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Eradication of Human Immunodeficiency Virus from Brain Reservoirs

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Abstract

Isolated cases in which HIV infection was claimed to have been eradicated generated renewed interest in HIV reservoirs in the brain particularly since attempts to reproduce the findings using genetically engineered stem cells and immune or myeloablation have failed. A clear understanding of the cell types in which the virus resides in the brain, the mechanism of viral persistence, restricted replication and latency and the turnover rate of the infected cells is critical for us to develop ways to control or get rid of the virus in the brain. The brain has several unique features compared to other reservoirs. There are no resident T cells in the brain; the virus resides in macrophages and astrocytes where the viral infection is non-cytopathic. The virus evolves in the brain and since the turnover rate of these cells is low, the virus has the potential to reside in these cells for several decades and possibly for the life of the individual. This review discusses the HIV reservoirs in brain, issues related to eradication of the virus from sanctuaries in brain and current challenges faced by neuroscientists in finding a cure.

Keywords

compartmentalization; astrocyte; microglia; macrophage; antiretroviral; latency

Introduction

Even though the advent of combined antiretroviral therapy (cART) has been hailed as a major success in modern medicine for its ability to prolong the lives of individuals infected with the human immunodeficiency virus (HIV), it is becoming abundantly clear that neurocognitive manifestations cannot be fully controlled with the treatment[1]. It was originally thought that the virus had been eliminatied from at least one patient which led to the enthusiasm that a cure for HIV infection may be possible [2-4]. Subsequently it was found that HIV sequences could be found in this patient however HIV replication has not been detected, hence he is now referred to as “functional cure”. This enthusiasm has been tempered by the inability to reproduce this in other patient populations. While there may be several explanations for this failure, one likely possibility is that these strategies are ineffective in eradicating or controlling HIV reservoirs in the brain. Strategies developed to

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eliminate the reservoirs include the enhancement of immune responses against cells that harbor the virus and creating genetically engineered cells that would be resistant to viral infection with the hope that these cells will eventually replace the viral reservoirs [5]. However, for these strategies to be successful they need to take into account the fact that the brain is an important reservoir for HIV where the virus can reside indefinitely in glial cells. While the presence of the virus in the brain was shown soon after the discovery of the virus, important questions regarding the timing of viral entry into the brain and the mechanisms involved in viral entry, persistence and turn over still remain unanswered. It is hence important to address these issues as a prerequisite to finding a cure for HIV-1.

The mechanisms by which HIV causes damage to the brain seems to be multifactorial. In general, the number of productively infected cells in the brain is small compared to the amount of neuronal damage and the neurons are not infected with the virus. Extensive studies show that viral proteins released from HIV-infected cells can cause glial cell activation which may in turn cause neuronal injury. Activated macrophages and astrocytes produce factors with neurotoxic potential like free radicals, peroxynitrite, tumor necrosis factor-alpha and arachidonic acid metabolites [6] that appear to ultimately lead to glutamate-mediated toxicity or direct neuronal injury via oxidative stress [7]. Alternatively, HIV proteins may also directly interact with neurons to cause excitotoxicity or travel along neuronal pathways to cause injury at distant sites [8]. Several viral proteins have been implicated as having neurotoxic effects and in particular the effects of gp120 and Tat have been characterized well *in vitro* and in limited *in vivo* studies [9-13]. Co-morbidities are often associated with HIV infection such as hyperlipidemia, vasculopathies, drug, alcohol and nicotine abuse and even some of the antiretroviral drugs themselves may be neurotoxic. Host genetic factors have also been implicated neuronal vulnerability to the virus and other neurotoxic agents.

cART fails to completely control progression of HIV-1 associated neurocognitive disorders or viral pathogenesis in brain

Nearly one third of HIV-infected individuals develop neurocognitive deficits despite adequate cART and excellent virological control in blood [1]. These range of neurocognitive deficits are collectively referred to as HIV-1 associated neurocognitive disorders (HAND). Although, cART is successful in most cases in rapidly reducing HIV RNA to <50 copies/ml, the virus typically rebounds back quickly, sometimes within two weeks of cessation of therapy [14]. Highly sensitive assays capable of detecting 1 copy of HIV RNA /ml have revealed that around 80% of patients continue to have low level viremia of around 3-5 copies/ml despite several years of cART [1516]. Such studies have strengthened the belief that latent infections persist in certain cells within the host and that latent viral genomes can be reactivated to produce infectious viral particles [17-19] that perhaps are responsible for the rebound of the virus. Multiple mechanisms have been proposed as to how low level viral replication may lead neurocognitive disorders. These include neurotoxicity and glal cell activation by viral proteins such as gp120 and Tat. In particular antiretroviral drugs do not impact the production of Tat protein once the proviral DNA has been formed and the viral

reservoir has been established[20, 21]. Tat can also travel along neuronal pathways and thus have far reaching effects from the site of production (reviewed in[8]).

The failure to eradicate HIV from its reservoirs in host tissues is one of the major hurdles towards curing HIV infection and cells in the brain constitute one such reservoir. cART therapy is successful in controlling HIV-1 replication in active CD4+ immune cells but fails to target infected quiescent cells. Microglia, perivascular and meningeal macrophages, astrocytes, and neural stem cells are sites of viral infection in human brain [22-27]. It is now clear that a cure for HIV infection is not possible unless safe heavens of the virus are purged and total eradication of HIV from the host is achieved. Recognition of this stumbling block has prompted several investigators to focus their research efforts on the paramount issue of eradication of HIV from its reservoirs.

HIV infection of the brain

HIV may traffic into brain via blood monocytes termed the Trojan horse phenomenon early in the course of infection long before symptoms of AIDS appear [28]. In fact, the virus can be detected in the CSF soon after a primary infection [29]. Phylogenetic studies suggest that the virus enters the brain early in the course of infection and subsequent viral entry may be inhibited by establishment of an immune barrier [30]. Virus may enter the brain again in the later stages of infection when there is a general immune failure [31]. The ability of the virus to replicate depends on the cells type and its state of activation. If complete viral particles are formed by the cell, it is termed, productive infection. In the context of brain infection, if p24 immunostaining is present, it has been interpreted to mean “productive infection”. In contrast, “latent infection” means the presence of proviral DNA but the absence of any HIV proteins being formed. It is uncertain if this form of true latency exists in HIV-infected brain tissues since detection of such cells is technically challenging. The term “restricted infection” has been used to describe the production of some viral proteins in the absence of production of infectious viral particles. HIV infected astrocytes may immunostain for nef protein but not p24 and hence this term is most often used to describe these cells.

Once inside the brain parenchyma, it resides in perivascular macrophages and microglial cells that provide the site of productive replication and evolution for HIV. Importantly however, the virus appears to not enter the brain in all individuals. In a small study of 13 patients it was found that nearly 50% of patients do not have HIV in the brain as detected by PCR at the time of death [32]. While larger and more rigorous studies are needed to validate these findings, it would be critically important to try and identify these patients as they may have the best chance for curative therapies.

Eradication of HIV from human brain is challenging due to the selectively permeable blood brain barrier (BBB) that interferes with bioavailability of cART in brain. Poor penetration of most of cART drugs into brain is attributed to an highly efficient drug efflux systems in the brain [33, 34]. However, a recent study showed that further intensification of antiviral therapy with raltegravir, an integrase inhibitor which achieves high CSF concentrations [35], did not reduce HIV RNA levels in CSF or intrathecal immunoactivation [36].

Replication and non-replicating viral DNA in brain

Following infection, HIV RNA gets reverse transcribed into a strand of DNA that can either get integrated in to the chromosomal DNA or it may reside episomally either as a circular DNA or a linear strand of DNA. This differentiation is important since integrated viral DNA is capable of producing viral products and can be silenced by epigenetic changes whereas episomal DNA may either be non-functional or the cell may eventually release the DNA extracellularly. In a study from the pre-cART era, the amount of unintegrated DNA in the brain was found to be 6-81 fold compared to integrated DNA [37]. The unintegrated DNA was largely in a linear form and only 01-1% of the viral DNA was circular. Importantly, the levels of unintegrated DNA did not correlate with the amount of viral antigen in the brain suggesting that the unintegrated DNA was either latent or dysfunctional. In contrast, in T cells, HIV is predominantly in an integrated state however central memory T cells may harbor the virus in a non-induced proviral state. Since this cannot be easily induced replication competent, it poses a significant barrier to eradication of HIV[38].

DNA has been shown to modulate resting T cell activity [39]. The high levels of unintegrated DNA may be suggestive of reinfection or superinfection of cells potentially making the brain an important reservoir for the virus. Another study shows that the presence of unintegrated HIV DNA and detection of HIV proteins in the brain is associated with dementia [40]. Importantly, the viral load in the brain on patients on prolonged cART may be very low [41].

Evolution of HIV sequences in brain

Since the brain is a relatively immune privileged site and is devoid of resident lymphoid cells, the selective pressure on the virus is different compared to other lymphoid organs. The virus evolves in the brain over time and thus acquires unique genetic and functional features. For example, the envelope protein evolves to become more macrophage tropic [42] with unique brain specific mutations[43]. The macrophage tropic strains of HIV in the brain are functionally different compared to macrophage tropic strains derived from the immune system [44]. A database (http://www.HIVBrainSeqDB.org) of envelope sequences has been created which contains 2517 envelope sequences from 90 patients. 1272 sequences are from brain; the remaining are from non-brain tissues [45]. Similarly, the Tat protein of HIV also evolves in the brain and while it maintains its HIV activation properties [46] it may vary in its neurotoxic potential [47]. The *nef* gene acquires unique sequences in patients with dementia compared to those without [48]. Normalized nonsynonymous substitutions in the nef gene are more frequent in brain compared to lymphoid tissue[49]. The brain-specific nonsynonymous substitutions are in regions of functional importance resulting in efficient replication in macrophages[49]. Viral sequences derived from brain macrophages and astrocytes show compartmentalization suggesting that cell specific evolution occurs in the brain [50]. Viral sequencing shows that the meninges harbor virus from both the brain and peripheral tissues suggesting that HIV is capable of migrating out of the brain, and the meninges are the most likely primary transport tissue [51]. The effect of antiretroviral therapy on evolution of HIV in the brain is not well understood. Poor penetration of antiretrovirals across the blood brain barrier might result in low frequency of antiretroviral resistant sequences in the brain and

hence cART might drive the compartmentalization of HIV in the brain. cART has been shown to induce a switch HIV co-receptor usage from CRR5 to CXCR4 which appears later in the CNS compartment compared to the periphery [52]. Importantly, even in patients on antiretroviral drugs analysis maximal viral evolution occurs within brain tissues of individuals with dementia compared to without dementia [27].

Regional compartmentalization of HIV in brain

The virus can be found in any part of the brain. However, maximal viral loads have been found in the basal ganglia, in particular in the caudate and globus pallidus, as well as in the medial temporal lobes, the hippocampus, and the frontal lobes [53, 54] The reason for this predilection is not clear, though several viral encephalitides that are spread hematogenously also target these areas preferentially [55]Recent studies suggest that there is a substantial viral load in the meninges as well where there is a rich collection of macrophages [51]. These meningeal macrophages also get infected with HIV and this may be a yet important reservoir for the virus. It is also possible that some of the infected macrophages might traverse the perivascular spaces from the meningeal blood vessels into the brain parenchyma where a network of macrophages have been shown to communicate between the two spaces[56]. The infected cells may be present in foci called microglial nodules and in some patients the infected macrophages may fuse to form multinucleated giant cells.

Perivascular and meningeal macrophages have been shown to be sites of active viral replication in human brain. Most studies do not distinguish between macrophages and microglia in the brain and many of the commonly used cellular makers stain both cell types and infection of parenchymal cells has been interpreted as microglial cell infection[57]. In an SIV model using a panel of makers to differentiate subtypes of macrophages and microglia, it was claimed that the parenchymal microglia do not get infected [58]. However, using similar markers it was found that about two thirds of productively infected cells in patients with HIV encephalitis were parenchymal microglia. [59]. Unfortunately, cART is not as effective in controlling HIV replication in microglial cells [59].

In addition to microglia, astrocytes, the most abundant cells in brain, are sites for HIV latent or persistent infection. The evidence for harboring of HIV in astrocytes comes from detection of viral DNA and RNA in post mortem brain tissues from AIDS patients [60-62] . Astrocytes *in vivo* have been demonstrated to contain integrated HIV [26]. Astrocytes may be efficiently infected by cell to cell contact with HIV-infected lymphocytes [63]. Studies using laser capture dissection microscopy with amplification of viral genes have demonstrated the frequency of astrocyte infection to be up to 20%. The frequency of infection correlates with both the severity of HIV encephalitis and proximity to perivascular macrophages and multinucleated giant cells[64]. HIV proteins can be detected in astrocytes with over-expression of the Nef protein [25, 65]. Astrocytes produce new viral particles when challenged with inflammatory cytokines [66]. It remains unknown when in the course of HIV infection do these cells get infected. The timing of astrocyte infection may be critical, since these cells are considered to be long lived cells. Studies looking at astrocyte turnover are limited and a single murine study suggests a rate of 0.4% per day in the corpus callosum suggesting that it would take nearly half the life span of a mouse to turn over all the astrocytes [67]. However it

is not known if the turnover of perivascular astrocytes maybe different compared to parenchymal astrocytes and if the rate may be altered in pathological states.

Turnover of HIV reservoirs in brain

Once the virus has entered the brain and has infected resident brain cells, the turnover rate of these cells would be critical to determine if they can be replaced by uninfected cells if genetic approaches to making HIV resistant cells are to be employed. Animal studies suggest that the turnover rate of perivascular macrophages may be quite rapid and within 14 weeks all perivascular cells get replaced [68] How this timeframe translates from mice to humans is not known. Further, in sites of neuronal injury, these macrophages migrate from the blood to the site and assume microglial markers and morphology within three days suggesting that the turnover rate of microglia may also be high in case of neuronal injury [69]. This is important because HIV-infected patients may have ongoing neuronal injury and in the case of patients who are additionally treated with radiation and chemotherapy [3] there may be an acceleration of microglial turnover.

Strategies for eradication of viral reservoirs

Several strategies are currently being pursued (Table). The variety of approaches are rapidly expanding and hence it is prudent to take a closer look at them to see what impact they might have on the brain and HIV reservoirs in brain cells. A popular approach is to activate viral replication in the reservoirs in the presence of cART to prevent the virus from spreading to other cells. Viral proteins produced by these latent reservoirs will be recognized by the immune cells and reservoir would then be eliminated. Broadly, these include drugs that could modulate epigenetic changes such as histone deacetylase inhibitors or immune activation therapies [70]. A concern with this strategy might be that if the reservoir in the brain has been established then activation and production of viral products could lead to an infiltration of cytotoxic T cells. Infiltration of activated lymphocytes in the brain could be injurious to neurons [71] leading to a devastating encephalitis termed, CNS-immune reconstitution syndrome[72] . Further, similar strategies have failed to eliminate other persistent CNS viral infections such as JC virus and herpes viruses [72]. However, there may be a window of opportunity for viral eradication via this strategy before it enters the brain. Immune ablation is also being considered for elimination the reservoirs however, due to the associated toxicity and immune suppression it has been used only in HIV-infected individuals who have developed leukemia or lymphoma. This approach targets dividing cells hence brain cells that are terminally differentiated may not be eliminated. Yet another approach being used is to engineer HIV resistant stem cells by creating mutations in the chemokine receptor CCR5 [73]. However since HIV can enter cells using the CXCR4 chemokine receptor this mutation alone may not be sufficient and creating additional blocks for viral replication post-viral entry should be considered. Whatever strategy is used for either achieving a sterilizing or functional cure, it is critical that close attention be given to similarly controlling the CNS viral reservoir or else there would be a theoretical risk for re-seeding periphery with the virus from the CNS. Experimental studies to evaluate this risk are critically needed.

Imaging HIV reservoirs

If viral reservoirs are to be eliminated, then they need to be monitored in real time. Since tissue reservoirs including those in the brain cannot be sampled, imaging techniques will be critical. Although currently, there are no such available techniques, several methods are being considered. This includes developing ligands for positron emission tomography using molecular probes, antibodies to the envelope protein, gp120 or antiviral drugs. However, the ability of the probes and antibodies to cross the blood brain barrier is limited and if viral loads are low such techniques may not be sensitive enough, hence further research along these lines is necessary.

Summary and Future Directions

In summary, while substantial research is still necessary to understand how viral reservoirs in the brain can be manipulated, eradication of HIV may be possible under certain circumstances. In particular we need to identify when the virus enters the brain and if there are certain individuals in whom the virus does not enter the brain at all. Further, if patients are going to be treated with agents that will lead to viral activation, some measure of CNS reservoirs are necessary. This may require the development of techniques for imaging the reservoirs and patients should be closely monitored for any signs of CNS inflammation so that appropriate treatment can be initiated. Genetically modified cells that are resistant to HIV infection might be useful, if the turnover of the HIV-infected cells is brain is faster that the resident parenchymal cells. Alternatively, in patients where CNS reservoirs have been established the possibility of developing a functional cure should be considered. The immune system may be capable of maintaining the virus in a latent state. Certainly, the immune system keeps in check multiple viral and other microbial organisms that manifest themselves as opportunistic infections only when the immune system fails. This requires sustained immune responses against the organism. However, in HIV-infected individuals, in whom viral replication is controlled with cART, the immune responses wean [74], hence periodic immunization may be necessary to maintain a sustained immune response against the virus [75].

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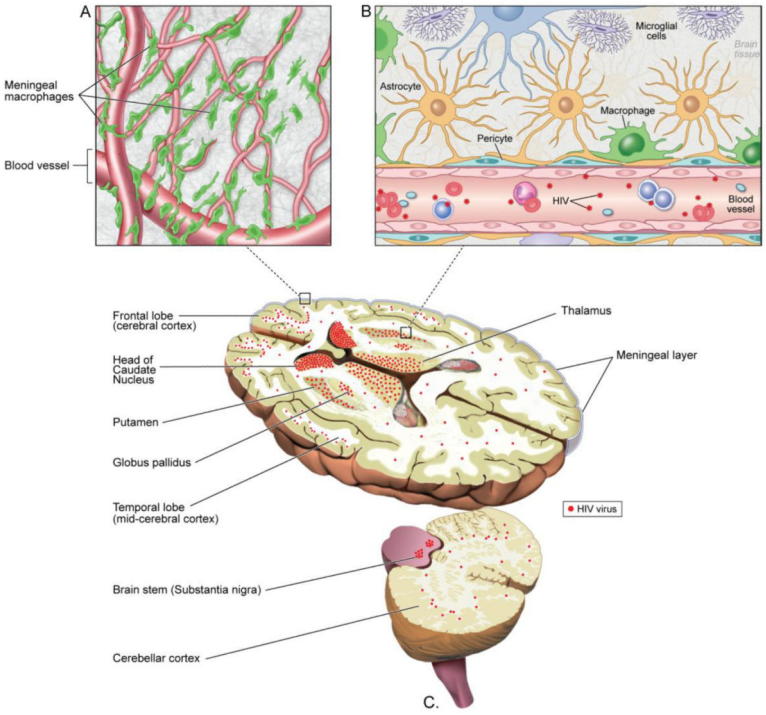

Sites of HIV Reservoir in the Brain: HIV in compartmentalized in the subcortical white matter and the midline structures of the brain. This includes the frontal and temporal lobe, the basal ganglia and the brain stem. The posterior parts of the brain are relatively spared. Within these regions HIV infects the macrophages/microglia and astrocytes most commonly located in the perivascular regions where they constitute the blood brain barrier. A previously under appreciated site of viral infection in the brain is the subarachnoid space where the meningeal macrophages form a rich plexus of cells surrounding the meningeal blood vessels. Viral sequencing suggests that the meninges harbor both brain and peripheral blood derived viruses, suggesting that this may be a route for viral transmission from brain to blood. Alternatively, the virus can spread along the meningeal macrophages to the brain parenchyma

Table

Approaches to curing HIV infection

Strategy		**Relevance for Brain Reservoirs**
Sterilizing Cure		
	Create HIV resistant cells	Need to create multiple blocks to HIV replication
	HIV excision using gene editing tools	Delivery to all infected cells in brain would be needed
	Blocking HIV entry into brain	Window of opportunity following primary infection needs to be defined
Functional Cure		
	Immune ablation	HIV resides in terminally differentiated cells in brain, hence unlikely to be effective
	Genetic silencing	Would need to take into account viral evolution in brain
	Enhancement of cytotoxic immune responses in presence of cART	Poses risk of immune reconstitution inflammatory syndrome in brain