AIDS (acquired immune deficiency syndrome) has acquired a state of notoriety from oblivion in less than two decades. But as more information became available on viral pathogenesis and better surveillance protocols were applied, AIDS emerged as a global killer. When HIV was initially isolated and characterized, it was believed to infect only CD4 lymphocytes and the effects were restricted to the suppression of the immune system. However in 1985, HIV was recovered from brain tissue, spinal fluid and peripheral nerves of patients. This observation provided evidence of the role of HIV in causing primary infection of the brain. Subsequent finding of HIV RNA and DNA in brain tissue and intrathecal production of antibodies against HIV served to show the relation between HIV infection and brain abnormalities. Primary neuropathological manifestations initiated by HIV leading to neuropsychological and behavioural dysfunctions are collectively referred to as HIV associated dementia or HAD. HIV dementia refers to a series of conditions such as mental slowness, forgetfulness, poor concentration and changes in behaviour, discombobulation, speech problem and decrease in spontaneity. In 1990s, the prevalence of HAD was as high as 30 per cent among those having advanced HIV associated disease. However, with the development of HAART (highly active anti retroviral therapy) the susceptibility has been reduced to about 10.5 per cent. The prevalence of HAD nowadays is estimated to be around 10 per cent among those having advanced HIV disease.
of HAD in India is, however, 1-2 per cent only. Nevertheless, considering the number of HIV patients in the world and plethora of symptoms and causes of HIV associated dementia, HAD seems to be one of the common neurological complication in HIV infected patients. HIV enters the brain compartment through the blood brain barrier (BBB) and its entry is probably through trans-endothelial migration. Though mechanism of viral transfer to cells in central nervous system (CNS) is still not clear, it is widely believed that a long-lived viral reservoir persists in macrophages and microglia in the brain despite antiviral therapy. Evidence, however suggest that astrocyte infection also occurs. The observation that the neurological manifestation is more of a pleiotropic affair rather than being caused by a single molecule is based on evidences showing participation of chemokines and excitotoxic molecule secretion by infected cells, blood brain barrier dysfunction or direct cellular damage. Brain perivascular macrophages release a battery of potentially neurotoxic substances which may include quinolinic acid, excitatory amino acids such as L-cysteine (Cys) and glutamate (Glu), arachidonic acid, platelet activating factor, N-tox, free radicals, tumour necrosis factor- α (TNF-α), tumour growth factor-β (TGF-β) and others.

The following exposition presents an overview of well known strategies used by HIV influencing cell types and cellular signaling pathways towards neuropathogenesis. With appropriate relevance, we have focused on specific interaction between viral and cellular factors to present examples of the distinct mechanisms by which HIV modulates neuropathogenesis.

**Cellular basis of HIV neuropathogenesis**

Pathological implication: Various neuro-imaging [for example, magnetic resonance imaging (MRI), computed tomography (CT), single photon emission computed tomography (SPECT) and positron emission tomography (PET)] studies indicated presence of virus in the brain even during early stages of infection. Patients develop cerebral atrophy, diffuse myelin pallor, multinucleated cell encephalitis and acute meningitis which are characterized by ataxia, seizures and altered mental state. The gliosis (which is a characteristic symptom of neuropathogenesis) is mainly restricted to white matter and does not reflect true demyelination. White matter shows relatively higher water content which may lead to cerebral oedema. Neurons and dendrites take up water from brain extracellular fluid (BECF) and appear swollen. This may be because of increased glutamate concentration in the BECF but the exact mechanism is still poorly understood. Glutamate activates ionotropic receptors that allow sodium ions to enter the cell. Water and chloride ions follow passively causing subclinical oedema. Ultimately, multinucleated giant cells (MGC) comprising microglia and macrophage gave the disease its assigned name: HIVE (HIV encephalitis). Though the presence of MGC and diffuse myelin pallor are specific to HIVE, it occurs in only 50 per cent of patients with dementia. Studies estimating viral burden in CNS correlated dementia with severe HIV encephalitis. Findings also indicated that HIV encephalitis exists for a period of time before the clinical symptoms develops. The localization of virus in the CNS indicated some specific sites predilection (mainly deep white matter and frontal cortex). In situ reverse transcriptase PCR showed the presence of HIV RNA in brain cells. Other clinical studies correlated cerebral metabolite abnormalities to clinical severity of HIV-1 associated neuropathogenesis. Recent studies showed that inefficient semantic and serial clustering of neuropsychological abnormalities in patients is a result of damage caused by HIV in brain basal ganglia. Most of these findings are related to HIV-1 clade B and very few reports are available as far as prevalence of HIV dementia in HIV-1 clade C infected population is concerned. However, prevalence of HIV dementia in Indian population may be higher than previously thought.

**Cells infected (Fig. 1):** As mentioned earlier, HIV preferentially infects perivascular macrophages and microglial cells in CNS. Establishment of HIV-1 infection in the CNS is thought to be caused during early infection that is particularly tropic for microglia-macrophage lineage. It is possible that neurotropic viruses adapt to grow in these cell types resulting in neuronal injury. It is widely believed that macrophages, microglia, and perivascular cells productively infected with HIV-1 lead to widespread activation of molecules residing in these cell types and causes CNS injury. Interestingly, HIV envelope proteins derived from brain tissue revealed reduced CD4 and CCR5 dependence which has been shown to be associated with increased macrophage tropism of HIV and simian immunodeficiency virus (SIV) by many studies. Occasionally, endothelial cells may be infected but infection of astrocytes and neurons
remains controversial. The damage to these latter cells is thought to occur through inflammatory mediators secreted by the infected cells. Beta amyloid precursor protein immunoreactivity and several other procedures indicated that the infection and associated brain damage is restricted to deep white matter only and much less in sub-cortical regions. Recent studies indicated that neural progenitor cells can also form HIV reservoirs in the brain. Multi-potent neural progenitor cell line infected with HIV showed the presence of viral Tat, Rev and Nef proteins. Nestin-rich periventricular cells (represented by progenitor cells) may also harbour HIV-1. Differentiation into an astrocytic phenotype has been shown to be associated with higher viral titre, as in stimulation with TNF-alpha.

Entry through the blood brain barrier (BBB) (Fig. 2): In 1900, Lewandowski showed that brain capillaries hold back certain molecules. The metaphor “Bluthirnschranke” came into existence which indicates the blood brain barrier. Cerebral capillaries are the major component of this barrier and are equipped with tight junctions, but their density does not prevent transport completely. Other peculiar features of these cerebral capillaries are non-feasibility of transcytosis (due to fewer endocytic vesicles), greater number of mitochondria, presence of thick basement membrane and presence of astrocytic end feet. On the other hand, capillaries from other regions of the body have small openings between the endothelial cells. Tight junctions present in the brain capillaries prevent water-soluble molecules and ions from passing. However, many alterations have been observed in brain microvasculature following HIV infection. HIV-1-infected mononuclear phagocytes downregulate tight junction protein and special polarized transport systems on brain microvascular endothelial cells (BMEC), as shown in human autopsy brain tissue and in severe combined immunodeficiency (SCID) mice with HIV. In addition, the down modulation of expression of members of tight junction proteins such as zonula occludens (ZO-1), occludin, and P-glycoprotein (P-gp), a transmembrane glycoprotein located on the apical/luminal membrane of brain microvascular endothelial cells (BMVEC) that transports endothelium-penetrating lipophilic molecules back into the blood were found in SCID mouse model of HIVE. Interestingly, SCID mice inoculated with macrophages and microglia with HIV-1 showed prominent neuropathological damages including astrogliosis. These observations were corroborated with findings that showed significantly higher than normal levels of serum proteins (fibrinogen and IgG) in postmortem brain tissue of infected individuals as compared to matched HIV seronegative controls. Thus, HIV infection results in increased
Transmigration: Transmigration is typically composed of four steps; rolling (by selectins), activation (by chemoattractant stimulus), adhesion (by integrins) and transendothelial migration. Varieties of molecules are responsible for this process. Endothelial cells possess leukocyte specific cell adhesion molecules (CAM). ICAM-2 (intercellular cell adhesion molecule-2) and PECAM-1 (platelet-endothelial cell adhesion molecule-1) are two such molecules. ICAM2 mediates transmigration independently and PECAM1 participates in diapedesis in a cytokine specific manner. CAMs may be expressed constitutively or are produced in response to HIV infection of perivascular space, thus, facilitating further entry of leukocytes. CAMs belong to four family of proteins - selectin (L, E and P), mucin like (CD34, MadCAM-1), integrin (CD18) and immunoglobulin (ICAM-1, VCAM-1) super family. For entry of cells through the BBB to take place the infected cell first binds to the endothelial cells with the help of mucin like CAMs. This results in weak attachment of the macrophage but the force of flowing blood can still roll it to some distance. This process is repeated many times and the HIV infected cell slows down a bit and is amenable to interact with chemokines present on the surface of endothelium. After strong attachment is established, the leukocyte extravasates to perivascular Virchow Robin space in nervous system. PECAM-1, CD11a/CD18 and JAM-1 (junctional adhesion molecule-1) are involved in this procedure. However, some reports showed that HIV-1 crosses brain endothelia by micropinocytosis dependent on lipid rafts and mitogen activated protein kinase (MAP-Kinase) pathway.

Tropism: CCR5-using HIV-1 predominantly colonizes the brain. This is believed to be the case as macrophage-microglial lineage cell types express predominantly CCR5 over other chemokine receptors that HIV-1 exploits for entry. It is believed that perhaps infected macrophages migrate through blood brain barrier and take the virus inside the CNS. Though contribution of other cell types resident in the brain towards neuropathogenesis is less clear, research evidences suggest that CD4+ astrocytes become infected, particularly in paediatric AIDS cases and CD4 independent infection of astrocytes using mannose receptors is also documented. Astrocyte infection is relatively unproductive with early HIV mRNAs (e.g., for rev and nef) detectable, but no late mRNAs encoding the structural gag and env proteins needed to produce progeny virus particles. Albeit mechanisms that upregulate HIV tropism for macrophages in the CNS are not well understood, a specific substitution of asparagine (Asn) 283 (N283) in the CD4-binding site of HIV envelope glycoprotein (Env) gp120 has been documented. Interestingly, this particular allocation of asparagine residue was found to be present at a high frequency in brain tissues from AIDS patients with HAD. It is suggested that N283 increases affinity of gp120 for CD4 by decreasing the gp120-CD4 dissociation rate thereby modulating the capacity of HIV envelope to use low levels of CD4 for virus entry and increasing viral replication in macrophages and microglia. Moreover, molecular modeling indicates that the enhanced capacity of envelope protein with N283 to use low levels of CD4 is likely to be due to a hydrogen bond formed with glutamine 40 (Gln 40) of CD4.

Neutralizing antibodies presumably targeting the CD4 binding site on gp120 might play a role in driving envelope evolution that could protect this site. It is generally believed that presence of neutralizing antibodies in CNS usually are at lower levels as...
compared to immune tissues, thus this environment perhaps would allow the evolution of variants that would interact efficiently with CD4 and can efficiently replicate in macrophage-microglial cells in the brain.\[^{42}\] Molecular basis of neuropathogenesis

**Viral replication** (Fig. 3): Quantitative tests for neuronal function including those utilizing ELISA, lactate dehydrogenase (LDH), calcium imaging, electrophysiology, neuronal apoptosis, glutamate receptor regulation, and reverse phase-high performance liquid chromatography (RP-HPLC) revealed that viral secretory products produce profound neurotoxicity in hippocampus and cortical neurons through both apoptosis and necrotic mechanisms. Analysis of surface area to volume ratio of neurons from different regions of brain indicated marked reduction in number of neurons following injection of viral proteins (Tat and gp120)\[^{43}\].

**Long terminal repeat (LTR):** Viral genome LTR is subdivided to regions namely TAR (Tat-response element), core (composed of initiator, TATA box and 3 sp1 binding elements), enhancer [which has a binding site for nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) and nuclear factor of activated T-cells (NF-AT)] and modulatory elements (with target sequences for variety of brain specific cellular transcription factors such as lymphoid enhancer binding factor, upstream stimulatory element, nuclear factor for interleukin-6 (NF-IL6), cAMP response element binding, Ets (E-twenty-six, etc.\[^{46}\].) Regulation of HIV gene expression involves a complex interplay between chromatin-associated proviral DNA, cellular transcription factors and the viral encoded trans-activator of transcription, Tat. Sequencing of LTR from –374 to +43 regions revealed significant sequence variation in brain, compared to other tissue sources. Majority of the variation was seen in LTR regions upstream from the two NF-kappa B sites. Distinct LTR populations with specific C/EBP site II configurations were found in different neuro-anatomical regions of the brain and these regions affect the rate of viral replication\[^{45}\]. Studies showed that sequence-specific interactions between cis-acting elements in the LTR, members of the CCAAT-enhancer-binding proteins (C/EBP) family of transcription factors, and the virion-associated proteins play important roles in the pathogenesis of HIVD; 89 per cent of LTRs derived from patients exhibiting clinical dementia contained C/EBP site I configurations that displayed a high relative affinity for Vpr\[^{46}\]. It is to be noted that two CCAAT/ enhancer binding protein (C/EBP) binding sites are critically important for efficient HIV-1 replication within cells of the monocyte/macrophage lineage, a cell type likely involved in transport of the virus to the brain. These sites are interacting with various other factors (such as ATF/CREB) or activating transcription factor/cAMP response element binding protein and as a result, regulating transcription\[^{47}\]. Sp1 gene family of transcription factors binds three GC boxes adjacent to TATAAA sequence. Sp1 and Sp3 are found to be expressed in the microglial cells. Some brain derived HIV-1 isolates contain NF-kB proximal binding sites which fail to bind sp factors and result in cell specific alteration of viral regulation\[^{48}\]. Sp1 interacts with Coup-Tf (chicken ovalbumin upstream promoter-transcription factor) in microglial cells. Coup-TF interacting protein (CTIP) is capable of repressing both initial as well as late phase of HIV-1 gene transcription in microglial cells\[^{49}\]. LTR sequence varies among HIV-1 clades. Indian clade C showed presence of additional NF-kB binding site (3 sites in clade C as opposed to 2 in clade B)\[^{50}\]. This characteristic is postulated to enhance clade C proviral transcriptional activation, but the correlation of this specificity to lower prevalence

![Fig. 3. Role of viral proteins: Viral proteins can directly or indirectly result in neuroinflammation. Top left: Viral gene transcription and translation is regulated by HIV-1 long terminal repeat (LTR) in infected cells nucleus. This LTR is in turn regulated by host NF-kB and viral transactivator protein-Tat. Middle: The nuclear transport of these transcription regulators is controlled by HIV-1 regulator protein-Rev. Viral Nef protein controls the production of host transcription regulators STAT-1 and NF-kB to name a few. Level of inflammatory cytokines such as IL-2 and TGF is regulated by HIV-1 tat. HIV -1 vpr promotes apoptotic protein production. Bottom right: The combined effect of these activities is release of complete HIV-1 particles, viral proteins and neuro-modulatory factors from the infected cells which can then effect nearby cells.](image-url)
of dementia in clade C infected population is not yet accounted for\(^5\).

**Env**: Demented and non-demented patients with AIDS differ in brain-derived human immunodeficiency virus type 1 envelope sequences. Comparison of HIV-1 env sequences of blood, CSF, brain and spleen isolates collected postmortem proposed compartmentalization of viral strains and all brain derived clones analyzed showed marked homology to macrophage tropic consensus sequence within the V3 loop\(^6\). HIV-1 infects microglia and perivascular macrophages by utilizing CD4 and chemokine receptors CCR5 and CCR3, while it infects astrocytes with the help of mannose receptor. To replicate in the cells of central nervous system HIV selects envelopes with reduced CD4 dependence and increased fusion activity\(^7\). In order to gain access to the cells of the CNS, HIV needs to pass through a layer of endothelial cells in brain microvasculature (BMEC) which compose the blood brain barrier. Proteoglycans heparin and chondroitin sulphate (HSPG and CSPG) are abundantly expressed on BMECs. In addition, several other receptors have been shown to aid HIV bind to cell surface of CD4 negative cells such as galactosyl ceramide, C-type lectins, dendritic cell-specific intercellular adhesion molecule-3-grabbing non-interigin (DC-SIGN), DC-SIGNR, langerin, etc\(^8\). HIV-1 binds to these molecules in a gp120 dependent manner with basic residues in gp120 binding sulphate groups of proteoglycans\(^9\). Recently, gp120 was found to decrease adult neural progenitor cell proliferation through checkpoint kinase-mediated cell cycle withdrawal and via G1 arrest\(^10\). Differences in molecular diversity in brain-derived sequences were dependent on the individual clade and domain within the env gene. The genetic sequence of the envelope gene (env) that attaches the virus to human cells can vary by as much as 35 per cent in virus samples from different clades which can have implications for prevalence and vaccine designing strategies\(^11\).

**Tat**: Tat when presented to neurons interacts with neuronal cell membranes to cause neuronal excitation and toxicity. Further, Tat mRNA and protein was detected in tissue extracts from patients suffering from HIV/AIDS\(^12\). Functional synergy between Tat and CD40 is believed to contribute towards neuroinflammation. Significantly higher levels of soluble CD40 ligand (CD40L) were detected in both cerebrospinal fluid and plasma from HIV-infected patients with neurological impairment characteristic of dementia, compared with their non-impaired counterparts, and this could account for neuroinflammation\(^13\). Tat enhances gene expression by binding TAR-RNA and then cyc-T1 which then recruits cdk-9. This forms P-TEFB (positive-transcriptional elongation factor b) which then phosphorylates carboxy terminus of RNA polymerase II. Cytokines and chemokines induced by Tat affect normal astrocyte action and result in damage to neurons\(^14\). In microglial cells, Tat results in prolonged production of cytokines which is in turn maintained by NF-κB activation. Tat induces nitric oxide synthase via NF-κB and C/EBP pathways in glial cells. Tat is also found to form a macromolecular complex of low-density lipoprotein receptor-related protein (LRP), postsynaptic density protein-95 (PSD-95), N-methyl-D-aspartic acid (NMDA) receptors, and neuronal nitric oxide synthase (nNOS) at the neuronal plasma membrane, and this complex is believed to trigger apoptosis\(^15\). On the other hand, immunoprecipitation studies revealed that Tat binds directly to p73 (a protein that is implicated in apoptosis and cell cycle control in CNS). This interaction may inhibit stimulation of HIV-1 promoter by Tat. Also, interaction of Tat with cyclin T1 is prevented in vitro following p73 binding\(^16\). By acting at the cell membrane, Tat stimulates the production of tumour necrosis factor (TNF) and chemokine (C-C motif) receptor 2 (CCL2) from monocytes and perivascular macrophages in CNS. However, a mutation of cysteine to a serine residue at position 31 of Tat significantly inhibits the ability of this viral protein to bind CCL2. It should be noted that this mutation is prevalent in clade C, which prompts C-Tat to be relatively less neurotoxic compared with B-Tat\(^17\). Tat on secretion affects nearby cells as well. It stimulates microglial cells to release pro-inflammatory molecules and accumulate free radicals. It causes endothelial cells to express E-selectin via NF-κB dependent mechanism\(^18\).

**Vpr**: Vpr is shown recently to induce apoptosis in various regions of brain including hippocampus and cortex\(^19\). Vpr plays multiple functions during virus replication, including an effect on the accuracy of the reverse-transcription process, the nuclear import of the viral DNA as a component of the pre-integration complex (PIC), cell cycle progression, regulation of apoptosis, and transactivation of HIV-LTR as well as host cell genes\(^20\). Also, Vpr is required for Nef expression from unintegrated human immunodeficiency type 1 DNA in brain\(^21\).

**Nef**: The lentiviral protein Nef plays an important role in HIV pathogenesis. Observations following
Cytokines and chemokines in neuropathogenesis: HIV-1 infection results in altered production of cytokine and chemokines which are capable of neuroinflammatory complications. Top left: Infected monocytes promote presentation of cell adhesion molecules VCAM-1 (Vascular Cell Adhesion Molecule-1) and ICAM-1 (Intercellular Cell Adhesion Molecule-1) on the surface of endothelial cells of blood brain barrier (BBB) which promotes the entry of HIV-1 as described earlier. Another chemokine RANTES (Regulated upon Activation Normal T cell Expressed and Secreted) is capable of controlling the entry step and it also alters the secretion of other cytokines such as TNF and IFN. These cytokines increase the production of other neuro-modulatory chemokines MCP-1/CCL-2 (Monocyte Chemoattractant Protein-1). Middle: Infected perivascular macrophages secrete cytokines such as IL-1, IL-2 and chemokines such as MIP-1α and MMPs (Matrix Metalloproteinases). These molecules on secretion affect the nearby cells and promote them to produce neuroinflammatory compounds. Bottom right: Infected cells also produce Arachidonic Acid. This eicosanoid precursor is capable of affecting the astrocytes by changing the level of Glutamate.

exposing human brain microvascular endothelial cells (HBMEC) to baculovirus expressed HIV-1 Nef protein revealed that Nef is capable of inducing apoptosis of these cells in a dose dependent manner. It upregulates production of factors that regulate LTR driven transcription such as NF-AT, NF-κB, AP-1 (activating protein-1), STAT-1, etc. Role of soluble Nef in inducing CD23 and ICAM from macrophages were proposed towards mediating their effects on expanding the cellular reservoir of HIV-1. Analysis of nef gene from patients infected with different HIV-1 clades revealed marked sequence conservatism.

Role of cytokines and chemokines in HAD (Fig. 4): Chemokines are low molecular weight substances which have been found to be expressed by the resident cells of the CNS. These molecules are responsible for normal brain functions. Chemokines have been implicated in regulation of the development of AIDS related dementia. The C-C chemokines RANTES, MIP-1α and MIP-1β are shown to be the natural ligands for CCR-5 and CXCR-4 receptors. These receptors are utilized by HIV for entering the cell. Cytokines are also correlated to neuropathogenesis caused by HIV. In situ RT/PCR showed a consistent profile of increased TNF-α and decreased IFN-γ and IL4 in HIV infected patients with neurological disease. IL1 did not increase in parallel with TNF-α. Major cytokines that exert their effect on the progression of ADC (AIDS dementia complex) are IL-1, IL-6, TNF-α, TGF-β and granulocyte monocyte colony stimulating factor (GM-CSF).

Chemokine

RANTES (regulated upon activation normal T cell expressed and secreted): High expression of RANTES was observed in the CSF of patients suffering with HIV associated dementia. RANTES stands for “regulated upon activation normal T cell expressed and secreted”, based on the location and function of the gene. It is believed to play both protective as well as destructive roles in HIV related neurological abnormalities. This chemokine acts as a strong chemoattractant for monocytes/macrophages and T lymphocytes which serves in a way to amplify the inflammatory response. In fact, immunohistochemical positivity for C-C chemokine RANTES in the brains of HIV positive patients is related to the presence of inflammatory infiltrates. Studies revealed that RANTES can inhibit the neurotoxic effect of viral gp120 in an indirect manner by binding to beta-chemokine receptors CCR1, CCR-2 and CCR-5. On the other hand, RANTES may be playing important role in cell mediated transmission of HIV infection in CNS. It may favour crossing of blood tissue barriers by indirect mechanisms involving membrane interactions between nonproductively infected and permissive cells. Endothelial cells, which form the blood brain barrier, are known to produce RANTES following stimulation by TNF-α and IFN-γ. It results in increased expression of cell adhesion molecules (VLA-4, ICAM-1, β2-integrins) on their cell surface. These molecules then serve as a site for binding of unstimulated peripheral blood lymphocytes followed by their transmigration into the CNS.

MIP (macrophage inflammatory protein): MIP-1α and 1β are produced during early stages of brain infection and is mainly immunoregulatory molecules. They have specific arrangement of cysteine residues which follows the sequence C-X-C-C. Like RANTES
these chemokines also have the ability to bind proteoglycans which may have consequences in recruitment and migration of leukocytes through the BBB. Its production is initiated in macrophages and microglial cells following stimulation by various cytokines mainly IL-1 and IL-2. Further there is also a negative control circuit between TGF-β and MIP-1α. Prominent expression of MIP-1α was found within areas of the brain containing the histopathological lesions. Also, encephalitic brain from SIV-infected animals has elevated immunohistochemical expression of the C-C chemokines, MIP-1α and 1β. The signaling mechanism initiated by MIP activates phospholipase C that results in arachidonic acid synthesis. Arachidonic acid is responsible for generating cytokine responses in the astrocytes.

**MCP-1/CCL2**: Monocyte chemoattractant protein (MCP-1)/CCL2 are believed to mediate trafficking of HIV-activated leukocytes into the CNS. Measuring MCP-1 levels in CSF and plasma indicated three-fold increases in initial as well as final amount of MCP-1 in CSF. Studies using various biomarkers correlated brain injury to MCP-1. MCP-1 expression is under the control of variety of cytokines viz., TGF-β, TNF-α and IL-1β, but not IFN-γ. TGF-β along with TNF-α caused an additive increase in MCP-1 mRNA, but not protein. CCL2, but not other chemokines, is found to play key role in infiltration of HIV-infected leukocytes into the CNS and the subsequent pathology characteristic of NeuroAIDS.

**IP-10**: Interferon-inducible protein 10 (IP-10), a CXC chemokine, is found to significantly enhance HIV LTR-driven gene expression in U38 monocytic cells. This chemokine is capable of binding to cell surface proteoglycans and thus, it can inhibit endothelial cell proliferation. IP-10 has been shown to be linked to expression of caspase 3 and hence apoptosis in macro and human brain sections.

**Chemokine mediated activation**

**MMP**: Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes that function primarily in degrading components of the extracellular matrix. Elevated expression of these molecules has been observed in brain following infection by HIV-1. This observation suggests a possible role of MMPs in neuropathogenesis. Factors that regulate MMP transcription are elevated during virus replication such as the cytokines, TNF-α and alpha interferon (IFN-α) and the β-chemokines RANTES and MIP-1α. Induction of several MMPs by mediators of inflammation or viral proteins involves activation of specific transcription factors, such as AP-1 and NF-κB. Further, HIV env sequence variation is found to be responsible for differential expression of MMPs and other associated molecules in brain. MMP-1 has been shown to interact with HIV Tat protein. This interaction prevents HIV long terminal repeat transactivation. Similarly, MMP-2 and MMP-9 also have neuroprotective roles. The activation of MMPs is mediated by MAPKp38 activation and inhibition of this pathway could result in complete abrogation of induction of MMP-9 pathogenic factor which can prevent demyelination caused due to MMPs.

**Cytokines**

**IL-1**: Activities of IL-1 and TNF-α overlap with each other to a great extent. IL-1 transcription is induced by calcium ionophores and adhesion to matrix components. Viral gp120 and gp41 are shown to induce production of IL-1 as antibodies to specific epitopes of these viral proteins can block IL-1 production. IL-1 is main endogenous pyrogen which can account for the fever in early stages of dementia. It may also result in production of MMPs. Endothelial cells of cerebral capillaries express IL-1R1, receptor for IL-1 and thus may be affected. Increased concentration of cAMP and DAG (diacyl glycerol) has been reported following IL-1 exposure. IL-1 is also a potent stimulus for nitric oxide synthase expression and apoptosis. IL-1 activated cells are shown to express Fas ligands.

**TNF**: Though tumour necrosis factor (TNF) is cytotoxic on transformed cell lines, under certain conditions it affects the endothelial cells and astrocytes in nervous system. TNF can induce necrotic or apoptotic cell death. TNF is capable of opening a paracellular route for HIV into the brain by affecting the integrity of BBB. Also, TNF-α has been shown to enhance the replication of HIV by activating NF-κB. TNF related apoptosis inducing ligand (TRAIL) is one of the prime candidates for inducing such neuronal apoptosis in HIV.

**IL-6**: Interleukin-6 (IL-6) is another cytokine which shows diverse manifestations in the HIV infected brain. Histological and ultrastructural studies of brain cells revealed increased production of IL-6 in response to gp120. IL-6 can also induce large cytoplasmic vacuoles in neural cells which indicate apoptosis.
IL-6 is transcriptionally regulated by AP-1, CREB (cAMP response element binding), and NF-kB which are in turn controlled by other cytokines. IL-6 is produced by macrophages, endothelial cells and astrocytes. It promotes astrogliosis, HIV-1 replication and neural differentiation. It was found to regulate the amount of TIMP-1 (tissue inhibitor of metalloproteinase-1) and MMPs which may have role in enzymatic degradation of endothelial cell layer, thus allowing entry of polymorphonuclear leukocyte (PMN)\(^9\).

**GM-CSF:** Granulocyte macrophage colony stimulating factor or GM-CSF is predominantly produced by astrocytes and is believed to promote activation of microglia. GM-CSF and M-CSF induce MIP and RANTES expression in brain. **In vivo**, GM-CSF expression was localized to activated astrocytes and some inflammatory cells in HIV, suggesting paracrine activation of microglia through GM-CSF\(^9\). In association with other cytokines, it promotes HIV replication within macrophages. In addition to these cytokines, IFN-\(\gamma\), TGF \(\beta\) and some other molecules have significant roles to play with respect to HIV neuropathogenesis.

**Altered transmitter systems**

Pathways of synaptic signaling in the brain are called as transmitter systems. Biochemicals that modulate signaling plays vital role in maintenance of normal physiology of many compartments including CNS. For instance, arachidonic acid is a component of phospholipid membrane of cells. Calcium ions and nitric oxide are indispensible signaling molecules and glutamate is a key molecule in cellular metabolism. Dysregulation in the transmission of some of these molecules results in the complications linked with HIV associated neuropathogenesis\(^7\).

**Arachidonic acid:** Most common eicosanoid precursor in humans is polyunsaturated arachidonic acid which is stored in cell membranes as C-2 ester of phosphatidylinositol. Arachidonic acid is released by the action of phospholipase A2. CC chemokines such as RANTES, MIP-1\(\alpha\) and 1\(\beta\) have been implicated in the release of arachidonic acid. High performance liquid chromatography revealed large amount of arachidonic acid production which was shown to be correlated to cytokines\(^9\). Specific inhibitors of the arachidonic cascade markedly diminished the cytokine response suggesting regulatory relationships between these factors. Arachidonic acid can either diffuse out of the cell, reincorporated to membrane phospholipids or be metabolized to other eicosanoids that can act as second messengers. Interaction between perivascular macrophages and astrocytes is due to arachidonic acid, at least in part. It is worth noting that astrogliosis is a histological feature usually observed in HIV-associated dementia. Histological sections of brain tissues obtained at necropsy from patients suffering from HIV dementia indicated increased amount of activated astrocytes [as found by measuring the marker glial fibrillar acidic protein (GFAP), which is an indicator of astrocyte activation]\(^9\).

**Calcium ions:** Intracellular calcium ions [Ca\(^{2+}\)] are shown to have wide ranging effects. They aid in fusion of diverse kind of membranes and thus may play a role in cell-cell fusion\(^100\). Calcium may bind to calmodulin proteins. Each protein molecule binds four Ca\(^{2+}\) ions. Ca\(^{2+}\) binding induces conformational change in these molecules permitting them to bind other proteins which activate a number of signaling cascades such as cAMP, NOS (nitric oxide synthase) and protein kinase C (PKC). Rise in [Ca\(^{2+}\)], may be brief or long-term, spread in spirals or waves and may be spread to nearby cells through junctions or indirectly by spilling into the BECF. Gp120 and Tat are capable of disrupting neuronal calcium homeostasis by perturbing calcium-regulating systems in the plasma membrane and endoplasmic reticulum\(^10\).

**Glutamate:** Dicarboxylic acid, glutamate is most important excitotoxic amino acid in the nervous system. RP-HPLC and other fluorometric studies revealed increased glutamate levels in CSF and plasma of patients with HIV dementia\(^102\). It is present in milimolar levels in the neurons. Astrocytes are important mediators in the metabolism of glutamate. Neurons can manufacture glutamate from glutamine or glucose. However, glutamine pathway appears to be more prevalent. Glutamine is synthesized by astrocytes with the help of enzyme glutamine synthetase. This glutamine travels through the BECF and is taken up by the neurons. Glutaminase in the presynaptic terminals of neurons converts glutamine to glutamate. Some of this is taken back by astrocytes thus completing the glutamine-glutamate cycle. Because of HIV infection and activation of various signaling cascades as discussed, there might be a sharp drop of ATP within the cell blocking the Na\(^+\)/K\(^+\) pump. As a result, the concentration of Na\(^+\) inside and K\(^+\) outside greatly increases. This results in membrane depolarization with glutamate release from presynaptic terminals. The
ability of astrocytes to take up glutamate from BECF decreases and, in fact, unfavourable gradient can cause the transporter to run in opposite direction, thus further increasing glutamate in BECF. On the other hand, recent studies in humans with HIV infection show that activated microglia and brain macrophages express the glutamate transporter, EAAT-1 (excitatory amino acid transporter-1) and this expression varies according to the disease stage. This glutamate uptake is coupled to glutathione synthesis and glutamate uptake. It is generally believed that these accessory cells might play neuroprotective role under unfavourable conditions.

Nitric oxide and N-methyl D-aspartate receptor (NMDAR): These are glutamate receptors which are closely associated with neuronal nitric oxide synthase. Presynaptic glutamate release stimulates calcium entry through NMDA glutamate receptor channels or voltage gated Ca$^{2+}$ channels. Entry of calcium results in synthesis of nitric oxide by activation of nitric oxide synthase that is in turn activated by calmodulin. It is synthesized from L-arginine in the presence of tetrahydrobiopterin and NADPH. It can diffuse freely from presynaptic neurons to postsynaptic ones and vice versa. NO synthesis requires increase in Ca$^{2+}$, however, its release does not, as it is not packaged into vesicles. Also excessive Ca$^{2+}$ stimulates protein kinase A (PKA) pathway that generates reactive oxygen species (ROS) in addition to NO. NO may react with ROS to form toxic free radicals. Cellular lipid metabolism also changes. Viral proteins like Tat potentiates excitatory amino acid (glutamate and NMDA) triggered calcium flux.

**Apoptosis (Fig. 5 a & b)**

HIV-1 infection in the brain induces neuronal apoptosis leading to dementia in some cases. In some countries the prevalence of HIV dementia is so high that it ranks second only to Alzheimer’s disease among diseases with cognitive dysfunction. Researchers have identified differences in various virus subtypes and difference in patients’ immune responses as the reason for differential potential of virus to cause disease of CNS. Infiltration of HIV-1 infected cells into the brain perivascular region results in neuronal apoptosis. These neurons were found frequently to be in close proximity to HIV-1 infected macrophages that express TRAIL. Most of the evidence cited in the literature do not support that HIV infects neurons directly. The appellation of the principle molecules involved is still a matter to argue. Multiple theories of neural injury have been proposed which relate the involvement of viral proteins to apoptosis. Viral proteins such as Vpr, Tat, Nef and Env are regarded to be...
of significance in this regard. Tat causes caspase activation through PI3k pathway. It also increases the concentration of IL-8, tyrosine kinase and nitric oxide synthase. HIV coat protein gp120 can interact with several members of chemokines receptor family and direct neuronal injury, could be because of chemokine receptor signaling. Also, very low concentrations of gp120 have been shown to bind glycine binding site of NMDAR. Vpr could be directly neurotoxic because of its ability to form cation-permeable channel. Nef has been shown to be essential in viral replication and disease pathogenesis. Nef allows HIV-1 to avoid immune surveillance via active and passive mechanisms. Also, N-terminal myristoylated Nef has been shown to have cytopathic effects probably via interaction with calmodulin. Neurons treated with cell free media from infected macrophages in culture induced cell death whether virions were present or depleted by ultracentrifugation. Apoptotic neurons do not co-localize with infected microglia indicating that neurodegeneration is perhaps due to release of soluble factors. Neuronal apoptosis, after excitotoxic insult, involves NMDAR and results in Ca\(^{2+}\) overload, activation of p38 MAP kinase and p53, cytochrome C release from mitochondria in addition to other molecules such as apoptosis inducing factor (AIF), activation of caspases, free radical formation, lipid peroxidation, etc. As stated earlier, scaffolding protein PSD-95 (post synaptic density-95) is found to link the principle subunit of NMDAR to nNOS and thus brings the latter into close proximity to calcium. Excessive [Ca\(^{2+}\)] stimulates nNOS and protein kinase cascades with subsequent generation of deleterious levels of free radicals, including ROS and NO. NO can react with ROS to form cytotoxic peroxo compounds. In addition to these effects, NO is shown to have many extracellular effects as well. For instance, It was shown to activate MMP-9 after S-nitrosylation and oxidation which may disrupt the attachment of the cells to neighbouring matrix and other cells. TNF-α and IL-1β stimulates release of L-cysteine which can stimulate NMDARs and result in neuronal apoptosis. TNF-α has the ability to activate caspase 8. Thus, it could possibly be involved in cell death. It has been suggested recently that upregulation of some cell surface factors on some peripherally activated monocytes leads to neurotoxicity. One such factor is TRAIL or Apo 2 ligand. It is closely related to Fas ligand. It can interact with at least five different kinds of receptors present on a variety of cell types. Some of these receptors have death domains and they can induce cellular apoptosis following ligand binding. TRAIL signaling can act by mitochondria or by death receptors. Mitochondrial pathway is initiated by stress signals that damage mitochondria. Some of the key cellular factors that play critical roles through transcriptional activation, post translational modifications, proteolytic cleavage are proposed to be Bcl-2, Bcl-XL, Bax, Bak, Akt and PUMA. In the death receptor pathway, perhaps TRAIL-R undergoes oligomerization and recruits FADD (Fas associated death domain). This complex has the capacity to recruit procaspase-8 which on activation mediates action of downstream caspases and hence, apoptosis.

**Summary**

Not surprisingly, HIV has evolved strategies that exploit specific host machineries towards causing pathogenesis that is cell type-dependent. As laid down in this exposition, these strategies are themselves limited by the nature of the virus-host interplay and their concerted efforts in the process of such manifestation that modulates neuropathogenesis. With the improvement in the knowledge about the pathogenesis and molecular mechanisms involved in the HIV propagation and specific colonization, many new molecules that take part in the development of HAD, came into light. New experimental strategies can be employed to find way to block some of these molecules. But as stated, the molecules seem to behave in a cascade manner with one molecule inhibiting and being inhibited by another and vice versa. The elucidation of the indispensable molecules would be much helpful in developing potential therapeutic strategies. NMDAR blockers, Ca\(^{2+}\) channel blockers, MAPK blockers, G protein coupled receptor blockers could be of particular significance. Also, chemokines can serve as potential therapeutic agents for HAD as they can compete with the virus for the binding site. Blocking the transcription factors can also have effect on viral propagation. But, these transcription factors can affect a variety of cells, so, attempt can be made to block the viral proteins which have been shown to have effect on transcription such as Tat, Vpr and others. It seems that brain responds in more or less similar fashion to a variety of external insults and hence exploring for more insights of crucial pathways causing HIVE would definitely help in understanding mechanisms that could potentially be an attractive target for effective intervention.

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