Journal of NeuroVirology, 8(suppl. 1): 79–86, 2002
 ISSN 1355–0284/02 $12.00+.00
DOI: 10.1080/13550280290050073

Viral neuropathogenesis

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Synthesis and assembly of JCV virions: progenies are efficiently generated in discrete regions of the nucleus in the presence of major and minor capsid proteins

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JC virus (JCV), a round or filamentous structure, is identified in the nucleus of infected oligodendrocytes in the brains of progressive multifocal leukoencephalopathy (PML). The virions likely consist of major capsid protein VP1 and minor capsid proteins VP2 and VP3 in an appropriate ratio. We have previously reported that VP1, VP2, and VP3 are synthesized from polycistronic mRNAs downstream of the agnoprotein, and VP1 is transported from the cytoplasm to the nucleus cooperatively with VP2/VP3 (Shishido-Hara *et al*, J Virol 2000). However, functions of the agnoprotein, VP2, and VP3 are not well investigated due to lack of antibodies, and how the virus generates progeny virions in the nucleus is still largely unknown. In the present study, to further investigate synthesis and assembly of virions, we prepared antibodies for the agnoprotein, VP2, and VP3. The viral proteins were expressed in COS-7 cells, and their distributions were investigated by confocal microscopy. Assembly of virus-like structures were analyzed by immunoelectron microscopy using anti-VP1 antibody. When only VP1 was expressed, VP1 was diffusely distributed both in the cytoplasm and the nucleus. No virus-like structure was detected in more than 20 VP1-positive cells. In contrast, when the three capsid proteins were expressed together, VP1 was predominantly localized to the nucleus, and accumulated to the discrete regions with VP2 and VP3. Discrete accumulation of the capsid proteins was more distinct in the presence of the agnoprotein. Assembly of virus-like structures was always seen in discrete regions where the gold particles were clusterized, either in the presence or absence of the agnoprotein; while the cells that formed virus-like structures tended to degrade more severely in the presence of the agnoprotein. These results suggest that the major and minor capsid proteins accumulate to discrete regions in the nucleus for efficient production of virions. The agnoprotein is not essential for assembly of virions, but may potentially influence the viral virulence as a human pathogen.

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Productive infection of cerebellar neurons by JC virus, the agent of progressive multifocal leukoencephalopathy

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Introduction: JC virus (JCV) is the etiologic agent of Progressive Multifocal Leukoencephalopathy (PML), a disease which occurs in immunosuppressed individuals. The hallmark of PML is a lytic infection of oligodendrocytes leading to lesions restricted to the white matter (WM). In a few PML cases, areas of focal cell loss have been observed in the internal granular cell layer (IGCL) of the cerebellum. However, infection of the cerebellar neurons by JCV has never been demonstrated.

Case report and results: We report here, for the first time, a productive JCV infection of neurons. We describe the case of an HIV-1-infected patient who died from PML. Postmortem examination showed typical lesions of PML in the WM of both frontal lobes. In the cerebellum, there was a focal destruction of the IGCL, with a relative preservation of adjacent Purkinje cells and intermixed myelinated axons. Immunostaining for JCV VP1 protein was positive in the oligodendrocytes of the WM of both frontal lobes, as expected, but also in the enlarged granule cells in the areas of focal cell loss in the cerebellar IGCL. These enlarged JCV-infected cells were positively stained by the specific neuronal marker Neu N. Staining for polyomavirus VP1 protein was negative in other regions of the brain, including cerebellar oligodendrocytes and Purkinje cells. To confirm the presence of JCV DNA in enlarged granule cells neurons, clusters of these cells were selected by laser capture micro-dissection (LCM). PCR amplification of a 181 bp fragment of JCV VP1 gene was positive in JCV-infected oligodendrocytes of the WM of both frontal lobes and in the enlarged neurons of the cerebellar IGCL, but not in other control regions of the brain. JCV regulatory region was sequenced from fresh frozen cerebellum tissue showing a tandem repeat pattern, which was not different from the sequence found in the WM of the frontal lobes.

Conclusion: This is the first convincing demonstration of a productive infection of neuronal cells by JCV. These findings are of particular interest since recent reports incriminate

JCV as a possible etiology of cerebellar tumors, in particular medulloblastoma.

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Successful rapid autopsy isolation of human microglia from a patient with HIV-1 encephalitis: identification of immune dysfunction in brain cells during disease

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Mononuclear phagocyte (MP) infection and immune activation underlies the neurodegeneration of HIV-1 associated dementia (HAD). The dynamics of this process are poorly understood. To address this issue, we established a rapid autopsy program for isolation, purification and study of primary neural cells from brain tissue. An autopsy was performed within 4 hours of death on a patient, a 44 y/o male with AIDS and a CD4+ T cell count of 25 cells/mm^3 and a viral load of 270,000 copies/ml. Pre-mortem neuroimaging showed brain atrophy and neuropsychological testing showed mild cognitive impairment with an AIDS Dementia Complex (ADC) score of 1. Brain tissue was harvested for the isolation of microglia from diverse brain regions including basal ganglia, white matter, cortex, cerebellum and anterior frontal cortex. Plasma, serum, and cerebrospinal fluid were also obtained. Highly pure preparations of microglial cells were obtained as demonstrated by CD68 antigen expression. Multinucleated giant cells were observed in cultures starting at 8–10 days after cell cultivation. Within two weeks basal ganglia, cortex and white matter microglia demonstrated significant cytopathic effects. Viral replication was confirmed by RT activity. Peripheral nerve and CD40 ligand were used to study microglial responses to immune stimuli. Tumor necrosis factor alpha (TNF-alpha) served as an indicator of immune activation. Microglial cells produced high levels of TNFalpha after activation and responded readily to injury stimuli. Cellular extracts of microglial cells were analyzed on ProteinChip, to obtain bio-marker profiles. This is the first report of a rapid autopsy on a patient with HIV-1 encephalitis and these studies on primary, *ex vivo* neural cells provide novel tools to study the role of microglial activation in HIV-1-associated dementia.

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Use of a novel multipotential human progenitor cell culture system to define molecular factors involved in JC Virus and HIV-1 susceptibility in neural cells

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The factors regulating neural cell susceptibility to both JC virus (JCV) and HIV-1 were studied using multipotential human central nervous system progenitor cells. These undifferentiated cells, isolated from human fetal brain tissue under selective growth conditions, were grown as attached cell layers or differentiated into highly purified astrocyte or neuron populations. Exposure to JC virions and receptor-independent transfection of infectious JC DNA both revealed that GFAP positive astrocytes were most susceptible to infection; progenitor cells were moderately susceptible, and neurons were nonpermissive for JCV. Susceptibility to JC infection correlated with higher levels of the transcription factor NF1-X in astrocytes and progenitors as compared to neurons. Transfection of an NF1-X expression vector into the neuronal cells initiated JC viral protein production. Similarly, transfection studies using the infectious HIV-1 clone pNL4-3 showed that progenitor-derived astrocytes were highly susceptible to HIV-1. Astrocyte infection was productive for 4–5 days but diminished to very low levels by 8 days post-transfection (as measured by p24 ELISA). Transfection of pNL4-3 into progenitor and progenitor-derived neuronal cells produced very low levels of infection. However, the pro-inflammatory cytokine TNF-alpha stimulated HIV-1 protein production in progenitors and neurons, and reactivated HIV-1 protein production in astrocytes 8 days post-transfection, suggesting that the inflammatory response may interact with intracellular factors regulating HIV-1 latency. Collectively, these results indicate that control of susceptibility to JCV and regulation of HIV-1 infection in neural cells occurs at the molecular level. This novel cell culture system of multipotential human progenitor cells will be useful for identifying factors that regulate viral susceptibility in different neural lineage pathways.

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Functional interaction between cyclin T/cdk9 and puralpha determines the level of TNFalpha promoter activation by Tat in glial cells

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In addition to its stimulatory effect on transcription of the HIV-1 LTR, the early protein of HIV-1, Tat, exhibits detrimental effects on the CNS by de-regulating expression of several cytokines and immunomodulators including TNFalpha. Activation of the viral promoter by Tat requires several cellular proteins including cyclin T1 and its partner, cdk9, which upon association with TAR sequence of the LTR, forms a complex that enhances the activity of RNA polymerase II. Here, we examined the involvement of cyclin T1/cdk9 in Tat-mediated transcriptional activation of the TNFalpha promoter which has no TAR sequence. Results from transfection of human astrocytic cells revealed that both cyclin T1 and cdk9 stimulate the basal promoter activity of TNFalpha, although, the level of such activation is decreased in the presence of Tat. Ectopic expression of Puralpha, a brain-derived regulatory protein which binds to Tat, enhanced the basal level of TNFalpha transcription, yet exerted a negative effect on the level of Tat activation of the TNFalpha promoter. The antagonistic effect of Puralpha and Tat upon the TNFalpha promoter was diminished in the presence of cyclin T and cdk9, suggesting a cooperativity between Puralpha with cyclin T and cdk9 in Tat activation of the TNFalpha promoter. Results from protein-protein studies showed the interaction of Puralpha with both cyclin T1 and cdk9 through distinct

domains of Puralpha which are in juxtaposition with each other. Interestingly, the site for cyclin T1 binding whithin Puralpha is adjacent to the region which is important for Tat:Puralpha association. In light of these observations, we propose a model which ascribes a bridging role for Puralpha in assembling Tat, cyclin T, and cdk9 around the promoter region of TAR-negative genes such as TNFalpha which is responsive to Tat activation.

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Evidence for dysregulation of cell cycle by human polyomavirus, JCV, late auxiliary protein

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The leader sequence of the human neurotropic JC virus, JCV, late genome encompasses a short open reading frame of 71 amino acid, which is produced during infection and accumulates in the late stages of lytic infection of glial cells. This Agnoprotein, whose function yet to be described, localizes in the cytoplasmic perinuclear region with a minor fraction found in the nucleus. In this study we demonstrate that the expression of Agnoprotein in the absence of other viral proteins slows progression of cells throughout the cell cycle. Cells which transiently or constitutively express Agnoprotein exhibit a delayed exit from G1 and an unusual accumulation in G2/M stage. Examination of various cyclins and cdks revealed a decrease in the activity of cyclin A and B in cells expressing Agnoprotein. Results from Western blot showed that while the level of p27 remained unchanged, a noticeable increase in the level of p21 is observed in cells expressing Agnoprotein. Evaluation of p53, the upstream activator of p21, showed no difference in the level of this protein in the control and the Agnoprotein producing cells. Interestingly, results from co-immunoprecipitation and GST pull-down experiments indicated the ability of Agnoprotein to associate with p53, suggesting that the interaction of Agnoprotein and p53 may alter the ability of p53 to orchestrate the various steps of the cell cycle. These observations suggest a functional role for Agno in de-regulating the host cell cycle by altering activity and/or expression of cyclins and tumor suppressors which are important for G1 and G2/M phases of the cell cycle pathway.

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Tubulin binding and nuclear shuttling of JC virus agnoprotein

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JC virus, the causative agent of progressive multifocal leukoencephalopath y (PML), encodes for small protein, designated as agnoprotein. To clarify the function of this protein, the intracellular localization of JCV agnoprotein in the JCV producing human neuroblastoma cell (IMR-32) was investigated using a specific antibody to agnoprotein. In addition, recombinant GFP-fused agnoprotein was transfected into COS-7, and its distribution was analyzed by laser confocal microscopy. Agnoprotein was observed mainly in the cytoplasm and the peri-nuclear region of JCV infected cells, and double immunostaining revealed that the agnoprotein was co-localized with the cytoskeletal protein tubulin. In addition, GFP-agnoprotein was similarly distributed in the peri-nuclear region and cytoplasm. Immunoprecipitation analysis showed that agnoprotein was co-precipitated with tubulin, and gel filtration chromatography confirmed that the molecular weight of the JCV agnoprotein complex is approximately 120 kDa, suggesting that agnoprotein (8 kDa) combines with the heterodimer of tubulin (55 kDa). Thus, JCV agnoprotein was mainly localized in the cytoplasm of the JCV infected cells as a complex with tubulin. Next, we made agnoprotein mutants fused with GFP and compared the intracellular distribution with wild-type (wt) GFP-agnoprotein. The GFP-agnoprotein mutant lacking the C-terminus was expressed in the nucleus, suggesting that agnoprotein has the nuclear exporting signal (NES) at the C-terminus region. We also found that agnoprotein has the functional nuclear localization signal (NLS). Thus, it is hypothesized that agnoprotein is a 'shuttle' protein which can be translocated into the nuclei in a situation which changes the function of its NLS and/or NES.

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Interplay between cdk9 and NF-kappa B factors determines the level of HIV-1 gene transcription in astrocytic cells

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Basal transcription of the HIV-1 genome is controlled by a variety of ubiquitous and inducible regulatory factors, some with the ability to associate with the viral DNA sequences within the promoter spanning the long terminal repeat (LTR). In this report we demonstrate that activation of the HIV-1 promoter through the inducible NF-kappa B factors can be affected by cdk9 in human astrocytic cells. Results from transfection experiments revealed that while similar to the p50/p65 subunits of NF-kappa B, cdk9 augments the basal activity of LTR, induction of the NF-kappa B pathway tempers the ability of both cdk9 and p50/p65 to stimulate viral gene transcription in the transfected cells. Results from DNA-binding and protein-protein interaction studies led us to conclude that the observed antagonistic activity between cdk9 and p50/p65 is mediated through the interaction of cdk9 with both p50 and p65 proteins, which results in the dissociation of p50 and p65 from their target DNA sequence within the LTR. Expression of the viral transactivator, Tat, alleviates the negative interplay between cdk9 and NF-kappa B factors, and causes high level transcription of the LTR in PMA-treated cells or cells with ectopic expression of cdk9 and p50/p65. Results from binding studies indicate that the interaction of cdk9 with p65, but not p50, is disturbed by Tat, suggesting that dissociation of p65 from cdk9 permits its interaction with the kappa B motif of HIV-1. Altogether, these observations provide evidence for an alternative pathway involving cdk9 in Tat activation of the HIV-1 LTR via the kappa B motif in human astrocytes.

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Gp120 induces PECAM-1 phosphorylation and permeability increase in human brain microvascular endothelial cells

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Encephalopathy represents a serious manifestation of HIV-1 infection, especially in children, but its pathogenesis is unclear. Possibly, HIV-1 proteins, e.g. gp120 play a role in the activation of the endothelium of the blood brain barrier BBB thereby increasing influx of HIV into the brain. Previously, we showed that gp120 activates human brain microvascular endothelial cells (HBMEC) derived from children, e.g induces increased cell adhesion molecule expression and monocyte transmigration via CD4. To further address the underlying mechanisms of gp120 induced HBMEC activation, we determined the effect of gp120 on the phosphorylation of PECAM-1, a junctional protein in relation to endothelial permeability and monocyte transmigration. We found that children's HBMEC were responsive to gp120 in upregulating PECAM-1 phosphorylation. Gp120 induced PECAM-1 phosphorylation was inhibited by anti-gp120 and anti-CD4 antibodies and inhibitors of both protein kinase C and tyrosine kinases. Since PECAM-1 is involved in maintaining the junctional integrity of the endothelium we tested whether gp20 would affect permeability as well. Permeability of HBMEC monolayers was studied using our in vitro model of the blood brain barrier. Gp120, added to the upper compartment induced an increase in 3H-Inulin permeability, which was inhibited by antibodies against CD4 and gp120.

This demonstrates that gp120 affect HBMEC PECAM-1 phosphorylation and permeability via CD4, and tyrosine kinases are involved in the signaling mechanisms. These pathways of HIV-1 protein gp120 activation of brain endothelium may contribute to the development of HIV-1 encephalopathy in children.

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Identification, cloning, and preliminary evaluation of human astrocyte genes displaying altered expression after HIV-1 infection

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The neurodegeneration and dementia caused by HIV-1 infection of the brain are common complications of AIDS. Among viral targets in the brain, astrocytes are acquiring prominence for their effects upon neuronal function and viability. HIV-1 induced astrocyte dysfunctions considered in this context are disruption of glutamate transport and aberrant secretion of inflammatory cytokines, both of which can lead to excitotoxicity. The present study was designed to identify global alterations in astrocyte gene expression induced by HIV-1 as a first step to determine the complex of changes in astrocyte gene regulation and cellular functions impinging upon neurons. Using rapid subtraction hybridization, we cloned and identified messenger RNAs in primary human fetal astrocytes either up-regulated or down-regulated by native HIV-1 infection or exposure to envelope glycoprotein, gp120. RNA was isolated at multiple time points after virus or gp120 treatment permitting distinction of transient changes in gene expression, cloned and sequences compared to the annotated human genome database. 15 known or novel astrocyte elevated genes (AEG) were identified that display early or late expression kinetics following HIV-1 or gp120 exposure. Aberrant expression of selected AEG mRNA was correlated with their protein expression. The comparable pattern of regulation of AEG by infection or gp120 suggest that astrocytes respond to HIV-1 by changes in the expression of at least 15 genes even in the absence of productive infection. In analogous studies, 10 genes was identified so far that are suppressed by HIV-1 or gp120 exposure, these genes were termed astrocyte suppressed genes (ASG). Taken together our findings define a set of about 35 genes regulated in astrocytes by HIV-1, forming the basis of definition of new pathways through which HIV-1 may contribute to neuropathogenesis.

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Neuronal alterations in hypothalamus induced by persistent infection with morbillivirus: implication in the onset of a delayed mouse obesity

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Neurotropic viruses could be involved in numerous pathological processes of the central nervous system, triggering transient or irreversible disorders, such as neuroendocrine diseases linked to viral replication or viral persistence in selected brain nuclei. The exact mechanisms are still unknown but the first events of cell-virus interactions may explain the late neurological pathologies. In a previously described model, we reported that infection of mice with a neurovirulent strain of canine distemper virus (CDV), a virus closely related to human measles virus, results in acute encephalitis followed in a substantial number of surviving animals by an obesity syndrome characterized by a plasmatic hyperleptinemia and hypothalamic receptor downregulation. This phenotype presumably results from the hypothalamus infection, a brain area strongly involved in food intake and energy expenditure. Indeed, CDV early targets and replicates in restricted hypothalamic nuclei (PVN, AN, VMH, DMH and LA) inducing local inflammatory process (pro-inflammatory cytokines, infiltrating T cells) and impaired balance of the metalloproteinases/endogeneous inhibitors (TIMPS). Such change could modify expression of extra cellular matrix components (deposits and neosynthesis) suggesting cell-cell communication perturbation. Actually, hypothalamic functioning was also modified and numerous early changes in neuronal genes expression were pointed out in both direct responsive neurons (NPY, POMC, CART) and downstream neurons (MCH) to leptin action (seen using ICC, real time PCR and mouse DNA array). Changes in the STAT/JAK pathway and its main physiological inhibitor SOC (suppressor of cytokine signaling) are currently under investigation. We

speculate that injured hypothalamic neurons, as a consequence of modified molecular machinery, may contribute to events such as excitotoxicity and homeostasis imbalance and in fine to the delayed neuroendocrine disorders.

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Differential effect of HIV Tat protein on astrocytes and neurons

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HIV infection frequently causes a dementing illness. While microglia/macrophages are productively infected, astrocytes have a restricted infection. The role of astrocytes in pathogenesis of HIV dementia is poorly understood. Multiply spliced Nef and Tat transcripts are often seen in astrocytes. Nef is localized in the cells while Tat is secreted and enters other cells. In this study, we addressed the effects of Tat on astrocyte functions and its subsequent effects on neurons. We established stably transfected rat glioma (C6) with Tat or Tat-GFP driven by CMV promoter and human astrocyte cell line SVGA with Tat-86 driven by tetracycline inducible promoter (tet). Tat expression was monitored by RT-PCR, LTR-CAT and immunostaining. Cultures of human fetal neurons were exposed to Tat expressing cells by co-culture or by transwells. The toxicity was evaluated by JC-1 assay for mitochondrial dysfunction and trypan blue exclusion for cell death. Gene expression profile of Tat expressing astrocytes was analyzed using superarray apoptosis kit. Tat was localized to the nucleus, nucleolus and small amounts were present in the cytoplasm in astrocytes. However, upon release Tat trasnsactivated HIV-LTR. Cultures of SVGA tet inducible Tat cells or C6 Tat cells with primary neurons in transwells as well as cultures of neurons with supernatants from these cells decreased mitochondrial membrane potential to $81\% + 2.5\%$ ($p < 0.001$) in 4 days and $65\% + 2.4\%$($p < 0.001$) in 7 days in neurons. Co-culture of SVGA tet inducible Tat cells and C6 Tat / C6 Tat-GFP cells with primary neurons induced neuronal cell death of $7\% + 2\%$ ($p < 0.02$) in 4 days and $12\% + 2\%$ ($p < 0.02$) in 7 days. No toxicity was seen in astrocytes. Tat expressing astrocytes revealed upregulation of DAXX and TNF alpha mRNA. It is concluded that intracellular Tat from astrocytes play a dual role by protecting astrocytes while causing neurotoxicity.

+ = Plus Minus

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Microglia infected with SIV/HIV recombinant viruses induce neuronal cell death

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Neurological complications and cognitive impairments often occur in both adults and pediatric AIDS patients, and generally lead to HIV-associated dementia (HAD). Evidence suggests that neuronal cell death and dysfunction are the underlying causes of these neurological complications and the clinical syndrome of HAD. However, HIV does not directly infect neurons and the causes of neuronal cell death have not been elucidated. In addition to brain macrophages, microglia constitute the cells primary infected by HIV in the brain and play a major role in viral persistence in the central nervous system (CNS). Chimeric simian-human immunodeficiency viruses (SHIV) containing HIV envelope genes are very useful for studying HIV-infection and subsequent disease sequelae in non-human primates. Our objective was to study the effect of SHIV-infected microglia on primary cultures of baboon cerebral cortical neurons (CCN). Neurons were either co-cultured for 24 hours with primary microglial cells infected with SHIV-KU or SHIV-89.6P, or were incubated (for 24 hours) with conditioned media from SHIV-infected microglia. Cytopathic effect was assessed by microscopy and cytotoxicity measured by the release of lactate dehydrogenase (LDH) in culture medium. Our results showed that infection of baboon microglia with SHIV-KU or SHIV-89.6P induced neuronal cell death. Following co-culture of CCN with microglia infected with SHIV-KU, 48% of the neuronal population died. Similarly, co-culture of neurons with microglia infected with SHIV-89.6P killed 45% of neurons, compared to the 22% cytotoxicity observed when neurons were co-cultured with non-infected microglia. Direct exposure of conditioned media from infected microglia induced even higher toxicity. Conditioned media from microglia infected with SHIV-KU and SHIV-89.6P killed respectively 78 and 71% of neuronal cells. These results suggest that once HIV crosses the blood brain barrier and invades the CNS, infection of microglia lead to neuronal cell death and is an important step in the pathogenesis of HAD.

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Ultrastructural observation of cultured adult mouse sensory neurons infected by rabies virus

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Adult mouse DRG sensory neurons provide an interesting model for the study of rabies virus (RV) infection. Such importance is centred on these cells being one of the routes used by the virus to get to the central nervous system. The object of this work was to describe sensory neuron ultra-structure in RV infected cultures, using a pre-embedding electron immunomicroscopy technique. Sensory neurons are highly susceptible to infection, but in none of those post-infection times studied were virions found to be assembled in the cytoplasm or associated with plasmatic membrane. On some occasions similar fibril matrices were found to those ribonucleoprotein accumulations described in neurons from infected brain. A large quantity of immunoreactive vesicles of about 500 nm diameter were found when using a polyclonal antibody for the detection of viral protein. No evidence was presented of completely assembled virions in neurons positive for the virus, but we proved that, when inoculated by either intracerebral or intramuscular route, the infected cell homogenate or its supernatants produced infection and death in mice, indicating their infective capacity. The transport of viral material or non-assembled virus through the vesicles has been described for other viruses; our results show immunoreactivity for RV in vesicles in sensory neurons, without complete virus being observed in them. This could then be a much more efficient mechanism for reaching the central nervous system.

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Evidence for regulation of LTR transcription by Wnt signaling factors in human astrocytic cells

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The Wnt signaling pathway plays an important role in neural cell development and function. The key components of this pathway, beta-catenin and its partner, TCF-4/LEF-1 exert their effects on transcription by entering the nuclei where they associate with the TCF-4/LEF-1 DNA motif positioned in the promoter of several important genes. Here we examined the role of Wnt factors upon transcription of the HIV-1 promoter in human astrocytic cells. Our results showed that while TCF-4 slightly decreased the basal activity of the LTR promoter, co-production of TCF-4 and beta-catenin enhanced the overall basal transcription of the LTR. In the presence of Tat, the potent viral transactivator, either TCF-4 or beta-catenin had a negative effect upon LTR transcription. However, co-production of TCF-4 and beta-catenin alleviated the negative effect of either protein upon Tat transactivation, suggesting that intracellular levels of beta-catenin and TCF-1 may be important for the optimum level of viral gene activation by Tat. Results from promoter deletion studies revealed that the minimal promoter sequence of the LTR with no binding site for TCF-4/LEF-1 remained responsive to regulation by Wnt factors. Results from GST pull-down and combined immunoprecipitation and Western blot assays revealed a strong interaction of Tat with TCF-4. Subcellular examination of TCF-4 and Tat in cells expressing either protein alone showed exclusive nuclear accumulation of these proteins. However, in cells which co-expressed TCF-4 and Tat, these proteins were found in the cytoplasm as well. Altogether, these observations provide evidence for the cooperative interaction of the Wnt signaling factor with Tat that may determine the level of viral gene transcription in human astrocytic cells.

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Identification of site-specific HIV integration in CNS specimens

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Introduction: The pathogenesis of HIV-associated dementia (HAD) remains unknown in spite of improved therapy for HIV infection. While a critical step towards developing HAD could be an increase in monocyte trafficking into the brain, other factors may accelerate HAD during late-stage infection, including HIV integration into specific cellular sites, with adverse consequences. Analysis for viral integration was undertaken to determine if site-specific integration may be present in HAD.

Methods: DNAs from 3 brain (deep white matter, DWM) and 1 bone marrow (BM) specimens were obtained from autopsy cases (JHU collaboration). 7 CSF specimens were obtained from patients entered on a longitudinal study (UH NASNRP). Brain specimens were selected based on the abundance/distribution of macrophages in paraffin-embedded sections (anti-CD68 immunohistochemistry). Sections showing focal accumulations of macrophages, potentially indicative of macrophage proliferation, were identified. DNA from serial sections of these specimens, and from CSF cells was digested with PstI, and amplified using inverse PCR with nested primers: gag1: CTTTAAATGCATGGGTAAAAGTAGT(AG)G and LTR1: TTGTTGGCTTCTTCTAACTTCTCTGG; and gag2: TGATACCCATGTTTTCAGCATTATCAG and LTR2: TGGTACTA(AG)CTTG(AT)AGCACCATCCA. Products were cloned, sequenced, and analyzed.

Results: Sequences from BM and 1 DWM showed HIV integrated within the integrin beta 4 subunit gene (ITGB4). The DWM had integration within intron 12 (Genbank #Y11107) while integration in the BM was within intron 26. HIV integration from the other 2 DWM was found but the sites are currently unknown human genomic regions. Analysis of 4 CSF specimens was negative for site-specific integration.

Discussion: Our observation is the first to describe site-specific HIV integration in CNS and BM within the ITGB4 gene. Because HIV integration is generally thought to be a random process, mapping 2 cases within the same region suggests that a positive selection pressure may be involved. This preliminary observation requires analysis of other cases to determine the significance of viral integration within this region or other regions. Supported by NIH 1U54NS43049.

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A neuroprotective role for activated microglia in retroviral infection of the brain

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Objectives: Microglial activation occurs sooner than neuron death in AIDS. We thus hypothesized that microglia and macrophages, that produce neurotoxins, may also protect neurons in asymptomatic HIV infection. We studied if they could participate in protection against glutamate toxicity and oxidative stress in vitro, and assessed microglial expression of protection effectors in vivo in SIV-infected monkeys.

Methods: Tissular or monocyte-derived macrophages (MDM), and microglia were isolated and cultured. We measured glutamate and cystine uptake with radiolabelled aminoacids and EAAT gene expression by RT-PCR. Glutathione level was quantitated by an enzyme assay. Neurotoxicity of supernatants was assessed on mouse cortical neurons. We stained EAATs and GS on paraffin embedded brain sections from asymptomatic SIVmac251 infected macaques and uninfected ones (antibody probes and peroxidase). We also performed double staining of CD68 and GS.

Results: MDM expressed functionnal EAATs in vitro, as astrocytes do. Tissue macrophages, including microglia, also took up glutamate through EAATs when activated.

Neurotoxicity experiments showed that macrophages efficiently cleared extracellular glutamate. This glutamate clearance via EAATs trans-stimulated cystine uptake through xc- transporter, which led to an increased glutathione synthesis (+80%). Compared to controls, infected macaque brains exhibited variable levels of microglial activation. No sign of SIV encephalitis was detected, no typical apoptotic neuron was observed by simple histological examination. In infected

animals, microglia and perivascular macrophages expressed EAAT and GS. Of note, satelite microglia located close to neurons were brightly stained.

Conclusion: Beside their neurotoxic properties, in vitro activated macrophages also clear extracellular glutamate and enhance their antioxidant capacity. Moreover, the fact that microglia and perivascular macrophages express EAATs and GS in SIV infection strongly suggests neuroprotective and neurotrophic properties in vivo. Alltogether, these data might explain why neuronal death is not exactly correlated with microglial activation in HIV infection.

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Viruses and dementia, and the role of apolipoprotein E

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Background: We established that a high proportion of brains of Alzheimer's disease (AD) patients and of age-matched normals harbour latent herpes simplex virus type 1 (HSV1), and that HSV1 in brain and carriage of the type 4 allele of the apolipoprotein E gene (apoE-e4) together confer a strong risk of AD. We showed also that apoE-e4 is a risk factor for herpes labialis. We suggested that localised damage occurs on virus reactivation and is greater in apoE-e4 carriers. A strikingly similar apoE-virus connection was shown also in HIV-infected people pre-AIDS.

Two recent discoveries were that apoE-e2 is a risk factor for herpes simplex encephalitis, and that in HSV1-infected mice, vaccination with mixed viral glycoproteins (which were known to protect against HSV1 latency in the trigeminal ganglia) prevented the establishment of viral latency in brain.

Objective and methods: To investigate the role of viruses in dementia using PCR and immunological techniques.

Results: (1) Detection of intrathecal antibodies (not attributable to damage to the blood-CSF barrier) has confirmed our PCR results demonstrating HSV1 in brain, and indicates also that reactivation has occurred. (2) Human herpesvirus type 6 and cytomegalovirus are present in a high proportion of brains of AD patients and vascular dementia patients, respectively; whether the association is causal in either disease is not known. (3) The extent of damage caused (in liver) by hepatitis C virus (HCV) is determined by apoE, apoE-e4 being strongly protective. Neither susceptibility to infection, nor clearance of the virus, nor damage caused by other factors such as alcoholism is influenced by apoE genotype.

Conclusions: (A) The HIV and HCV results strongly if indirectly support our findings that HSV1 and apoE-e4 confer a strong risk of AD. Also, in a current trial using an amyloid peptide for immunotherapy of AD patients, the response of certain patients suggests the involvement of HSV1 in brain. (B). Vaccination against HSV1 to prevent establishment of viral latency in brain, and usage of anti-viral agents for treatment of AD, seem feasible.

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Neurotrophin anti-transcriptional and anti-replicative effect on dorsal root ganglion cultures infected with rabies virus

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Previous work by our group found that Nerve Growth Factor and Neurotrophin 3 modulate in vitro infection by rabies virus in sensory neurons. Different types of treatment with neurotrophins (NT) were tested in this work, before (PRE-treatment), during (TRANS-treatment) and after infection (POST-treatment), to evaluate in which step of the infection process this antiviral effect was being produced. Viral genomic RNA and messenger RNA for nucleoprotein, neural cell adhesion molecule (NCAM) and low affinity neurotrophin receptor (p75NTR) in treated cultures was quantified using real time PCR. NCAM and p75NTR expression were also evaluated in cultures treated with neurotrophins, these two proteins have been reported as virus receptors. Treatment with NT for four days prior to infection produced a significant increase in the quantity of virus adsorbed into the cultures and a concomitant increase in genomic RNA. Contrarily, treatment with NT during and after infection (TRANS- and POST-treatment) induced a strong decrease in the quantity of viral genomic and messenger RNA present in cultures. Treatment with NT did not change p75NTR and NCAM transcription rates, but it did increase protein p75 NTR expression in culture. It is well-known that the beginning of virus transcription and replication in neurons depends on protein phosphorylation by cell kinases; it is therefore possible that neurotrophins are affecting some metabolic pathway implicated in viral cycle producing an antiviral effect or that there is competition between rabies virus and neurotrophins. Adult mouse dorsal root ganglion cultures have provided a very useful model for investigating basic phenomena regarding rabies virus biology, detection of possible therapeutic targets and evaluation of experimental antiviral drugs.

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Inflammatory reaction of the brain reduces HSV-1 neurovirulence

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The neurovirulence of HSV-1 in the brain can be estimated determining the LD50 of intracerebral inoculations of virus. Intracerebral inoculations made a few days after a peripheral inoculation show that the herpesvirus neurovirulence is reduced. In order to understand the brain mechanisms responsible for the neurovirulence reduction, we studied an experimental model of HSV-1 (strain SC16) brain infection in albino Swiss mice. Experimental mice, previously inoculated intranasally, were inoculated intracerebrally, and control mice were inoculated intracerebrally without any previous inoculation. The experimental mice showed a reduction

of neurovirulence of the order of 4 log units, accompanied by a reduction of virus titer and virus spread in the brain, but at the same time they displayed an enhanced inflammatory reaction characterized by T-cells and macrophages infiltrating the tissue around microvessels and a few B-cells in the leptomeninges. We suggest that the enhanced inflammatory reaction partakes of neurovirulence reduction and helps safeguarding vital brain functions.