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Glial Responses to Virus Infection

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Introduction

The nonneuronal cells of the central nervous system (CNS) that provide support to and promote normal neuronal function are termed glia or glial cells. As described elsewhere in this encyclopedia, astrocytes and oligodendrocytes arise from ectodermal tissue just like neurons. Microglia, the immune cells of the CNS, are derived from the bone marrow and migrate into the nervous system at various points during embryonic development and beyond. Many viruses target cells of the central and/or peripheral nervous systems, and glial cells, infected or not, respond with a battery of mechanisms that to a large extent determine, together with the specific characteristics of the pathogen, the eventual outcome of the infection. The final result depends on both the cell type and the virus causing the infection; different cell types have a variable response to different pathogens, and even to the same pathogen, and different pathogens induce different responses in various cell types. We provide an overview of these responses together with a more detailed description of the glial response to human immunodeficiency virus infection of the CNS.

Glial Cells

Glial cells, neuroglia, or simply glia, the most abundant cells in the nervous system, have been described as nonneuronal cells that provide support and nutrition, regulate the internal environment of the brain (especially the fluid surrounding neurons and their synapses), form myelin, and participate in signal transmission in both the peripheral nervous system and the CNS. They also have important developmental roles and are active participants in synaptic transmission (e.g., regulating clearance of neurotransmitters from the synaptic space). Information regarding the biology and functions of astrocytes or astroglia, the most abundant type of glial cell, and oligodendrocytes is provided in other articles in this encyclopedia. Microglia are the resident brain macrophages and, together with the perivascular macrophages that are found around blood vessels in the brain, they perform immune surveillance in the CNS and are capable of phagocytosis and initiating the inflammatory and immune response to most insults. Despite their mesodermal origin, they are commonly categorized as glia because of their supportive role for neurons. Their roles are discussed in more detail later. Perivascular macrophages and microglia arise from bone marrow elements that settle in the CNS at various times during embryonic development and throughout adult life. Perivascular macrophages and perhaps macrophages associated with other CNS regions such as the choroids plexus and the meninges have a relatively short life span within the CNS and, therefore, a considerable turnover in adult life. The parenchymal microglial cells are long-lived and estimated to have an extremely low turnover on the basis of murine and human studies. This has important implications considering that pathogens that establish persistent infections in cells such as microglia ensure their replication for long periods of time after the initial infection.

Glial Responses

Microglial cells are quiescent in the normal CNS and are generally considered to be unable to perform effector functions such as antigen presentation to other immune cells until they are activated by injury or infection. At that time, microglia become the first line of defense. In vitro studies have shown that microglia can present antigens and perform other effector functions when activated by proinflammatory cytokines. In addition, infection of mice with Thiel’s murine encephalomyelitis virus (TMEV; a natural mouse pathogen that causes an autoimmune demyelinating disease associated with a persistent infection of the CNS and that is used as a model for multiple sclerosis) has been shown to be persistent in murine microglial cells and perivascular macrophages. In this model, chronic demyelinating disease is dependent on CD4+ T cells responding to myelin epitopes presented by microglial cells, suggesting that microglia most likely play an effector role and are critical in establishing an inflammatory response in autoimmune diseases and during viral infection.

The innate immune response is the initial antigen nonspecific response to insults such as traumatic injury and viral or microbial infection. It leads to the rapid production of chemokines and proinflammatory cytokines and subsequent expansion of the intra-CNS immune response. Cells of the innate immune system use the toll-like receptors (TLRs) to recognize ‘danger’ signals, microbial structures, and pathogen-associated molecular patterns (PAMPs), which are molecules or molecular structures expressed by various classes of pathogenic organisms that are not normally expressed...
by eukaryotes. TLRs are members of an ancient superfamily of proteins, also present in invertebrates and plants, that not only activate the innate immune response but also orchestrate the development of adaptive immune responses. By discriminating between diverse stress or danger molecules, microbial structures, and PAMPs, TLRs are able to mount tailored or signal-specific cellular immune responses.

At least 11 such TLRs have been identified in humans and mice, and some of their known ligands are listed in Table 1. TLR2 recognizes peptidoglycan (PG) and associates with TLR1 to recognize bacterial triacyl lipopeptides and with TLR6 to recognize diacyl lipopeptides. TLR3 recognizes double-stranded RNA (dsRNA) which is produced during the replication of many RNA viruses, whereas TLR4 binds to lipopolysaccharide (LPS) from gram-negative bacteria in association with the LPS-binding protein, CD14. TLR9 recognizes unmethylated CpG DNA primarily found in bacteria. Upon engagement of the TLRs by their ligands, TLR-induced signaling leads to the induction of NF-κB, ultimately resulting in upregulation of transcription of chemokines, proinflammatory cytokines, and cell surface molecules involved in the initiation of adaptive immune responses to pathogens. TLRs are present on a wide variety of cells, but specific cell types show preferential expression of certain TLR molecules, probably related to the specific functions that the particular cell types will execute. Monocytes/macrophages have been shown to express most TLRs (with the likely exception of TLR3) during different stages of maturation, whereas dendritic cell expression of TLRs seems to be dependent on both the cell subset and the maturation state: immature dendritic cells express TLR1, -2, -4, and -5 (responding to LPS and PG with upregulation of expression of proinflammatory cytokines such as interleukin (IL)-12 and costimulatory molecules B7-1 and B7-2) and their expression decreases upon maturation, whereas TLR3 expression is increased. Finally, in addition to the recognition of dsRNA by TLR3, other TLRs have been identified as being capable of sensing and mediating responses to viral infections. Thus, TLR2 binds to a cytomegalovirus protein and to the hemagglutinin protein of wild-type, but not vaccine strain, measles virus; TLR4 interacts with the fusion protein of respiratory syncytial virus and with the envelope protein of mouse mammary tumor virus; TLR9 binds to herpesviral genomic dsDNA in mouse cells; and TLR7 in murine dendritic cells and TLR8 in human dendritic cells and macrophages bind to viral ssRNA.

Most TLR molecules signal through a common adaptor protein, MyD88, which binds to the conserved

| TLR | Ligands | CNS cell types |
|-----|---------|---------------|
|     |         | Microglia | Astrocytes | Oligodendrocytes |
| TLR1 | Triacyl lipopeptides (associated with TLR2) | +++ | ++ |
| TLR2 | MALP-2 (mycoplasma) | +++ | ++ | + |
|     | LAM (mycobacteria) | | | |
|     | Lipoproteins (gram-positive bacteria) | | | |
|     | Zymozan (yeast) | | | |
|     | Hemagglutinin protein of wild-type measles virus<sup>a</sup> | ++ | ++ | + |
|     | Virion protein of cytomegalovirus<sup>a</sup> | | | |
| TLR3 | Viral dsRNA<sup>a</sup> | ++ | +++ | + |
| TLR4 | LPS (gram-negative bacteria) | +++ | ++ | |
|     | Hsp60, fibronectin, hyaluronan (host-derived factors from stressed or damaged cells) | | | |
|     | Protein F of respiratory syncytial virus<sup>a</sup> | | | |
|     | Envelope protein of mouse mammary tumor virus<sup>a</sup> | | | |
| TLR5 | Bacterial flagellin | ++ | ++ |
| TLR6 | Diacyl lipopeptides (associated with TLR2) | ++ | + |
| TLR7 | Small antiviral compounds (e.g., Imidazoquinolines) | | | |
|     | ssRNA (in murine dendritic cells)<sup>a</sup> | +++ | + |
| TLR8 | GU-rich ssRNA (in human dendritic cells and macrophages)<sup>a</sup> | ++ | - |
| TLR9 (intracellular) | Bacterial unmethylated CpG DNA | | | |
|     | Herpesviral genomic dsDNA (in mice)<sup>a</sup> | | | |
| TLR10 | Unknown | +/− | |
| TLR11 | Unknown | ? | |

<sup>a</sup>Viruses or viral molecules that bind to these receptors.
cytoplasmic domain on the TLRs. Following stimulation, MyD88 activates the IL-1R-associated kinase, which associates with tumor necrosis factor receptor (TNFR)-associated factor 6, leading to activation of the NF-κB family of transcription factors. MyD88-independent pathways unique to TLR3 and TLR4 have also been identified, including the activation of interferon regulatory factor-3 and subsequent upregulation of expression of several interferon-inducible genes. Thus, engagement of the TLRs leads to transcriptional activation of cytokines, chemokines, effector molecules, costimulatory molecules, and major histocompatibility complex (MHC) class I and II molecules.

Research has begun to elucidate the expression of TLRs in microglia, as well as their activation by various stimuli. Studies conducted directly on human tissue showed that the brain expressed all TLR molecules except for TLR6 and TLR8, although at levels lower than those observed in a peripheral organ such as the spleen. Slightly different results have been obtained with isolated adult human microglia, which expressed all TLR molecules except for TLR9. Human brain tissue specimens also showed that astrocytes and oligodendrocytes only expressed TLR2 and TLR3. Similarly, it has been shown that cultured neonatal murine microglia expressed all the TLR molecules, although expression in in vitro culture and in the CNS environment may vary, thus explaining the slight differences. In vitro stimulation of quiescent murine microglia with various TLR agonists, including LPS (TLR4), PG (TLR2), polyinosinic-polycytidylic acid (poly[I:C]) (TLR3), bacterial CpG DNA (TLR9), as well as infection with TMEV, induced the cells to upregulate innate and effector immune cytokines and chemokines (e.g., CCL3/MIP-1α, CCL2/MCP-1, and CCL5/RANTES) that are required for attraction of peripheral macrophages and T cells into the CNS. In addition, TLR stimulation upregulated MHC class II and some costimulatory molecules (B7-1, B7-2, CD40, and ICAM-1), enabling the microglia to effectively present myelin peptide to CD4+ Th1 cells.

Interestingly, microglial cells apparently develop a stronger response to infection with viable TMEV than when stimulated directly with TLR ligands, including poly(I:C), which signals through TLR3. This suggests that the virus may activate innate immunity via multiple TLRs or through other mechanisms that trigger innate immune recognition, such as through dsRNA-activated protein kinase, which binds intracellular viral dsRNA and induces expression of type I interferons. TLR molecules other than TLR3 have been shown to recognize viruses, viral proteins, or viral nucleic acids. Examples are the TLR4-dependent inflammatory immune response to respiratory syncytial virus protein F and to mouse mammary tumor virus envelope protein, or the TLR2/CD14-dependent inflammatory cytokine production in response to human cytomegalovirus infection. Therefore, it is apparent that a more robust innate immune response can be achieved by stimulation/recognition through several TLR molecules (although a combination of LPS and poly(I:C) does not seem to result in a stronger response than either ligand alone in microglial cells). Genetic susceptibility to herpes simplex virus encephalitis has been mapped to UNC93B, a protein involved in the mediation of the response to TLR3, TLR7, and TLR8, and it has been suggested that a deficient interferon response is specifically responsible for this phenotype. The available data therefore suggest that microglia play a key role in both innate and adaptive immune responses, providing the CNS with the means to rapidly and efficiently respond to a wide range of pathogens.

Astrocytes and oligodendrocytes may also aid in the innate and adaptive immune responses, although microglial cells appear to be the most potent CNS resident antigen presenting cells. Studies on oligodendrocytes are more limited, but TLR expression seems to be low and limited to TLR2 and TLR3. In comparison with microglia, astrocytes also express a somewhat limited set of TLR molecules, with predominant expression of TLR3; adult human astrocytes in culture also express TLR4 to high levels.

The astrocytic response to TLR ligands is similar to that of microglia, with some notable differences. For example, TLR-mediated activation of microglial cells results in positive feedback for TLR2 and TLR3 expression but in downregulation of TLR4 expression. However, astrocytic TLR3 ligation and subsequent activation of astrocytes has been shown to result in upregulation of expression of all TLR2, TLR3, and TLR4. In addition, TLR3 can be found in astrocytes both within the cytoplasm and at the cell surface, whereas it is localized almost exclusively in the former in microglial cells. Another striking difference is that although other TLR molecules may be expressed on astrocytes, cellular activation of in vitro systems is limited to TLR3 ligands. The underlying reason for the absence of an astrocytic response to other TLR ligands is unknown, but it may be related to expression levels. Finally, activation of adult human astrocytes through TLR3, but not through TLR4, has resulted in suppression of astrocyte growth, promotion of endothelial cell growth, and enhancement of neuronal survival, providing a comprehensive neuroprotective response rather than a polarized proinflammatory reaction. Some differences in TLR expression and responsiveness have been observed in studies on murine and human astrocytes, although data on murine and human microglia appear to be much more consistent.
Thus, the evidence suggests that more cell type-specific, and possibly species-specific, differences in the responses mediated by TLRs are likely to be found as knowledge accumulates. In addition, in a disease context, multiple TLRs may be engaged by multiple ligands in multiple cell types and the ultimate functional outcome will be dependent on the timing of stimulations and cross-regulation of TLR-mediated signaling.

**Viral Infections of the CNS**

Viruses from many diverse RNA and DNA families and genera can infect the CNS. Some are only neurotropic – they can infect the nervous system – but are not neuropathogenic in normal circumstances, for example, in individuals who have intact immune systems. Others target the CNS principally, and their importance as pathogens is limited to their neurological disease. Some of these are listed in Table 2. In many ways, viral infection of the CNS is more complex than that of peripheral tissues. First, the presence of the blood–brain and blood–cerebrospinal fluid barriers limits viral trafficking into and out of the CNS. Primarily for this reason it was thought for many years that the neural route was the major route of viral entry into the nervous system, and experimental systems documented the retrograde axonal transport along the peripheral nerves of rabies virus, herpes simplex virus, and some nervous system-adapted strains of polioviruses. The olfactory mucosa is a unique anatomical site where distal processes of neural cells are directly exposed to the environment while the processes at the opposite site of the same neural cells establish connections within the CNS. This extremely specialized mucosal site is exploited by some aerosol-borne and respiratory infections to spread to the olfactory bulbs directly from the olfactory mucosa. Importantly, in most natural and experimental infections, viruses invade the brain from the bloodstream (in the so-called hematogenous route). When present at sufficiently high titers in the blood, viruses may infect endothelial cells and spread from them into the nervous tissue, be transported across the endothelial cells within vesicles, or be carried inside the brain within infected cells such as leukocytes migrating into the brain across the blood–brain barrier (BBB).

Once in the CNS, some viral infections in the brain, such as rabies and polioviruses, involve only neurons, and spread within the CNS most likely occurs through dendrites and other neuronal processes. The type and extent of neuropathology also relate to the target cell population and type of infection. Thus, polioviruses infect motor neurons and cause flaccid paralysis, whereas rabies virus infects neurons of the limbic system and cortical neurons leading to behavioral abnormalities in addition to eventual paralysis. Others, such as herpes simplex virus, infect both neurons and glial cells and cause a more focal encephalitis, preferring the temporal lobes. In contrast, some arboviruses (arthropod-borne viruses) cause generalized encephalitis by widespread infection of neurons and other cells. Murine and human cytomegaloviruses preferentially cause lytic infection in immature glial cells during brain development but mainly establish persistent infection in neuronal cells. Finally, the polyomavirus JC virus is an example of selective infection of oligodendrocytes that can lead to a focal or multifocal demyelinating disease, as seen in progressive multifocal leukoencephalopathy.

Cell damage due to viral infection in the nervous system may occur through necrosis induced directly by the virus, as a by-product of the immune response to the infection, or through apoptosis – a mechanism of programmed cell death that is critical in the normal development of the nervous and lymphoid systems, two organs in which excess cells are generated. At the same time, postmitotic cells such as many neuronal populations are not replenished easily and have mechanisms that are designed to protect them from

| Table 2 | Representative neurotropic viruses |
|---------|-----------------------------------|
| DNA viruses | α, β, and γ herpesviruses |
|           | Herpes simplex virus |
|           | Cytomegalovirus |
|           | Epstein–Barr |
|           | Human herpesvirus 6 |
|           | JC virus (polyomavirus) |
| RNA viruses | Retroviruses |
|           | HIV-1 |
|           | HIV-2 |
|           | Human T lymphotropic virus type 1 |
| Coronaviruses | Enteroviruses (polioviruses, coxsackieviruses, cardioviruses) |
| Arboviruses | Alphaviruses |
|           | Western/Eastern equine encephalitis |
|           | Flaviviruses |
|           | West Nile virus, St. Louis encephalitis virus |
|           | Bunyaviruses |
|           | La Crosse virus, Rift Valley fever virus |
| Arenaviruses | Lymphocytic choriomeningitis virus, Lassa fever virus |
| Paramyxoviruses | Mumps virus (rubulavirus) |
|           | Measles virus (morbillivirus) |
|           | Henipaviruses (Nipah virus, Hendra virus) |
|           | Rabies viruses (Lyssavirus) |
|           | Rubella virus (Rubivirus) |
premature apoptosis, such as expression of antiapoptotic genes. Moreover, they do not express histocompatibility antigens, which exempts them from most T cell-mediated cytotoxicity. In many instances, the balance between these two competing effects results in moderate or no cytopathology, leading to mild or slowly progressing disorders instead of a rapidly fatal encephalitis. In contrast to neurons, virus-infected glial cells can be destroyed not only by the virus but also by immune-mediated mechanisms since they have the capacity to express viral antigens on the surface and are subject to both humoral and cell-mediated immune responses. Neurons have been shown to initiate an innate immune response through poly(I:C) and viral dsRNA interaction with neuronal TLR3 with subsequent induction of β-interferon.

Finally, the association of HIV-1 infection with neurological diseases has raised the significance of viral infections of the nervous system to a new level. The extent of the HIV-1 epidemic and the fact that the virus is present in the CNS of the majority of infected patients have made HIV-1 the most prevalent neuropathogenic viral infection ever recognized. HIV/AIDS-related neurological complications are among the most frequent neurological diseases worldwide. In the developed world, highly active antiretroviral therapy has decreased the incidence of, but not eradicated, the most severe forms (e.g., HIV-1-associated dementia and vacuolar myelopathy), whereas the prevalence of milder cognitive disorders remains high and may even be increasing due to the longer survival and more advanced age of HIV-infected individuals. Because of the intensity and breadth of the many studies that have analyzed HIV infection of the CNS, this lentiviral complication now serves as a paradigm for the relationship between a virus and brain cells, specifically glia, since HIV does not infect neurons in significant levels.

**Neurotropism of HIV-1**

The CNS is susceptible to infection by retroviruses of various species and in particular by members of the lentivirus family, such as HIV-1. The specific requirements for entry into the brain and the variety of cell types within the CNS increase the complexity of virus–cell interactions in this organ, but the BBB is not an absolute barrier for many viral pathogens, such as HIV-1, since it is selectively permeable rather than impermeable. Cells of the immune system can cross the BBB and enter the brain within the context of immune surveillance in a process that is carefully regulated. Using the lentivirus visna virus of sheep as a model, Haase first proposed that HIV-1 and other lentiviruses enter the CNS as passengers in cells trafficking into the brain as part of normal immune surveillance mechanisms (the ‘Trojan horse’ hypothesis). For HIV-1, which infects CD4+ T cells and monocytes in the circulation, this model is the most intuitively appealing and, unlike alternative mechanisms, has the most compelling evidence.

**Figure 1** schematically shows the potential mechanisms of entry of HIV-1 into the brain. In situ hybridization and immunohistochemical analyses have demonstrated virus accumulation in perivascular regions, principally in CD14+ cells. CD14 is a LPS receptor present as a membrane-bound form at the surface of myeloid cells, including monocytes, macrophages, and granulocytes. Therefore, the bulk of HIV-1 entry into the brain takes place within infected monocytes crossing the BBB and migrating into the brain to replenish the population of perivascular macrophages (Figure 1, A). In addition, phenotypic analysis of HIV-1-infected monocytes demonstrates an activation pattern that promotes migration into tissues such as the brain. The infected monocytes differentiate into macrophages and release new virions into the brain’s extracellular space, spreading infection to additional perivascular macrophages and microglial cells present within the brain parenchyma (Figure 1, B). Microglial cell activation may also play a role in neuropathogenesis.

T cells also travel into the brain as part of the immune surveillance process, and HIV-1-infected T cells could therefore bring virus into the brain (Figure 1, C). However, the contribution of virions produced by infected CD4+ T cells migrating into the brain to the pool of replicating virus present in the CNS may not be significant since genotypic and phenotypic analyses show that HIV strains from brain are closer to those from monocytes/macrophages than to those from T cells.

There is very limited pathological evidence of HIV-1 infection of endothelial cells, although BBB abnormalities due to HIV infection have been demonstrated (Figure 1, D). Other mechanisms, such as macrophagocytosis, transcytosis, or localized loss of BBB functionality, might constitute additional pathways for viral penetration of the BBB, but they are unlikely to account for the bulk of the virus entering the brain.

Several mechanisms for HIV-1 infection of astrocytes have been described (Figure 1, E); however, it is accepted that infection of these cells is mostly nonproductive and that they do not contribute significantly to viral replication in the brain since few astrocytes express viral antigens or RNA. The mechanism of viral entry into astrocytes is also unclear since these cells are CD4 negative and do not express large amounts of the major HIV coreceptors (although detection of corresponding mRNAs has been reported). However,
it seems likely that the very limited HIV-1 gene expression in astrocytes, the astrocytosis induced in HIV-associated dementia, and the deregulation of cytokine and chemokine signals due to infection of macrophages and microglia all play a role in the alteration or loss of astrocytic functions and brain homeostasis leading to neuropathogenesis.

The HIV-1 envelope glycoproteins expressed on the surface of infected cells mediate cell-to-cell fusion with receptor- and coreceptor-positive cells; in the brain, cell-to-cell fusion involves macrophages and microglia and results in the formation of large multinucleated giant cells or syncytia that also produce virus before they eventually die (Figure 1, F). Their life span after undergoing fusion is unknown, but it is estimated to be days to weeks.

There is no in vivo evidence of HIV infection of oligodendrocytes, although in vitro infection has been reported. However, binding of gp120 to galactosylceramide or other proteoglycans present on the membranes of oligodendrocytes may reduce myelin synthesis and increase intracellular Ca^{2+} levels, which could lead to apoptosis.

Finally, neurons, as major effectors of cognitive and motor function, must be involved in HIV neuropathogenesis, and indeed there is significant accumulated neuronal cell death in HIV-infected brains. However, neuronal infection has been reported only by some investigators using very sensitive techniques, in agreement with the fact that neurons lack expression of the main HIV-1 receptor CD4. However, different neuronal subsets express some of the chemokine receptors that serve as coreceptors for HIV-1 infection, where they may be involved in cell migration.

### The Chemokines and Chemokine Receptors Network

In the CNS, chemokines and their receptors are involved in cell migration, differentiation, activation, and proliferation of glial and neuronal cells. They are essential components of glial and neuronal physiology and play a crucial role in the balance between neuroprotection and neurodegeneration by counteracting the apoptotic signals that may be produced in response to injury and infection. CXCR4 and its only ligand, CXCL12/SDF-1α, are both widely expressed in the normal CNS and may be implicated in neuropathogenesis since they appear to be elevated in the cerebrospinal fluid and brain of HIV-1-infected individuals. Members of the CCR subfamily of receptors have also been detected in physiological conditions and upregulated during inflammatory and neurodegenerative diseases (including in the CNS of simian immunodeficiency virus (SIV)-infected macaques and HIV-1-infected patients), mostly in perivascular macrophages and microglia. Although β chemokines are only weakly or not expressed at all in the normal human brain, altered expression is observed in SIV and HIV encephalitis and could contribute to neuronal
degeneration and dysfunction. However, no neurotoxic effects of β chemokines have been demonstrated. In contrast, several studies have shown that CCL4/MIP-1β and CCL5/RANTES can protect cultured neurons against apoptosis induced by gp120. Therefore, reported alterations in expression of chemokines and their receptors in the lentivirus-infected CNS may have either protective or deleterious effects.

The primary protective role of β chemokines and CCR5 in viral infections of the CNS seems to be better established in the case of West Nile virus (WNV) encephalitis. CCR5 presumably functions normally in antimicrobial host defense because it mediates leukocyte chemotactic responses; however, evidence of antimicrobial functions for CCR5 in humans has been elusive. In a mouse model of WNV infection, CNS expression of CCR5 and its ligand CCL5/RANTES were prominently upregulated by the virus, and this was associated with CNS infiltration of CD4+ and CD8+ T cells, natural killer cells, and macrophages expressing CCR5. Infection of mice engineered to knock out CCR5 expression resulted in increased viral burden and was rapidly and uniformly fatal. This indicated that CCR5 is a critical antiviral and survival determinant in WNV infection of mice that acts by regulating trafficking of leukocytes to the infected brain. In addition, an increased frequency of homozygosity for CCR5Δ32, a defective CCR5 allele found predominantly in Caucasians, has been described in two independent cohorts of patients with laboratory-confirmed, symptomatic WNV infection (4% and 8% vs. 1% in a healthy control population), suggesting that CCR5 also mediates resistance to symptomatic WNV infection in humans. Thus, the same chemokine receptor can protect from fatal encephalitis or mediate infection, depending on the virus. Following WNV infection of the CNS, inflammatory chemokines are expressed by trafficking leukocytes and resident cells (microglia and astrocytes), and chemokine-dependent T cell recruitment to infected CNS tissues is likely to modulate viral pathogenesis. WNV infection also seems to induce neuronal production of CXCL10, which will recruit effector CD8+ T cells through its cognate receptor, CXCR3. Also, a genetic deficiency in CXCL10 has been shown to correlate with reduced T cell trafficking to the CNS, greater viral loads, and enhanced mortality. Although CCR5 is a logical target for drug development in HIV/AIDS, blocking CCR5 could result in an increased risk of WNV disease for these patients.

**HIV-1 Neuropathogenesis**

Despite highly active antiretroviral therapy, HIV-associated dementia and especially milder psychomotor and cognitive disorders remain an important complication of HIV-1 infection in the developed world and even more so in the developing world, where access to therapy is limited. One potential explanation for the increased prevalence of mild to moderate HIV-associated neurological disorders in developed countries is that low-level viral replication, consistent with all but the most successful antiretroviral regimens, together with the longer life span of treated patients and the insufficient penetration of at least some of the currently used antiretroviral drugs into the brain, leads to slow, progressive neurodegeneration. Absent direct infection of neurons, microglia/brain macrophages and astrocytes will be the key cell types involved in HIV-1-associated neurodegeneration. Two non-mutually exclusive, probably coexisting mechanisms have been proposed (Figure 2):

1. **Direct Injury Mechanism**

   In *in vitro* experiments have shown that the HIV-1 surface glycoprotein gp120 seems to interact with chemokine receptors in neurons leading to neuronal injury. However, this interaction likely requires previous CD4 engagement, at least for infection, and CD4 is absent in neurons, putting into question its *in vivo* relevance. Alternative scenarios have been proposed since gp120 may induce neuronal cell death through direct interaction with the N-methyl-D-aspartate receptor or by further activation/stimulation of macrophages/microglia leading to increased TNF-α production and triggering of caspase-mediated cell death in neurons.

   Two other viral proteins, Tat and Vpr, have also been suggested as potential effectors in HIV neuropathogenesis. Tat, a transcriptional transactivator, if secreted from infected cells (as shown *in vitro*) could alter tight junction protein expression and BBB function, upregulate expression of inflammatory mediators such as CCL2/MCP-1 promoting monocyte infiltration, and result in neurotoxicity by multiple intracellular signaling pathways. Viral protein Vpr is involved in cell cycle arrest, transcription, and
integration, and it has been shown to activate replication in latently infected cells and to induce caspase-dependent apoptosis of bystander cells such as neurons.

**Bystander Effects: Toxicity of Glial Products**

HIV encephalitis is associated with immune activation that is seemingly out of proportion to the amount of virus present, due to the alteration of the secretory functions of microglia and brain macrophages. Glutamate, cytokines such as TNF-α and IL-1β, chemokines and other soluble products, macrophage activation markers, quinolinic and arachidonic acids and related metabolites, as well as free radicals, have all been related with astrocytic proliferation and apoptosis and with the activation of uninfected cells, probably contributing to the amplification of HIV-induced neurotoxicity. In addition, some of these macrophage products modify the permeability of the BBB and promote further monocyte migration into the brain.

Glutamate, the major excitatory neurotransmitter, and other excitatory molecules are thought to be involved in HIV-induced neurotoxicity. Increases in extracellular glutamate concentration mediate uncontrolled Ca2+ influx and loss of cellular homeostasis, leading to neuronal death through excitotoxicity in a mechanism common to many neurodegenerative disorders. The clearance of extracellular glutamate involves high-affinity uptake by several excitatory amino acid transporters (EAATs), which are mainly expressed in astrocytes which therefore play a key role in neuroprotection against glutamate excitotoxicity. However, in the course of HIV infection, expression of certain astrocytic EAATs is reduced, resulting in a greatly decreased glutamate uptake by astrocytes. On the other hand, expression of certain EAATs has been described in human macrophages and macrophages and microglia in rat models and in simian and human AIDS, suggesting that they may also exhibit neuroprotective properties. This expression seems to be modulated by macrophage/microglia activation and infection, but it suggests that macrophages/microglia can have a protective role against glutamate excitotoxicity during HIV infection, contributing to the maintenance of homeostasis and the prevention of neuronal degeneration.

TNF-α seems to be clearly involved in neuropathogenesis, primarily by promoting additional production of inflammatory cytokines by macrophages and astrocytes, which results in amplification of glutamate release and decrease of glutamate uptake mainly by astrocytes. However, *in vitro* studies have shown that TNF-α can be neurotrophic as well. It has been reported that TNF-α has a neuroprotective role by preventing or attenuating Ca2+ accumulation in the
cytosol of neurons as well as through the activation of the antiapoptotic kinase Akt (a major component of pro-survival signaling pathways) and NF-κB signaling (which promotes expression of genes vital for the response to insults).

Finally, β chemokines and CX3CL1/Fractalkine have also been shown to protect neurons from gp120 toxicity upon receptor interaction, again through Akt and NF-κB, suggesting that neuronal chemokine receptors mediate the neurotrophic effects of β chemokines and Fractalkine. Since TNF-α induces production of β chemokines by activated glial cells and of Fractalkine by astrocytes, it is likely that these cytokines and chemokines are involved in an extremely complex network of both paracrine and autocrine interactions between neurons and glial cells, with outcomes and functional consequences that are difficult to predict.

See also: Astrocyte: Identification Methods; Axon Guidance by Glia; Glia and Stroke; Glial Cells: Astrocytes and Oligodendrocytes During Normal Brain Aging; Glial Cells: Microglia During Normal Brain Aging; Glial Responses to Injury; Inflammation in Neurodegenerative Disease and Injury; Microglia Identification Methods; Microglia Properties; Microglial Response to Injury; Neural Repair and Regeneration: Inflammatory Mechanisms and Cytokines.