Cell-specific temporal infection of the brain in a simian immunodeficiency virus model of human immunodeficiency virus encephalitis

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Increasing evidence supports early brain infection by human immunodeficiency virus (HIV). Definitive temporal studies determining when and within which brain cells viral DNA is present are lacking. This study utilized simian immunodeficiency virus (SIV)-infected macaques sacrificed at days 10, 21, 56, and 84 post inoculation. Laser-microdissection isolated pure perivascular macrophage, parenchymal microglia, and astrocyte populations. Nested polymerase chain reaction (PCR) and sequencing determined the presence and characteristics of SIV V3 and V1 env DNA from each population. At day 10, SIV DNA was detected in perivascular macrophage and astrocytes but not parenchymal microglia. gp41 expression was restricted to perivascular macrophage. At day 21, SIV DNA was not detected in any cell type. At day 56, SIV DNA was detectable in perivascular macrophage from one of two macaques, with no gp41 expression detected. At day 84 (morphologic and clinical encephalitis), SIV DNA was detected in all cell types, gp41 was only detected in perivascular macrophage and parenchymal microglia. The neurovirulent molecular clone, SIV/17E-Fr, was the only genotype identified in the brain cell populations. Early, productive brain SIV infection was transient and restricted to trafficking perivascular macrophage. During the nonencephalitic stage, there was a period of time when no SIV DNA could be detected in the brain cell populations. SIV was then seen to reenter the brain via infected perivascular macrophage, leading to productive infection of brain parenchymal macrophage/microglia with a terminal phase of encephalitis. These data challenge current notions of a HIV reservoir within latently infected, semipermanent brain cells and has significant implications for the timing and design of therapies to prevent HIV encephalitis (HIVE). Journal of NeuroVirology (2009) 15, 300–311.
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Introduction

The introduction of highly active antiretroviral therapy (HAART) has significantly decreased the incidence of acquired immunodeficiency syndrome (AIDS), human immunodeficiency virus encephalitis (HIVE), and of HIV-associated dementia (HAD) (Dore et al., 1999; Sacktor, 2002). HAART is frequently not initiated until well after acute infection when HIV is disseminated throughout the body, including the brain (Gray et al., 1996). Thus the virus may have already entered and established infection in the brain, resulting in a latent viral infection in long-lived cells, sequestered from immune surveillance. The brain may then act as a reservoir of latent virus that current therapeutics are unable to eliminate, and that could reactivate later in disease. Additionally, ongoing persistent infection of brain cells may result in the inability of the infected cells to perform normal functions critical for brain functioning (Overholser et al., 2003).

Limited studies of acute HIV infection suggest that virus enters the brain during acute infection (Bell, 2004; Bell et al., 2006; Gonzalez-Scarano and Martin-Garcia, 2005), but it is not clear whether virus that enters the brain during acute infection persists there for life or whether this is cleared by the host and HIV develops as a result of later introduction of new virus into the brain. It is crucial to understand the events that lead to establishment of virus in the brain and the eventual development of HIVE. Studies of HIV infection of the human brain are necessarily limited by difficulty in accessing acutely infected individuals and the inaccessibility of the human brain for sampling during early infection in life. SIV-infected macaques may provide a useful model to study when and in which brain cells retroviral infection occurs. Macaques can be inoculated with well-characterized virus strains to identify specific viral genes that are important in the development of organ-specific disease, such as the demonstration that env and nef genes contain molecular determinants of neurovirulence (Mankowski et al., 1997). Body fluid and tissues can be repeatedly sampled at different times during the course of infection to measure virus replication and evaluate the hosts’ immune responses. Finally, infected animals can be euthanized at different stages of infection to reconstruct the complete kinetic picture of infection. Previous work utilizing a SIV model combined immunohistochemistry (IHC) and in situ hybridisation to show that SIV infection was localized to perivascular macrophage at the earliest stage and was found in perivascular macrophage and microglial cells scattered through the brain parenchyma at later stages (Chakrabarti et al., 1991). Another study analyzed envelope sequences from individual brain multinucleated giant cells of simian immunodeficiency virus (SIV) encephalitic macaques; however, interpretation of temporal pathogenesis is limited in that the study analyzed only six multinucleated giant cells (MNGCs) from one macaque with end-stage disease (Ryzhova et al., 2002).

Clements et al (1994) previously developed a SIV/macaque model that causes consistent, accelerated HIVE in >90% of infected animals by 84 days post inoculation (Clements et al., 1994; Zink et al., 1997, 1998). Pigtailed macaques (Macaca nemestrina) are inoculated with a neuroviral molecular clone, SIV/17E-Fr (Flaherty et al., 1997) and an immunosuppressive biological isolate, SIV/DeltaB670, that consists of at least 20 different genotypes (as determined by sequencing of the V1 region of env) (Babas et al., 2003). The high incidence of HIVE in this model provides an opportunity to correlate host and viral events in the brain during the acute infection with the later development of HIVE (Zink et al., 1997, 1998, 1999).

In this study, laser microdissection (LM) was utilized to isolate specific brain cell populations from macaques infected with SIV and sacrificed at different time points following infection. Morphological analysis of the tissue sections at each time point was performed to assess for evidence of encephalitis. Triple-nested polymerase chain reaction (PCR) was utilized to determine the presence and genetic characterisation of SIV V3 env DNA sequences from the cell populations. This enabled examination of which cells were infected as early as 10 days post SIV infection, and how the infection in cells proceeds over the 3-month time span. Additionally, by cloning and sequencing the V1 envelope region of SIV DNA, it could be determined which of the inoculated SIV genotypes were present in the brain cells and whether they differed between cell populations.

Results

Brain histopathology

IHC and hematoxylin and eosin (H&E) stains performed on the brain sections were microscopically examined by a neuropathologist (C.A.M.) (Table 1). At day 10 post SIV inoculation, SIV gp41 positivity (indicative of productive infection by SIV) was only detected in perivascular macrophage. A moderate inflammatory response was noted (Figure 1A and B). At day 21, SIV gp41 IHC was not detected in...