HIV Neuropathogenesis:
Persistent Infection, Persistent Questions

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The central nervous system is a critical reservoir of HIV-1. While the introduction of HAART has reduced the mortality associated with neuropathologic manifestations of HIV-1 infection, milder neurologic disorders persist whose cumulative effects are unclear. HIV-1 neuroinvasion appears to involve brain microvascular endothelium, with the virus then being transmitted to macrophages and microglia. There, infection by select strains of macrophage-tropic and T-cell-tropic HIV-1 appears to induce the production of viral and cellular products that cause neuronal apoptosis. The CNS also might provide refuge from antiretroviral drugs, which may allow more neurovirulent strains of HIV-1 to evolve as well as the reseeding of the systemic circulation. Innovative strategies aimed at restoring and protecting neuronal function are under investigation.

SCI & MED 10(2):112-123, 2005.

Publication date: 3 Nov 2005

HIV-1 invades the central nervous system (CNS) of humans during the early stages of infection and persists until death. In the advanced stages of HIV-1 infection, complications associated with CNS infection are often manifested as a dementing process known as the AIDS-dementia complex (ADC) or HIV-1-associated dementia.

ADC is an AIDS-defining illness, according to the definition of AIDS by the Centers for Disease Control & Prevention. Approximately 20 to 30% of AIDS patients eventually manifest ADC, and almost half of these patients develop ADC within 2 years of AIDS diagnosis, with dementia being the AIDS-defining illness in 3% of them.

Although cognitive dysfunctions are prominent in ADC, vascular myelopathy predominates as HIV-1-associated myelopathy. ADC and HIV-1-associated myelopathy are the severe manifestation of neurologic disease and are AIDS-defining illnesses. A milder version, known as HIV-1-associated minor cognitive/motor disorder (formerly HIV-1-associated neurocognitive disorder or HIV-1-associated neurobehavioral abnormalities), is not an AIDS-defining illness.

An array of factors influence the development of ADC, including the vigor of the host immune defense to suppress viral replication in the CNS and qualitative differences in the infecting viral strains.

The widespread introduction of highly active antiretroviral therapy (HAART) in the developed world has ensured prolonged survival of patients despite induction of ADC. Prior to HAART, ADC was associated with high AIDS mortality, and survival rates were particularly poor when ADC developed during advanced stages of AIDS. The median survival has increased from 5 months in 1993-95, to 38 months in 1996-2000, following an ADC diagnosis in patients with equivalent CD^{4+} T-cell counts of 100×10^6 cells/L.

In the short term, HAART seems to reverse neurocognitive deficits and ADC in nonelderly adults with HIV-1 infection. Yet, while HAART has reduced the occurrence of ADC, milder neurologic disorders caused by HIV-1 persist during limited HIV-1 replication in the CNS.

The lasting effects of HIV-1 on brain function as patients age are not known. Aging increases the risks of neurodegenerative disease and dementia independently, and combined with HIV-1 infection, such risks could increase synergistically. Despite HAART, involvement of the brain is frequently found in autopsies of AIDS patients.

Whereas HAART enhances the recovery of immune function and protection against AIDS-related opportunistic infections, ADC still remains a critical issue in the developed world, and ADC is likely to remain associated with high AIDS mortality in poorer regions lacking steady access to HAART.

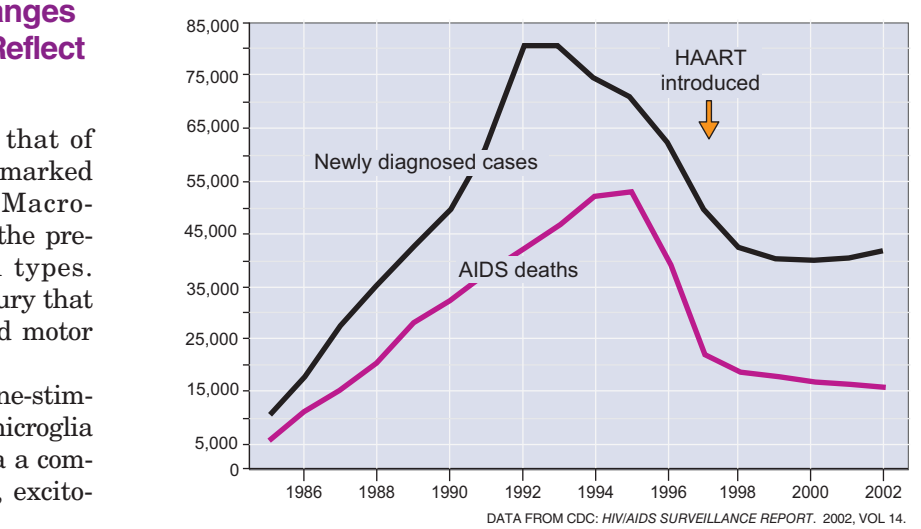
Neuropathologic Changes Do Not Consistently Reflect Dysfunction

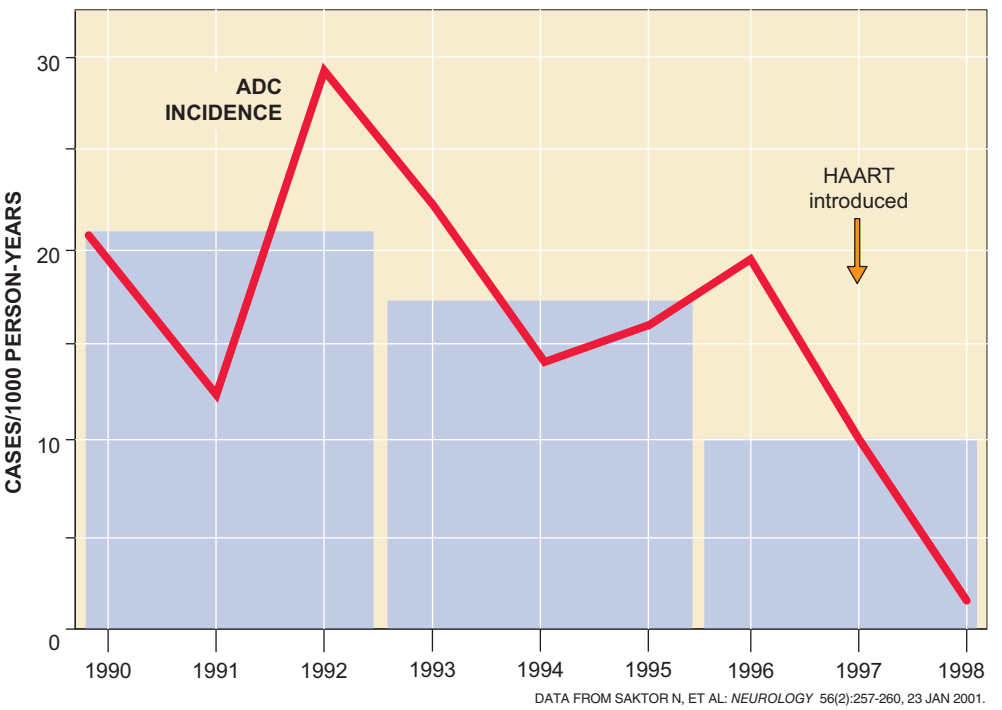
The pathology of ADC is that of chronic encephalitis with marked macrophage activation. Macrophages and microglia are the predominantly infected cell types. However, it is neuronal injury that underlies the cognitive and motor dysfunction seen in ADC.

HIV-1-infected or immune-stimulated macrophages and microglia inflict neuronal damage via a complex network of cytokines, excitotoxins, viral proteins, and free radicals. Overstimulation of neuronal NMDA receptors and increased intraneuronal Ca^{2+} play a critical role in ultimately causing neuronal apoptosis.

Neuropathologic features seen with ADC include widespread reactive astrocytosis, diffuse myelin pallor, macrophage infiltration, multinucleated giant cells, and microglial nodules. Microglial nodules are scattered throughout the gray and white matter of the brain, appearing frequently in white matter and subcortical gray matter of the thalamus, basal ganglia, and brainstem. These nodules are inflammatory and contain macrophages, lymphocytes, and microglia.

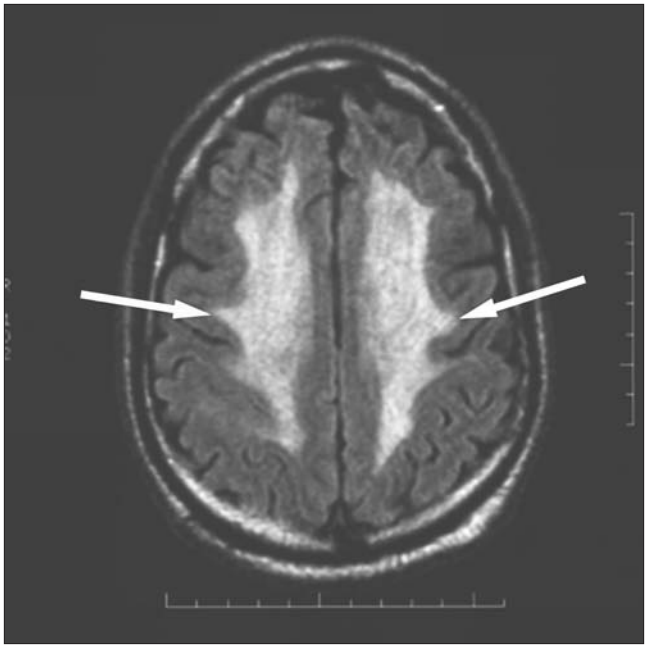
The presence of microglial nodules is not limited to HIV-1 infection, and numbers of nodules do not seem to correlate with the severity of dementia. Interestingly, a direct correlation seems to exist between the frequency of multinucleated giant cells and the degree of dementia as well as the detection of HIV-1 DNA. Diffuse myelin pallor corresponds to perturbations in the blood-brain barrier and results from edema due to leakage of serum proteins, and not myelin loss.





Prevalence of AIDS and the numbers of deaths increased until the early 1990s. With the widespread introduction of HAART beginning in 1997, both deaths and the incidence of HIV-1-associated neurologic disease have declined. Despite the decreased incidence of HIV-1 dementia, milder neurologic disorders persist during limited HIV-1 replication in the CNS. The horizontal bars correspond to the mean incidence rates of ADC during those years.

Neuronal damage is manifested by neuronal loss, dendritic pruning, and simplification of synaptic contacts. Apoptosis of neurons, astrocytes, and endothelial cells contributes to neuronal damage as well; however, the correlation with the severity of neurologic disease is not particularly strong.



Axial T2-weighted MRI scan of the brain of a 38-year-old man with advanced HIV-1 disease and encephalitis. Signs of viral encephalitis and cortical atrophy can be seen, along with marked and diffuse signaling (myelin pallor) surrounding the lateral ventricles (*arrows*). White matter changes extend into the centrum semiovale, pons, cerebellum, and thalamus. Cortical atrophy is manifested by generalized prominence of the cortical sulci and by enlarged ventricles.

Neuropathologic changes seen at the cellular level are substantially validated by analysis of structural damage to the brain, particularly by imaging studies. Imaging studies are critical in the evaluation of suspected ADC to exclude opportunistic processes.

Cerebral and cortical atrophy as well as white matter abnormalities are often observed in radiologic examinations of patients with ADC. Calcifications of the basal ganglia are frequently seen on CT scans in children.

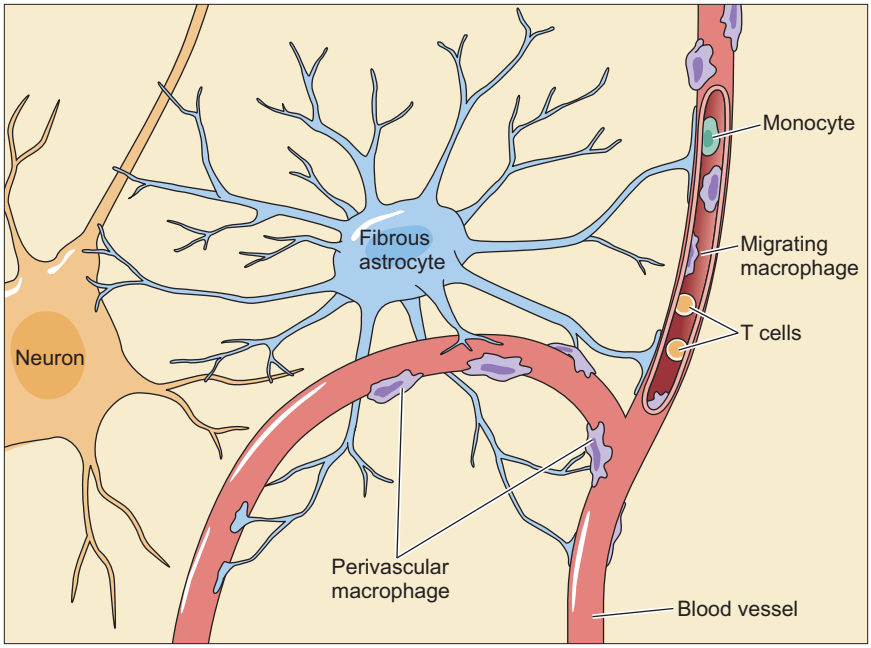
At times, diffuse cerebral atrophy parallels clinical deterioration, but some studies have shown no relationship between the degree of atrophy and cognitive impairment. On the other hand, cerebral atrophy has a predominantly frontal-temporal distribution. Atrophy, particularly of the basal ganglia, is seen on MRI scans, often with a diffuse or fluffy, multifocal increase in T2 and proton density signal in the white matter and basal ganglia.

Whereas these imaging techniques reveal structural damage to the brain, magnetic resonance spectroscopy confirms the observations from histologic examinations. Brain choline levels precede the onset of ADC, probably reflecting an increase in glial cell number or neuronal membrane damage. As well, *N*-acetyl aspartate levels decline in severe ADC, confirming the neuronal loss found in histologic examinations.

Unlike evaluations in systemic disease, assessing the impact of viral infection on neurologic and cognitive damage is complex. The severity of systemic illness correlates with viral load in the blood. However, the quantity of viral RNA or DNA in the CNS does not bear a direct relationship to the degree of neurologic or cognitive involvement. Furthermore, neuropathologic observations do not correlate strongly with the degree of cognitive dysfunction.

The most critical factor that determines neurologic disease seems to be severe deficiency in cell-mediated immunity. ADC predominates during the late stages of AIDS and correlates with extremely low CD^{4+} T-cell counts.

In approximately 80% of autopsies of AIDS patients, typical neuropathologic changes are present. These changes are not present in HIV-1-positive subjects without AIDS. No substantial differences in the prevalence of such changes are observed in patients who are in the early or late stages of AIDS,



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Only some cells of the CNS make direct contact with BMVECs. Perivascular macrophages and astrocytes are in contact with BMVECs, whereas neurons do not make a direct contact with BMVECs. It is believed that HIV-1 initially enters BMVECs and is then transmitted to perivascular macrophages and microglia in the CNS.These cells are the site of productive HIV-1 infection in CNS. Productive infection of astrocytes and neurons remains contentious.

nor in those who do or do not have dementia.

Selective susceptibility and loss of specific neuron populations at certain sites might be associated with cognitive dysfunction. An increase in caudate atrophy on MRI scans correlates with worsening of some aspects of neuropsychologic function, especially motor domains.

HIV-1 Enters the CNS Via the Brain Microvascular Endothelium

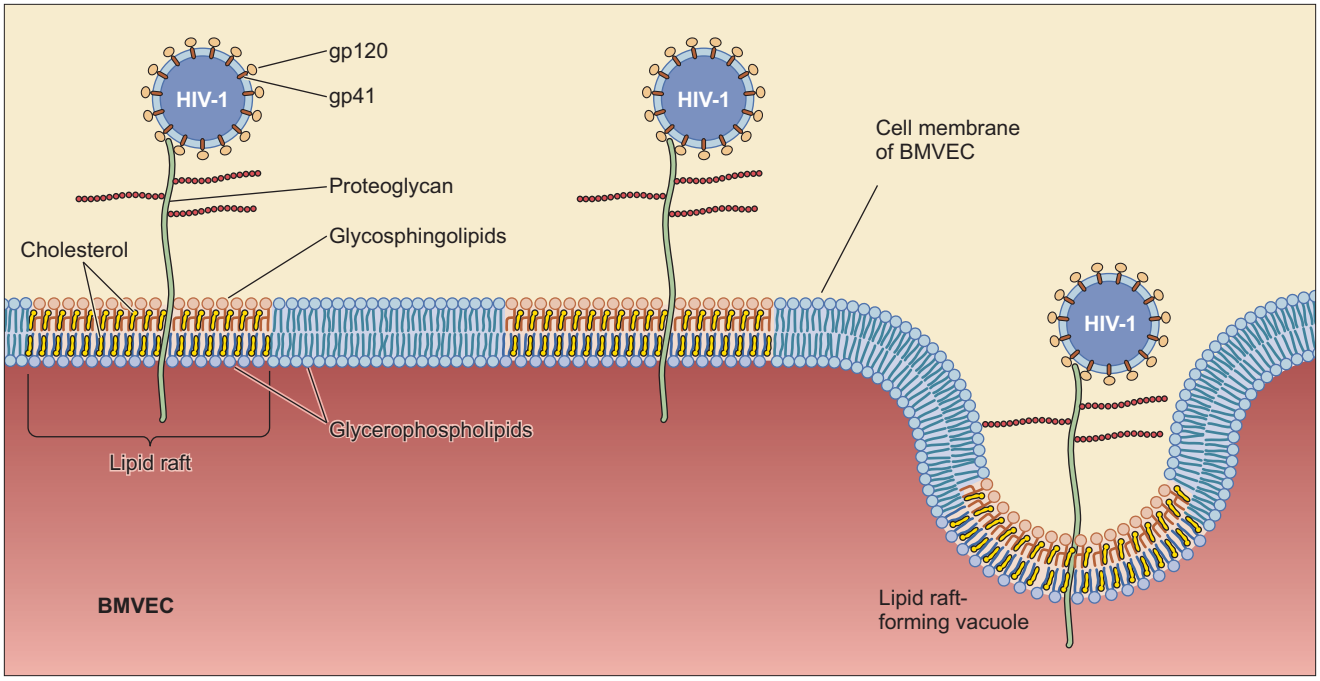
HIV-1 neuroinvasion has been investigated extensively, yet the mechanisms remain ambiguous. HIV-1 enters the CNS early after the primary infection, but disruption of the blood-brain barrier (BBB) does not seem to be an early event. HIV-1-infected CD^{4+} T cells and cell-free virions are the two most likely sources of neuroinvasion, as they accumulate to high levels prior to the development of the antiretroviral immune response.

The BBB presents a strong barrier to pathogens invading the CNS. It is widely perceived that HIV-1 crosses the BBB via transcytosis through the barrier, by infecting the BBB, or by passing through breaches in the barrier.

Infection of brain microvascular endothelial cells (BMVECs) in vitro remains a critical but contentious issue. In recent years, two major hypotheses have been proposed concerning BMVECs. A study by Milan Fiala's group, at the University of California Los Angeles, concluded that the virus enters BMVECs via lipid rafts, whereas a study led by Roger Pomerantz, at Thomas Jefferson University in Philadelphia, claims no involvement of lipid rafts.

Both studies suggest involvement of proteoglycans. The Pomerantz group used enzymes that degrade specific proteoglycans, and it demonstrated the specific involvement of cell-associated chondroitin sulfate proteoglycans for viral entry. On the other hand, Fiala's group used extensive transmission electron microscopy and confocal microscopy to show that HIV-1 enters BMVECs by vesicular uptake through surface invaginations.

Strong support for the fate of virus inside the BMVECs comes from Fiala's study, since they used DNase I-treated viruses in their analysis of polymerase chain reaction products. Their observations suggest that BMVECs do not support productive HIV-1 replication



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Cell-surface proteoglycans play a critical role in HIV-1 neuroinvasion. HIV-1 interacts with the cell-surface proteoglycans and is one of the initial interactions between BMVECs and viral particles. Such interactions lead to viral entry into BMVECs via micropinocytosis. Although proteoglycans play a critical role, the role of lipid rafts (regions of cell membrane enriched in cholesterol and glycerophospholipids) in this process is disputed.

but do support restricted reverse transcription. Reverse transcription was restricted to R/U5 transcripts; no U3/R and *pol* transcripts were detected in BMVECs.

Reverse transcription is a critical step towards productive infection of HIV-1. Restricted reverse transcription indicates viral entry, but not productive infection, of BMVECs.

Hence, although the involvement of lipid rafts remains disputed, involvement of proteoglycans is clearly critical in viral entry into BMVECs. However, the restricted nature of viral transcription, in conjunction with rapid lysis of endocytosed virus, fails to explain the consequence of these interactions for neuroinvasion.

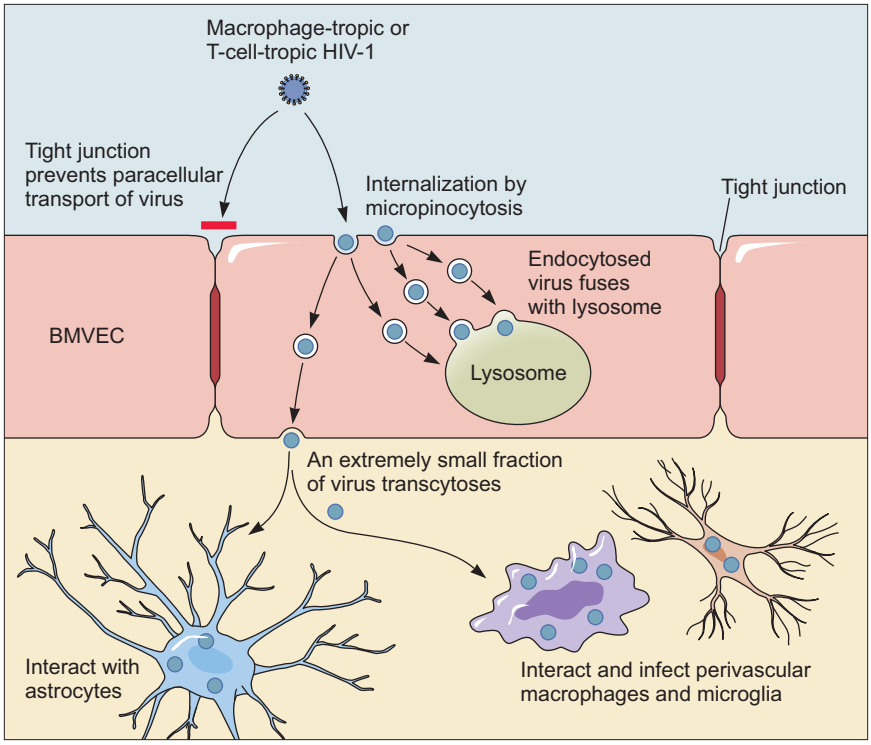
BMVECs appear to play a passive yet critical role in neuroinvasion, but infection of these cells by HIV-1 might be inconsequential. This might be possible if the physiology of BMVECs is altered on contacting HIV-1, such that they gain the ability to home macrophages and microglia on either side of the blood-brain barrier and thus allow viral entry into the CNS.

Alternately, HIV-1-infected monocytes and perivascular macrophages might make random contacts with BMVECs and create an environment conducive for neuroinvasion. Several studies from different laboratories support such a scenario.

Almost a decade ago, Stephen Spector's group, at the University of California San Diego, reported that contact between macrophages and BMVECs (as well as umbilical vein endothelial cells) upregulated HIV-1 production of macrophage-tropic strains. More importantly, such contact also upregulated replication of T-cell-tropic strains of HIV-1 that would not ordinarily replicate in macrophages.

The critical requirement for this enhanced HIV-1 replication is contact with BMVECs. Cocultures of macrophages with glioblastoma, astrocytoma, cortical neuron, fibroblast, and placental cells failed to increase HIV-1 replication. This study highlighted the role of leukocyte function-associated antigen-1 (LFA-1), a macrophage-endothelial cell adhesion molecule.

A more recent study from Andrew Henderson's laboratories at Pennsylvania State University has corroborated the critical nature of macrophage-endothelial cell contact and highlighted the



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HIV-1 enters brain microvasculature endothelial cells (BMVECs) via micropinocytosis. Both macrophage-tropic and T-cell-tropic HIV-1 enter BMVECs by this process. Most of the endocytosed virus fuses with the lysosome; however, an extremely small fraction of virus is able to transcytose across BMVECs, and then it might interact with astrocytes, perivascular macrophages, and microglia. It might also infect macrophages/microglia. Paracellular transport of virus via tight junctions does not occur when the integrity of the blood-brain barrier is intact.

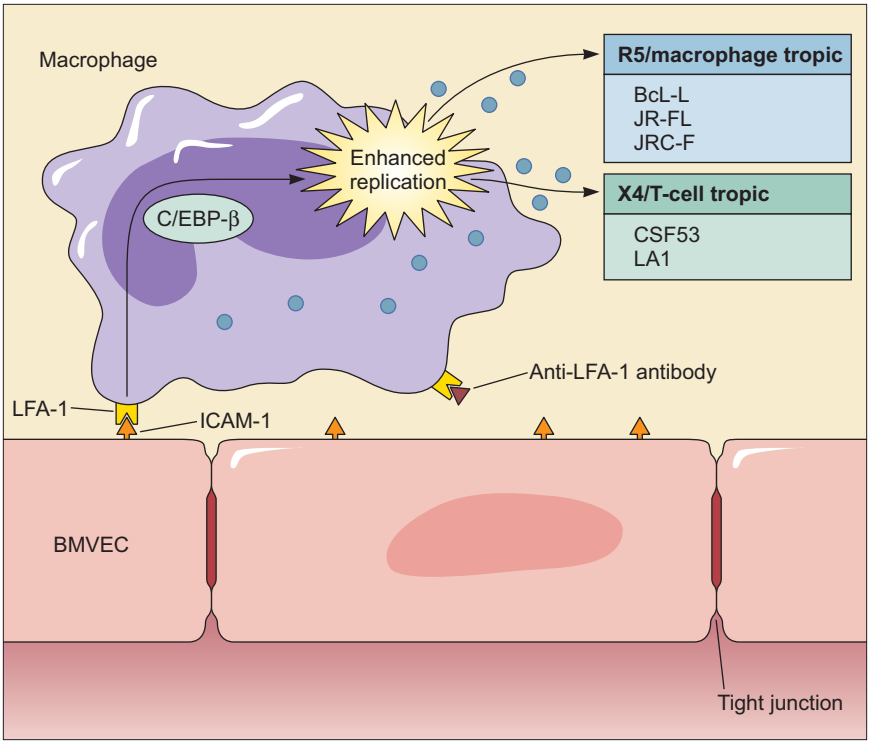
involvement of the transcription factor C/EBP-β (CCAAT enhancer binding protein-β, also known as NF-IL-6) in enhancing HIV-1 replication in macrophages. Although this study highlights the importance of macrophage-endothelial cell–cell contacts, it is notable that human umbilical vein endothelial cells, and not BMVEC, were used in it.

Hagen von Briesen's group, at Frankfurt am Main, Germany, used an alternate approach to study interactions between macrophages and BMVECs. They used non-contact cocultures and concluded that HIV-1-infected macrophages do not interfere with the permeability of the BMVECs, suggesting that an immediate consequence of a proinflammatory response generated due to HIV-1-infected macrophages does not include disruption of the blood-brain barrier.

Several aspects of HIV-1 neuroinvasion in the context of the BMVEC–macrophage interaction remain unresolved. Whether interaction of HIV-1 viral particles with BMVECs plays a role in enhancing its potential to home macrophages and whether proinflammatory conditions generated by HIV-1-infected macrophages in direct contact with BMVECs directly affect the blood-brain barrier integrity remain unclear.

The involvement of proteoglycans in HIV-1 neuroinvasion has immense implications, and how they might affect the outcome is being actively investigated. Several studies from Philippe Gallay's group, at Scripps Research Institute in La Jolla, support the notion that cell-surface proteoglycans serve as major HIV-1 receptors on BMVECs. Although proteoglycans enhance the binding of HIV-1 viral particles and infected macrophages to BMVECs, this does not translate as transmigration across the blood-brain barrier and entry into the CNS.

Despite substantial and specific information implicating proteoglycans as substitute receptors that interact with HIV-1 gp120, this role cannot be unequivocally resolved. These studies do not take into account that proteoglycans can be incorporated in the viral particles, and that *env*-deficient



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Interaction of BMVECs and macrophages enhances HIV-1 production. The adhesion molecule leukocyte function-associated antigen-1 (LFA-1), on the surface of macrophages, interacts with intracellular adhesion molecule-1 (ICAM-1) on BMVECs. This interaction leads to enhanced production of both macrophage-tropic and T-cell-tropic HIV-1 via a C/EBP-β-dependent mechanism. Blocking the interaction of LFA-1 and ICAM-1 with anti-LFA-1 antibody prevents enhanced viral production.

viruses might fail to incorporate them. Hence, the possibility of cell-surface and viral-associated proteoglycans interacting without gp120 involvement exists.

Post-Infection Mutations in HIV-1 Appear to Enhance Neuropathogenesis

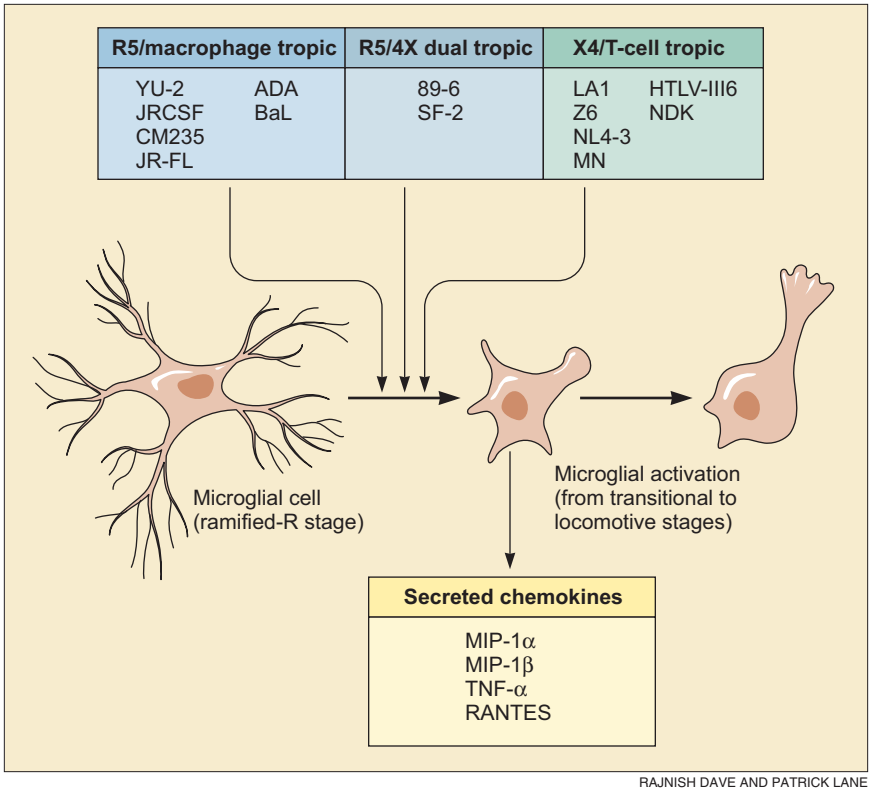
HIV-1 viral tropism does not seem to determine neuroinvasion. Subsequent evolution of the virus enables it to replicate in the CNS and thereby contribute to HIV-1 neuropathogenesis.

The determinants of neurotropism are not clearly defined. They most likely involve the variable regions of *env*, elements of the long terminal repeat (LTR), as well as the ability of these viruses to activate macrophages/microglia, induce apoptosis, and hinder the normal functions of astrocytes, neurons, oligodendrocytes, and BMVECs. That all neurotropic strains are not neurovirulent adds another layer of complexity.

The relevance of viral tropism in the initial stages of neuroinvasion also is not well defined. Most likely, the cellular microenvironments create conditions that are conducive for viral replication.

For example, in addition to the augmented replication of macrophage-tropic HIV-1 strains, viruses that traditionally would be considered syncytium-inducing T-cell-tropic HIV-1 strains, incapable of replicating productively in macrophages, are induced to replicate efficiently in macrophages when they interact with BMVECs. This suggests that most, if not all, strains of HIV-1 may play an important role in HIV-1 neuroinvasion.

However, with disease progression, viral strains capable of more efficient replication in macrophages/microglia are predicted to have the greatest impact in neurodegeneration, as macrophages and microglia are the only cell types that are productively infected by HIV-1 in CNS. These cells have the ability to express all the viral proteins at appropriate stages of the replicative cycle, and this ultimately leads to production of infectious viral particles. More importantly, macrophages/microglia act as a viral reservoir throughout the course of infection.



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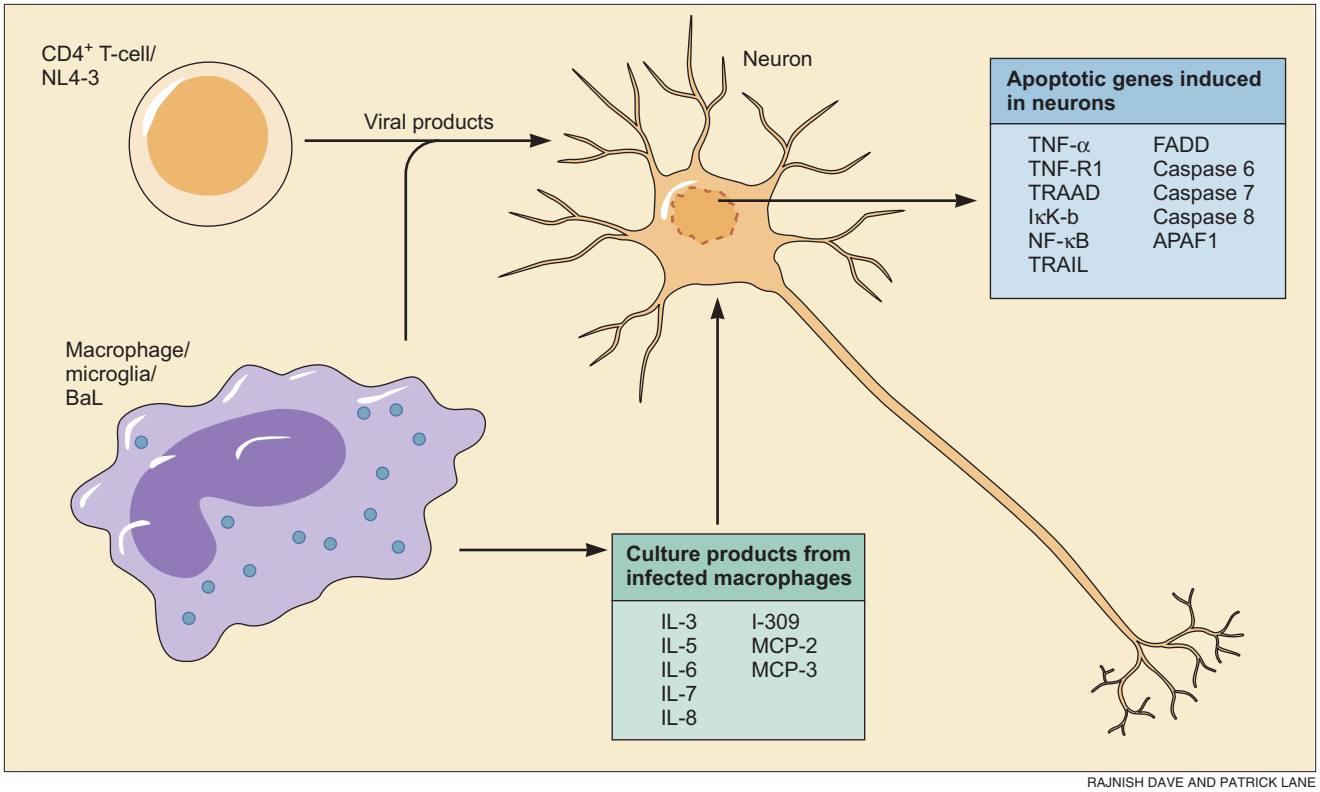
HIV-1 can activate macrophages and microglia independent of their coreceptor phenotype. Select strains of HIV-1, both macrophage-tropic and T-cell-tropic, are able to induce rapid and extensive β-chemokine synthesis via Ca^{2+}-mediated signal transduction.

How relevant is viral tropism in inducing the inflammatory condition and subsequent neuronal dysfunction? The receptor usage phenotype of HIV-1 and the ability to replicate in macrophages are not linked to the potential of these strains to induce an inflammatory response.

Mary Jane Potash's group at Columbia University in New York has demonstrated in a series of studies that macrophages are induced to secrete chemokines (MIP-1α, MIP-1β, RANTES, and TNF-α) by select T-cell-tropic and macrophage-tropic isolates of HIV-1 and their envelope glycoprotein. The T-cell tropic viruses seem to contribute to both astrocytic and neuronal dysfunction by HIV-1 gp120–CXCR4 receptor interactions. Astrocytes might at best support limited HIV-1 infection. However, the binding of HIV-1 to CXCR4 receptor on the surface of astrocytes affects proper functioning of these cells, which are critical for protecting neuronal activity and survival.

In a recent study, David Volsky's group used rapid subtraction hybridization and gene microarray analysis with Affymetrix U133A/B gene chips and determined that HIV-1 alters globally and significantly the overall program of gene expression in astrocytes, including changes in transcripts coding for cytokines, G-coupled protein receptors, transcription factors, and others. As well, interaction of HIV-1 gp120 or HIV-1 impaired the ability of the cells to transport L-glutamate, and this was related to transcriptional inhibition of the EAAT2 glutamate transporter gene.

The influence of viral tropism on neuronal function is more complex, as was recently shown in an interesting manner in a study from Roger Pomerantz's group. Both viral and host cell products, released from HIV-1-infected cells, have been implicated as inducers of neuronal cell apoptosis. The authors emphasize the involvement of viral proteins in inducing neuronal apoptosis. Interestingly, T-cell-tropic viruses induced neuronal apoptosis, while products secreted from cells infected with these viruses did not. However, in the case of cells infected with macrophage-



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Viral products induce neuronal apoptosis. Neuronal apoptosis is induced by an array of cell products secreted from HIV-1-infected macrophages. HIV-1 and gp120 from both macrophages and CD4+ T cells can also induce neuronal apoptosis.

tropic viruses, neuronal apoptosis was induced by viral products as well as by cellular products secreted by the infected cells.

The irrelevance of viral tropism in neuroinvasion and to lesser extent in inducing an inflammatory response in macrophages and disruption of astrocytic and neuronal functions does not undermine the fact that certain strains of HIV-1 are responsible for neurovirulence and the neurodegenerative manifestations of AIDS.

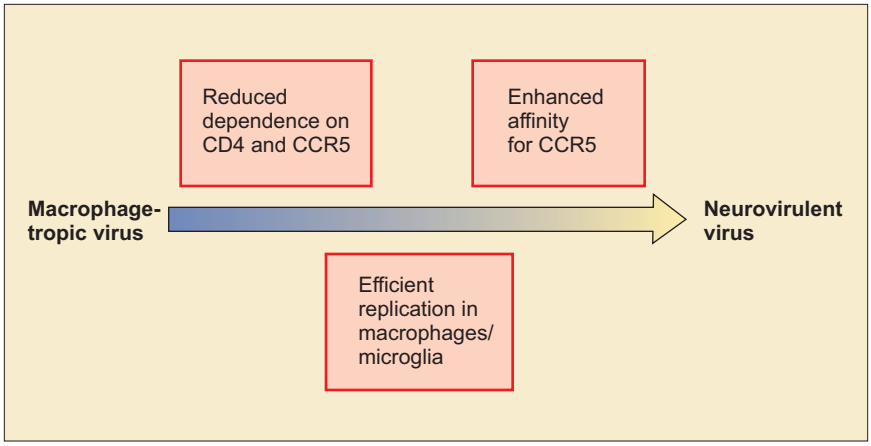
The initial infection of macrophages/microglia plays a critical role throughout the course of HIV-1 infection. These cells act as a viral reservoir in the CNS and a source of drug-resistance mutations due to poor penetration and suboptimal concentrations of antiretroviral drugs into the CNS.

Only a small number of antiretroviral agents can efficiently penetrate the blood-brain barrier and attain optimal concentrations in the CNS. Unique anatomic structures limit the distribution of these drugs in the CNS, including the blood-brain barrier located between the blood and brain tissue and the blood-cerebrospinal fluid barrier, primarily formed by the choroid plexus.

Limiting HIV-1 replication to macrophages and microglia and the suboptimal levels of antiretroviral drugs create a unique environment for HIV-1 to evolve in the CNS. Resultant viruses might evolve into neurovirulent strains and thereby exert considerable influence on the outcome of neurologic disease. More importantly, such viruses might have the potential to reseed in the systemic circulation on failure of therapy.

Substantial effort has been invested in identifying subtle changes in HIV-1 *env* that contribute to neurotropism and the propensity to grow in macrophages. CNS-derived HIV-1 isolates (with a few exceptions) use CCR5 as a coreceptor and have a non-syncytium-inducing phenotype.

This was corroborated by the findings of Nitin Saksena's group at the University of Sydney, who isolated HIV-1 from the right and left parietal, occipital, and frontal



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Factors contributing to neurovirulence. The transition from a macrophage-tropic virus to a neurovirulent virus involves the ability to efficiently replicate in macrophages/microglia. It is also predicted to involve a reduced dependence for CD4 and CCR5 receptors. Enhanced affinity for CCR5 (without independence from CD4) is also involved in this transition.

lobes of the brain cortex of three HIV-1-infected patients, two with ADC and one without. HIV-1 strains from diverse areas of the CNS showed a higher propensity to grow in macrophages of patients with ADC, as opposed to patients without ADC, suggesting that the emergence of primary cell type-adapted isolates during ADC may play a crucial role in the development and progression of ADC.

In another study, from Dana Gabuzda's group at Harvard Medical School in Boston, a different stance was taken. Their results suggested that macrophage-tropic isolates with increased affinity for CCR5 and a reduced dependence on CD4 and CCR5 (but not independence from CD4) may represent a pathogenic viral phenotype contributing to the neurodegenerative manifestations of AIDS.

Adaptation to grow in microglial cells might allow viral strains to proliferate and replicate terminally and perhaps contribute to neurodegeneration. A study from M. Christine Zink's group, at Johns Hopkins in Baltimore, used a macaque simian immunodeficiency virus (SIV) model in which more than 90% of pig-tailed macaques co-inoculated with SIV/17E-Fr and SIV/DeltaB670 developed encephalitis.

Two macrophage-tropic, neurovirulent viruses, SIV/17E-Fr and SIV/DeltaB670 CI-2, predominated in the brain RNA of macaques with encephalitis, comprising 95% of the genotypes detected. The same two viral genotypes were present at the same frequencies in microglial cell RNA, suggesting that microglia are pivotal in the selective replication of neurovirulent viruses.

Restoration and Protection of Neuronal Function May Be Possible

Our understanding of the neurodegenerative manifestations of HIV-1 infection, despite being far from complete, is substantial enough to devise methods to monitor neurodegeneration and to develop therapeutic alternatives that would supplement HAART in order to restore neuronal functions.

The accumulation of host and viral proteins within the neurons, microglia, and astrocytes or in their extracellular milieu is a common feature of HIV-1 neuronal degeneration. These molecules often serve as markers of underlying molecular pathology. For this reason, the genes encoding these proteins and the mechanisms by which they contribute to neurodegenerative changes are an active area of investigation.

Optimal functioning of synapses is the key to normal functioning of neurons. Synaptic dysfunction may occur due to loss of critical proteins and protein turnover via the ubiquitin-proteasome apparatus.

Benjamin Gelman's group, at the University of Texas in Galve-

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ston, observed an increase in ubiquitinated protein aggregates and diffuse extracellular plaques containing the β-amyloid (Aβ) fragment of the amyloid precursor protein in the hippocampal region of the brains of older AIDS patients. The loss of synaptic proteins in AIDS, and its correlation with increased acute phase inflammatory protein, suggests that chronic inflammation, in the setting of long-term survival and persistent HIV-1 infection, could exacerbate senile dementia by disturbing synaptic protein turnover through the proteasome.

In another study, the same group studied the gene expression profile of the human brain cortex in persons with ADC and noted that several ionic channel genes were overexpressed. This increase in gene expression was not correlated with a generalized increase of inflammatory cell transcripts, and such changes occurred in people with dementia with and without HIV-1 encephalitis. These shifts in gene expression were attributed to neurons, as they were not detected in gyral white matter.

These changes in gene expression are predicted to disrupt neuronal excitability. Ion-channel dysfunction as a result of HIV-1 infection in the brain might have severe consequences leading to substantial brain dysfunction, including long-term potentiation and synaptic strength, which are highly relevant to clinical dementia. If ion-channel function could be restored upon suppression of HIV-1 replication in macrophages and microglia, it might have a significant impact on restoring at least some neuronal functions.

Although the spectrum of HIV-1-induced neurodegeneration ranges from microglial activation to disruption of astrocytic and neuronal functions, macrophages and microglia are still the primary sites of HIV-1 replication in the CNS. It is crucial to determine the impact on CNS regeneration after successful inhibition of viral replication in these cells.

Recent advances in small interfering RNA (siRNA)-based technologies and gene transfer into the CNS are allowing design of experimental approaches to contain the virus. In our laboratory, we have devised siRNA-based approaches to target neurotropic HIV-1 isolates in the CNS.

In macrophages, HIV-1-specific siRNAs were able to potently inhibit HIV-1 infection for sustained durations. Sustained inhibition of macrophage-tropic strains of HIV-1 in macrophages also has been reported by Premlata Shankar's group at Harvard. Whether such approaches will lead to CNS regeneration is yet to be ascertained.

Recent advances in the use of mouse models have allowed researchers to study HIV-1 neuropathogenesis and develop strategies for neuroprotection. In a recent study, David Volsky and Mary Jane Potash used a chimeric HIV-1 to infect conventional mice. The chimeric HIV-1 acquired the ability to infect mice when the coding region for gp120 was replaced with that of gp80 from ecotropic murine leukemia virus.

The uniqueness of this model is in its simplicity. Three weeks after tail vein inoculation, virus was detected in the brain tissues. This approach seems to overcome the limited infectivity in rat or mice models transgenic for human CD4 and either CCR5 or CXCR4. More importantly, this model system will provide an excellent means to study HIV-1 neuroinvasion.

In an alternate approach, severe combined immunodeficiency (SCID) mice reconstituted with human peripheral blood lymphocytes have been widely used. Viral encephalitis is induced in these models by intracranial injection with autologous HIV-1-infected, monocyte-derived macrophages.

Such models have been used for several studies at the University of Nebraska. Larisa Poluektova used

such a model to demonstrate brain tissue restoration following elimination of HIV-1-infected human monocyte-derived macrophages. This model system also was used by Yuri Persidsky to manipulate immunosuppressive activity of indoleamine-2,3-dioxygenase. Such manipulations are predicted to enhance the elimination of HIV-1 infected macrophages in brain.

With increasing understanding of how HIV-1 induces cell death in the CNS, several neuroprotective strategies have been investigated to ameliorate excitotoxic damages, free-radical toxicity, oxidative stress, and changes associated with increased intracellular calcium levels.

Numerous compounds with the potential to exert neuroprotection and to reverse neuronal damage have been tested. These include nimodipine (a Ca-channel antagonist), memantine (NMDA antagonist), OPC-14117 and α-lipoic acid (antioxidants that counteract excessive glutamate receptor activation), lexipafant (platelet activation factor receptor antagonist), and sodium valproate (inhibitor of glycogen synthase kinase-3α). Caspase inhibitors, p38 MAPK antagonists, α-chemokine receptor antagonists, and similar compounds are being studied because of their involvement with the neurodegenerative processes discussed above.

There seems to be a shift in focus in studies of HIV-1 neuropathogenesis, with an increasing focus on neuroprotection and restoration of neuronal function. Innovative use of mouse models is expected to provide greater insights into both HIV-1 neuropathogenesis and neuroprotection.

While neuropathologic manifestations of HIV-1 are localized, the deficits in neuronal functions are global. Overcoming this challenging aspect of HIV-1 neuropathogenesis most likely will be the key to successful development of therapeutic interventions to reduce or eliminate the neurologic manifestations of HIV-1.

Acknowledgments

A large group of researchers are involved in understanding HIV-1 neuropathogenesis, and we acknowledge their contributions. We apologize those whose work could not be cited or discussed due to space limitations. The authors wish to thank Rita M. Victor and Brenda O. Gordon for secretarial assistance.

This work was supported in part by USPHS grants MH074359 and AI052732 to Dr. Pomerantz.

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