HIV-1 entry and *trans*-infection in astrocytes: implications for cure and eradication

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Background: HIV-1 penetrates the central nervous system (CNS) during early infection, establishing a viral reservoir in macrophages and astrocytes. We recently demonstrated that astrocytes are extensively infected and may represent a significant HIV-1 reservoir within the CNS. Whilst direct infection of astrocytes maybe a long-term reservoir, short-term astrocyte reservoirs may exist by storing, protecting and concentrating cell free virus. This latter concept is presently referred to as *trans*-infection, where a cell can bind and harbor virus to be transferred to a recipient. Here, we characterised HIV-1 entry and *trans*-infection of astrocytes. Elucidating both is essential to understanding the HIV-1 CNS reservoir, and for development of eradication strategies.

Methods: Astrocytes were pulsed with non-saturating amounts of HIV-1 BaL and virus half-life determined by p24 ELISA or co-cultured with different ratios of JLTRG cells (T-cell line expressing LTR-EGFP) and *trans*-infection determined by FACS analysis of EGFP in the T-cells. Astrocytes were infected with an EGFP content-labelled HIV-1 $YU2_{ciGFP}$ and immunofluorescently stained for endosomal markers (CD63/CD81/CD107/EEA1) to characterise the compartment harboring virus. Endosomal compartments were modified by using inhibitors (dynasore, dyngo-4a) or by shRNA silencing of CD81.

Results: Astrocytes bind and harbor virus in the short-term, with virus detectable out to 72 hours and an initial half-life of 1.2 hours. Astrocytes can transfer HIV-1 to JLTRG cells with similar efficiencies observed for 1:1 and 1:10 astrocyte:JLTRG ratios. The virus-containing compartment required 37°C to form and was trypsin-resistant. HIV-1 and CD81 demonstrated clear co-localization, while CD63, CD107b and EEA1 did not. SVG-lowCD81 cells were generated using shRNA silencing of CD81 (42% reduction). Experiments with SVG-lowCD81 revealed no loss of co-localization between CD81 and HIV-1, despite reduced CD81 levels.

Conclusion: The CD81 compartment observed herein, has been shown elsewhere (in other cell types) to be a relatively protective compartment. Within astrocytes, this compartment may be actively involved in virus entry and/or spread. The ability of astrocytes to transfer virus, without *de novo* viral synthesis, suggests they sequester and protect virus and thus facilitate not only viral dissemination in the CNS but add further complexities to HIV eradication strategies within this compartment.