Entry Barrier to HIV Infection of Astrocytes Can Be Overcome by Immature Virus via A CD4-independent, CXCR4-dependent Mechanism

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ABSTRACT

Studies show that astrocytes are a critical reservoir for HIV in the brain. However, HIV infection in astrocytes is extremely inefficient *in vitro*. We found that there was no significant intrinsic barrier for restricting HIV replication in astrocytes because the viruses could be constantly produced following either infection with VSV-G pseudotyped HIV-1 or transfection with HIV-1 proviral DNA. As expected, HIV was able to efficiently infect the cells pre-transfected with a CD4 plasmid. The barrier of viral entry could also be overcome to result in persistent infection by infections with reporter virus NLENG1 while astrocytes were simultaneously treated with lysosomotropic agent, chloroquine (ChQ) and a fusion peptide bearing Tat basic domain and influenza HA2. In fact, we could consistently detect low levels of CD4 mRNA in primary fetal astrocytes and neuroprogenitor-derived astrocytes and small amounts of CD4 protein by immunoprecipitation. Interestingly, CD4 was able to be significantly up-regulated by pro-inflammatory cytokines at level of mRNA, but not at level of protein. Therefore, increased infection of HIV was not consistently seen in the cells pre-treated with pro-inflammatory cytokines. However, the efficient, productive infection of astrocytes was consistently demonstrated in the transwell-cultures with HIV-infected T lymphocytes. A novel CD4-independent, CXCR4-dependent mechanism was further identified, by which immature HIV particles are able to directly bind to CXCR4 on astrocytes in the process of viral budding or immediately after viral release from the infected lymphocytes and may trigger virus-cell fusion during or after the process of viral maturation. These observations indicate that HIV may infect astrocytes using alternative mechanisms *in vivo* rather than a classical mechanism.

METHODS & RESULTS

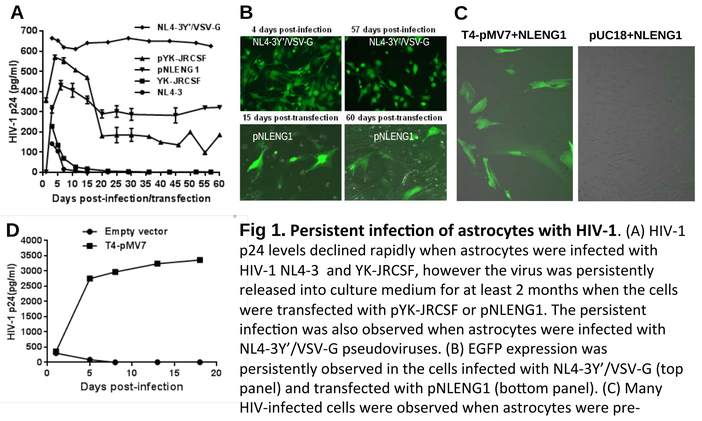

Fig 1. Persistent infection of astrocytes with HIV-1. (A) HIV-1 p24 levels declined rapidly when astrocytes were infected with HIV-1 NL4-3 and YK-JRCSF, however the virus was persistently released into culture medium for at least 2 months when the cells were transfected with pYK-JRCSF or pNLENG1. The persistent infection was also observed when astrocytes were infected with NL4-3Y'/VSV-G pseudoviruses. (B) EGFP expression was persistently observed in the cells infected with NL4-3Y'/VSV-G (top panel) and transfected with pNLENG1 (bottom panel). (C) Many HIV-infected cells were observed when astrocytes were pre-transfected with a plasmid encoding CD4 receptor and then infected with HIV-1_{NL4-3} based reporter virus NLENG1 (left), but no infected cells were observed in the cells pre-transfected with an empty vector and infected with NLENG1 (right). (D) HIV-1 p24 was quantified in the supernatants from the cultures of astrocytes infected with NLENG1 described in (C).

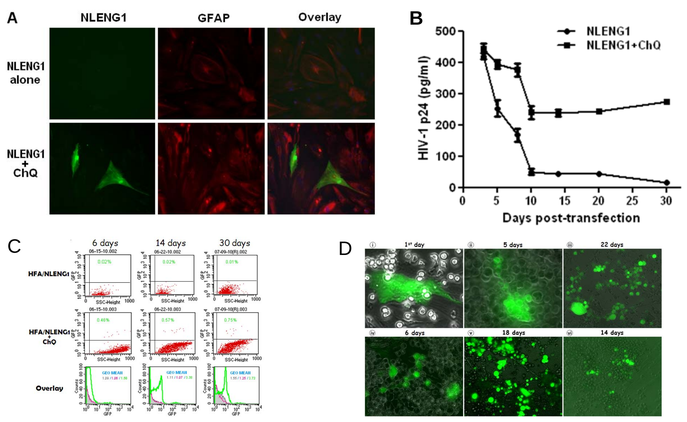

Fig 2. Persistent infection occurred in the astrocytes infected with HIV-1 and simutalneously treated by lysosomotropic agent. (A) HIV infection was observed 5 days post-infection in the astrocytes infected with NLENG1 while the cells were treated with chloroquine (ChQ) simultaneously. However, EGFP-expressed cell was not observed in the cells infected with NLENG1 alone. (B) The persistent replication was established in HFAs with NLENG1 and simultaneously treated with ChQ. (C) The results were further confirmed by analysis of flow cytometry.

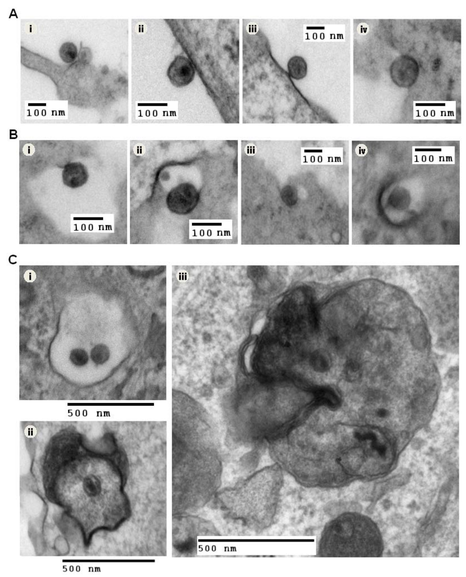

Fig 3. Endocytosis of HIV in astrocytes was shown by electron microscopy. (A) Increased density on the cell membranes appeared at the sites of attachment of HIV to astrocytes (i-iv), that might extend to the surrounding area of attachment on the cell membrane (ii and iii) or form a crescent border at the site of invagination between the cell membrane and viral particle (iv). (B) HIV particles were seen partially or completely endocytosed into astrocytes. High-density signal at the site of viral attachment to the membrane was seen in the endocytic vesicles (i-iv). A bridge-like connection formed between viral particle and the cell membrane (iv). (C) HIV particles were observed in endosome (i) and endolysosomes (ii-iii) of the infected astrocytes.

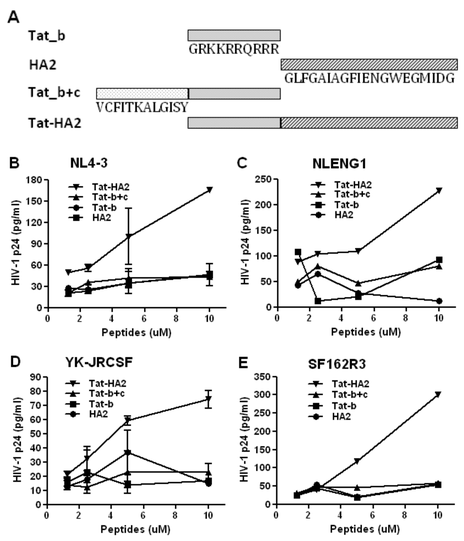

Fig 4. Tat-HA2 enhanced HIV infection in astrocytes by aiding HIV escape from degradation in the endosome/lysosome. (A) Diagrammatic representation of synthetic peptides and their sequences. (B) Infection of astrocytes with NL4-3 was significantly increased by Tat-HA2 in a dose-dependent manner, but not by other peptides. HIV-1 p24 was quantified 3 weeks post-infection and done in the same way in other graphs. Similar results were obtained from the infections with NL4-3 based reporter virus NLENG1 (C) , HIV-1 strain YK-JRCSF (D) and R5-tropic virus, SF162R3 (E).

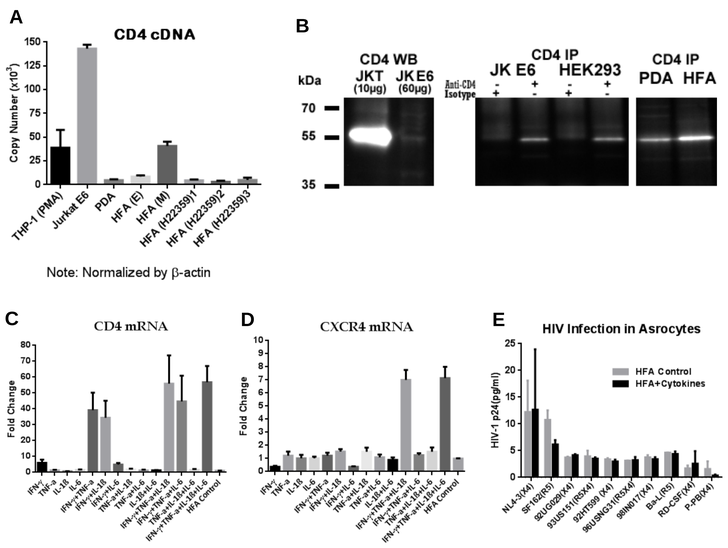

Fig 5. Residual level of CD4 could be detected in human astrocytes. (A) Low level of CD4 mRNA was detected in HFAs and progenitor-derived astrocytes (PDA). (B) Residual CD4 was detected in astrocytes by immunoprecipitation (IP). (C) CD4 mRNA was up-regulated by pro-inflammatory cytokines. (D) CXCR4 mRNA was up-regulated by pro-inflammatory cytokines. (E) HIV infection was not significantly increased in HFAs pre-treated with cytokines because CD4 was not increased at protein level.

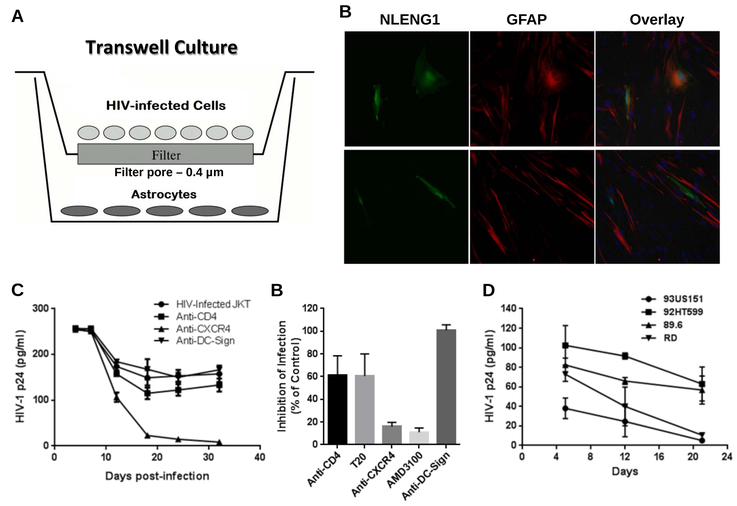

Fig 6. Infection of astrocytes by immature，cell-free HIV particles via a CD4-independent, CXCR4-dependent mechanism. (A) A transwell-culture device was used to determine if newly produced HIV particles could infect astrocytes. Astrocytes were pre-seeded in the wells of culture plate and HIV-infected lymphocytes were seeded on top of the membrane filter of transwell insert, with pores of 0.4 μm in diameter. (B) NLENG1-infected astrocytes were easily observed 3-5 days post-transwell culture. The infected astrocytes were be GFAP+ (top panel) or GFAP- (lower panel). (C) Astrocytes were incubated respectively with 20 μg/ml of anti-CD4, CXCR4 or DC-Sign antibodies for 1 hr prior to the placement of transwell inserts with 4 x 10^5 NLENG1-infected Jurkat T cells. The inserts were removed off 3 days post-culture and astrocytes were washed with PBS. HIV-1 p24 was quantified in the culture media at different time-points. (D) Antibodies (anti-CD4, CXCR4, DC-Sign), fusion inhibitor T20 (500 nM) and CXCR4 antagonist AMD3100 (50-100 μM) were used to determine their effects on NLENG1 infection of astrocytes in the transwell cultures. The inserts were removed off 3-5 days post-culture and HIV-1 p24 was measured in the culture media after 3-4 weeks. Data represent mean ± SEM from three experiments. * p<0.05, ** p<0.01. (E) The same experiments were performed to test whether other HIV-1 strains could also infect astrocytes via the transwell cultures. The inserts were removed 5 days post-culture. Data represent mean ± SEM from three experiments.

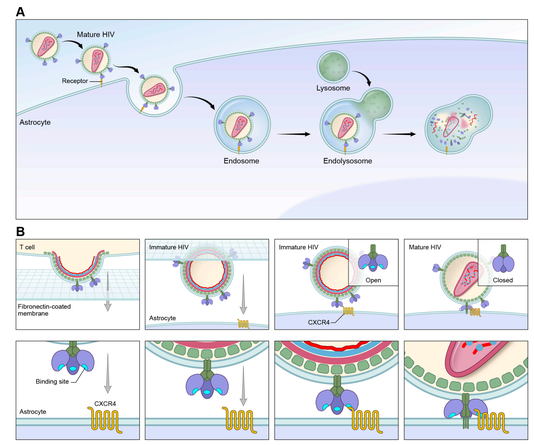

Fig 7. Model of HIV infection in astrocytes. (A) Cell-free HIV (pre-prepared, mature virus) enter astrocyte by endocytosis, but is detained in endosomes/endo-lysosomes and finally degraded. (B) Immature viral particle can infect astrocyte via a CD4-independent but CXCR4-dependent mechanism.

SUMMARY & CONCLUSION

1) *There is no significant intrinsic obstacle for HIV persistent infection in astrocytes, but the major barrier for the infection with cell-free HIV in vitro is at viral entry level.*
2) *HIV can enter astrocytes by endocytosis, but is typically detained and degraded in endosomes/endolysosomes because residual level of CD4 expression is not sufficient to trigger the process of HIV endosomal fusion.*
3) *Pro-inflammatory cytokines can significantly upregulate expression of CD4 and CXCR4 in astrocytes at the mRNA level but not at the protein level, hence doesn't increase HIV infection.*
4) *Productive infection of HIV can be achieved at the circumstance by which immature viral particles are produced and shortly attach to astrocytes prior to completion of viral maturation.*