HIV Encephalopathy

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Abstract
HIV encephalopathy is one of the most complex viral diseases. HIV-infection is mainly restricted to macrophages and microglia in HIV-infected brains, although HIV-induced damage extends to neurons and oligodendrocytes. Accumulating evidences suggest that HIV-encoded factors and other host factors are involved in the development of this disease: however, the precise mechanism remains unclear. In order to investigate this mechanism, we developed an HIV-1-infected human cell-transplanted mouse model (hu-PBMC-NOD-SCID mouse) and a coculture system with HIV-1-infected macrophages and murine or rat brain cells. Using these models, we have successfully determined that certain host factors are involved in the neuronal damage. Additionally, we are developing a screening system to identify the host factors that provide protection against HIV-1-induced encephalopathy. Our study will contribute to the development of a new therapeutic strategy for HIV encephalopathy and other CNS diseases.

Key words HIV encephalopathy, Macrophage, hu-PBMC-NOD-SCID mouse, A lentiviral screening system, Host factors, CNS diseases

Introduction
The human immunodeficiency virus type 1 (HIV-1) causes acquired immunodeficiency syndrome (AIDS) by the destruction of the immune system.1–3 However, the target tissues are not restricted to those of the immune system. This virus invades the central nervous system (CNS) and induces a neurological disease called HIV encephalopathy. Most cases of this disease are diagnosed several years after the primary infection, and by then, the number of CD4+ cells in the peripheral blood is significantly lower. The clinical symptoms of this disease include cognitive, behavioral, and motor dysfunction: these symptoms are characteristically found in subcortical dementia and commonly develop over a period of few months. In the early stage, forgetfulness and reduced concentration are frequently encountered and these are typically followed by short-term memory loss, carelessness and mental slowing. Motor disturbance in the form of leg weakness is sometimes observed at this stage. These symptoms often occur along with behavioral symptoms such as personality changes. Ataxia, tremor, pyramidal sign, and paresis are also found. On disease progression, the patient shows behavioral changes such as social withdrawal, apathy, akinetic and mute state.

New Problems After the Introduction of HAART
In 2004, approximately 40 million people worldwide were estimated to be already infected with HIV. However, a very effective anti-viral therapy called highly active antiretroviral therapy (HAART) that comprises a combination of HIV reverse transcriptase and protease inhibitors has been available since around 1997. Following the availability of this therapy, the number of deaths

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occurring due to AIDS has decreased, particularly in advanced countries. Although the incidence of HIV encephalopathy has markedly decreased due to this therapy, in 2004, it was estimated that approximately 3 million patients worldwide continued to die of AIDS. Further, a less severe form of HIV encephalopathy that comprises a milder cognitive and motor disorder (MCMD) is now a potentially serious problem. This syndrome is characterized by a much less pronounced state of memory loss and a decrease in computational and other higher cortical functions. The clinical presence of MCMD has been thought to be associated with the extent of pathological changes observed in the CNS due to HIV invasion. A potential explanation for the development of MCMD is that low level viral replication, as shown even in cases of highly HAART regimens, leads to the gradual progression of neurodegenerative damage.

The CNS of young children appears to be more vulnerable to the effect of HIV than that of adults. This is probably because in young children, the CNS is still in the developmental stage and contains many undifferentiated cells. The progression of pediatric AIDS is rapid and children do not respond to HAART. In addition, clinical analysis reveals that congenitally HIV-infected children frequently develop progressive encephalopathy, which is complicating microcephaly, spastic paraparesis, and delayed developmental milestones. After the introduction of HAART in many areas, the maternal-fetal transmission of HIV has been reduced successfully, thereby reducing the prevalence of progressive encephalopathy.

Currently, more than 6,500 people in Japan have been confirmed to be infected with HIV, and the number of HIV-infected persons is gradually increasing at a rate of 780 patients per year. Although the number of HIV encephalopathy patients clearly decreased after the introduction of the HAART, new problems such as the emergence of HAART-resistant viruses and the side effects of HAART are becoming apparent. Since subclinical MCMD patients appear to be increasing in many countries, HIV encephalopathy may also become a serious disease in Japan.

**HIV and HIV Encephalopathy**

HIV is virologically classified into HIV-1 and HIV-2. HIV-1 was initially isolated from a French patient in 1983, and subsequently, it has been identified as the causative agent of AIDS in many other countries. Currently, the HIV-1 epidemic has spread worldwide. It has been demonstrated that HIV-1 originated from chimpanzees:
however, infected chimpanzees are resistant to the development of this disease. In 1986, HIV-2 was independently isolated from some patients in West Africa. Interestingly, in the case of HIV-2, it has been demonstrated that it originated in a small monkey species, such as the mangabe, and its potential for causing pathogenesis or acting as a pathogen in humans was clearly low. HIV-1 belongs to the retrovirus family, and its virion structure comprises 100-nm ball-like particles. Two viral RNAs, viral structural proteins, core protein p24, matrix protein p17, nucleocapsid p7, and the accessory protein Vpr are packed into its capsid, and the capsid is enveloped by two viral-encoded glycoproteins, namely, gp120 and gp41, and a plasma membrane-derived lipid (Fig. 1). When the HIV particles attach to the target human cells, gp120 on the viral surface specifically binds to the CD4 molecule on the plasma membrane of the target cells and subsequently to CXCR4 or CCR5, which are the physiological receptor molecules for different chemokines. In vivo, HIV replicates in the CD4$^+$ T cells and macrophages because both these cells express CD4 and CXCR4 or CCR5. Although these receptor positive cells get distributed in many lymphoid tissues such as the peripheral blood and lymph nodes, they rarely reside in the healthy brain. An examination of the autopsy samples of HIV encephalopathy patients revealed that HIV was predominantly found in the macrophages and microglia but not in the CD4$^+$ T cells located near the vessels. It is thought that the CD4$^+$ T cells are depleted in the peripheral blood at the time of development of HIV encephalopathy, and that they invade the brain very little. The typical pathological features of parenchymal infections include the activation of macrophages and astrocytes, cortical and central atrophy, diffuse myelin pallor, multinucleated giant cells, and microglial nodules. Furthermore, the macrophage-tropic virus, which uses CCR5
as a coreceptor, is frequently isolated from the HIV-infected brain. However, the T-tropic virus, which uses CXCR4, could not be detected. Interestingly, HIV does not replicate in neurons and oligodendrocytes, which are found to be severely damaged in the infected brains. Therefore, it has been postulated that macrophages and microglia are the key cell types that are affected in HIV, while the neurons and glia cells are damaged by the factors released from the infected macrophages and microglia. HIV-encoded proteins and host factors from the macrophage and glial cells may mutually influence the function and fates of neurons. Based on studies using an animal model, it is thought that HIV can enter the brain early after systemic infection. Although the CNS is physiologically separated by the blood-brain barrier (BBB), the mechanism of action of the BBB in HIV-infected brains and the critical factors that lead to the development of HIV encephalopathy remain unclear.

Several chemokines and their receptors have been the focus of the studies on the pathogenesis of HIV encephalopathy. It has been reported that CXCL8 (IL-8), CXCL10 (IP10), CXCL12 (SDF-1α, β), CCL2 (MCP1), CCL3 (MIP1α), CCL4 (MIP1β), CCL5 (RANTES), CCL7 (MCP3), and CX3CL1 (Fractalkine) are involved in the development of HIV encephalopathy. CXCR1, CXCR2, CXCR3, CXCR4, CCR1, CCR2, CCR3, CCR4, CCR5, and CX3CR1 are known to be expressed in the CNS and might have a various role in maintaining the balance between neuroprotection and neurodegeneration.

The infection of perivascular macrophages and microglia may cause a disruption in the normal neurological functions either by producing viral proteins, such as gp120, Tat, and Vpr, or by exerting an indirect or bystander effect via some neurotoxic factors. In addition, it has been proposed that after the inflammatory process, due to the establishment of a self-sustaining chain reaction, viral infection might play a more limited role in the degenerative process. Although both these mechanisms are not mutually exclusive and might coexist, the bystander theory is probably more consistent with most of the evidence (Fig. 2).

Recently, a hypothesis that the CXCR4-using X4 virus emitted to the neurons has been suggested. Although the X4 virus may act as a neurotoxic factor in vitro thus far, there is little evidence indicating that the X4 virus plays a critical role in the human CNS. HIV-1 has certain characteristics that differ from other simian-related viruses, namely, simian immunodeficiency virus (SIV) and simian human immunodeficiency virus (SHIV): the latter is a recombinant virus that is obtained by replacing the SIV envelope with an HIV envelope. The X4 virus frequently infects the neurons in SIV- and SHIV-infected models.
By contrast, in the case of HIV-1, only the viruses using CCR5 have been found in the infected brain.

**New Models of HIV Encephalopathy**

We have developed an animal model of HIV encephalopathy. We used the NOD-SCID mouse, which represented severe immunodeficiency acquired through heredity, and produces human chimeric mice by the intraperitoneal transplantation of human peripheral blood mononuclear cells (PBMCs). HIV-1, which uses CCR5, was then inoculated intraperitoneally. After establishing systemic HIV-1 infection, a bacterial component lipopolysaccharide was injected intraperitoneally (Fig. 3). Infiltration of human T cells and macrophages was induced in the mouse brain and many of these cells were found to be infected with HIV-1. Further, the astrocytes and microglia were activated. Importantly, the apoptosis of neurons was frequently detected near the human macrophages infected with HIV-1. On the other hand, using the X4 virus, we observed that significant neuronal death was not detected in the brain. The TRAIL molecule, which is one of the death-inducing ligands, was found to be predominantly expressed in the HIV-1-infected macrophages in the brain. When a neutralizing antibody against human TRAIL was injected intraperitoneally, neuronal apoptosis was significantly inhibited. This suggests that the TRAIL molecule is important for guiding the apoptosis of neurons in HIV encephalopathy. Therefore, we propose that the TRAIL molecule may play a role in HIV encephalopathy. Our proposal was further confirmed by the examination of human pathological autopsy samples and cultured human neurons.

Based on these results, we are focusing on the analysis of HIV pathogenesis in the CNS using small animals such as mice and rats. These animals are very useful in neuroscientific studies because a considerable amount of scientific knowledges has been amassed using these animals. Several experiments using the cells of these small animals have been reported in HIV research.
Novel investigations have been carried out to determine the factors associated with this virus using a lentiviral vector system; this system was originally generated from HIV itself. We transduced the human leukocyte cDNA library into a human T cell line and then infected them with X4 HIV-1. After analyzing the gene that provides anti-HIV activity in the surviving cells, the CD14 gene and an N-terminal deletion mutant of CD63 gene, namely CD63dN, were isolated (Fig. 4). CD14 appears to partially inhibit HIV-1 entry and provides resistance to HIV-1-induced cytopathic effect. CD63dN inhibits the surface expression of CXCR4. CD14 is one of the marker molecules of monocytes, and although the expression of CXCR4 can be seen in these cells, X4 virus cannot replicate efficiently in these cells. Further, since CD63 downregulates the activity of CXCR4 and the expression of CD63 is augmented in activated macrophages and microglia, it was suggested that CD63 might preferentially inhibit X4 HIV-1 infection in the infected brain. This explanation may be supported by the fact that it is difficult to detect X4 HIV-1 in the HIV-1-infected brain.

Further, we are trying to identify the genes that function against the cytopathic effect of HIV encephalopathy. An organic culture of the rat brains was cocultivated with the human macrophages with HIV infection. RNA was then collected from the samples of this culture, and was analyzed using a microarray system. Based on this analysis, the cDNA of a candidate gene was transduced into the rat brain cells and was cocultivated with HIV-infected human macrophages (Fig. 5). These experiments are currently in progress.

**Conclusion**

To date, the research on HIV encephalopathy has been carried out by analyzing the brain tissues of clinical specimens and by infecting experimental animals with SIV or SHIV. In addition to these
models, a novel approach has been initiated using small animals such as mice and rats. Based on previous reports, it is thought that many host factors as well as viral factors are closely involved in the pathogenesis of the HIV encephalopathy, and the elucidation of its mechanisms is very important. Further, new types of researches that aim at identifying additional host factors have been initiated by using a lentiviral vector. They can be applied to devise powerful new medical treatments. Finally, it is important to improve these systems using small experimental animals and lentivirus for various central nervous system diseases.

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