HIV reservoirs \textit{in vivo} and new strategies for possible eradication of HIV from the reservoir sites

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Abstract: Even though the treatment of human immunodeficiency virus (HIV)-infected individuals with highly active antiretroviral therapy (HAART) provides a complete control of plasma viremia to below detectable levels (<40 copies/mL plasma), there is an unequal distribution of all antiretroviral drugs across diverse cellular and anatomic compartments \textit{in vivo}. The main consequence of this is the acquisition of resistance by HIV to all known classes of currently prescribed antiretroviral drugs and the establishment of HIV reservoirs \textit{in vivo}. HIV has a distinct advantage of surviving in the host via both pre- and postintegration latency. The postintegration latency is caused by inert and metabolically inactive provirus, which cannot be accessed either by the immune system or the therapeutics. This integrated provirus provides HIV with a safe haven in the host where it is incessantly challenged by its immune selection pressure and also by HAART. Thus, the provirus is one of the strategies for viral concealment in the host and the provirus can be rekindled, through unknown stimuli, to create progeny for productive infection of the host. Thus, the reservoir establishment remains the biggest impediment to HIV eradication from the host. This review provides an overview of HIV reservoir sites and discusses both the virtues and problems associated with therapies/strategies targeting these reservoir sites \textit{in vivo}.

Keywords: HIV, HAART, reservoirs, compartmentalization, elimination strategies for reservoirs, AIDS

Introduction

Since the discovery of human immunodeficiency virus (HIV) type 1 and the recognition that HIV is the causal agent of acquired immunodeficiency syndrome (AIDS) in 1983,\textsuperscript{1} over 25 million people have died of AIDS. It is estimated that 33 million people are now currently living with the deadly disease.\textsuperscript{2} Among the 33 million infected 92.7\% are adults, 46.4\% are females, and 7.5\% are children. In the year 2007, the World Health Organization estimated that 2.7 million people were newly infected with HIV and a further 2 million people died of AIDS-related diseases that year. Of the 33 million currently living with AIDS, 68\% of the individuals are living in the sub-Saharan Africa. Following that, South East Asia is the next largest region affected by HIV and accounts for an estimated 12\% of the globally infected population. Individuals with AIDS, who live in North America, Latin America and Eastern Europe to central Asia, account for an estimated 4\% each. It is therefore not surprising that the majority of people living with HIV inhabit the world’s poorest regions. This is due to the lack of access to HIV prevention and treatment among the poor and marginalized populations.\textsuperscript{3} This has resulted in the continual dissemination of the virus among marginalized populations.
The introduction of highly active antiretroviral therapy (HAART) has provided HIV patients with a prolonged life and has led to a considerable decline in co-morbidities and mortality in HIV patients. Even though a significant proportion of HIV-infected individuals receiving antiretroviral treatment can achieve below detectable limits (BDL) of plasma viremia (<40 copies/mL), the acquisition of drug resistance by HIV to practically all currently prescribed classes of antiretroviral drugs is the biggest impediment to the clinical management of HIV patients and the successful outcome of antiretroviral therapy.

HIV can continue to replicate at very low levels even during effective HAART. Persistent infection of nonactivated T-lymphocytes (where low levels of viral replication occurs) has been detected in individuals on successful HAART with BDL. Thus, it is apparent that HAART exerts considerable selection pressure on HIV, which provides partial restoration of the host immune system. In this tussle HIV is a winner, as it is able to conceal itself by establishing reservoir and sanctuary sites, which show disparate and suboptimal drug penetration. As a consequence the suboptimal drug concentrations, that reside in these diverse reservoir sites, encourage the emergence of drug-resistant HIV strains. In addition, HIV is also able to safeguard its survival in the face of HAART and strong host immune selection pressure through proviral integration into the host genome. This provirus being inert to the host immune system is ideal for latent reservoir establishment in vivo. Latent cellular reservoirs may hinder attempts at HIV eradication. Latency is a common feature of all retroviruses and is one of the most important host cell-virus interactions whereby the virus can survive and persist in the face of the immune response and antiviral therapy. Of major concern is the re-emergence and spread of HIV-1 variants from reservoirs after the withdrawal or failure of HAART because the emerging variants are as fit as, or in some cases fitter than the wild type virus.

Although much has been learnt about the mechanisms and sites of HIV persistence and reservoir establishment during drug therapy, the failure to eliminate latent viral reservoirs is a major shortcoming of the current HAART regimens. New strategies are being developed to flush virus out of these reservoirs, which may provide the complete eradication of HIV in vivo in the future.

The present review is focused on HIV reservoirs and the eradication of HIV from them. It attempts to provide a comprehensive overview of what we have achieved so far in the understanding of HIV reservoirs, strategies to overcome the establishment of and viral elimination from these reservoirs; in addition to approaches and products in the pipeline for eliminating HIV from these reservoir sites.

**Definition of a viral reservoir**

A viral reservoir is a cell type or anatomical site, where a replication-competent form of HIV can accumulate and persist stably. The definition of a viral reservoir should have two vital elements for it to be termed “reservoir”. To be biologically significant, a reservoir must preserve some replication viable virus, which can provide for the replenishment of the population of infected cells in the future. The second element for qualifying as a viral reservoir is stability; where viruses can persist either as virions or in infected cells in order to safeguard the future continuation of viral progeny in the host.

**Tissue and cellular reservoirs of HIV**

Two major reservoirs for HIV have been recognized: anatomical and cellular. A viral reservoir may be defined as an anatomical site or cell type in which a replication-competent form of HIV persists, accumulating with more stable kinetic properties than the circulating pool of actively replicating virus. A reservoir can be classified as latent, if the infected cells are not producing virus but retain the capacity to do so. HIV has been shown to target many different cell types, and a number of major tissue reservoirs of the virus are evident within infected individuals. The ability of the virus to colonize diverse cellular and tissue targets contributes strongly to persistence in the setting of HAART, and is responsible in part for the emergence of antiretroviral drug resistance.

**Tissue reservoirs**

A number of anatomical sites may act as reservoirs of HIV replication including the lymphoid tissue, gastrointestinal tract, brain/central nervous system (CNS), genital tract, semen, and the lung. These structures are immunologically sheltered or separated by a barrier from the blood and lymphoid systems. Some anatomical sites may be non-permissive to immune surveillance and effective drug penetration, thus serving as potential sites of persistent HIV replication eg, the respiratory, gastrointestinal, and reproductive tracts. These reservoirs are either established early in the course of HIV infection or during HAART. Differential viral evolution in these sites as compared to the blood compartment has been observed, and may be indicative of the significance of these sites to whole body HIV...
replication.\textsuperscript{23} The optimal delivery of antiretroviral therapies to these different anatomical sites is also important in the context of both viral eradication and preventing the emergence of drug-resistant HIV variants.

Peripheral lymphoid tissue

HIV generally enters the human host via mucosal surfaces and is subsequently disseminated throughout the lymphatic tissues, which then become a major reservoir of virus throughout the course of infection.\textsuperscript{17–19} Initial colonization of lymphoid tissue is thought to be mediated by dendritic cells (DCs) and macrophages, which associate with the virus at the mucosal surfaces and transport it to the lymph nodes where the initial infection of CD4\(^+\) T lymphocytes occurs. Although lymphoid tissues are considered the primary site of CD4\(^+\) T cell infection over the course of the disease, follicular dendritic cells (FDCs) are the major source of viral RNA in lymphoid tissue. Measurement of viral pools in lymphoid tissue during the asymptomatic phase of infection has revealed an extremely large FDC pool of approximately 1011 copies of viral RNA, exceeding the viral RNA in productively infected cells by more than 50 fold.\textsuperscript{20} The level of HIV-1 RNA in virions associated with FDCs is also two orders of magnitude greater than plasma levels during this phase of infection.\textsuperscript{20} This indicates that the measurement of plasma HIV levels greatly underestimates the viral burden and that lymphoid tissue is a major reservoir site where HIV is stored. In the later stage of infection, there are comparable quantities of virus stored in the FDC pool,\textsuperscript{21} although earlier in infection the FDC pool is somewhat smaller and closer in size to the viral load in productively infected cells. In addition to viral burden, lymphoid tissue is the major site of HIV production in the body.\textsuperscript{20,21}

The pattern of productive infection in lymphoid tissue is consistent with cell-to-cell transmission from one cell to another activated T cell in its vicinity.\textsuperscript{22} The localized nature of virus production in lymphoid tissue is implied by observations of foci of infected cells,\textsuperscript{17} and evidence of founder effects in viral populations derived from single white pulps.\textsuperscript{22,23} The inhibitory effect of HAART on both viral replication and the levels of virus in the blood and in lymphoid tissues is well documented, and has been consistent over a number of studies.\textsuperscript{21,24–29} Little or no viral RNA is detectable in the FDC pool, in total RNA extracted from lymphoid tissue, or in cells isolated from lymphoid tissue, when plasma viremia is suppressed to below detectable levels. However, chronically and latently infected CD4\(^+\) T cells persist\textsuperscript{24} and represent potential focal points of viral reproduction upon cessation of therapy.\textsuperscript{2}

In addition, chronically infected cells and replication of less fit drug-resistant viruses result in the continued evolution of genotypic drug resistance.\textsuperscript{27}

The gastrointestinal tract

The gastrointestinal tract mucosa is the largest lymphoid organ in the body.\textsuperscript{22} Due to a large population of activated CXCR4\(^+\) CCR5\(^+\) target cells, the gastrointestinal mucosa represents a favored target for HIV-1 infection\textsuperscript{29} and appears to support enhanced HIV-1 replication in comparison to other body compartments.\textsuperscript{30–32} With the exception of parenteral transmission, almost all vertical and homosexual transmission of HIV occurs via the gastrointestinal tract. Virus inoculated into the gastrointestinal tract of the fetus or infant is likely to enter the gut associated lymphoid tissue through tonsillar and/or upper intestinal mucosa. Homosexual transmission of the virus occurs through orogenital or anogenital contact. The efficiency of HIV transmission across the gastrointestinal mucosa depends upon factors including: donor blood viral levels;\textsuperscript{33,34} the pre-existence of genital tract infection and local inflammation;\textsuperscript{35} mucosal integrity; genetic predisposition; and behavioral factors. Potential cellular routes in the translocation of HIV across the epithelium include: mucosal cells,\textsuperscript{36,37} DCs,\textsuperscript{38} and epithelial cells.\textsuperscript{39} Epithelial cells appear to be the most predominant viral transfer mechanism, selectively transporting R5 tropic viruses, which are the predominant phenotype of the majority of transmitted viruses.\textsuperscript{39}

The gastrointestinal mucosa contains a large proportion (40%–60%) of the body’s lymphocyte population\textsuperscript{40} and is the largest reservoir for macrophages in the body.\textsuperscript{41} Furthermore, the majority of mucosal CD4\(^+\) T lymphocytes are of the activated memory phenotype\textsuperscript{42} providing a ready target for HIV-1 infection.\textsuperscript{43} This heightened state of cellular activation arises from elevated levels of pro-inflammatory cytokines released in response to foreign antigens and bacterium. Several studies have demonstrated that in addition to similar levels of CXCR4 expression, mucosal mononuclear cells have a higher predominance of CCR5 expression than peripheral blood cells, thus increasing susceptibility to infection by primary HIV-1 isolates.\textsuperscript{44} Although large numbers of intestinal macrophages exist in the subepithelial lamina propria, these differ markedly in phenotype and function from blood monocytes,\textsuperscript{45,46} and are poorly permissive to HIV infection.\textsuperscript{47} Although the prevalence of HIV-infected macrophages in the mucosa is low (0.06% of lamina propria mononuclear cells),\textsuperscript{48} the extremely large size of the gastrointestinal mucosa makes intestinal macrophages a prominent reservoir of HIV. In contrast, epithelial lymphocytes express CXCR4 and CCR5.
and support replication by X4 and R5 viruses, and it is likely that these are the initial target cell for HIV after upper gastrointestinal tract inoculation. From the mucosa, virus is disseminated to systemic sites initiating large-scale depletion of CD4+ T cells first in the intestinal lamina propria and subsequently in the blood.

The central nervous system

The CNS is a key anatomical reservoir of HIV-1 in both therapy-experienced and therapy-naive patients. The CNS is protected by two formidable barriers: the blood–brain barrier which tightly segregates the brain from the circulating blood, and the blood–cerebrospinal fluid barrier in the epithelium of the choroid plexus, which limits the passage of molecules and cells into the cerebrospinal fluid. This barrier-mediated separation of the CNS gives rise to an altered immunological environment, and also provides an obstacle to therapeutic measures such as antiretroviral therapy. For these reasons, the CNS represents a unique site for viral replication in HIV-infected patients, particularly in the setting of HAART.

HIV enters the CNS early in the course of systemic infection, and is present at all subsequent stages. As a result of CNS infection, patients may suffer from a range of clinical symptoms, the most notable being dementia. Prototype HIV-1 isolates from the CNS are typically R5 nonsyncytium inducing M tropic strains. Macrophages and microglia are the principle target cells for productive infection in the CNS. Both infection and activation of these cell types appears to be critical for the development of dementia.

Recently, HIV DNA has been detected in cortical and basal ganglia-derived astrocytes, and it is believed that astrocytes may serve as a viral reservoir in the brain. Gene microarray analysis has shown that HIV-1 alters the programme of gene expression in astrocytes, including changes in transcripts encoding cytokines. HIV infection also inhibits the capacity of astrocytes to clear glutamate, a feature likely to affect neuronal activity or survival and contribute to HIV neuropathogenesis. Although there is no firm evidence that neurons can support HIV-1 infection, recent experiments have demonstrated the ability of neurons to sustain transient productive infection for brief time periods.

The mechanisms of HIV-1 penetration into the CNS are uncertain. Some evidence exists that HIV-infected bone marrow-derived monocytes may transport HIV across the blood–brain barrier. Blood-derived microglia have also been suggested to transport HIV across the blood–brain barrier. A second putative mechanism of HIV entry into the CNS is a mechanism involving the passage of HIV from the blood to the cerebrospinal fluid, then into the choroid plexus and ventricles and thus into the brain. Supporting this, productive viral infection occurs in the choroid plexus, predominantly in stromal macrophages and DC. The incidence of HIV-1 infection in the choroid plexus exceeds that of the brain, and also occurs prior to the onset of AIDS and immunosuppression. It is thought that the greater vascular permeability of the choroid plexus may render it more susceptible to HIV-1 infection, and may facilitate heterogeneous dissemination of the virus to the brain. Supporting this, studies have shown that choroid plexus HIV sequences are admixtures of brain-and blood-derived isolates and are common in asymptomatic HIV-infected patients. Due to the presence of the blood–brain and blood–cerebrospinal fluid barriers, suppression of HIV populations in the CNS with antiretroviral drugs is problematic. Drug penetration into the CNS is poor, and the maintenance of effective drug concentrations is difficult. These are some of the problems influencing the treatment of HIV infection in the CNS.

Reproductive tract

Both the male and female genital tracts are potential reservoirs for HIV. Each has a distinct microenvironment that permits viral replication independent from the systemic circulation. The male genital tract possesses unique vascular features, including the blood–testis barrier, formed by specialized tight junctional complexes between Sertoli cells that prevent free ingress of substances from the testicular interstitium to the seminiferous epithelium. In addition to cell-free HIV particles, T lymphocytes and macrophages isolated from semen of HIV-infected men harbor provirus. A differential source of virus in semen and blood has been suggested by a number of studies, providing evidence that the male reproductive tract is a distinct reservoir of virus in HIV-infected men. An absence of correlation between plasma and semen viral loads has been reported by several investigators suggesting independent viral replication. HIV infection of the testis has been described, and CD4 is detectable on the cell surface of lymphocytes and macrophages infiltrating the testis, suggesting these cells are potentially infectable by HIV. HIV-infected cells of the lymphocytic/monocytic type have been found in seminiferous tubules and interstitium of the testis, and these cells were also observed in semen. Other possible targets of HIV infection in the testis include spermatagonia, spermatocytes and spermatids, and residual germ cells.
Multiple cells and tissues in both the upper and lower female reproductive tract are susceptible to HIV infection,\(^{79}\) and histopathological studies have clearly demonstrated the genital tract of HIV-infected women is an active site for viral replication, especially in the submucosa of the cervical transformation zone.\(^{80}\) HIV infection has been demonstrated in a range of tissues within the female genital tract, including: epithelial and stromal cells of the uterus and fallopian tube; cervix; and ectocervix.\(^{79}\) Virus in the genital tract of HIV-infected women also has its own dynamics that are partly independent of the systemic compartment, supporting the female genital tract as an HIV reservoir.\(^{80}\) Genital tract shedding has been found to occur in women on HAART with less than 500 viral copies per milliliter of plasma, suggesting a separate reservoir of HIV-1 replication.\(^{81}\)

**The lung and kidneys**

Both the lung and kidneys have been suggested to act as reservoirs of virus in HIV-infected individuals, however, the significance of these organs during the course of disease remains uncertain. HIV can be detected frequently and recovered from both alveolar macrophages and alveolar lymphocytes.\(^{82,83}\) HIV is most commonly recovered from the alveolar lymphocytes indicating that the virus is carried into the lungs by these cells as they migrate from the blood in response to opportunistic infections. Although distinct populations of the virus have been detected between the blood and lung, more homogeneous populations have been detected in the lung with low copy number suggesting a lack of replication. The low level of RNA detected in alveolar macrophages also suggests the lung is of limited importance as a site of HIV replication.\(^{83,84}\) However, larger amounts of HIV RNA have been detected in bronchoalveolar lavage fluid.\(^{85,86}\) Some discordance between drug resistance mutations in the RT gene of HIV from bronchoalveolar lavage fluid and plasma has also been reported.\(^{86}\)

The kidney represents another site of localized HIV replication, and has been an organ of particular interest due to the occurrence of HIV-associated nephropathy. Renal glomerular and tubular epithelial cells have been shown to harbor both HIV DNA and mRNA, suggesting productive infection.\(^{87,88}\) Circularized viral DNA has also been found in kidney biopsies suggesting active replication in renal tissue, although infiltrating infected leukocytes harbor more viral mRNA than renal epithelium.\(^{87}\) Productive HIV infection in renal epithelial cells was recently confirmed by in situ hybridization polymerase chain reaction (PCR), and phylogenetic analyses of kidney-derived sequences conducted in the same study revealed evidence of tissue-specific evolution when compared to those of peripheral blood mononuclear cells.\(^{88}\) The combination of productive and independent HIV infection in a localized manner within the renal tissue suggests the existence of a renal viral reservoir.

**Cellular targets and reservoirs**

A wide-range of cell types distributed amongst a number of different tissues are susceptible to HIV infection. CD4\(^+\) T lymphocytes\(^{89}\) and macrophages\(^{89}\) are the primary cellular targets of HIV. Other infectable cell types include: monocytes, CD8\(^+\) T lymphocytes; natural killer (NK) cells; peripheral blood and FDCs; B cells; and an array of specialized cell types derived from various tissue reservoirs of HIV (eg, renal, mucosal, and cervical epithelial cells; astrocytes and microglia in the CNS; skin fibroblasts; and bone marrow stem cells). However, the infection of a number of putative cellular HIV targets remains controversial, and a contribution to HIV pathogenesis is often unclear.

**CD4\(^+\) T lymphocytes**

The hallmark of HIV-1 infection is the progressive depletion of CD4\(^+\) T lymphocytes. CD4\(^+\) T cell tropic HIV strains are designated X4 viruses as they predominantly use the CXCR4 co-receptors for cellular entry. X4 viruses are associated with increased replication, and syncytium inducing infection.\(^{91}\) Reflecting this, HIV infection of CD4\(^+\) T cells is cytopathic and leads to extensive cell death.\(^{92}\) CXCR4 is widely expressed and present on both naive and memory CD4\(^+\) T cells.\(^{93}\) HIV entry and subsequent replication in CD4\(^+\) T cells requires cellular activation,\(^{94}\) which leads to an increase in the size of the nucleotide pool required for viral DNA synthesis and the level of transcription factors.\(^{94}\) The majority of CD4\(^+\) T lymphocytes in the blood are in a resting non-activated state and thus are not susceptible to infection.\(^{95}\) Despite this, CD4\(^+\) T lymphocytes are still the principle cellular reservoir of HIV.

HIV-1 infection of CD4\(^+\) T lymphocytes is thought to occur predominantly in lymphoid tissue, where there is close contact with other infected leukocytes including antigen presenting cells.\(^{19,96}\) The pattern of productive infection in lymphoid tissue is consistent with cell-to-cell transmission, from one cell to another activated T cell in its vicinity.\(^{22}\) Within lymph nodes, germinal centre CD4\(^+\) T cells represent an important site of HIV replication *in vivo*.\(^{97}\) Lymphoid tissue biopsies from HIV-infected patients have also suggested that CD4\(^+\) T cells remain the major source of HIV-1 production in the end-stage of the disease.\(^{98}\) The
gastrointestinal tract is a major site of CD4+ T cell depletion and HIV replication. A large proportion of the body’s lymphocyte population resides in the gastrointestinal mucosa, and the intestine contains an abundance of activated memory CD4+ T cells, which are favored targets of HIV infection. CD4+ T cells are also likely to become infected in the thymus, and the depletion of CD4+ thymocytes through the destruction of CD4+/CD8+ precursor cells may play a significant role in the pathogenesis of HIV-1 disease. Analyses have demonstrated the emergence of cytopathic and tissue-specific HIV variants in the thymus. Furthermore, CXCR4 expression is modulated during T-lymphoid differentiation such that immature thymocytes display an increased frequency and higher surface density of the coreceptor than do more mature cells. High levels of both primary receptor and coreceptor may therefore be responsible for the efficient infection of the thymus, and could in part account for the rapid disease progression observed in HIV-infected children, where the thymus is actively involved in the production of new T lymphocytes.

Much debate exists over the mechanisms of CD4+ T cell depletion by HIV. At the outset, it is important to realize that the fall in CD4+ numbers is not only a result of CD4+ T cell destruction, but also stems from impaired production. Early suggestions of cytopathic destruction of CD4+ T cells were complicated by observations that there were many more cells dying than there were infected cells. It was soon realized that a number of other mechanisms were responsible for CD4+ T cell death and the subsequent decline in numbers. Both the destruction of mature CD4+ T cells and the impaired production of new cells can be attributed to both direct and indirect effects of the virus.

One of the central immunological defects in HIV-infected individuals is a weak or absent CD4+ T helper proliferative response. When present these responses correlate directly with decreased plasma viremia. The clinical stages of HIV disease are also firmly linked with the CD4+ T cell dynamics over time during the infection. CD4+ T cell numbers decline sharply during the acute phase of infection, partially rise again and then slowly decline once more during the asymptomatic phase until counts fall below 200 cells per microliter of blood, which marks the progression to AIDS. The emergence of CD4+-tropic (X4) viruses is associated with rapid CD4+ T cell decline and disease progression. In the years after chronic infection is established, X4 strains emerge in approximately 50% of infected individuals. The association between X4 virus and acceleration of HIV-1 disease progression has been attributed to the expanded spectrum of CXCR4+ precursor cells susceptible to infection by X4 strains. It has also been postulated that the decline of the host immune system associated with clinical AIDS may allow X4 viruses to evolve and replicate more freely in later stages of infection. The establishment of latent HIV infection in resting memory CD4+ T cell subsets early in infection is of primary importance in the persistence and continued evolution of HIV, particularly in the setting of potent antiretroviral therapy.

Emerging concepts on central memory and transitional memory CD4+ T cells

New data shows that the central memory (TCM) and transitional memory (TTM) CD4+ T cells define major cellular reservoirs for HIV, where viral persistence occurs by two different mechanisms. Primarily, HIV persists in the TCM cells in subjects showing reconstitution of the CD4+ compartment upon HAART. The maintenance of this reservoir is through T cell survival and low-level antigen-driven proliferation, which is slowly depleted over time. In contrast, preferential detection of proviral DNA is a feature of TTM cells from aviremic individuals with low CD4+ counts and higher amounts of interleukin-7-mediated homeostatic proliferation. This mechanism ensures persistence of these cells in vivo. Chomont and colleagues have identified two viral reservoirs within the memory CD4+ T cell subsets of virally suppressed subjects. The TCM reservoir is the major long-lasting reservoir in immune responders to HAART. As TCM cells are characterized by their extremely low degree of cellular proliferation, and, because of their intrinsic capacity to survive for decades, these cells provide a long-lasting cellular reservoir for HIV-1.

The second paper, by Brennan and colleagues, uses genetic analyses of HIV sequences to show that there is a reservoir of virus that seems to be coming from a cell type other than memory CD4+ T cells. This study shows that in most cases, the residual virus detectable in individuals on suppressive ART is genetically distinct from the virus found in the memory CD4 T cells. Therefore, if much of the residual viremia in HIV patients on HAART arrives from other reservoir sites, as suggested, the eradication strategies need to be revisited for purging reservoir virus.

CD8+ T lymphocytes

Although debate still exists over the significance of HIV infection of CD8+ T lymphocytes, there is little doubt that HIV can productively infect these cells. Reports of CD8+ T cell infection have occurred on a regular basis since the
early stages of the epidemic. Initially, CD8+ T cell infection was described in vitro. These reports were followed by evidence that CD8+ T cells harbor and express HIV-1 in vivo, and may express small amounts of CD4+ RNA.

Much of the research conducted on the HIV infection of CD8+ T cells has focused on the mechanism of viral entry, and the origins of CD8+ cell infection. Evidence suggests that CD8+ T cells become infected through a conventional CD4-dependent mechanism during their maturation in the thymus, at the double-positive (DP) stage where CD4 is co-expressed with CD8. Implants of human thymic tissue containing infected DP thymocytes in severe combined immunodeficiency (SCID) mice have been shown to produce infected single positive (SP) CD8+ T lymphocytes in the peripheral circulation. HIV-1 proviral DNA is also preferentially distributed in the naïve (CD45RA-) subset of CD8+ T cells, further supporting the thymus as a source of CD8+ T cell infection.

In addition to possible intrathymic mechanisms, stimulation of highly purified CD8+ T cells with mitogens, allogeneic DC or anti-CD3 and anti-CD28 antibodies in vitro leads to de novo synthesis of CD4 and susceptibility to HIV-1 infection. Activated subsets of circulating CD8+ T lymphocytes express high frequencies of CD4 in vivo, rendering these cells vulnerable to viral destruction. Reports of CD8+ mediated CD4 independent entry into CD8+ T cells have also been provided by Saha and colleagues. Multiple CD8+ clones generated from patients with AIDS were characterized and it was found that several were CD8 single positive and endogenously infected by HIV. Subsequent biological characterization of these isolates demonstrated CD8-mediated cell entry without the requirement for any known chemokine coreceptor. Recent work by this group has lead to further characterization of CD4-independent entry.

In addition to clarification of CD8+ T cell entry mechanisms, several studies have focused on quantitative aspects of CD8+ infection by HIV. Several reports have sought to compare the abundance of HIV provirus in CD8+ cells with those of other leukocytes. It has been concluded that CD8+ T cells harbor substantial amounts of provirus. Livingstone and colleagues found that in the late stages of the disease, infection in CD8+ T cells accounts for between 66% and 97% of total proviral DNA; there is an inverse relationship between CD8+ T cell counts and the frequency of CD8+ T cell infection. Similarly, more recent reports have found that CD8+ T cells contain significant amounts of provirus. Additional studies have also attempted to clarify the distribution of proviral DNA in specific CD8+ T cell subsets. One report demonstrated preferential distribution of HIV in the naïve (CD45RA-) subset of CD8+ T cells compared to the memory/effector (CD45RO-) population. In contrast to all previous findings, one recent report has suggested that naïve and memory CD8+ T cells are rarely infected by HIV.

Currently there is debate over whether the infection of CD8+ T cells contributes significantly to the immunodeficiency observed in AIDS. This stems from the existence of a number of other possible mechanisms that could account for the numerical decline and functional impairment of CD8+ cells observed in the progression of the disease. These include increased susceptibility to apoptosis from alterations in the cytokine milieu in lymphoid tissue; bystander effects from neighboring productively infected CD4+ T cells, or toxicity from the release of HIV derived proteins such as gp120 or Tat. Loss of CD4+ helper function leading to impaired clonal expansion and function of CD8+ T lymphocytes on antigenic pressure also contributes. Thymic destruction of CD8 precursor cells has been proposed as an explanation for the eventual failure of CD8 homeostasis; the decline in circulating numbers of first naïve and then memory CD8+ T cells upon disease progression; and the recovery in naïve CD8+ numbers on commencement of antiretroviral therapy.

**Macrophages**

Macrophages represent a key target of HIV in addition to CD4+ T lymphocytes. Although CD4 surface expression on macrophages is lower than on CD4+ T cells, and the number of infected macrophages in the body is relatively low in comparison, macrophages still produce large amounts of virus. HIV in macrophages is produced on the complex surfaces between cells, on free surfaces, and in cytoplasmic vacuoles of the Golgi apparatus. In addition to producing new virions, macrophages spread viral particles to bystander CD4+ T lymphocytes through a fusion mechanism, and activation of the oxidative pathway in infected macrophages may also lead to apoptotic death of noninfected bystander cells.

Macrophages are a primary agent of viral dissemination with a widespread distribution, and are the principal infected cell type in many tissues (eg, CNS). Macrophage HIV isolates (M-tropic) are predominantly R5 strains, associated with slower and nonsyncytium-inducing infection. The infection of macrophages is predominantly mediated through CCR5, and M-tropic variants are...
preferentially transmitted.\(^{127}\) Although macrophages have been shown to express CXCR4,\(^{128}\) they are believed to impose a restriction on the replication of X4 virus at the intracellular level.\(^{129}\)

Macrophages provide a different environment for HIV that is distinct from the milieu in lymphocytes. In contrast to lymphocytes, macrophages are in a stage of terminal differentiation and have a limited potential for proliferation.\(^{130}\) They are insensitive to the cytopathic effect of the virus, and the long life span of infected macrophages probably compensates for their relatively low number, substantiating their contribution to viral production.\(^{131}\) Short-term dynamics in productively infected CD4\(^+\) T cells are characterized by a rapid exponential increase of virus replication followed by extensive cell death. In contrast, macrophages can produce and release high levels of the virus over an extended time period,\(^{132}\) categorizing them as chronically and persistently infected cells.\(^{5}\) Signal transduction pathways in macrophages differ substantially from those of T cells. Such pathways can be modulated by HIV and contribute to the regulation of cell susceptibility to infection.\(^{133}\) For example, different CD45 isoforms are expressed by naive and memory CD4\(^+\) T cell subsets. The signal transduction molecule p56lck is present on CD4\(^+\) T cells but absent on macrophages, correlating with CD4 endocytosis in macrophages.\(^{134}\) There are differences in the infectivity of viruses produced by macrophages,\(^{135}\) and limited nucleotide precursors in macrophages can result in an increased time period for viral replication.\(^{135}\) However, some studies have shown similar kinetics in both macrophages and lymphocytes,\(^{136}\) while others have demonstrated that primary isolates replicate at higher levels in macrophages compared to T cells.\(^{137}\)

Although macrophages are most likely to be a significant source of HIV during the second, slower phase of decay,\(^{138}\) it has been suggested that latent infection may be established in macrophages. Latently infected macrophages have been observed in large numbers throughout the lymphoid system from the early to late stages of infection.\(^{139}\) In addition, the unusual dynamics of replication in macrophages and the unique characteristics of these cells result in altered responses to antiretroviral therapy.

**Monocytes**

HIV infection of circulating blood monocytes was reported early in the epidemic.\(^{140}\) These initial reports made it unclear as to whether monocyte infection is latent or productive. Subsequent studies found levels of proviral DNA in monocytes to be relatively low or undetectable in comparison to T cell compartments.\(^{141}\) However, more sophisticated approaches using in situ hybridization coupled with simultaneous surface immunophenotyping revealed a higher incidence of monocyte infection, and demonstrated the production of viral mRNA in monocytes indicating productive infection.\(^{142}\) Despite the apparent production of viral mRNA, other in vitro studies indicated that HIV replication was blocked prior to reverse transcription and integration.\(^{143}\) Following this, replication-competent virus was shown to be recoverable from blood-derived monocytes upon stimulation and differentiation into macrophages.\(^{144}\)

Recent studies have investigated the levels of cell-associated viral RNA, mRNA and the genetic evolution of HIV over the course of infection.\(^{145}\) Monocytes isolated from blood have been shown to contain unintegrated circu-

lar viral DNA and multiply spliced RNA suggesting their infection is recent and transcriptionally active rather than latent.\(^{146}\) Zhu and colleagues observed that blood-purified monocytes harbored HIV DNA over time in both untreated and therapy-suppressed individuals.\(^{145}\) Viral decay in monocytes was considerably slower on average than that in activated and resting CD4\(^+\) T cells. In addition, the average half-life of HIV DNA in monocytes was considerably longer than the estimated mean intermitotic lifespan of monocytes and macrophages,\(^{138}\) suggesting renewal of the virus as a result of continued viral replication.\(^{146}\) A significant genetic evolution in monocytes was also observed, and in some individuals on suppressive therapy monocyte strains were phylogenetically linked with circulating plasma variants.\(^{145}\) These findings suggest that monocytes may constitute a continuing source of infectious virus during HAART regardless of the length of treatment.

The circulating monocyte population is heterogeneous consisting of several subsets. The majority express high levels of CD14 and little or no CD16, described as CD14hi.\(^{147}\) The normal pathways of monocyte migration and trafficking may also define a mechanism by which HIV is distributed to various compartments around the body. After leaving the bone marrow, monocytes remain in circulation for between two and four days before migrating through the endothelial walls of capillaries and undergoing differentiation into tissue macrophages. Alternatively they may differentiate into DCs and enter the lymphatic system. HIV-infected monocytes can thus migrate to a variety of different sites around the body, and are likely to be responsible for colonization and continued turnover in diverse tissue compartments such as the CNS.\(^{148}\) The early establishment of HIV infection in
monocytes and the ongoing replication and persistence of HIV in this compartment represents a considerable challenge for antiretroviral drug regimens.

Dendritic cells

Dendritic cells (DCs) are professional antigen-presenting cells that play a major role in HIV pathogenesis. Langerhans cells, a DC subset residing in epithelial surfaces such as the skin, act as one of the primary, initial targets for HIV infection. Myeloid DCs (MDCs) and plasmacytoid DCs (PDCs), also professional antigen-presenting cells, are additional targets of HIV. As part of the normal immune response, DCs capture virions at the site of transmission in the mucosa, and migrate to the lymphoid tissue where they are responsible for large-scale infection of CD4+ T lymphocytes. Thus, dendritic–T cell interactions in lymphoid tissue, which are critical in the generation of immune responses, are also a major catalyst for HIV replication and expansion. Exposure of HIV to DCs may also impair the antigen-presenting capacity of these cells resulting in inadequate expansion of HIV-specific T cell responses.

Langerhans cell progenitors are derived from the bone marrow,149 and home to epithelial surfaces where they remain in a resting or immature state until they encounter antigens. Immature Langerhans cells possess a highly active endocytic system for efficient antigen processing.150 Langerhans cells were the first DCs to be reported as target cells for HIV infection.151

Viral attachment to DCs occurs via cell surface interactions that are dissimilar to those of regular HIV infection. Viral attachment to the surface of DCs is mediated by C-type lectins including: DC-SIGN (dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin);152 langerin; and the mannose receptor.153 Viral attachment via these receptors can be followed by internalization and/or subsequent transfer to permissive T cells.154 Internalized HIV can be stored in early endosomal compartments without degradation, while surface-associated HIV can remain infectious for several days and can be transmitted to CD4+ T cells.152,154 The migratory nature of DCs and their ability to recruit numerous T cells to lymphoid tissue identifies them as strong candidates for a central role in spreading HIV within the host.155 A large number of DCs accumulate rapidly in the lymphoid tissue in the first weeks after infection,156 coinciding with a massive increase in the numbers of productively infected CD4+ T cells in the lymphoid tissue.157 In addition, HIV replicates in clusters of DCs and CD4+ T cells in lymphoid tissue throughout the course of the disease.158 Reflecting this, the FDC network in lymphoid tissues is a major site of HIV storage in both presymptomatic and the late stages of disease, and FDCs are recognized as the major source of viral RNA in this compartment.16

B cells

During infection with HIV, a large number of virions are detectable in the follicular areas of lymphoid tissues. Most of this virus is trapped on the surfaces of FDCs as immune complexes along the network of dendrites, which play a critical role in antigen mediated interactions with both T cells and B cells.159 FDC networks in the germinal centers account for the bulk of HIV RNA.160 It has recently been demonstrated that B cells in both lymphoid tissue and the peripheral blood carry replication competent virus attached on their surfaces.160 The mechanism of B cell binding to the virus is similar to that of DC, involving the binding of HIV-containing immune complexes to CD21 on the B cell surface.160,161 Virus bound to B cells can efficiently infect activated CD4+ T cells through cell-to-cell contact,160 and HIV in the form of immune complexes bound to B cells is more stable and more efficiently passed on to CD4+ T cells than free viral particles. These studies led to the suggestion that B cells may represent an important link between trapped virus on FDCs and virus replicating in CD4+ T cells.

A more recent study examined the genetic relationships between HIV bound to B cells and viruses in both CD4+ T cells and plasma.162 HIV bound to peripheral blood B cells was found to be closely related to virus in CD4+ T cells, and more divergent from virus in plasma.162 HIV bound to lymph node-derived B cells and CD4+ T cells were similar, and showed an equivalent divergence from HIV bound to peripheral blood B cells. In addition, both were more distantly related to circulating CD4+ T cells.162 These findings gave evidence of a close association between virus circulating on B cells and virus replicating on CD4+ T cells, and a significant degree of trafficking between viruses from B cells and CD4+ T cells of the lymph nodes. Thus it is likely that in addition to FDCs, B cells may also contribute to ongoing replication by serving as an extracellular reservoir of HIV.

Natural killer cells

Natural killer (NK) cells are defined phenotypically as a CD3 negative lymphocyte subset that functions as a first line of immune defense. NK cells express surface
markers including CD56, CD16, CD57, NKR-P1, however these markers may also be expressed on T lymphocytes.\textsuperscript{163} The majority of NK cells can be discriminated on the basis of CD56 expression, with the majority being CD56dimCD16\textsuperscript{+}, which have a higher cytotoxic potential. The smaller CD56brightCD16dim/neg NK cell subset has higher cytokine producing potential. Resting CD56bright and CD56dim subsets also show differences in their expression of NK receptor repertoires.\textsuperscript{164} NK cells are pivotal in innate immunity against viruses and tumors. They can spontaneously kill virally infected cells without prior antigen stimulation and thus participate in early immune defense mechanisms prior to the establishment of adaptive responses. NK cells have direct cytotoxic and also perform non-cytolytic functions such as the secretion of cytokines which later modulate adaptive responses.\textsuperscript{164}

Recently a subset of CD56\textsuperscript{+} CD16\textsuperscript{+} CD3\textsuperscript{-} NK cells was identified which express both CD4 and the chemokine coreceptors CCR5 and CXCR4.\textsuperscript{165} This previously uncharacterized NK cell subset was shown to be productively infected \textit{in vitro} by both X4 and R5 strains via a CD4-mediated mechanism. Further characterization of NK cells purified from HIV-infected individuals receiving HAART demonstrated the presence of viral DNA, and virus could be recovered from these cells in culture.\textsuperscript{165} Longitudinal analysis revealed persistent infection of this NK cell subset suggested a substantial contribution of viral DNA to the total pool in peripheral blood mononuclear cells, and showed that current antiretroviral therapies fail to eliminate these cells in culture.\textsuperscript{165} These findings raise the possibility that early defects in innate immune responses of HIV-infected individuals,\textsuperscript{166} may derive at least in part from the HIV infection of NK cells.

### Tissue compartmentalization of HIV

#### The central nervous system

The CNS is one of the best examples of HIV compartmentalization. The CNS is a key anatomical reservoir of HIV in both therapy-experienced and therapy-naïve patients, and independently evolving HIV variants have been detected in diverse areas of the CNS, and which are genetically distinct from those circulating in the blood.\textsuperscript{167–169} HIV gene sequences in the V3 loop may confer neurotropism as well as neurovirulence.\textsuperscript{49} Brain-derived isolates are macrophage tropic and their env sequences tend to exhibit negative or neutral charges compared to those in the blood.

As discussed previously, the CNS is protected by the blood–brain barrier which tightly segregates the brain from the circulating blood, and the blood–cerebrospinal fluid barrier in the epithelium of the choroid plexus, which limits the passage of molecules and cells into the cerebrospinal fluid. This barrier-mediated separation of the CNS gives rise to an altered immunological environment, and also provides an obstacle to therapeutic measures such as antiretroviral therapy. Much of the HIV compartmentalization arising in the CNS relates directly to antiretroviral drug resistance. This primarily stems from inadequate drug penetration into the CNS, a major shortcoming of current HAART regimens. Inadequate drug penetration into the CNS is of concern firstly as the CNS may form a drug sanctuary for HIV, and secondly because the suboptimal concentration of drugs in this compartment favors the emergence of drug resistance.

HIV protease inhibitors have limited penetration into the brain. This poor transport through the blood–brain barrier is mainly due to active efflux by proteins; such as P-glycoprotein (P-gp), which prevent drugs from clearing virus from the CNS.\textsuperscript{170,171} Most protease inhibitors (PIs), including ritonavir and saquinavir, are P-gp substrates.\textsuperscript{170,171} P-gp is an active drug-transporter of the ATP binding cassette transporter family and possesses a wide substrate range.\textsuperscript{172} As P-gp transports its substrates in an outward (extracellular) direction, it blocks PIs from moving across the blood–brain barrier.\textsuperscript{170,173} Humans have only one drug-transporting P-gp, MDR1, which seems to carry out the same functions as the mouse Mdr1a and Mdr1b P-gps.\textsuperscript{170} The poor penetration of different antiretroviral drugs extends to different regions within the brain, which also show variability in the distribution of antiretroviral drug resistance.\textsuperscript{174} Regional variability in the incidence and frequency of HIV encephalitis lesions have also been described,\textsuperscript{175} and localization of viral RNA and DNA in the brain has also been reported.\textsuperscript{175} However the mechanisms for this viral localization within the brain are still unclear.

### Semen and the reproductive tract

Semen is a complex mixture of fluids from several organs including the testis, epididymis, seminal vesicles, prostrate and seminal ducts.\textsuperscript{176} The fluid component of semen is derived from the accessory glands, and the levels of seminal HIV RNA usually correlate with blood plasma RNA levels, although it has been shown to increase with genital tract inflammation from sexually transmitted diseases.\textsuperscript{177} HIV-infected cells in semen include lymphocytes and macrophages.\textsuperscript{70} The male
genital tract contains unique vascular features such as the blood–testis barrier. The function of the blood–testis barrier is to protect germ cells from harmful influences; however, it also impedes the delivery of chemotherapeutic drugs to the testis. The barrier has three components: firstly it contains a physicochemical barrier consisting of continuous capillaries; Sertoli cells in the tubular wall, connected together with narrow tight junctions; and a myeloid-cell layer around the seminiferous tubule. Secondly, it has an efflux-pump barrier containing P-gp and multidrug-resistance-associated protein 1 (MDR1); and thirdly, it has an immunological barrier consisting of Fas ligand on Sertoli cells.

These features provide a distinct environment for HIV replication, and hinder access of PIs into the germ cell compartment. Compartmentalization of HIV in the male genital tract has been described by numerous studies based on phylogenetic comparisons between blood and semen and the rate and pattern of the emergence of drug resistance in blood and semen. Distinct resistance patterns are likely to arise from the compartmentalization of viral replication and suboptimal concentrations of drugs in semen.

Studies have found genotypic differences between HIV in blood and HIV in female genital tract secretions. The mechanisms of this compartmentalization are uncertain, particularly as there is no anatomical barrier to entry in this compartment as in the male genital tract. It is probable that the genetic differences in this location arise from a founder effect, whereby virions migrate to a new tissue and proliferate, followed by localized evolution. However, selection may also shape the distinct viral populations and immune pressures are also likely to contribute. Local conditions including sexually transmitted infections and co-receptor expression on host cells may also be a factor.

Gastrointestinal mucosa

In addition to the distinctive cellular characteristics of lymphocyte populations in gastrointestinal mucosa, genetic and molecular differences in HIV-1 quasispecies isolated from this compartment have also been demonstrated. Viral isolates from gastrointestinal mucosa have been shown to have a greater propensity to induce cytopathology than blood derived isolates and may differ phenotypically with respect to syncytium induction. Genotypic differences in the env, pro and RT genes have also been reported, supporting the presence of anatomically distinct, independently evolving viral quasispecies in this compartment. Despite this, a recent study reported that there were no significant differences in viral tropism or co-receptor utilization between isolates from mucusal and co-receptor utilization between isolates from mucusal and peripheral blood mononuclear cell (PBMC) compartments. The pattern of antiretroviral drug resistance in gastrointestinal mucosa has been demonstrated to be similar to that of the PBMC compartment, and in most cases those of plasma viral populations.

Kidney epithelium and lungs

Recent molecular characterization of HIV quasispecies in the renal compartment has revealed evidence of tissue-specific evolution. Phylogenetic trees constructed from HIV V3-loop or gp120 sequences of HIV DNA derived from renal epithelial cells and PBMC showed that kidney-derived sequences clustered separately within the radiation of blood mononuclear cell-derived viral sequences. This is best explained by a stochastic event representing a founder effect following the initial seeding of kidney epithelium by a blood-derived variant.

Discordant mutation patterns in the reverse transcriptase and protease genes have been observed in HIV isolates derived from plasma and cell free bronchoalveolar lavage fluid. Likewise, HIV DNA recovered from PBMC and cells from bronchoalveolar lavage fluid showed discordant patterns of drug resistance mutations in some cases.

Cellular compartmentalization of HIV

Plasma and PBMC

The archiving of HIV in PBMC complicates the relationship between cell-associated and cell-free HIV circulating in the blood. There are multiple sources of circulating plasma virus in addition to PBMC, and the antiretroviral therapy is likely to suppress viral replication more efficiently in some compartments than others. Longitudinal analyses of plasma HIV RNA and proviral DNA have demonstrated significant differences between the frequencies of envelope sequence variants in the plasma and PBMC populations at any given point-in-time in asymptomatic patients. Similarly, continual evolution and compartmentalization of HIV drug resistance between plasma and PBMC has been observed over time during HAART. Another study found that therapy was able to reduce plasma HIV RNA to below the level of detection, but PBMC-associated RNA was not suppressed. In addition, CD4 counts correlated more with PBMC HIV RNA levels. More recently, a report comparing HIV derived from lymph nodes and blood concluded that most of the virus in plasma originated from
sources other than CD4+ T cells in the peripheral blood and lymph nodes.162

Resting memory CD4+ T cells

The most notable example of HIV compartmentalization at the cellular level is the latent CD4+ T cell reservoir. Compartmentalization of HIV results from these cells remaining inactivated and persisting for long periods of time, which significantly impacts on the ability of HIV to turnover and evolve. Persistently infected, inactivated CD4+ T lymphocytes191 are also a major obstacle to viral eradication. Resting CD4+ T cells harboring provirus have been demonstrated in patients receiving HAART with undetectable plasma virus concentrations for extended time periods, and replication competent virus can be recovered from these cells after CD8+ T cell depletion in vitro.192 The reservoir is established soon after primary HIV seroconversion, and can be activated by proinflammatory cytokines and bacterial products in vitro and potentially in vivo. The latent replication-competent virus is predominantly found in resting memory (CD4+ CD45RO+) cells and at significantly lower levels in resting naïve (CD4+ CD45RA-) cells. Evidence has shown that virus in the latent CD4+ T cell reservoir is mainly CCR5 tropic,193 and contains fewer drug resistance mutations than circulating plasma strains.24 These findings are therefore in agreement with the reservoir being established soon after infection. The presence of unintegrated HIV-1 DNA in infected resting CD4+ T cells from patients with undetectable plasma viremia also suggests persistent and active viral replication in vivo.191 This is most likely to be responsible for the continued low-level viral turnover and evolution observed in patients thought to be fully suppressed by HAART.194

Macrophages

Macrophages produce and release high levels of virus over an extended time period,132 categorizing them as chronically and persistently infected cells.6 They are insensitive to the cytopathic effect of the virus and have an extended life span. It is unclear whether HIV populations in macrophages are truly compartmentalized, but several features of their infection warrant interest. First and most notably, latent infection is widespread in macrophages throughout the lymphoid system, from early to late stages of infection.139 Secondly, it has been shown that there are differences in the infectivity of viruses produced by macrophages,125 indicating that HIV may evolve differently in these cells. Finally, the unusual dynamics of replication in macrophages and the unique characteristics of these cells result in altered responses to antiretroviral therapy. Although nucleoside reverse transcriptase inhibitors (NRTIs) are potent inhibitors of HIV replication in macrophages,195 PIs show reduced activity requiring much higher dosages for a desirable effect; a feature that could be attributable to P-gp-mediated drug clearance.

Drug penetration, viral reservoirs and drug resistance

Poor penetration of antiretroviral drugs in the CNS

There is considerable evidence showing that unique anatomical structures, which limit the distribution of antiretroviral drugs into the CNS, the blood–brain barrier and the blood–CSF barrier primarily formed by the choroid plexus. High plasma protein-binding of protease inhibitors and their unidirectional efflux by P-gp membrane proteins in the blood–brain barrier limit CNS penetration and absorption of antiretrovirals.170,171 Thus, the CNS represents a site where ongoing viral replication may occur. Mutations conferring resistance to multiple antiretroviral drug classes may also predominate in compartments where drug levels are suboptimal.

Suboptimal drug penetration also influences the emergence of multiply drug-resistant variants, which may also predominate in this anatomical reservoir. Discordant changes in the peripheral blood and CSF HIV-1 RNA levels have been reported in response to antiretroviral therapy. Similar and discordant patterns of antiretroviral drug resistance have been detected in the RT and protease genes of isolates from the blood compartment and the CSF of the same patient.196 Better understanding of how drug-resistant mutations emerge in HIV populations in vivo, and the development of more efficacious antiretroviral drugs is of paramount importance to achieve and maintain consummate therapeutic drug levels in blood or brain.

Drug penetration in blood leukocytes

A similar scenario of differential penetration and bioavailability of anti-HIV drugs also applies to blood leukocytes. To help ensure that adequate drug concentrations are achieved throughout the dosing interval, therapeutic drug monitoring of the anti-HIV drugs (such as PIs) in plasma is commonly used. The drugs show differential accumulations within lymphoblastoid cell lines and peripheral blood mononuclear cells of virologically suppressed patients in vivo, with:
nelfinavir > saquinavir > lopinavir > ritonavir > indinavir. Furthermore, drug concentrations in vivo can also vary considerably between cell/tissue types during HAART.\(^{197}\) Differences in drug concentrations can be attributed to the variable penetration of antiretroviral drugs which, in turn, is influenced by multispecific drug transporters such as: organic anion transporting polypeptides (OATPs); the MDR1 (P-gp); as well as multidrug resistance-associated proteins (MRP). It is also known that cell lines expressing high levels of P-gp significantly reduce the accumulation of HIV-PI, and are less sensitive to HIV-PI antiviral activity than cell lines not expressing P-gp.\(^{198}\) These proteins work in concert with detoxification enzymes to protect the organism/living cells from potentially harmful compounds.\(^{16,199}\)

Possible relationship between drug transporter concentrations and drug levels

Recently, Lucia and colleagues\(^{200}\) demonstrated differences in the levels of P-gp and MRP in T lymphocytes of HIV patients. They have also shown that protease inhibitors contribute to P-gp efflux function in major cell targets for HIV-1 such as CD4\(^+\) and CD34\(^+\) progenitor cells. These data suggest that drug level studies in different cell types of HIV patients are vital to gain a clear understanding of the expression of drug transporter proteins and their relationship with the emergence of cellular drug resistance and drug levels.

New approaches to eradicate viral reservoirs

It is now apparent that HAART does not provide a complete elimination of HIV from the infected host. If the treatment is stopped, residual virus concealed in several potential reservoir sites can rapidly expand, thereby allowing disease progression to occur. Therefore, investigations concerning the sources of persistent replication-competent HIV during HAART and strategies to purge these reservoirs remain the top priority in HIV treatment. Memory cells are long-lived host cells and HIV can successfully tamper with this unique feature of memory cells and can then persist for decades with its inert and metabolically inactive viral cargo. Upon receiving a stimulatory signal the cell activates, which concomitantly induces the production of viral progeny from the latent HIV genome. T cells also decay very slowly during HAART, with an average half-life of 44 months, and thus it is estimated that with the currently prescribed regimen to HIV patients, complete eradication of this reservoir would take over 60 years. Thus, viral reservoir establishment in HIV patients is a serious obstacle to the long-term success of antiretroviral treatment, especially in those reservoirs that restrict the penetration of various antiretroviral drugs.

There has been considerable interest in developing potential approaches for diminishing, containing and eliminating latent reservoirs of HIV in the infected hosts; however, to date, all these approaches have received skeptical reception at best because of the nature and complexity of viral reservoirs in the human host. Although no successful approach has emerged as a winner in effectively targeting viral reservoir sites, several options for treatments that are targeted at eliminating HIV-infected cells capable of producing replication-competent virus in specific anatomical and cellular reservoirs are presently being explored. As a vaccine is still some distance away from being used as a preventative measure against HIV infection, additional therapies to HAART that target viral reservoirs are clearly needed. These are the treatments that will be aimed at eliminating viral reservoirs or at least preventing their long-term establishment, which can provide a glimmer of hope for the future. Some of these key strategies are discussed below.

Activating persistently and latently infected resting CD4\(^+\) T cells

Given the complexity of pre-integration and post-integration latency in HIV reservoirs, it is difficult to devise a strategy that can provide sustainable eradication of HIV from the reservoir sites, where most of the latent virus is concealed. Most strategies targeting reservoirs involve activating the latently infected cells in order to induce expression from the HIV genome. As a consequence, the cell will become infected and killed either via cytopathic effects induced by HIV or by host immune effector mechanisms. This, in turn, can be a viable platform to halt virus spread through HAART during the stimulation phase. Though a few stimulants are known, interleukin-7 (IL-7), in particular is able to rekindle latent virus from the reservoir site. IL-7 causes a substantial increase in the expression of latent HIV-1 from both human thymocytes and peripheral T cells with minimal effects on T cell phenotype. Thus IL-7 remains a viable candidate in the fight for clearance of viral reservoirs. New therapies should target pathways downstream of homeostatic proliferation, including inhibitors of the IL-7 pathway or pathways associated with self-renewal and ‘stem cell-ness’, such as those being developed for treating of leukemias and cancers. Indeed, by limiting immune activation and affecting long-lived infected CD4\(^+\) T cells by targeting IL-7-dependent proliferation and
the self-renewal of memory T cells, in association with HAART, will pave the way for the eradication of virus in aviremic individuals.106

Other molecules, which are capable of activating latent provirus include agents such as the non-tumor-inducing phorbol ester prostratin. Prostratin inhibits HIV infection and viral spread at the entry/fusion step of viral life cycle. The lack of tumor promotion of prostratin coupled with its ability to up-regulate latent HIV-1 provirus expression and inhibition of viral infection are important features that could be exploited as an effective therapy to eliminate latent reservoirs.201 Histone deacetylase inhibitors such as valproic acid202 and certain modulators of cellular microRNAs,203 can activate latent provirus. Unfortunately, to date, none of the strategies have translated clinically in effectively purging the latent virus from the reservoir sites. The caveat with these technologies is that robust viral activation can also lead to undesirable immune activation. Thus, further refinement is needed to their clinical translation.

Shock to kill approach using histone deacetylases

Savarino and colleagues have studied the so-called ‘barrier of latency’, which has been the main obstacle to eradicating HIV from the body.203 Cells harboring a quiescent virus are responsible for HIV persistence during therapy. They have found a new way that could drive out the stubborn virus from infected cells so that the body’s immune system or drugs have a chance to kill them.204 A class of inhibitors called histone deacetylases, which keep HIV in its dormant state, only work at toxic doses. Histone deacetylase (HDAC) is a host mediator of gene repression, which inhibits HIV gene expression and virus production thereby contributing to quiescence of HIV within resting CD4+ T cells.204 A recent proof of concept study by Lehrman and colleagues202 showed that combination therapy with an HDAC inhibitor and intensified HAART safely accelerates the clearance of HIV from resting CD4+ T cells in vivo, suggesting a new and practical approach to eliminate HIV infection in this persistent reservoir. However, extensive patient-based studies are required to support this proof of concept study. Moreover, the beneficial effects of this drug are visible on HIV only when used in toxic concentrations.

Furthermore, they have also shown203 that adding a second drug called buthionine sulfoximine along with HDAC ‘awakens’ the infected cells at lower doses, while leaving the virus-free cells intact. It appears that at non-toxic quantities, class I HDAC inhibitors were able to induce the ‘awakening’ of a portion of cells within a latent infected cell population. The researchers then repeated the experiment adding a drug inducing oxidative stress, buthionine sulfoximine (BSO). The results showed that BSO was able to recruit cells non-responsive to the HDAC inhibitors into a responding cell population. Clearly evident was that the infected cells’ ‘awakening’ was followed by cell death, whereas the non-infected cells were spared by the drug combination.

Enhanced killing approach

Methods for enhanced killing of recently activated HIV-infected cells are also being investigated. One such example is immunotoxins, which are composed of a targeting domain derived from a monoclonal antibody linked to a toxic moiety. For this purpose, the immunotoxin specifically targeting cells expressing the HIV envelope protein have been used to deplete both latently infected T cells205 and infected macrophages206 after upregulation of HIV gene expression with stimuli. ‘Activation-elimination’ strategies such as this may therefore accelerate clearance of HIV from its various cellular reservoirs. Moreover, if this type of approach were used in conjunction with post-exposure vaccination or genetically modified stem cell immune reconstitution strategies, it might prove even more effective in decreasing or eliminating latent and persistent viral reservoirs. While many of the approaches outlined above are still at the developmental stages and are not without limitations, it is hoped that some of these nascent strategies will rapidly advance to the point that they can provide benefits to patients.

Gene-based induction therapy targeting latent reservoirs of HIV-1

One approach to eradication of the latent reservoir is induction therapy, whereby latently infected cells are activated to initiate viral replication. Herpes virus saimiri protein StpC has been shown to enhance HIV-1 replication in MOLT4 cells, suggesting it may be a useful tool for induction therapy.207 An induction vector containing StpC and a suicide gene such as Herpes simplex virus thymidine kinase (TK) may be utilized as a novel treatment targeting latently infected cells. Turner and colleagues207 have shown that the transfection with a suicide vector based on StpC and TK increased HIV-1 replication compared to cells transfected with a vector containing TK alone. The addition of ganciclovir resulted in reduced HIV-1 p24 levels in the cells transfected with the StpC-TK vector. This study provides proof of concept that gene induction therapy using suicide vectors encoding StpC to target cells latently infected with...
HIV-1 may offer a new approach to treating latent HIV-1. These studies will lead to a better understanding of viral latency and its disruption; and will assist development of improved techniques towards targeting latent reservoirs.

**Genetic therapies against HIV**

In the absence or presence of chemotherapy, gene therapy offers some hope of combating HIV infection via sustained interference with HIV replication in vivo. It is believed that the emergence of viral resistance and the establishment of reservoirs seen during HAART are the biggest impediments to successful HAART. These may possibly be addressed by gene therapies that use combinations of genetic agents for inhibiting both viral and host gene targets. Several of these strategies have already been or are being tested in planned clinical trials. These include, RNA-based agents (ribozymes, antisense, RNA aptamers and small interfering RNA (RNAi)), protein-based agents, (mutant HIV Rev protein M10, fusion inhibitors, zinc-finger nucleases, dominant-negative proteins, intrabodies, intrakines). Recent advances in T-cell–based strategies include: gene-modified HIV-resistant T cells; lentiviral gene delivery; CD8+ T cells; T bodies; and engineered T-cell receptors. HIV-resistant hematopoietic stem cells have the potential to protect all cell types susceptible to HIV infection.

Overall, of several gene therapies tested so far none have fared successfully. Viral escape is a major problem, which will and has confounded every gene therapy approach tried to date. Although cellular targets are far less prone to mutational escape, the long-term side effects are unclear. Another major confounding factor is the specific targeting of the anti-HIV gene to have maximal effect against HIV-1 coupled with minimal cellular toxicity.

**Conclusions**

Even though eradication of HIV in the infected host appears to be extremely complex and challenging with the currently prescribed antiretroviral drugs, researchers should not abandon the goal of finding ways to challenge HIV, and developing strategies for either the long-term containment of HIV replication and prevention of immune dysfunction during HIV infection, or flushing out HIV from the reservoir sites in vivo. Although the latter has proven extremely difficult, it will need a coordinated effort and innovative thinking from scientists and clinicians alike.

**Disclosures**

The authors declare no conflict of interest relevant to this research.

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