HIV-1 target cells in the CNS

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Abstract HIV-1 replication in the central nervous system (CNS) is typically limited by the availability of target cells. HIV-1 variants that are transmitted and dominate the early stages of infection almost exclusively use the CCR5 coreceptor and are well adapted to entering, and thus infecting, cells expressing high CD4 densities similar to those found on CD4+ T cells. While the "immune privileged" CNS is largely devoid of CD4+ T cells, macrophage and microglia are abundant throughout the CNS. These cells likely express CD4 densities that are too low to facilitate efficient entry or allow sustained replication by most HIV-1 isolates. Examination of CNS viral populations reveals that late in disease the CNS of some individuals contains HIV-1 lineages that have evolved the ability to enter cells expressing low levels of CD4 and are well-adapted to entering macrophages. These macrophage-tropic (M-tropic) viruses are able to maintain sustained replication in the CNS for many generations, and their presence is associated with severe neurocognitive impairment. Whether conditions such as pleocytosis are necessary for macrophage-tropic viruses to emerge in the CNS is unknown, and extensive examinations of macrophage-tropic variants have not revealed a genetic signature of this phenotype. It is clear, however, that macrophage tropism is rare among HIV-1 isolates and is not transmitted, but is important due to its pathogenic effects on hosts. Prior to the evolution of macrophage-tropic variants, the viruses that are predominately infecting T cells (R5 T cell-tropic) may infect macrophages at a low level and inefficiently, but this could contribute to the reservoir.

Keywords Macrophage tropism · HIV-1 · Pathogenesis · Host range variants · NeuroAIDS · CNS

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Introductory summary

HIV-1 pathogenesis has been a major focus of research for the last 30 years. The central feature of HIV-1 disease is the loss of CD4+ T cells leading to immunodeficiency and the appearance of opportunistic infections and virus-driven cancers. In addition, untreated HIV-1 infection can lead to HIV-associated dementia (HAD) in a significant proportion of subjects. Viral replication in and destruction of CD4+ T cells is only part of the story of viral pathogenesis. To maintain replication in the face of the host immune system, HIV-1 has the capacity to undergo rapid evolution that results in the outgrowth of immune escape mutants, escaping the selective pressure of both humoral and cellular immune responses. This capacity for rapid evolution underlies the ability to generate drug-resistant variants in the presence of incomplete suppression of replication. However, these are not the only places where the effects of viral evolution can be seen.

The rapid evolution of HIV-1 also generates host range variants, i.e., variants of the virus that have evolved to

replicate in new cell types. In addition to the typical virus, which uses CD4 and CCR5 to enter activated T cells, there are two well-recognized variants. One variant switches from using CCR5 as the coreceptor to using CXCR4 for entry, in addition to CD4, and these viruses have been termed X4 viruses. X4 viruses likely represent an expansion of viral replication into naive CD4+ T cells, which express high levels of CXCR4 and little to no CCR5 (Zamarchi et al. 2002). The second variant retains the requirement for both CD4 and CCR5 but evolves to enter cells that have lower levels of CD4. The obvious target cell for this variant is the macrophage, which has significantly lower levels of CD4 than CD4+ T cells, and these variants have been termed macrophage-tropic (or M-tropic) viruses. The appearance of X4 variants has been associated with a more rapid loss of CD4+ T cells (Connor et al. 1997; Koot et al. 1993), although the question of cause or effect persists (Arrildt et al. 2012). Viral replication in both naive CD4+ T cells and macrophages may be slower than in activated memory CD4+ T cells, thus allowing some level of immune surveillance prior to immunodeficiency. Alternatively, the virus may be more likely to encounter activated memory CD4+ T cells with their high levels of CD4 and CCR5, thus driving the selective pressure for replication in this cell type until it declines in number with advanced disease. This review will focus on macrophage-tropic variants, their evolution, and their role in pathogenesis. As interest in pathogenesis morphs into questions about viral persistence and latency on therapy, a different set of issues arise about the role of macrophage-tropic viruses.

Nomenclature

"Words have meaning and names have power." Cervantes

There is an unfortunate twist in the history of HIV-1 research that has led to great confusion about the nature of macrophage-tropic HIV-1. Initial attempts to grow HIV-1 in CD4+ T cell lines (transformed cells typically isolated from lymphomas or leukemias) showed that only a fraction of viral isolates were capable of infecting these cells and quite naturally the viruses that could were called T cell-tropic. Of all the isolates not able to infect these T cell lines, some of them could efficiently infect macrophages and the entire group of "non-T cell-tropic" viruses have come to be called macrophage-tropic. The subsequent identification of CCR5 and CXCR4 as coreceptors revealed an important flaw in this dichotomous designation, and as a field we are now struggling to redefine these viruses and integrate how the new definitions impact our understanding of HIV-1 pathogenesis and persistence.

Specifically, we now know that most transformed CD4+ T cell lines do not express CCR5 and therefore the "T cell-tropic" viruses are now appropriately called X4 viruses for their use of CXCR4. The confusion comes with the "macrophage-tropic" viruses, which we now know are a collection of two distinct types of CCR5-using viruses. Although a small minority of the CCR5-using viruses can enter cells with low levels of CD4 and infect macrophages efficiently, the vast majority of these viruses require high levels of CD4 to enter cells and only infect macrophages poorly. Thus, it is a serious conceptual mistake to refer to all of them as macrophage-tropic. We have developed the following designations to describe the host range of most HIV-1. (1) *R5 T cell-tropic*: these variants use CCR5 and require high levels of CD4 to enter cells, and represent the vast majority of HIV-1, including transmitted/founder viruses. (2) *X4 T cell-tropic*: T cell-tropic variants that have switched to use the CXCR4 coreceptor. (3) *Macrophage-tropic*: variants that still use CCR5 but can enter macrophages and other cells expressing low levels of CD4. We review the evidence for these designations below, but want to point out that it is essential to view HIV-1 as these three different types of host range variants—the normal R5 T cell-tropic virus and the two host range variants, X4 T cell-tropic viruses and macrophage-tropic viruses—to place HIV-1 transmission, evolution, pathogenesis, and persistence in their proper context.

There are two (at least) types of HIV-1 that do not fit neatly into our basic nomenclature. First, some T cell-tropic viruses evolve the ability use CXCR4, while retaining the ability to use CCR5. It is unknown whether these dual-tropic variants use both coreceptors in vivo, but the extent to which the virus favors one co-receptor or the other largely predicts its sensitivity to coreceptor antagonists in cell culture assays (Toma et al. 2010). Second, some macrophages express low levels of CXCR4 (Lee et al. 1999), raising the possibility that CXCR4-using viruses could evolve macrophage tropism. CXCR4-using viruses have been isolated from the macrophage-rich central nervous system (CNS) (Mefford et al. 2008; Yi et al. 2003), but it is unknown whether these variants are macrophage-tropic as defined by the ability to enter using low levels of CD4. One of these variants was shown to infect macrophages differentiated in culture (Yi et al. 2003), but differentiation methods are known to strongly influence expression of CXCR4 and CD4 (Lee et al. 1999), making it difficult to know if this variant would be likely to infect macrophages in vivo. At a minimum, putative X4 macrophage-tropic viruses are exceedingly rare, indicating that either CXCR4-using viruses cannot adapt to replicating in macrophages or do so infrequently.

The nomenclature that we have adopted indicates the cell type in which viruses are replicating and adapting. This is similar to the "lymphocyte-R5" nomenclature that has been proposed by Goodenow and Collman (2006). A limitation of both approaches is that "R5 T cell-tropic" viruses (or "lymphocyte-R5" viruses) have not been shown to be better adapted at replicating in T cells than are "macrophage-tropic" variants. In order to avoid any implication that one type of

virus is better able to infect T cells than another, some researchers (Peters et al. 2006) simply divide viruses into "macrophage-tropic" and "non-macrophage-tropic". The disadvantage of this approach is that it provides no information about where the vast majority of HIV-1 (i.e., "non-macrophage-tropic" HIV-1) replicates. We have chosen to distinguish viruses based on the cells in which they are replicating, and we acknowledge that we have an incomplete understanding of the relative replication rates of different viruses in T cells and T cell subsets.

Note that tropism is often assessed based on the ability of a HIV-1 variant to enter a specific host cell, but replication blocks that arise after entry may also influence cellular tropism. HIV-1 entry is determined by the HIV-1 Env protein. The Env precursor protein is cleaved into two subunits by a host furin-like protease. The resulting subunits, gp120 and gp41, stay non-covalently associated as heterodimers and are organized as trimers on the surface of the virion, with gp41 being a transmembrane protein anchoring the trimer in the viral envelope/membrane. Entry into the host cell is initiated when the CD4 binding site of gp120 binds a CD4 molecule on the surface of the host cell, which results in conformational changes that expose the coreceptor binding site on gp120. Binding either the CCR5 or CXCR4 coreceptor causes the extracellular domain of gp41 to form a six-helix bundle and generate a fusion pore between the viral and host membrane that allows the capsid to enter the host cell (reviewed by Wilen et al. 2012). Thus, functional or genetic analyses of Env proteins are effective tools for assessing the ability of an HIV-1 variant to enter a target cell. However, other factors may also influence cellular tropism after entry. For example, the concentration of dNTPs in macrophages is approximately 100-fold lower than that of activated CD4+ T cells and efficient replication in macrophages requires that reverse transcriptase be able to catalyze DNA synthesis when the dNTP concentration is low (Diamond et al. 2004). We would argue that the viral proteins that act against intracellular innate host responses evolved to do so in T cells, but it is possible that these viral proteins also adapt to carry out similar functions, or the long terminal repeats (LTRs) adapt to different host transcription factors, in the environment of different cell types.

HIV-1 in the blood is R5 T cell-tropic

There are three types of evidence indicating that the vast majority of HIV-1 is R5 T cell-tropic. The first type of evidence comes from studies showing that T cells are the most commonly infected cells in the blood. This is illustrated by the fact that HIV-1 proviral DNA and replication competent viruses can be readily recovered from CD4+ T cells isolated from HIV+ subjects both on (Brenchley et al. 2004; Chun et al. 1997; Finzi et al. 1997; Ho et al. 2013; Wong et al. 1997; Yukl

et al. 2013) and off antiretroviral therapy (Brinchmann et al. 1991; Psallidopoulos et al. 1989; Schnittman et al. 1989). The only other cell type in the blood reported to be infected is monocytes. A number of studies report the detection of HIV-1 DNA in monocytes (Wang et al. 2013; Xu et al. 2008; Zhu et al. 2002) and the isolation of replication competent virus (Lambotte et al. 2000; Sonza et al. 2001; Wang et al. 2013) from monocytes, but T cells are clearly infected with much greater frequency than monocytes (Fulcher et al. 2004; Lambotte et al. 2000; Spivak et al. 2011) and some investigators have failed to find viral DNA in monocytes above levels that could be accounted for by T-cell contamination (Spivak et al. 2011).

The second line of evidence that CD4+ T cells are the primary targets of HIV-1 in vivo comes from the observation that initiating highly active antiretroviral therapy (HAART) results in exponential decay of HIV-1 in the blood (Ho et al. 1995; Perelson et al. 1996) (Fig. 1). Since antiretroviral therapy blocks infection of new cells, viral decay reflects the death of infected cells and can be used to calculate the half-life of HIV-1-infected cells. Exponential viral decay after the initiation of antiretroviral therapy indicates that most viruses are produced by short-lived cells such as infected T cells (Ho et al. 1995; Perelson et al. 1996; Simon and Ho 2003; Wei et al. 1995).

Viral Decay Upon Initiation of HAART
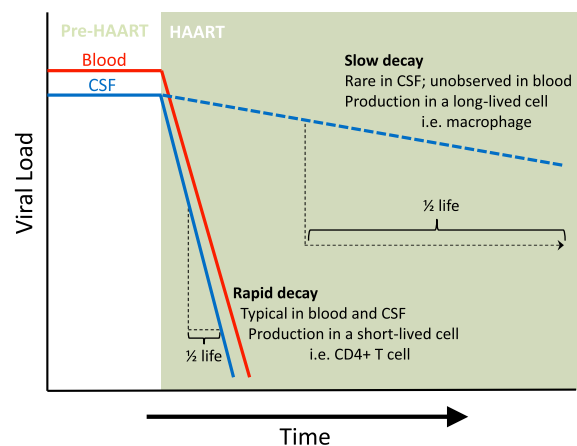
Fig. 1 Successful HAART blocks new infection but does not affect cells already infected with HIV-1. Therefore, the remaining viral production (and viral load) is directly related to the lifespan of the cells infected prior to initiating HAART. In essentially all patients infected with HIV-1, viral load (VL) decays rapidly upon initiation of HAART with a half-life of approximately 1 to 2 weeks in the blood (indicated by the *solid red line*) and cerebrospinal fluid (*CSF*; *solid blue line*), which is consistent with virus produced from infected CD4+ T cells in both compartments. In rare cases, patients will have the same rapid decay of VL in the blood but will have a much slower decay in the CSF (*dashed blue line*). This indicates that in these patients viruses are being produced in CD4+ T cells in the blood but are being produced by a longer-lived cell, likely perivascular macrophages or microglia, in the CSF.

The final type of evidence comes from in vitro analyses showing that most HIV-1 isolates are well adapted to replicating in CD4+ T cells and poorly adapted to replicating in macrophages, the rationale being that an HIV-1 lineage will be well-adapted to the cell type in which it typically replicates. Multiple studies have shown that infectious molecular clones (Li et al. 2010; Ochsenbauer et al. 2012; Salazar-Gonzalez et al. 2009) and env gene clones (Isaacman-Beck et al. 2009) generated from transmitted/founder viruses encode surface Env proteins that do not allow efficient infection of monocyte-derived macrophages (MDM). A limitation of this approach is that MDMs are highly variable in their susceptibility to infection by HIV-1 both across donors and from different time points (Joseph et al. 2014; Naif et al. 1998; Sonza et al. 1995), which may cause a virus to appear well adapted to infecting MDMs in one experiment and poorly adapted at another.

There is another, more reproducible approach to examining whether viruses are adapted to entering macrophages by examining whether viruses are adapted to entering cells with similar CD4 receptor levels compared to macrophages. Monocyte-derived macrophages and CD4+ T cells express similar numbers of CD4 molecules (Joseph et al. 2014; Lee et al. 1999), but their larger surface area causes MDMs to have a 20-fold lower CD4 density than do CD4+ T cells (Joseph et al. 2014). Using this approach, we (Joseph et al. 2014; Ping et al. 2013) and others (Alexander et al. 2010; Parrish et al. 2012; Peters et al. 2006) have found that blood-derived Env proteins from all stages of infection require high densities of CD4 to facilitate entry, similar to those levels expressed on CD4+ T cells (Joseph et al. 2014). In vitro assays have also revealed that the vast majority of blood-derived Env proteins require CCR5 (Alexander et al. 2010; Isaacman-Beck et al. 2009; Keele et al. 2008; Parrish et al. 2012; Wilen et al. 2011), but it is unclear whether they differ from CNS-derived, macrophage-tropic Env proteins. We previously observed no difference in how paired macrophage-tropic and T cell-tropic Env proteins from five patients interact with CCR5 (Joseph et al. 2014). In contrast, another study suggested that macrophage-tropic viruses are more "CCR5-dependent" (Salimi et al. 2013); however, their analysis may have confounded CCR5 usage with CD4 usage.

Because most blood-derived viruses are well adapted to entering CD4+ T cells and use the CCR5 coreceptor, most HIV-1 isolates are R5 T cell-tropic. This situation changes with the appearance of X4 T cell-tropic and/or dual-tropic viruses appearing in the blood of approximately one half of subjects infected with subtype B HIV-1 as they progress to late in disease (Berger et al. 1999).

While T cells are the most commonly infected cells in the blood and most viruses in the blood are R5 T cell-tropic, there is evidence that tissue macrophages may represent important viral reservoirs and may be detected in the blood under some circumstances. It has long been noted that after initiating antiretroviral therapy, the plasma viral load initially decays very rapidly but then enters a period of slow decay that corresponds to the release of virus from longer-lived cells (Perelson et al. 1997). One potential source of this plasma virus is infected macrophages in tissue. In some patients, CNS macrophages are known to be productively infected by HIV-1 (see below). It is still unknown how often HIV-1 infects macrophages in different tissues and what conditions favor those events, but some studies suggest that coinfections may promote HIV-1 infection of macrophages in tissue. An analysis of macrophages in lymphoid tissue found that macrophages were infected with HIV-1 in subjects with opportunistic infections (OIs), but not in subjects lacking OIs (Orenstein et al. 1997). Further, a study of subjects coinfected with HIV-1 and *Mycobacterium tuberculosis* found that both macrophages and T cells contributed to virus in pleural fluid (Lawn et al. 2001). However, when the slow decay virus was examined using the heteroduplex tracking assay to assess its genetic complexity, it was found to be the same as the virus in the blood plasma indicating that this component of virus, at least in the subjects examined, does not represent a distinct population (Ince et al. 2009).

The CNS is an "immune privileged" site containing a unique mix of target cells

The CNS has long been viewed as an "immune privileged" site where T cells are rare and antigens do not induce a strong adaptive immune response. The foundation of this concept can be traced as far back as 1921 when it was observed that a rat sarcoma tumor grew well if transplanted into the mouse brain but failed to grow when transplanted outside of the CNS (Shirai 1921). Subsequent studies were able to show that growth of the tumor in the CNS was possible because the CNS shielded it from immune surveillance (reviewed by Galea et al. 2007).

How the immune system achieves this "privilege" is generally attributed to the blood–brain barrier (BBB) and the blood–cerebrospinal fluid barrier (BCSFB), which restrict the movement of cells and other materials from the peripheral blood into the CNS (Ousman and Kubes 2012). The BBB lines blood vessels in the brain and consists of endothelial cells expressing tight junctions. In the average adult, this barrier has a large surface area of between 12 and 18 m^2 (Nag and Begley 2005), presenting many potential points of weakness where substances in the blood might gain direct access to the brain parenchyma. Alternatively, substances can cross the BCSFB at the choroid plexus. Ependymal cells within the choroid plexus secrete CSF by processing the peripheral blood (Brown et al. 2004) and the resulting CSF flows into the brain ventricular system and circulates through

the subarachnoid spaces surrounding the brain. Substances in the CSF can enter the brain parenchyma and spinal cord by crossing ciliated ependymal cells that line the ventricles and subarachnoid spaces (Del Bigio 2010). Thus, there are different barriers separating the blood from the CNS and multiple ways for substances to breach those barriers and reach the brain parenchyma.

Immune privilege functions to maintain the proper conditions for neuronal and glial signaling (Verkhratsky et al. 1998) and protect the delicate cells of the CNS. The efficiency of these barriers is well illustrated by the observation that the concentration of T cells and HIV-1 in the cerebrospinal fluid (CSF) is typically less than 1 % of that found in the blood. These barriers also appear to alter the ratio of cells. For example, neutrophils, the most common leukocytes in the blood, are rarely observed in the CSF, and the ratio of CD4+ to CD8+ T cells is higher in the CSF than in the blood (Ransohoff et al. 2003). Thus, the BBB and BCSFB considerably limit movement from the blood into the CNS and also select for specific cells.

There are a number of ways for T cells may to enter the CNS (Ransohoff et al. 2003). An analysis of fluorescently labeled T cells injected into the peripheral blood of mice showed that 2 h after being injected, the cells could be observed entering the CNS through both the choroid plexus and meninges (Carrithers et al. 2002). This is supported by the observation that T cells are clustered in the choroid plexus and meninges of human autopsy tissue (Kivisakk et al. 2003). Another study found that CD4+ T cells that have been primed to attack myelinated nerves primarily enter the CNS at the fifth lumbar cord (Arima et al. 2012). Interestingly, this study also found that this point of entry could be blocked by reducing the expression of the chemokine CCL20 (MIP3A) (Arima et al. 2012), and other studies have shown that inflammatory cytokines such as tumor necrosis factor α (TNFα), interleukin (IL)-1β, and IL-6 reduce the integrity of the BBB (deVries et al. 1996). Thus, permeability of the BBB varies both by anatomical region and immunologic state.

HIV-1 in the CNS can be either R5 T cell-tropic or R5 macrophage-tropic

The CNS contains three groups of CD4+ immune cells that are likely to be infected by HIV-1: CD4+ T cells, macrophages, and microglia. The CNS also contains CD4− astrocytes which have been proposed to be infected by HIV-1. Unlike CD4-mediated infection of T cells, macrophages, and microglia, infection of astrocytes would require a CD4-independent pathway. Here we review the evidence that HIV-1 infects these cells in vivo.

CD4+ T cells

CD4+ T cells are the most commonly HIV-infected cells in the human body overall (see above) and are major targets of HIV-1 infection in the CNS. The CSF of a healthy individual contains as many as 300,000 CD4+ T cells in a total volume of approximately 150 ml (Ransohoff and Engelhardt 2012), the majority of which are CD4+ CD45RA− CD27+ CD69+ activated central memory T cells (Kivisakk et al. 2002, 2003). Given that CD4+ memory T cells are the primary target of HIV-1 in the blood (Brenchley et al. 2004; Douek et al. 2002; Sleasman et al. 1996), the T-cell population in the CSF is likely to be highly susceptible to infection by HIV-1.

Direct identification of HIV-infected CD4+ T cells in the CNS is challenging given that CD4+ T cells are primarily confined to the CSF, where they are present at low concentration. Similarly, the concentration of CD4+ T cells in the brain parenchyma is likely to be very low. Thus, identifying HIV-infected CD4+ T cells in the CNS requires less direct methods, such as assessing whether viruses replicating in the CNS are well adapted to entering T cells and not adapted to entering macrophages or microglia. In an analysis of eight subjects diagnosed with HAD, we observed three subjects with CSF viral populations that were genetically distinct compared to virus in the blood (i.e., compartmentalized) (Schnell et al. 2011), poorly adapted to entering macrophages (Schnell et al. 2011) and other low CD4 cells (Schnell et al. 2011) and decayed rapidly when the subject initiated therapy (Schnell et al. 2009). The observation that some viral populations in the CSF decay rapidly after initiating therapy and are poorly adapted to entering macrophage/microglia indicates that the virus was replicating in CD4+ T cells at the time of sampling and had been replicating in CD4+ T cells for many generations. Whether CNS inflammation and pleocytosis are necessary to support ongoing replication in CD4+ T cells in the CNS is unknown.

Microglia

Examinations of microglia and macrophages in the CNS reveal that they are frequently infected by HIV-1 despite expressing low levels of CD4 (Dick et al. 1997; Wang et al. 2002), levels that are well below those expressed on CD4+ T cells in the blood (Dick et al. 1997). Assessing the relative susceptibility of these two types of myeloid cells to HIV-1 infection is complicated by the fact that they express many of the same surface markers. Prior to activation, these two cell types are easily distinguished based on morphological differences, but activation by infection, trauma, etc. causes microglia to take on macrophage-like characteristics (reviewed by Kettenmann et al. 2011). Despite these similarities, the glucose transporter 5 protein (GLUT5) is expressed on both resting and activated microglia, but not on macrophages

(Horikoshi et al. 2003; Maher et al. 1994; Vannucci et al. 1997), and has been used to stain microglia in healthy (Kitamura et al. 2010) and damaged brains (He and Crews 2008; Kitamura et al. 2010; Sasaki et al. 2004). Similarly, antibodies to the mannose receptor stain macrophages, but not microglia, in inflamed and normal human (Fabriek et al. 2005) and mouse brains (Galea et al. 2005). Given how difficult it is to distinguish these cells by morphology and/or immunohistochemistry, they are often identified based on their localization within the CNS.

Microglia are the resident macrophages in the CNS. They are the only myeloid cells located within the CNS parenchyma (i.e., the brain tissue proper, which does not include the CSF spaces, blood vessels, or meningeal coverings) (Ransohoff and Cardona 2010) and they represent 5–20 % of the adult brain cells (Polazzi and Monti 2010). In their non-activated or "resting" state, the surface of microglia are made up of many branched processes that are continuously surveying the brain, but activation causes them to transition to an amoeboid, macrophage-like appearance (Kettenmann et al. 2011). Recent studies have shown that unlike most myeloid cells, microglia are not derived from hematopoietic stem cells in the bone marrow, but rather they are derived from precursors that colonize the CNS during embryonic development (reviewed by Perdiguero et al. 2013). Microglia share many functions with macrophages such as phagocytosis, secretion of proinflammatory cytokines, and antigen presentation (Polazzi and Monti 2010), but they also have protective roles that promote proper brain function and development (Tremblay et al. 2011).

HIV-1 has been shown to infect microglia both in vivo and in vitro. At autopsy, infected microglia can be identified by labeling brain tissue for HIV-1 RNA (Stoler et al. 1986; Wiley et al. 1986) and/or protein (Cosenza et al. 2002; Neuenjacob et al. 1993; Wiley et al. 1986). However, these analyses are typically performed on subjects who died with severe neurocognitive impairment and HIV-1 infection of microglia may be less common earlier in disease. HIV-1 has also been shown to infect microglia in culture (Albright et al. 2000; Ioannidis et al. 1995; McCarthy et al. 1998; Watkins et al. 1990), but it is unclear how culturing these cells influences their susceptibility to infection. Together, these studies provide strong evidence that microglia are susceptible to HIV-1 infection but provide little information about the frequency of microglial infection in subjects that do not have severe neurocognitive impairment.

It is clear that the viruses replicating in the CNS can make an evolutionary transition from replicating in T cells to replicating in macrophages or microglia. This transition can be assessed by determining whether viruses replicating in the CNS are well adapted to entering cells expressing low levels of CD4 and whether viral RNA load decays slowly in the CSF after initiation of antiretroviral therapy. One caveat of this approach is that microglia and macrophages are both long-

lived cells with low surface densities of CD4. As a result, replication in and adaptation to either cell type would likely result in viral populations that decay slowly and are well adapted to entering cells expressing low levels of CD4. In our analysis of eight subjects diagnosed with HAD, we observed five subjects with CSF viral populations that were genetically distinct from virus in the blood (i.e., compartmentalized) (Joseph and Kincer unpublished data; Schnell et al. 2011), well adapted to entering macrophages and/or other low CD4 cells (Schnell et al. 2011) and decayed slowly after initiation of therapy (Joseph and Kincer unpublished data; Schnell et al. 2009). This suggests that the CSF lineages had been replicating in long-lived, low CD4 cells in the CNS (either microglia or macrophage) for many generations to allow evolution of this host range variant.

It is important to make a distinction about two different circumstances under which cells with low levels of CD4 may become infected. There is not an absolute block to infection of CD4-low cells by R5 T cell-tropic viruses. These viruses will infect MDMs in culture but at very low efficiency compared to M-tropic viruses (K. Arrildt unpublished observation). Thus, there could be low levels of infection of macrophages in vivo throughout infection. Such levels may be enhanced by juxtaposition of an infected T cell to a macrophage (cell–cell transmission). However, this type of infection is distinct from the situation where the virus actually evolves to infect cells with low levels of CD4, i.e., macrophage tropism, signaling a change in the predominant cell type that is being infected locally.

Choroid plexus, meningeal, and perivascular macrophages

There are three types of CNS macrophages found outside of the brain parenchyma: perivascular macrophages, choroid plexus macrophages, and meningeal macrophages (Ransohoff and Cardona 2010). These bone-marrow-derived macrophages are named for anatomical region in which they reside. Perivascular macrophages reside in the perivascular (Virchow–Robin) space of cerebral vessels and are likely to be exposed to HIV-1 that crosses the BBB. In contrast, choroid plexus macrophages are located in the stroma of the choroid plexus on the peripheral side of the BCSFB. Thus, choroid plexus macrophages are likely exposed to HIV-1 in the blood being used to form CSF and meningeal macrophages would be exposed to HIV-1 that reaches the CSF. It is unclear whether HIV-1 migrates readily between these macrophage populations or whether they represent independent sites of viral replication. These questions have been addressed by experiments infecting macaques with highly neurotropic SIV. One study identified genetically distinct viral lineages in the meninges and brain parenchyma of animals with normal disease progression, but failed to detect compartmentalization in animals that progressed rapidly (Matsuda

et al. 2013). This indicates that viruses can replicate independently at different anatomical sites or mix freely throughout the CNS, and it provides suggestive evidence that these viral states are associated with different outcomes.

Most of what we know about the susceptibility of CNS macrophages to HIV-1 is based on immunohistochemistry of autopsy tissue, which most often identify infection of perivascular macrophages (Cosenza et al. 2002; Fischer-Smith et al. 2001). Their position in the perivascular (Virchow–Robin) space of cerebral vessels may increase their exposure to HIV-1 and increase their probability of being infected. This is consistent with the observation that perivascular macrophages are productively infected more often than microglia both in humans with HAD (Fischer-Smith et al. 2001) and in macaques infected with SIV (Williams et al. 2001). Similarly there is some evidence of HIV-1 infection in the meninges, although it is often localized to the perivascular region (Lamers et al. 2011), suggesting that perivascular macrophages are more often infected than other meningeal cells. Two studies observed that 50 % of HIV$^+$ subjects had infected choroid plexus macrophages (Falangola et al. 1995; Petito et al. 1999), but another study failed to find infected cells in the choroid plexus (Zhou et al. 2008). Further, we (Joseph et al. 2014; Schnell et al. 2011) and others (Gorry et al. 2001, 2002; Koyanagi et al. 1987; Li et al. 1999; Peters et al. 2004) have shown that some CNS-derived HIV-1 Env proteins can enter monocyte-derived macrophage in culture, though it is unclear how closely cultured MDMs represent macrophage in vivo. Together, these findings suggest that HIV-1 infects different types of CNS macrophages in vivo and that HIV-1 can adapt to replication in these cells.

Astrocytes

Astrocytes are the most abundant cell type in the brain (Nedergaard et al. 2003) and they express little or no CD4 (Harouse et al. 1989; Liu et al. 2004); also, there is no obvious functional reason for this cell type to express CD4, so its absence could be expected based on first principles. However, several groups of researchers have identified the presence of viral DNA in astrocytes, especially in autopsy samples taken from subjects with HIV-associated neurological disease (Nuovo and Alfieri 1996; Bagasra et al. 1996; Takahashi et al. 1996). These initial observations were confirmed in subsequent studies that showed in some areas of the brain up to 10–20 % of astrocytes have viral DNA (Churchill et al. 2009), although in a non-productive infection (Churchill et al. 2006; Gorry et al. 2003; Thompson et al. 2004). These observations represent a challenge to put in the context of a virus that requires the presence of CD4 for entry, including the M-tropic variants (Joseph et al. 2014). One possible explanation may lie in the recently appreciated capacity of astrocytes to engage in phagocytosis in the context of removal of neuronal debris (Cahoy et al. 2008; Chung et al. 2013; Tasdemir-Yilmaz and Freeman 2014). If these pathways were also active in the context of cell death during CNS infection, then evidence of HIV-1 in astrocytes could be coming from cell debris of infected CD4+ cells.

The evolution of macrophage tropism in the CNS

There is clear evidence that HIV-1 replication in the CNS is associated with neurocognitive impairment. However, our understanding of this relationship has been limited by the fact that HIV-infected cells can only be directly observed in autopsy tissue and may be difficult to detect if they are latently infected, producing low levels of virus, and/or buried deep in the tissue. Despite these limitations, a study of autopsy samples from 39 HIV$^+$ subjects both on and off antiretroviral treatment found that most had HIV-associated encephalitis (HIVE) as characterized by features including multinucleated giant cells, microglial nodules, astrocytosis, and myelin pallor (Cherner et al. 2002). Of those subjects with neurocognitive impairment in their last 18 months of life, almost all had HIVE (95 %) at autopsy (Cherner et al. 2002), and other studies have shown that neurocognitive impairment is more common in HIV$^+$ subjects than in HIV$^-$ subjects (Antinori et al. 2007). Thus, HIV-1 infected individuals often have elevated CNS damage and neurocognitive impairment.

Macrophages and microglia are the predominant CD4-expressing cells in the CNS and are known to be infected by HIV-1 (see above). Further, we have shown that between 40 % and 70 % of subjects with HAD have macrophage-tropic HIV-1 in their CNS (Schnell et al. 2011; Joseph and Kincer unpublished data). This strongly suggests that infection of macrophages and/or microglia contributes to neurocognitive impairment, making it essential to understand how and when macrophage-tropic variants evolve and to develop methods for identifying these variants.

As soon as 8 days after transmission, HIV-1 RNA can be isolated from the CSF (Valcour et al. 2012), but it is unclear whether the CNS is initially colonized by free virus or by infected cells that subsequently release virus into the CNS/CSF. In a subset of infected individuals, there is minimal CNS viral burden early (Spudich et al. 2011; Sturdevant et al. under review); thus, HIV-1 is likely entering the CSF/CNS at low levels via incomplete partitioning of virus at the BBB, or background levels of trafficking of immune cells, including small numbers of infected CD4+ T cells. Often, HIV-1 viral RNA loads within the CNS are elevated during primary infection, and this can be associated with higher levels of CSF pleocytosis (Sturdevant et al. under review). Thus, the CNS viral burden early may result from the release of virus from increased numbers of infected CD4+ T cells trafficking

from the periphery into the CNS in response to local HIV-1 replication in the CNS or another inflammatory condition.

In the CSF of a subset of HIV-infected subjects, it is possible to detect compartmentalized CSF viral populations that are undergoing independent replication (Dunfee et al. 2006; Harrington et al. 2009; Ohagen et al. 2003; Pilcher et al. 2001; Schnell et al. 2009). These CSF viral populations are genetically distinct from virus in the periphery. Studies using both heteroduplex track assays and single genome amplification have shown that compartmentalized CSF populations can be detected during primary infection (Harrington et al. 2009; Schnell et al. 2010; Sturdevant et al. under review), suggesting that viruses can begin replicating in the CNS very early and in the absence of overt neurological symptoms. CNS viral populations detected early are often clonally amplified populations, observed in the CNS as early as 2 to 6 months post-infection (Sturdevant et al. under review), but can also be characterized by more genetically diverse variants, indicative of persistent replication beyond a single clonal amplification event (Schnell et al. 2010; Sturdevant et al. under review). Additionally, in both vertical (Sturdevant et al. 2012) and horizontal transmission (Sturdevant et al. under review), CNS compartmentalization can also be established via the early sequestration of one of multiple transmitted variants in the CNS shortly after transmission, with replication of a second transmitted variant occurring predominantly in the periphery. We have shown that viruses in the CSF during primary infection are unable to efficiently infect cells expressing low levels of CD4 (Sturdevant et al. under review) and so are not adapted to infect macrophages/microglia. Little is known about how viral populations in the CSF transition from being R5 T cell-tropic during primary infection to being macrophage-tropic in some subjects late in disease.

Many groups have used env sequences from HIV-1 genomes isolated from macrophages or from the CNS of patients with HIV-associated neurocognitive disorders to locate the genetic determinants of macrophage tropism. Although these studies have had some success in identifying genetic determinants that work in patient-matched viruses, these same genetic changes do not occur in all or even most other macrophage-tropic env genes (Table 1), and a previous study found that the genetic determinants of macrophage-tropism can be complex and/or do not translate between patients (Rossi et al. 2008). Because of this complexity, comparisons of much larger sets of patient-matched macrophage- and R5 T cell-tropic env sequences will need to be compiled with differences tested as functional determinants before we are able to develop a full understanding of the evolutionary pathway(s) that create the macrophage-tropic phenotype.

Table 1 Mutations proposed to confer macrophage tropism are not observed in a well-validated collection of macrophage-tropic clones

Patient	Tropism	Env amino acid changes thought to convey macrophage tropism										
		153	167	238[b]	240	283	308	317	326	373	386	396
Consensus (sub. B)	T cell	E	D	P	T	T	H	F	I	M	N*	N*
Proposed mutation	Macrophage	G[a]	N[a]	K[b]	K[b]	N[c,d]	P[e]	L[d]	(M→I)[b]	K[f]	X[f, g,]	X[h]
4013	T cell	–	–	L	R	–	P	–	–	–	–	K
4013	Macrophage	–	–	–	–	–	–	I	–	–	–	–
4051	T cell	–	–	–	–	–	P	–	–	T	–	–
4051	Macrophage	–	–	L	K	–	P	–	–	T	T	–
4059	T cell	–	N	E	K	–	N	W	–	–	–	T
4059	Macrophage	–	N	E	K	–	S	W	–	–	–	T
5002	T cell	–	–	–	–	I	–	–	T	–	–	–
5002	Macrophage	D	–	–	–	–	–	–	–	–	–	–
7115	T cell	–	–	–	K	–	–	–	–	–	–	–
7115	Macrophage	–	–	–	K	–	–	–	–	–	–	–

[a] Musich et al. (2011)

[b] Cashin et al. (2011)

[c] Dunfee et al. (2006)

[d] Duenas-Decamp et al. (2009)

[e] Thomas et al. (2007)

[f] Duenas-Decamp et al. (2008)

[g] Dunfee et al. (2007)

[h] Ouyang et al. (2014)

Eradicating HIV-1 reservoirs in the CNS

HIV-1 infection of long-lived macrophage, microglia, and astrocytes may present a major barrier to eradication strategies. One such strategy for eradicating latent viral reservoirs is to "shock and kill" them by inducing expression of latent proviruses so that HIV-infected cells will be killed by virus production or eliminated by the immune system (Archin and Margolis 2014). The most promising method of inducing this reservoir is the use of histone deacetylase inhibitors (HDACi) (reviewed by Archin and Margolis 2014). Expression of HIV-1 proviruses in latently infected cells is inhibited by deacetylation of histones on the HIV-1 LTR (reviewed by Wightman et al. 2012). The addition of HDACi facilitates acetylation of histones and expression of the proviral genome. A recent study found that addition of the HDACi vorinostat increased HIV-1 expression approximately 5-fold in resting CD4+ T cells isolated from the blood of HIV$^+$ subjects who were virologically suppressed by antiretrovirals (Archin et al. 2012). This finding suggests that HDACi may be able to stimulate the latent reservoir which could subsequently be targeted by "kill" strategies.

If a "shock and kill" strategy is developed that can effectively induce latent reservoirs in the periphery, it is still unclear whether the same strategy would be effective at eliminating latent cells in the CNS. The first barrier will be the development of therapeutics that can reach the CNS. Studies examining the ability of vorinostat to treat Huntington's disease (Hockly et al. 2003) and cancer (Palmieri et al. 2009) suggest it is able to reach the CNS in mice, but it is unclear whether it could reach to human CNS at concentrations necessary to induce HIV-1 reservoirs. In contrast, the HDACi belinostat penetrates the CNS very poorly in macaques (Warren et al. 2008). A more fundamental question is whether HDACi are able to induce HIV-1 expression in CNS cells. Vorinistat has been shown to stimulate host gene expression in astrocytes (Nuutinen et al. 2010), but testing of HDACi for induction of HIV-1 expression in HIV-infected macrophage, microglia, or astrocytes has not been reported. A final concern is whether the immune system would be capable of eliminating HIV-infected cells from the CNS after induction. This may be a problem given the low concentration of T cells in the CNS. Further, efforts to target T cells to that compartment could damage the CNS.

Conclusions

A new assay that provides a more quantitative measurement of the ability of HIV-1 to enter cells with low levels of CD4 has now provided a clearer view of macrophage-tropic variants. This new view is forcing a reassessment of the role of macrophage-tropic HIV-1 variants in transmission and disease progression. The molecular determinants of macrophage tropism are as yet poorly understood. This more rigorous definition of macrophage-tropic HIV-1 will allow an assessment of the role these viruses play in the persistence of HIV-1 during suppressive antiviral therapy.

Conflict of interest statement The authors declare that they have no conflict of interest.

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