In vitro studies on HIV-1 infection of Astrocytes.

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THESIS SUMMARY

HIV-1 infection of astrocytes is involved in HIV-1 induced neurological diseases and is a possible source of viral persistence. *In situ* studies of post mortem brain tissue indicate that HIV-1 infection of astrocytes does occur, but is restricted. Previous *in vitro* studies have revealed intrinsic intracellular blocks to HIV-1 transcription and translation in astrocytes. Co-culture of infected astrocytes with permissive $CD4^+$ cells has been shown to "rescue" infectious HIV-1 from the restricted infection of astrocytes, resulting in transmission of infection to the $CD4^+$ cells. However, the early viral replication steps of entry, reverse transcription and integration have not been previously characterised in detail in astrocytes, and are the focus of this study.

In this thesis, two routes of initiation of *in vitro* HIV-1 infection of astrocyte cell lines were employed; i) "cell-to-cell" infection (involving coculture of astrocytes with HIV-1 infected macrophages or T-cell lines), and ii) "cell-free" infection (direct application of cell-free virus to the astrocytes). In the cell-to-cell infection model, the process of HIV-1 reverse transcription was investigated but could not be clearly demonstrated to occur within the astrocyte cell population. The cell-free infection model permitted a more detailed analysis of the interaction between astrocytes and HIV-1, focussing on the entry and post entry HIV-1 replication steps of reverse transcription and integration. In cell-free infection, uptake of HIV-1 by astrocytes occurred within vesicles. Although infectious virus was subsequently released from these cells, there was no evidence that viral replication had occurred. Extrachromosomal viral DNA could be detected in these cells, however the level of viral DNA associated with the astrocytes declined with time and was unaffected by inhibitors of HIV-1 reverse transcription. This astrocyte-associated viral DNA was subsequently shown to be due to HIV-1 DNA in the viral inoculum. Using specific assays to detect integrated forms of HIV-1 DNA, no evidence was found for viral integration in these cells. Unspliced viral RNA was present, and declined with time post infection. Despite the striking absence of integrated HIV-1 DNA in these cells, the majority of infected astrocyte cultures sporadically released infectious virus. This demonstrated that an alternative, replication-independent pathway of HIV-1 infection and transmission, previously reported in dendritic and epithelial cells, can also occur in astrocytes.

The identification and characterisation of this replication-independent pathway of astrocyte infection in this thesis may have significant ramifications for our understanding of the entry and spread of HIV-1 through the central nervous system (a major viral reservoir). The possibility that this pathway of infection occurs in astrocytes in the CNS may also impact upon the management of viral persistence and anti-retroviral therapy evasion by the virus. The effect of this replication-independent process on astrocyte function, and the relevance of this in the pathogenesis of HIV-1 associated dementia, remains to be determined.