurological syndromes such as HIV-associated dementia (HAD) and HIV encephalitis demonstrated at autopsy.\textsuperscript{67} However, as Honeycutt et
the challenge for more studies aimed at determining these processes is the difficulty associated with obtaining brain tissues leading to reliance on interpretations from post mortem tissues, CSF and animal models.

Respiratory tract

The respiratory tract has not received much attention as an HIV reservoir although HIV has been shown to infect alveolar macrophages and T lymphocytes,70–72 and the virus has long been demonstrated in both cell free bronchoalveolar lavage and bronchoalveolar cells.73, 74 Primate studies indicate that the lungs are infected during primary infection,75 and have high viremia during HAART.29 The importance of HIV in the respiratory tract is perhaps best demonstrated by the high number of pulmonary infections including opportunistic infections and lung disorders that affect HIV positive individuals, an indication that HIV impairs cellular immunity in the lower respiratory tract.70, 76 Lung infections by other microorganisms may also act as the initial cause of HIV spread to this compartment as HIV infected lymphocytes from the blood stream move to the lungs in response to these infections. The lymphocytes would then infect alveolar macrophages through direct cell-to-cell spread in the lungs. Macrophages are the most common cell type in the lungs and have favorable characteristics to act as the major HIV reservoir in this compartment, which includes their resistance to apoptosis and reduced bioavailability of ARVs compared to T lymphocytes.77, 78 Discordance in drug resistance patterns in the HIV reverse transcriptase and protease genes have been reported between bronchoalveolar lavage fluid and plasma,79 and HIV evolution in the lungs was detected in 56% of individuals by comparing the C2- V5 region of the envelope gene in sequences from paired blood and lung samples.80 HIV evolution in the lung may be occurring independently as shown by these cited examples. However, there is no barrier to viral trafficking and leaking to the blood due to the closeness of the circulatory and
cardiopulmonary systems, which may limit the importance of the lungs as a strictly compartmentalized HIV reservoir.

**Liver**

There is recent evidence to classify the liver as an anatomical reservoir. Independent HIV replication in the liver has been supported by identification of a number of unique amino acid mutations in HIV variants from the liver.\(^84, 85\) HIV infection has also been associated with liver damage and has been shown to interfere with the classic Hepatitis B virus serology patterns in HIV-HBV co-infected individuals.\(^86, 87\) There also seems to be a relationship between HIV viral load and liver damage in patients with no hepatitis virus co-infection.\(^88\)

The virus can infect and replicate in several liver cell types including Kupffer cells, endothelial sinusoidal cells, stellate cells and hepatocytes.\(^89-91\) Hepatotoxicity of antiretroviral drugs may necessitate administration of lower dosages for patients with severe hepatic insufficiency which may result in persistent replication in the liver of such patients.

**Kidney**

HIV infection of renal tubular epithelial cells has been detected in patients on HAART,\(^93\) and infection of kidney allografts after transplantation was also detected in 13 out of 19 HIV positive recipients with undetectable plasma HIV RNA at transplantation.\(^94\) HIV DNA was detected in biopsies done 3 months after transplantation and was still detectable in biopsies done 12 months after transplantation. This could demonstrate insufficient drug levels in the graft and highlights the kidney cells as potential HIV reservoirs in virally suppressed individuals on HAART. Analysis of env sequences in paired PBMC/plasma and urine pellet/urine samples from viremic patients with normal kidney function showed distinct
compartmentalization of virus in the urine,\textsuperscript{95} highlighting the potential for HIV to independently evolve in the genitourinary tract.

**Cellular reservoirs**

Resting memory CD4+ T lymphocytes are the major cellular HIV reservoir and this source is considered a major barrier to HIV eradication during HAART.\textsuperscript{96} These cells harbour inactivated HIV proviral DNA which persist for long periods despite treatment. The latent CD4+ T lymphocyte reservoir contains variants which are mainly CCR5 tropic,\textsuperscript{97} indicating early establishment and lack of further evolution. This reservoir is suspected to be the source of persistent low level viremia in patients on HAART who otherwise seem to have suppressed the virus.

Macrophages may also be a source of persistent HIV viremia during HAART although their role is still controversial. These cells can withstand the cytopathic effects of viral infection which gives them an extended period of existence during which they produce and release large numbers of viruses. Viruses produced by macrophages also seem to be different in terms of infectivity and response to HAART probably due to reduced ARV uptake with a probability of independent evolution in these cells.\textsuperscript{77, 98} Follicular dendritic cells are also a potential cellular reservoir as they can trap infectious HIV on their surfaces for months and lead to CD4+ T lymphocyte infection.\textsuperscript{99}

Cellular reservoirs in the brain may include non CD4+ cells such as astrocytes and microglia.\textsuperscript{56} HIV replication in astrocytes is known to be very low and only a few astrocytes are infected,\textsuperscript{100} but since astrocytes are an abundant cell type in the brain, the infection of a small percentage of these cells will have significant results in terms of neurotoxicity and brain
cells damage. The slow turnover of glial cells also means that infected cells could potentially harbor the virus for up to several years.\textsuperscript{101, 102} In the study by Thompson et al,\textsuperscript{56} HIV DNA was detected in macrophages, astrocytes and microglia cells from treatment-naïve individuals who died from non-HIV related causes with no symptoms of HIV Encephalitis (HIVE) showing that the reservoirs are established before the onset of HIVE. It is still not clear which of the brain cells act as a viral reservoir but they all seem to have a part to play.

HIV can also infect hematopoietic precursor cells which include mast cell progenitors, multipotent hematopoietic progenitor cells (HPCs), and monocytes with suggestions that HPCs can act as a cellular reservoir.\textsuperscript{31, 103 - 105} A study in which CD133+ HPCs were shown to harbor HIV genomes in virally suppressed individuals who had been on HAART for more than 8 years supports that HPCs can act as a long term viral reservoir,\textsuperscript{106} although other studies could not detect HIV infection of HPCs.\textsuperscript{107, 108}

**Mechanisms of HIV persistence, immune cell trafficking and HIV spread**

Figure 2 shows how HIV spreads to various tissues and persists in anatomical reservoirs despite HAART. Several underlying mechanisms have been suggested to contribute towards ongoing low levels of HIV replication and HIV persistence during HAART. Understanding the contributions of different cellular reservoirs and anatomical compartments to viral persistence is very important for developing strategies to reduce the size or eliminate the HIV reservoir. Some strategies that have been used or have been considered to reduce the reservoir size include early therapy initiation,\textsuperscript{110, 111} therapy intensification,\textsuperscript{112 - 114} stem cell transplants and gene therapy,\textsuperscript{115, 116} and the use of latency reversing agents that target proviral HIV genome activation,\textsuperscript{117} or cytotoxic CD8+ T lymphocytes and broadly neutralizing antibodies that suppress HIV viral replication after the cessation of therapy,\textsuperscript{118, 119} but much work needs
Figure 2: Immune system cell trafficking and HIV spread. Suboptimal drug concentrations in anatomic reservoirs due to penetration barriers and efflux pump mechanisms together with chronic immune activation, presence of HIV latently infected and long lived cells and T cell homeostatic proliferation allows HIV to replicate and persist despite ART. Virus cell-to-cell spread in these tissues also protects the virus from the effects of drugs in the extracellular environment and allows for more efficient infection of new cells. Green arrows show HIV and immune cells trafficking and blue arrows show ARV drugs movement. (CVF: Cervicovaginal fluid, AZT: Zidovudine, d4T: Stavudine, DDI: Didanosine, 3TC: lamivudine, NVP: Nevirapine, IDV: Indinavir, RGV: Raltegravir, ATV/r: Atazanavir, DRV: Darunavir, LPV: lopinavir).
to be done to test and advance successful and clinically viable options that can be implemented on a large scale.

Latency in resting CD4+ T lymphocytes
Models that explain HIV latent infection in resting CD4+ memory T cells have been proposed. The pre-activation latency model proposes that subsets of resting CD4+ T lymphocytes can be directly infected by the HIV. The post-activation latency model suggests that activated CD4+ memory T cells are infected but escape cell death and return to the resting state. After infection of CD4+ T cells, mechanisms which result in HIV latency include DNA hyper-methylation and modification of histone proteins and DNA in the HIV long terminal repeat (LTR) which arrest HIV transcription. Other mechanisms include suppression of host transcription factors in resting CD4+ T lymphocytes and low levels of the transactivator of HIV gene expression (Tat) protein in these cells. The role played by the site of HIV integration into the host genomic DNA in affecting transcription rates may also be important. The latent provirus can through mechanisms and stimuli that have not as yet been identified be reactivated and produce infectious virions.

Cell-to-cell spread
HIV cell-to-cell transmission as the major method of HIV dissemination was established after investigators realized that shaking of lymphocyte cultures in vitro slowed down HIV replication compared to stationery cultures. In cell-to-cell spread there is a higher chance for a virion to successfully attach to a new cell and cause infection as it does not have to diffuse through the extracellular space to infect new cells, and the virus is also shielded from the effects of antiviral drugs in the extracellular space. Reduced cellular drug sensitivity has been reported in cell-to-cell virus transmission as the cause for ongoing viral replication.
and persistence during HAART. However, using cocktails of antiretroviral drugs is more potent against cell-to-cell HIV transmission when compared to the same drugs used individually which supports the effectiveness of combined ART. This also highlights the importance of reaching optimum drug concentrations at all replication sites in different anatomic and cellular reservoirs.

Incomplete drug penetration

Sub-optimal levels of some commonly used ARV drugs in different anatomical reservoirs may also serve to drive persistent viral replication and the development of drug resistant strains in patients on HAART. Compartmentalization provides an environment for independent viral evolution which negatively affects therapy progression and effectiveness. The compartment becomes a source of persistent viremia or circulating low-level minority drug resistant variants which can be seeded from these sites.

Chronic immune activation and T cell homeostatic proliferation

One of the challenges of HIV is the accompanying persistent immune activation characterized by the release of proinflammatory cytokines and this is also thought to fuel HIV replication and persistence. Persistent inflammation and depletion of gut-associated CD4+ T cells breaks down the integrity of the gut mucosa leading to translocation of bacterial products and prolonged immune activation. Immune activation is reduced during HAART but normal levels are not achieved leading to continued proliferation of CD4+ T cells. The result is persistence of a genetically stable HIV reservoir driven by interleukin-7-mediated low level homeostatic proliferation of infected central memory T cells.
Measuring the HIV reservoir size

While CD4+ T cell counts and plasma viral loads have been effective in monitoring treatment progress, these biomarkers do not measure the magnitude of replication-competent HIV genomes in latently infected cellular reservoirs. An important assay has been the single copy assay which provides an indication that the virus is suppressed but not eradicated. However there is no standard assay for measuring HIV reservoirs although assays that can measure various reservoir markers and their sizes or the ability of the latent reservoirs to be activated exist.\textsuperscript{133} Some assays that have been used include the quantitative viral outgrowth assay (Q-VOA),\textsuperscript{134, 135} which is considered to be the gold standard assay and PCR based methods that measure HIV DNA.\textsuperscript{136 – 138} The disadvantage of PCR based assays is that viral genome quantification using these methods includes genomes that are not replication competent and overestimates the HIV reservoir. RNA: DNA ratio has been used to act as an indicator of the number of infected cells harbouring replication competent HIV genomes.\textsuperscript{139} On the other hand Q-VOA assays, which measure the frequency of T cells carrying integrated replication competent HIV genomes that can be stimulated to reverse latency tend to underestimate the latent reservoir.\textsuperscript{140} Overall, available current assays are inadequate to accurately measure the viral reservoir and confirm curative therapy even when used in combination. This was demonstrated in the cases of the Mississippi baby,\textsuperscript{141} and in two adult patients,\textsuperscript{116} who all eventually rebounded after periods of cessation of HAART and undetectable virus levels confirmed using a battery of assays from different laboratories with samples obtained from different body tissues and compartments. The development of sensitive assays that can accurately measure the HIV reservoir therefore remain an important goal as they will be needed to evaluate the effectiveness of strategies aimed at eliminating the reservoir.
Discussion and Conclusions

Anatomic reservoirs are established early in primary infection and have an important role in persistent viremia during HAART. The virus hides in these immune privileged anatomic sites which are poorly penetrated by ARV drugs. Eradicating HIV directly from the reservoir sites has been difficult to achieve to date but new strategies targeting activation of the resting cells to induce expression of the HIV genome, which can then be targeted by the immune cells or HIV drugs while at the same time limiting immune activation are being explored. Histone deacetylase inhibitors such as vorinostat and panobinostat which are able to induce viral RNA production in latent cells, and recombinase enzymes developed to identify patterns within the HIV Long Terminal Repeats and remove the sandwiched HIV DNA, are some of the strategies that are still being investigated. In addition there are recent reports on use of vaccines as treatment in combination with HAART. The HIV-1 Tat protein was used as an immunogen to intensify HAART efficacy by restoring the immune function to levels capable of reducing the viral reservoir. Another study used CD4-mimetic compounds to sensitize HIV infected cells, which may be important in targeting cellular reservoirs that then may potentially be eliminated through antibody-dependent cell-mediated cytotoxicity as a host mechanism.

Anatomic reservoirs also play a major role in viral evolution which may frustrate drug and vaccine development efforts. Compartmentalization leads to restrictions in genetic flow which may lead to distinct homogenous populations in different tissues within a host. Understanding intra-host HIV diversity and evolution is therefore important to understand disease progression, improving response to HAART, limiting drug resistance and designing of more efficient therapies to reduce or eliminate the HIV reservoir. The extent to which the viral reservoirs contribute in the persistence of HIV during ARV treatment and the contribution of
different reservoir sites to the reservoir size need to be determined. Possible continued viral replication in these sites and seeding of low level drug resistant variants into the circulation highlights the need to test and monitor for the presence of minority drug resistant variants which may be originating from these sites. In conclusion, continued studies to better characterize intra-host HIV-1 diversity and evolution in different tissues are necessary in order to characterize all possible HIV reservoirs and to facilitate the development of new methods and drugs for targeting these reservoirs.

**Competing Interests**

The authors declare that they have no competing interests

**References**


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