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HIV Compartmentalization: Pathogenesis and Clinical Implications

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Abstract

Infection with HIV results in the dissemination of the virus to a number of target compartments which may act as sanctuary sites for the virus. Sanctuary sites for HIV may be divided into cellular and anatomical sites. Cellular sites for HIV include both productively and latently infected CD4+ lymphocytes, macrophages and dendritic cells while anatomical sites include blood, lymph nodes, central nervous system (CNS), genital tract, spleen and lung. Some of these sites may be inaccessible to some classes of antiretroviral drugs e.g. some protease inhibitors penetrate poorly into the CNS and genital tract. Mathematical models have been used to predict the importance of sanctuary sites and trafficking of virus between compartments on the pathogenesis of HIV infection. This review will discuss recent understanding of the role of sanctuary sites in the persistence of HIV in the host.

Key words

Introduction

Studies on the dynamics of HIV replication have demonstrated that the virus is highly active during the long clinically asymptomatic phase of the disease[1-3]. As many as 100 million virus particles are produced each day by HIV infected host cells and although the immune system may limit the infection, HIV is never eradicated from the host. The lymph nodes appear to be the most active sites for HIV replication,[4-6] and it is clear that the level of HIV replication in infected individuals is very important to the rate of progression to AIDS and death. The success of highly active antiretroviral therapy (HAART) has prompted interest in cellular and anatomical reservoirs of the virus which may evade antiviral immune responses and HAART[7]. Current models for HIV replication in vivo are based upon drug induced changes in steady-state levels of viral RNA in blood[1,2,7]. The half-life of virus in plasma has been calculated to be as little as 3 m, with the half-life of productively infected CD4 T-lymphocytes being 24 hours[7-9]. Intravenous inoculation of SIV into rhesus macaques indicated that viral particles were rapidly cleared from the blood compartment in less than 5 m. The cleared virus was predominately degraded but detectable levels of SIV RNA were recovered from the lymph nodes, spleen, lung and liver but not from other organs[9]. Once infected with HIV it is unlikely that the virus will be eradicated from the host because even if HAART is initiated during primary infection competent virus can still be isolated from purified peripheral blood CD4 T-lymphocytes after 3 years of therapy[10]. Therefore, a clear understanding of how HIV evolves once seeded within the target organs and sanctuary sites is of major importance in devising protocols for eradicating the virus from the host. Sanctuary sites for HIV may be divided into cellular and anatomical compartments[11].

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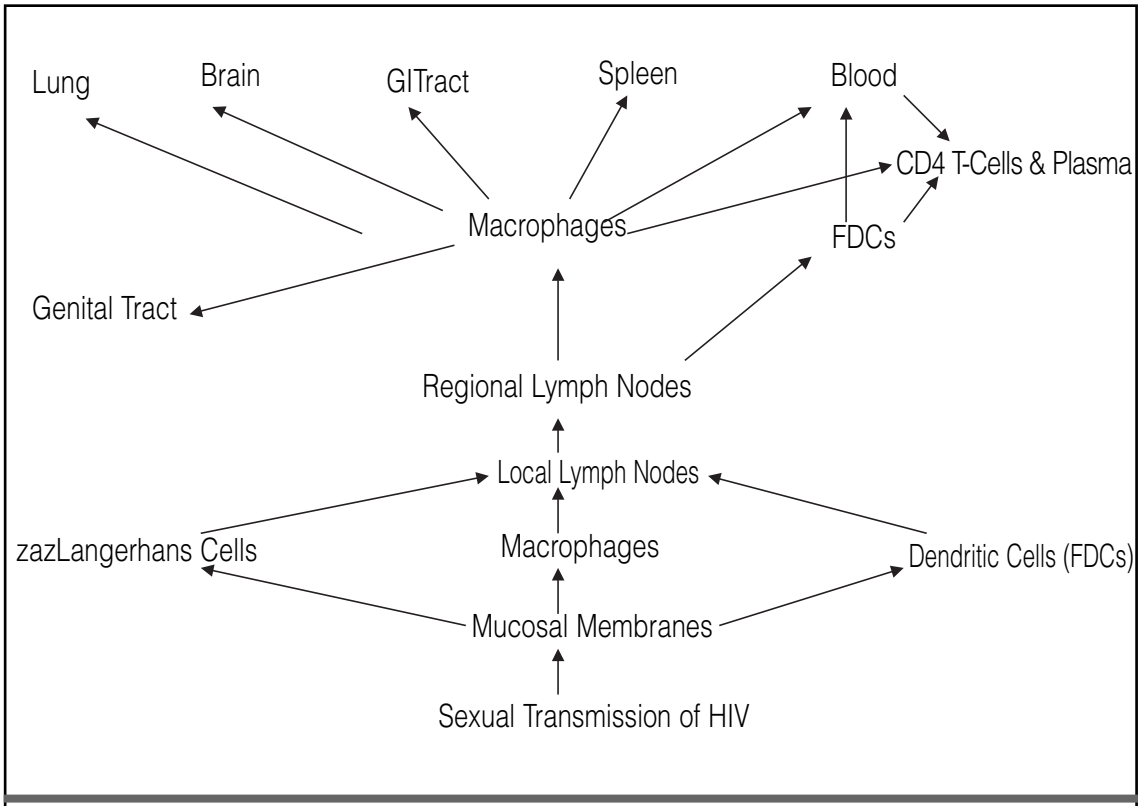

Fig. 1. *Dissemination of HIV following Sexual Transmission.*

Dynamics of HIV replication

Important insights into the understanding of viral reservoirs have come from the analysis of changes in levels of virus following initiation of antiretroviral therapy (reviewed in[7]). The biphasic nature in the rate of decay of plasma virus is due to differences in the kinetics of replication in different infected cell populations[7]. The major cell of HIV infection is the CD4 T-cell which accounts for over 99% of replicating virus within the host. However, there is some controversy over which cells are the most important target during primary infection. Infected macrophages while accounting for < 1% of total infected cell burden are important in disseminating HIV throughout the host and a possible mechanism of dissemination of HIV (shown in (Fig.1)[12]). CD34 progenitor cells are very susceptible to infection with HIV and distinct quasi-species of HIV have been detected in the bone marrow compared to the blood[13]. Macrophages have been shown to be a major target cell for HIV in the bone marrow[14]. In addition to macrophages, Langerhans and dendritic cells at the mucosal surfaces are important targets during the sexual transmission of HIV[15].

Within 7-14 days of starting antiretroviral therapy virus is cleared from productively infected CD4 T-cells which is accompanied by a 99% reduction in plasma HIV RNA viral load (reviewed in[7]). The slower rate of decay in the remaining 1% of virus infected cells is due to low levels of continuous viral replication in tissues[7]. Three cellular sources of virus are important for the persistence of HIV following the initiation of HAART: Extra-cellular virus trapped on the surface of follicular dendritic cells (FDCs) in the germinal centres of lymph nodes[5,7,16-18], virus replicating within tissue macrophages and latently infected CD4 T-cells[7]. The half-life of viable replicative competent virus trapped on the surface of dendritic cells was 14 days and following the initiation of HAART follicular hyperplasia and cell activation continued in the germinal centres of lymph nodes for at least 6 months[19]. A bi-phasic dissociation of HIV from FDCs has been postulated[20]. Virus trapped on the surface of FDCs provide a sanctuary site in which HIV is protected from the effects of HAART. Although, some new NNRTIs may be able to inhibit endogenous reverse transcriptase activity within virions[21] and this may be a necessary consideration in future HAART regimens, as in some instances HIV may be trapped on the surface of these cells for years[20]. If only a small proportion of this virus remained viable then sufficient virus would exist to re-infect CD4 cells if the antiretroviral therapy was withdrawn. This could explain why HIV viraemia rebounds so quickly after the cessation of antiretroviral therapy.

Macrophages

Mathematical models predict that infection of macrophages may be essential for the successful

establishment of HIV in the primary phase of the disease[22]. The number of infected macrophages appear to be low but macrophages are resistant to the cytopathic effects of HIV and are relatively long lived cells which would allow the constitutive release of virus over the life time of the cells[23-24]. The infection of cells from the monocyte/macrophage lineage enables the dissemination of HIV to target tissues and may provide a vehicle for the trafficking of viruses between target organs[12]. Infection of tissue macrophages increases late in the disease process during opportunistic infections resulting in an increase in the number of macrophages that are infected and the amount of virus produced from these cells[23,24,26,27]. Distinct differences have been detected in the quasispecies that infect CD4 T-cells and macrophages indicating that virus evolves separately in these cells. Heterogeneity in HIV proviral DNA from CD4 T-cells was much higher than that found in monocytes or in viral RNA from plasma[28]. Analysis of V3 loop sequences from plasma and PBMCs clearly demonstrated compartmentalization[29]. Acquisition of resistant mutations in lymphocytes may also differ from cells of the monocyte/macrophage lineage because nucleoside analogues show greater activity in monocyte derived macrophages due to the lower endogenous nucleoside pools within these cells[30]. The peculiar characteristics of HIV replication and efficacy of antiretroviral drugs in macrophages have a natural counterpart in lymphoid tissue where macrophages provide a major focus of infection for HIV[31]. Viral infection of macrophages should be considered in therapeutic strategies wanting to achieve optimal effects in all tissue compartments where the virus seeks sanctuary[31].

Decay rates of HIV-1 in latently infected cells indicate that eradication of HIV-1 following initiation of HAART may take as long as 60 years[32]. Therefore, latent infection of resting CD4 T-cells by HIV-1 provides a mechanism for life-long persistence of virus. In patients with incomplete suppression of viral replication there is clear evidence of evolution of HIV genomic RNA sequences (reviewed in[7]). However, in some patients with complete suppression of plasma viraemia, virus recovered following treatment failure was genotypically identical with baseline samples taken 2 years prior to failure, indicating the persistence of latently infected cells reviewed in[7].

Blood and lymph nodes

Despite suppression of plasma viraemia for 18 months by HAART, HIV-1 RNA expression could be detected in the lymphoid tissues of the majority of patients and HIV-1 DNA was detected in both PBMCs and lymphoid tissue of all patients[33]. Low levels of replication can still be detected in macrophages after > 6 months of HAART[34] and may be the reason that HIV RNA can still be detected in plasma in many individuals on HAART whose viral load is consistently below the detection limit of commercial viral load assays (< 20 copies/mL)[35].

In the majority of patients plasma viraemia becomes undetectable within 3 months after initiating HAART and this is accompanied by a significant reduction in the number of productively infected cells in the lymph nodes but HIV RNA expression could still be detected in these cells[36]. Others found no evidence of viral replication in blood or lymph nodes of patients with plasma viral load of < 20 copies/ml[37].

Compartmentalization

Kepler and Perelson in 1998 proposed a mathematical model in 1998 that predicted that the eradication of HIV from the host will depend on the capability of totally suppressing viral replication in all infected cells within the sanctuary sites and on the elimination of these cells either due to natural death or destruction by the cellular immune system[38]. In their model, partial suppression of HIV replication in sanctuary sites resulted in the evolution of resistant virus and treatment failure. In one compartment systems there was a relatively narrow window of drug concentrations that allowed evolution of resistant variants[38]. When the system was enlarged to consider two spatially distinct compartments held at different drug concentrations where penetration of drug into one is sub-optimal, the range of drug concentrations that permitted the development of resistance increased[38]. In the host the opportunity for the generation of resistance to occur was widened by having one compartment in which mutants were generated such as a sanctuary or region of low drug concentration that HIV can enter and a second compartment in which the drug concentration is high enough to give a selective advantage to the mutant. The process of generating mutants in one compartment and selecting them in another may be repeated a number of times to achieve a step wise accumulation of resistant mutants. This became a particular problem if there was significant trafficking of viruses between the two compartments. In a sanctuary where the drug penetrance is small, partially resistant strains can proliferate and produce more resistant strains which are then able to re-seed other compartments[38]. The role of sanctuaries in the evolution of drug resistance is likely to be even more central for HAART when 3 or more drugs are used as many mutations may be needed for treatment failure.

High rates of mutations and replication of HIV allows for the continuous generation of diverse genetic variants *in vivo*[39]. Selective pressures within the micro-environments of different anatomical compartments result in the emergence of dominant quasispecies which can be distinguished by their envelop sequences. It has not been established that comparable tissue-specific selective pressures lead to the independent evolution of *pol* sequences within different body compartments, nor is it known how differing rates of virus turnover in tissues might affect the pace of such evolution[39]. The same mutations that confer resistance to antiretroviral agents

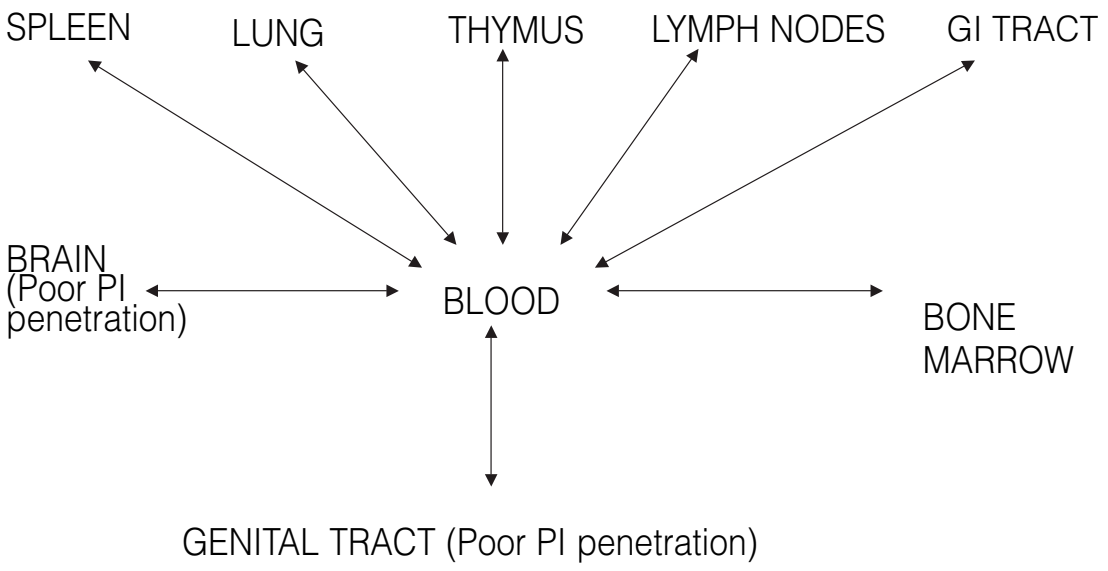

Fig 2. *Sanctuary sites and trafficking of HIV-1 between compartments.*

in the blood are also found in the other body compartments[40]. However, subtle differences in mutations detected in various compartments may prove to be important when assessing the effectiveness of therapy. Sequential sampling at body compartments is not always possible due to the invasive nature of some techniques.

The mechanisms responsible for parallel evolution of virus in different organs are poorly understood, but tissue specific tropism of different HIV quasispecies, variations in the replication rates of virus in the different tissues and local immune responses are all likely to be involved[41]. Caution should be used when interpreting genotypic data as one study showed that despite clear differences in the V3 loop sequences of the major variants recovered from different tissues all HIV variants showed identical cell tropism and replicative kinetics in primary cells *in vitro*[42]. These issues are important in formulating a model for the emergence of drug resistance *in vivo* and for understanding drug trafficking and virus turnover (Fig. 2). Several studies have noted a discordance in the distribution of quasispecies in PBMCs, plasma, spleen, brain, semen, lymph nodes and lung (Table 1). This indicates that there are anatomically distinct independently evolving HIV quasispecies at these sites[39].

The evolution of independent quasispecies may be even more localised as distinct genotypes of HIV have been recovered from different regions of the brain[43]. It is conceivable that antiretroviral drugs may penetrate some areas of organs with differing efficacies leading to variations in resistance patterns within a compartment[43]. Phylogenetic analysis of the V3-V5 envelop region of HIV confirmed tissue specific compartmentalization of blood-brain viruses but also showed that there was some trafficking of quasi-species between the two sites[44]. This makes the analysis of quasispecies recovered from different compartments complex.

Subtle changes in target cell maturation may also be important for the replication of HIV. Expression of CD4 and appropriate coreceptors correlated with HIV entry into thymocytes but factors related to activation and maturation were important for the replication of HIV[45]. Furthermore, quasispecies within the thymus were distinct from PBMCs[46].

Table 1. *Sanctuary sites where distinct HIV quasispecies have been identified.*

Sanctuary	Primary Sanctuary	Secondary References
PBMCs	plasma	29
blood	bone marrow	13
blood	brain	44
brain	brain	43
blood	thymus	46
blood	semen	63,68,69
blood	cervical secretions	71
blood	lung	72-75
brain	spleen	51

Pharmacokinetics

One concern with HAART is adequate penetration of drugs to all body compartments and sanctuary sites. AZT has been shown to penetrate all organ systems in mice but the same has not been established for man[47]. Protease containing regimens target HIV reservoirs in lymphoid tissue more effectively leading to a more profound restoration of lymph node architecture[48]. The blood brain barrier restricts the entry of antiviral agents into the central

nervous system thereby facilitating the creation of a reservoir of HIV that could potentially reinfect peripheral tissues. The lymphatic system could provide a potential pathway through which CNS reservoirs of HIV could directly reinfect lymphoid tissue[49]. It has been shown that protease inhibitors penetrate poorly into the male genital tract[50]. Durability of antiviral effect following removal or cessation of therapy varies between cell types and this is likely to be the case in individual body compartments. The long half-life of NNRTIs in plasma could result in patients who stop therapy receiving monotherapy with NNRTI for a period which could lead to the development of resistance against these drugs.

Brain and CSF

Sanctuary sites for HIV such as the brain have been recognised as contributing to the success of the infection[51]. HIV mediates a productive infection of brain macrophages/microglial cells[52]. HIV can be detected in the CSF during seroconversion and molecular analysis of the V3 loop shows substantial homogeneity between CSF and blood genotypes early in infection which is lost as disease progresses. Redistribution of HIV quasispecies to the brain from other sanctuary sites has been observed in patients who progress to AIDS dementia complex, although quasispecies also evolved independently in the brain when compared to the spleen[51]. Although, others found no differences between the V3 loops of clones from the brain and spleen of three individuals who died of HIV encephalitis and this may be due to the advanced nature of disease in these subjects[53].

The decay of HIV RNA viral load in CSF following the initiation of HAART was comparable to that observed in the plasma[54]. However, interruption of therapy resulted in a rapid rebound of viral load in the CSF. The same mutations associated with resistance to antiretroviral drugs in HIV quasispecies from plasma are also common in CSF[55]. Active efflux transport out of the CSF is a predominant mechanism limiting nucleoside analogue access to the central nervous system[5]6. Few studies exist that measured the half-life of drugs in tissues. The comparison of pharmacokinetics of AZT in CSF and plasma revealed that AZT penetrated into the CSF more slowly with a longer half-life[57]. Poor uptake of protease inhibitors into the CNS may result in the early breakthrough of HIV RNA in CSF of patients failing HAART[58]. Nelfinavir did not penetrate into the CSF[59] while indinavir was present in therapeutic concentrations[60]. Poor reduction in both CSF and plasma HIV-1 RNA levels may occur in some patients and this may be due to either sub-optimal therapy or lack of compliance[61]. However, epidemiological studies have reported that despite the success of HAART in reducing opportunistic infections and slowing the rate of disease progression there has been no overall reduction in the levels of AIDS dementia complex[62].

Genital Tract

It is well established that HIV is found in semen, either as cell free or cell associated virus[63], yet the source of the virus has not been established. Some individuals demonstrated disproportionally high levels of HIV RNA in seminal plasma compared to blood plasma[64-65]. HIV replication persisted in plasma and seminal fluid of nucleoside analogue experienced subjects who switched HAART regimens due to therapy failure[66]. Although in retroviral naive patients triple combination antiretroviral therapy with a protease inhibitor containing regimen was found to effectively reduce cell free HIV RNA in semen[67].

Protease gene sequencing from semen and PBMCs indicated that viral populations from the two sources are distinct[63,68]. In particularly, mutations associated with resistance to PIs were found in the blood but not semen. However, mutations associated with resistance to PIs have been recovered from the DNA of seminal cells and differences in the mutation patterns observed in PBMCs and seminal cells noted[68-69]. Mutations associated with resistance to antiretroviral therapy were also found in the female genital tract[70]. Distinct genetic evolution was reported in blood and cervical secretions of women infected with clade A viruses[71]. Some evidence was found for trafficking of viruses between compartments but once seeded at target sites virus evolved independently[71].

Lung

Comparison of the genetic sequence of the V3 loop of gp120 recovered from alveolar macrophages AMs and PBMCs has revealed tissue specific sequences[72-75]. Structural differences seen in this region are associated with HIV tropism for macrophage or T-cells[75]. Consequently, variation in the V3 sequences between AMs and PBMCs may merely be indicative of selectivity of the diffferent viral cell types. HIV strains from AMs, but not from peripheral blood cells, contain V3 domain nucleotide sequences with a greater degree of homogeneity in the C-terminal region[75]. This suggests that strains infecting AMs have evolved further from a presumed ancestral species than those infecting blood monocytes. It is possible that, as the disease progresses, viral strains in lung and blood are not in a state of unrestricted bidirectional traffic but, instead may involve independently. For complete eradication of HIV, it is essential that drugs exert optimal therapeutic effects and suppress replication in all infected body compartments[76]. In the lung, this involves the ability of drugs to penetrate alveolar spaces and alveolar macrophages. Differences in the mutations associated with resistance to nucleoside analogues have been found in the lung when compared to the lung[77-79]. However, it is extremely difficult to obtain sequential samples of lung tissue from patients receiving HAART therefore, to date it has not been possible to monitor the evolution of HIV quasispecies in the lung.

Conclusions

A clear understanding of how HIV evolves once seeded within target organs and sanctuary sites is of major importance in devising protocols for eradicating the virus from the host. Despite HAART low levels of HIV replication occur in various sanctuary sites and this virus may seep back into the circulatory system to re-infect CD4 cells. Viral infected cells trapped in these sanctuary sites may continue to produce virus for years. Predictions that virus trapped on the surface of FDCs may remain viable for years, if correct, would provide an inaccessible sanctuary site for HAART. Protocols need to be devised to eliminate virus from these target cells. However, the differential uptake of drugs into the CNS, genitals and lymph nodes is of grave concern if HAART is ever to be effective at eliminating HIV from sanctuaries. The ultimate goal is the elimination of HIV from the host but this seems increasingly unlikely even with immune restoration strategies.

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