Endocytosis of human immunodeficiency virus 1 (HIV-1) in astrocytes: a fiery path to its destination

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Abstract

Despite successful suppression of peripheral HIV-1 infection by combination antiretroviral therapy, immune activation by residual virus in the brain leads to HIV-associated neurocognitive disorders (HAND). In the brain, several types of cells, including microglia, perivascular macrophage, and astrocytes have been reported to be infected by HIV-1. Astrocytes, the most abundant cells in the brain, maintain homeostasis. The general consensus on HIV-1 infection in astrocytes is that it produces unproductive viral infection. HIV-1 enters astrocytes by pH-dependent endocytosis, leading to degradation of the virus in endosomes, but barely succeeds in infection. Here, we have discussed endocytosis-mediated HIV-1 entry and viral programming in astrocytes.

Introduction

In a significant number of HIV-1-infected patients undergoing suppressive antiretroviral therapy, residual viral activity in brain causes immune activation, which leads to HIV-associated neurocognitive disorders (HAND) (1,2). Astrocytes, the most abundant cells in brain, maintain homeostasis (3,4). In addition, in response to brain injury or viral infections such as HIV-1, astrocytes are activated to pathological state (reactive astrocytosis). Although HIV-1 in the brain productively infects myeloid lineage cells such as microglia and perivascular macrophages (5–12), only unproductive infection has been reported in astrocytes (13–24). Molecular investigations of HIV-1-infected brain tissues from post-mortem cases have demonstrated viral DNA in 3% to 19% of astrocytes (20,24–31). In vitro investigations of HIV-1-infected brain tissues and virus-infected astrocytes inferred

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unproductive HIV-1 infection from the presence of viral DNA and an absence of viral RNA and protein expression. However, limited HIV-1 infection in astrocytes has been reported and thought to occur because of intracellular restrictions (18,32). Several possibilities have been suggested for abortive viral infection in astrocytes; in particular, several intracellular host factors have been implicated in unproductive HIV-1 infection (33–38). However, several studies, including ours, have identified inefficient viral entry, which occurs because of the absence of CD4-receptor, as the major impediment to HIV-1 infection in astrocytes (19,39–45). The concept of inefficient viral entry is supported by the findings that use of vesicular stomatitis virus envelope (VSV)-pseudotyped HIV-1 or ectopic introduction of infectious viral DNA into astrocytes resulted in robust viral replication and release of infectious virus (39,42–44).

Viral entry into target cells occurs by viral envelope fusion at either the cell surface (plasma membrane fusion) or inside endosomes after endocytosis of viral particles (FAE) (46,47). Both of these fusion processes can be either pH-dependent or pH-independent. Viral entry into target cells occurs by several different endosomal pathways, such as clathrin-mediated endocytosis or caveolae-dependent endocytosis or macropinocytosis (48). In clathrin-mediated endocytosis, which is dependent on cytosolic GTPase dynamin, virus and its receptor are enclosed in clathrin-coated vesicles. Caveolae are invaginations in the plasma membrane that contain caeolin (49). In macropinocytosis, virus particles are internalized and transported to endosomes. In all of these processes, virus particles, once internalized, are routed to early and late endosomes and lysosomes (50). However, the endolysosomal path is destructive as well. HIV-1 infection in CD4+ lymphocytes uses both plasma membrane fusion and FEA (47,51). HIV-1 enters by endocytosis in epithelial and HeLa cells lacking CD4 receptor (52). HIV-1 entry into macrophages by macropinocytosis leads to degradation of virus in endolysosomal compartments, but allows a small number of virus particles to complete fusion. However, degradation efficiency is cell-type-specific. For example, VSV-envelope-pseudotyped HIV-1 (VSV-HIV-1), virus infection is least productive in macrophages (53), but produces extremely productive infection in astrocytes and other transformed cells (39,42,43). HIV-1 entry into astrocytes by endocytosis was proposed several years ago (23,54), but details of the mechanism by which this occurs have emerged only recently (43,45). Here, we have discussed the HIV-1 infection in astrocytes, in particular viral entry by endocytosis.

Natural endocytic entry of HIV-1 and viral infection in astrocytes

Lack of ample evidence on productive HIV-1 infection in astrocytes could be a result of the complexity of infection and failure to detect authentic viral infection. Although few studies have shown non-permissiveness of astrocytes to HIV-1 infection (23,55), several studies, including ours, have shown productive HIV-1 infection in astrocytes (32,41–44,56–60). Indeed, productive infection at the single-cell level was corroborated by viral p24 protein expression in HIV-1-infected astrocytes, even though viral activity was undetectable in culture supernatants after 10 days of infection (43,44). In particular, use of fluorescent HIV-1 reporter viruses unambiguously demonstrated barely productive infection by T- or M-tropic viruses, which was far below the limit of the viral p24 detection assay. Thus, irrespective of viral tropism, productive HIV-1 infection occurred in astrocytes. Minimal

HIV-1 infection in astrocytes occurs by endocytosis-mediated viral entry and is pH dependent (43). Only a few studies of HIV-1 infection in astrocytes have elucidated the viral kinetics of the infection process. The most impressive data emerged with the use of live fluorescence microscopy which demonstrated HIV-1 infection in astrocytes on Day 4 after infection, which peaked between 12–15 days after infection (Fig. 1). The overall natural HIV-1 infection in astrocytes was less than 0.025% (43). However, earlier in-vitro studies on astrocytes found that HIV-1 infection was higher than 1% (24,41,61). By monitoring viral kinetics in HIV-1-infected astrocytes, a phenomenon of transient viral adsorption has been observed. Astrocytes have been shown to adsorb the majority of HIV-1 particles through incomplete plasma membrane fusion. These incompletely fused HIV-1 particles from the plasma membrane of astrocytes are released extracellularly within 10 days after infection without having actually entered astrocytes. (41,43,55). On follow-up of HIV-1 infection in astrocytes from day 0 to 21 post-infection, extracellular p24 levels peaked at day 3, declined by day 10 and became undetectable at 3 weeks. These findings are similar to those of other studies (39,41,55).

Intriguingly, on monitoring the live fluorescence of recombinant yellow fluorescent reporter HIV-1 (NLENY1; Fig-1A) infected astrocytes, we found that during the first 10 days, intracellular viral infection did not correlate with extracellular viral p24 level. Authentic and pseudoviral activity were validated after 4 to 20 days of NLENY1 infection by green-positive astrocytes (Fig. 1B) in combination with viral p24 protein in culture supernatants (42,43). Similar results were obtained by infecting ectopically CD4-expressing human fetal astrocytes (HFA) or astrocytic reporter cells, recapitulating the scenario of HIV-1 infection in wild-type HFA; i.e., pseudoviral activity (residual). Thus, the presence of HIV-1 p24 protein in the culture supernatants of infected astrocytes between 3–10 days after infection is actually residual (pseudo) viral activity that did not correlate with viral replication (41,43,55). Accordingly, astrocytes transiently retain HIV-1. This suggested the presence of a major viral entry block, although one that was not complete for productive HIV-1 infection. Given the absence of HIV-1 classical receptor CD4 and co-receptors CCR5, but the presence of CXCR4 co-receptor in fetal astrocytes, virus infection is restricted at the viral entry level. Although few studies have claimed expression of CD4 or CCR5 in astrocytes (19,62,63), we and others could not confirm this in fetal astrocytes or transformed astrocytes (19,39–43,64–66).

Poor HIV-1 infection in astrocytes was observed with cell-free virus or transinfection studies. We (42,43) showed that this was because of the endocytic entry of the virus. However, earlier study suggested that cell-cell transinfection is efficient in astrocytes, but details on the mechanism of augmented infection were not provided (56). Interestingly, recent in-vitro study showed protrusions (synapse) from uninfected astrocytes acquiring virus from neighboring HIV-1-infected lymphocytes, but no details on efficiency of viral infection were provided (67). A few studies have suggested some HIV strain selectivity in infecting astrocytes (68–70). However, current knowledge does not explain strain selectivity beyond the major restriction at the viral entry level. In a recent study, it was concluded that HIV-1 infection in astrocytes is unproductive and nonpermissive, but that virus is retained after trypsinization of infected astrocytes (71). In this study, HIV-1 infected astrocytes were treated with trypsin to remove the attached virus after infection; this was followed by

immediate co-culture with lymphocytes. After a few days of co-culture, transiently adsorbed virus particles on astrocytes (resistant to trypsin washing), transinfected lymphocytes in co-cultures (71). In general, residual virus activity is lost in 10 days by itself in-vitro cultures, as previously described (43,55). However, Chauhan et al, (43) have shown authentic, productive HIV-1 infection in astrocytes. In their study astrocytes were infected with recombinant yellow fluorescent protein expressing HIV-1 (NLENY1) and, 15 days after infection, co-cultured with uninfected lymphocytes. On follow-up of HIV-1 infection by live fluorescence microscopy, viral transinfection to lymphocytes was seen only from productively HIV-1-infected astrocytes. Transinfection or cell to cell infection from HIV-1-infected astrocytes or lymphocytes to uninfected astrocytes was minimal and similar to cell-free virus infection (43), suggesting that cell-free virus infection and cell-to-cell infection (transinfection) were equally restricted and pointing toward viral entry restriction. In both of these scenarios, restricted HIV-1 infection in astrocytes occurred by endocytosis.

Endocytic entry of HIV-1 into astrocytes came to light when treatment with lysosomotropic drugs (chloroquine and bafilomycin A), the drugs which localize to lysosomes and endosomes, resulted in several-fold increases in viral infection. This increase in HIV-1 infection occurred equally in both T- and M-tropic HIV-1 viruses. Further, it was anticipated that lysosomotropic drugs confer their effects through change in endosomal function, increasing lysosomal pH and inhibition of endolysosomal fusion which was corroborated in our earlier study (42). Further, lysosomotropic drug CQ behaved as an agonist on chronically HIV-1-infected astrocytes, ensuring that CQ also abrogates degradation of viral components, but was not involved in direct activation of HIV-1 LTR promoter (42,43). Overall, these observations suggested that in natural HIV-1 infection of astrocytes, adequate numbers of HIV-1 particles are entrapped within endosomes in astrocytes, but still might establish little productive infection owing to heightened degradation or limited escape of virus particles from endolysosomes.

Mechanism of HIV-1 endocytosis in astrocytes

HIV-1, an enveloped virus, enters cells by fusion either directly at the plasma membrane or at the endosomal membrane (47,53,72). Endosomal membrane fusion occurs after receptor interaction with virus at the surface of the target cell and successful infection depends on escape from degradation in endolysosomes (73). The status of the endolysosomal pathway, which depends on the type and activation of the cell, has been studied in HIV-1 infected macrophages. HIV enters by CD4 receptor and co-receptor either CCR5- or CXCR4-dependent fusion with the plasma membrane (74–77). Also, HIV-1 can enter by CD4-mediated, but co-receptor-independent endocytosis. Entry by CD4 receptors has been reported in primary CD4^{+} lymphocytes (78), while dynamin and clathrin-mediated endocytosis has been shown for CD4+ HeLa cells (79). These results were corroborated by blocking lysosomal degradation, leading to increased HIV-1 infection (51,80,81). More precisely, HIV-1 infection of target cells is a multistep process that begins with initial binding of the viral gp120 subunit of viral envelope glycoprotein to CD4 and co-receptor CCR5 or CXCR4. Its interactions with CD4 and a co-receptor trigger conformational changes in the transmembrane part of envelope gp41 and initiate membrane fusion (75–77,82). Several viruses enter target cells through intracellular compartments in which low

pH and or cellular proteases activate the viral fusion proteins, permitting release of virus genome from intracellular compartments (reviewed in ref 83). Receptor and coreceptor-mediated HIV entry is pH-independent and does not rely on low (acidic) pH to infect host cells (84).

In general, the majority of HIV-1 enters through the classical pH-independent CD4-CXCR4 pathway in lymphocytes and is highly productive. However, limited pH-dependent viral endocytosis also seems to occur in lymphocytes (52,73,85). Another virus, VSV, enters target cells by pH-dependent endocytosis (46,86). VSV-envelope-pseudotyped HIV-1 (VSV-HIV-1) infection is highly productive in astrocytes (39,42–44). Interestingly, treatment with CQ or bafilomycin A severely impaired VSV-HIV-1 replication in astrocytes (43,44,51). Natural wild-type HIV-1 enters by pH-dependent endocytosis in CD4$^-$ astrocytes, but infection is barely productive. Treatment with CQ or bafilomycin A markedly increased wild-type HIV-1 infection in astrocytes (42,43). These differences between the VSV- and HIV-envelope seem to occur at the endolysosomal level, where the VSV envelope needs an acidic environment for activation, while the HIV-1 envelope remains inactivate in acidic environment.

Endocytosis is a complex cellular process involving endosomes and several proteins (reviewed in 87). Endosome functioning involves gaunosine triphosphatases (GTPases) of the Ras superfamily (Rab). Rab proteins (Ras-related proteins in brain) regulate specific steps of endocytosed vesicles from the plasma membrane to early endosomes (Rabs 4 and 5) (88), late endosomes and lysosomes (Rab7) (89), and vesicle-recycling endosomes (Rabs 4 and 11) (90,91). Chauhan et al (43) showed that HIV-1 infection in astrocytes via endocytosis involves early, late, and recycling endosomes. Using a molecular approach, RNAi-mediated ablation of Rab-5, -7, and -11 demonstrated their indispensible role in endocytosis-mediated HIV-1 infection in astrocytes. Leukocyte-specific protein 1 (LSP1), an F-actin binding protein, plays a role in endocytosis, as demonstrated by the finding that its depletion substantially decreases the rate of endocytosis (92). LSP1 has been shown directing HIV-1 particles to endosomes and proteasomes (93). Ablating LSP1 by siRNA, productive HIV-1 infection in astrocytes was severely impaired in the presence of lysosomotropic agents, indicating its intricate role in HIV-1 endocytosis (43). Despite the hostile environment in endosomes, productive HIV-1 infection in astrocytes succeeds, though to a minimum level. Minimum productive HIV-1 activity in astrocytes is a post-viral DNA-integration phenomenon, establishing the authentic replication from integrated viral DNA. Thus, although the endosomal route is indispensable for HIV-1 infection in astrocytes, it is, at the same time, detrimental, because endosomal internal machinery is least conducive to successful establishment of viral infection.

Perspective and conclusion

Normally, HIV-1 enters lymphocytes via a pH independent pathway (natural infection), using a classical receptor-coreceptor mechanism. CD4$^-$ astrocytes adsorb the majority of HIV-1 to the surface. In the absence of intracellular virus uptake, this partially fused virus at the cell surface is transiently retained, then released within 10 days. Few virus particles in astrocytes enter via pH-dependent endocytosis, resulting in barely productive HIV-1

infection (Fig. 1). Although most HIV-1 particles internalized by the vesicular pathway appear to be degraded in endolysosomes, a small number of virus particles escape the strongly acidic environment in endocytic vesicles to enter the cytoplasm. Inhibiting endosomal acidification or increasing pH by lysosomotropic agents such CQ or bafilomycin A, equally bolstered both M- and T-tropic virus infectivity irrespective of viral tropism (43). In addition to lysosomotropic drugs, proteasome inhibitor MG132 and an autophagy modulator slightly increased HIV-1 infection in astrocytes, but not to the extent that endosomal inhibitors did (43). None the less, inhibiting nucleases in astrocytes did not affect productive HIV-1 viral activity. However, ablating endosomal Rab proteins in astrocytes cripples productive HIV-1 infection, indicating viral entry by endocytosis. A few studies have suggested that there is some HIV-1 strain selectivity in infecting astrocytes efficiently (68,69). However, current knowledge does not explain why astrotropic strain selectivity will occur, given that the brain harbors HIV-1 permissive cells such as microglia and perivascular macrophages. The possibility that some strains may have overcome endolysosomal resistance and efficiently infect astrocytes needs further investigations. Chauhan et al. (43) have recently shown in co-cultures that HIV-1 infection does occur, but is not different from cell-free virus and was restricted, as is cell-free virus infection in astrocytes. In both of these HIV-1 infection scenarios, restricted HIV-1 infection in astrocytes occurs by endocytosis. Endocytic HIV-1 entry in astrocytes, albeit a fiery path on which the majority of viral particles are degraded, is the only natural way for HIV infection to occur in astrocytes and leads only to minimal survival of HIV-1.

Indeed, intracellular restrictions other than endosomal and proteasomal ones cannot be ruled out in non-CD4 astrocytes because HIV-1 infection could not exceed 0.5% after treatment with lysosomotropic drugs. However, bypassing natural viral entry by using VSV-HIV-1 or transfection of HIV-1 infectious DNA clone in astrocytes resulted in profound infection (39,42–44), ruling out any major intracellular restrictions for the virus. Mimicking natural receptor and co-receptor viral entry in astrocytes by ectopic expression of CD4 in human astrocytes, also resulted in profound HIV-1 infection, given that astrocytes naturally express CXCR4 viral co-receptor (43). Further, use of fluorescent reporter virus infection in astrocytes yielded other interesting observations, such as sustained production of the HIV-1 regulatory proteins Tat and Rev. These proteins regulate the viral life cycle at transcriptional and post-transcriptional levels. Alteration in their function cripples HIV-1 infection. Persistent production of Tat and Rev sustain chronic viral activity in limitedly dividing astrocytes. Corroborating this, Chauhan et al. (43) have demonstrated persistently productive HIV-1 infection in primary astrocytes for 160 days (43,44). Similarly, other studies have reported persistently productive HIV-1 infection in human astrocytes (39,94–96). Based on authors' perspective and the evidences available, whatever little HIV-1 infection occurs in astrocytes, occurs exclusively via pH-dependent endocytosis. HIV-1 infection is impeded at the viral entry level because of an absence of CD4 receptor, as well restriction imposed intracellularly at the endo-lysosomal level. The contribution of this miniscule HIV-1 infection in astrocytes to overall viral load in the brain seems insignificant, even considering the abundance of this cell type in brain. However, this infection could be an elusive HIV-1 reservoir of productive infection and cause of immune activation in the brain. Using lysosomotropic drugs in HIV-1-infected patients may augment viral infection in cells

lacking viral receptors. Although pH-dependent HIV-1 endocytosis is not an attractive target for therapy, it would be an impediment in efforts to purge virus from limitedly dividing astrocytes.

Acknowledgments

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Highlights

- HIV-1 infection in astrocytes is impeded at the viral entry level

- Astrocytes adsorb HIV-1 particles and release them within 10 days

- HIV-1 infects astrocytes minimally by pH-dependent endocytosis

- Inhibiting endosomal acidification or increasing endosomal pH by lysosomotropic agents, bolstered both M- and T-tropic HIV-1

A

RCOMBINANT YELLOW FLUORESCENT PROTEIN EXPRESSING HIV-1 VIRUS (NLENY1)
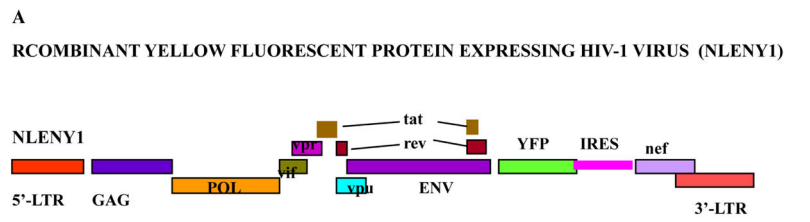
B

NLENY1 INFECTION IN PRIMARY HUMAN FETAL ASTROCYTES (13 DAYS POST INFECTION)
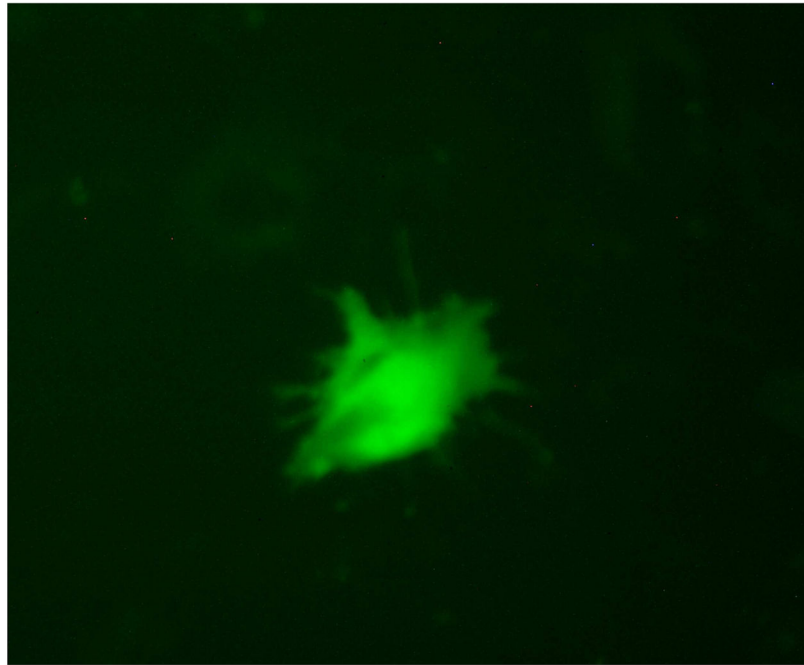

Figure 1. Natural HIV-1 infection in primary human fetal astrocytes
(**A**): Diagrammatic view of recombinant HIV-1 infectious molecular clone, NLENY1
showing yellow fluorescent protein (YFP) gene insertion. (**B**): Six-week-old human fetal
astrocytes (HFA) were seeded in six-well culture plates and, two days later, infected with
NLENY1 (1.0 µg/mL p24) for 2 h. Infected HFA shows green fluorescence on day 13 post-
infection.