BASIC SCIENCES
THE HIV-1 ENVELOP PROTEIN GP120 INDUCES NEURONAL APOPTOSIS IN HIPPOCAMPAL SLICES.
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The HIV-1 virus has been shown to produce brain damage associated with neuronal cell death. However, the exact mechanism of this pathology is presently unknown.

Several studies have demonstrated that the HIV-1 envelop protein gp 120 induces neuronal and glial cell death. However, the effects of gp 120 “in vivo” is more controversial.

In this report, we developed the culture of organotypic slices which preserve many features of “in vivo” structure including development of normal networks. We show clearly that gp 120 produces neuronal death by apoptosis with TUNEL technique and electron microscopy studies. This preparation provides therefore a good model to test the molecular and cellular mechanism of action of gp 120 in physiological conditions and avoid problems of diffusion and degradation of drugs.

HUMAN IMMUNODEFICIENCY VIRUS TAT GENE TRANSFER TO THE MURINE CENTRAL NERVOUS SYSTEM USING A REPLICATION-DEFECTIVE HERPES SIMPLEX VECTOR STIMULATES TRANSFORMING GROWTH FACTOR BETA 1 GENE EXPRESSION.
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The high incidence of neurological disorders in patients afflicted with acquired immunodeficiency syndrome (AIDS) may result from human immunodeficiency virus type 1 (HIV-1) induction of chemotactic signals and cytokines within the brain by virus-encoded gene products. Transforming growth factor beta 1 (TGF-B1) is an immunomodulator and potent chemotactic molecule present at elevated levels in HIV-1 infected patients, and its expression may thus be induced by viral trans-activating proteins such as Tat. In this report, a replication-defective herpes simplex virus type 1 (HIV-1) Tat gene transfer vector, dSTat, was used to transiently express HIV-1 Tat in glial cell in culture and following intracerebral inoculation in mice brain in order to directly determine whether Tat can increase TGF-B1 mRNA expression. dSTat infection of Vero cells transiently transfected by a panel of HIV-1 long terminal repeat (LTR) deletion mutants linked to the bacterial chloramphenicol acetyltransferase (CAT) reporter gene demonstrated that vector-expressed Tat activated the LTR in a trans-activating responsive (TAR)-dependent fashion independent of the HIV-mediated induction of the HIV-enhancer or the NF-kB domain. Northern blot analysis of human astrocytic glial U87MG cells transfected by dSTat vector DNA resulted in a substantial increase in steady-state levels of TGF-B1 mRNA. Furthermore, intracerebral inoculation of dSTat followed by Northern blot analysis of whole mouse brain RNA revealed an increase in levels of TGF-B1 mRNA similar to that observed in cultured glial cells transfected by dSTAT DNA. These results provided direct in vivo evidence for the involvement of HIV-1 Tat in the induction of TGF-B1 gene expression in brain. Tat-mediated stimulation of TGF-B1 expression suggests a novel pathway by which HIV-1 may alter the expression of cytokines in the central nervous system (CNS), potentially contributing to the development of AIDS-associated neurological disease.

IMPAIRMENT OF KYNURENINE METABOLISM IN BRAINS OF PATIENTS WITH HIV INFECTION
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Disturbance of tryptophan metabolism was suggested to be involved in human immunodeficiency virus (HIV) associated neurological deficits. Some reports have described lowered tryptophan, elevated kynurenine (KYN), and increased quinolinic acid (QA), a metabolite of KYN pathway and a well known endogenous neurotoxin and agonist at excitotoxins acid (EAAs) receptors. Kynurenic acid (KYN), an intermediate metabolite of KYN, is a broad-spectrum antagonist at EAAs receptors and able to block the neurotoxicity of QA. We examined the KYN and KYN biosynthetic machinery in the frontal cortex of patients with HIV infection (n=14) as compared with normal controls (n=14). We measured the content of KYN as determined by HPLC, and the activities of enzymes synthesising KYN, kynurenine aminotransferase I and II (KAT I, KAT II). KAT activity was determined at pH optima of 10.0 for KAT I and 7.4 for KAT II. In HIV brains, KYN levels were significantly reduced (51% of control, p<0.05). Also KATs were remarkably affected: activities were significantly lowered for KAT I in the presence of 2-oxoisocaproate (31.6 %, p<0.05) and pyruvate (24.8 %, p<0.01), and for KAT II in the presence of 2-oxoglutarate (49.2 %, p<0.01) and pyruvate (42.6 %, p<0.01). These data demonstrate prominent impairment of kynurenine metabolism possibly enhancing the susceptibility to excitotoxicity in the brains of HIV patients.

HIV-1 INFECTION IMPAIRS GLUTAMATE UPTAKE BY HUMAN FETAL ASTROCYTES. M. BENCHERICH*, H. GELBARD*, M. CANITI, J. SPARROW, T. FLYN*, M.J. POTAHER, D.J. VOLSKY*, S. Lukin/Rosewell Hospital, University of Rochester, Cornell University, New York, NY, USA.

HIV-1 infection causes neurological disease in more than 70% of the individuals infected. The routes to CNS dysfunction are not fully understood, but they are likely to involve direct infection by HIV-1 of cells resident in the nervous system. We have investigated the impact of HIV-1 replication in astrocytes, on one of their critical functions in signal transduction, the uptake of the neurotransmitter, glutamate. Human fetal astrocytes were transfected with HIV-1 DNA of 14 different strains or control plasmid DNAs or were infected with different HIV-1 strains including primary isolates from the eye. Virus replication was monitored by the level of core antigen p24 or viral DNA. Astrocyte function was monitored by their ability to take up 3H glutamate. Virus replication varied from strain to strain, reaching a peak of over 10,000 pg p24 per ml and then declining. Glutamate uptake after transfection was inhibited completely by HIV-1 infection or transfection of viral DNA but not control DNA. Inhibition was detected by 5 days after infection or transfection, reached 80-100% by 2 weeks and was stable for three months. Similar data of HIV-1 infection of a novel target for HIV-1, pigmented retinal epithelial cells, will be presented. These results indicate that human astrocytes are susceptible to low productive, persistent replication of HIV-1 and that such viral replication is deleterious to astrocyte function.
SYNCYTlUM-INDUCING VARIANTS DERIVED FROM THE CEREBROSPINAL FLUID (CSF) OF AN HIV-1 SUBTYPE D-INFECTED PATIENT WITH AIDS DEMENTIA COMPLEX (ADC)

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Recently, genetic determinants associated with ADC were identified in the V3 region of the HIV-1 gp120. However, these results were obtained from the study of HIV-1 subtype B isolates.

Here, we analyzed sequence variability of the V3 loop and SU/NS1 phenotype of HIV-1 subtype D isolates and HIV-1 subtype B isolates derived from the plasma and the CSF of two ADC and two non-ADC patients.

CSF-derived virus population in both clinical groups was more homogeneous than in plasma. The predominant V3 variant found in the CSF was also detected in the plasma although its frequency differed between these two compartments.

When comparing the CSF-derived V3 sequences in ADC and non-ADC patients, we found no marked distinctions between both groups of patients. Thus, specific amino acid residues in this region of the viral genome are probably not sufficient to predict an ADC.

Interestingly, the CSF-derived variant of the ADC patient with an HIV-1 subtype D genotype did not show the ADC-associated mutations and was characterized by an SI phenotype. It would be interesting to study the neuropathology of such isolates since the main biological property of HIV-1 isolates infecting the central nervous system is the macrophage tropism, essentially correlated with the NS1 phenotype.

CEREBROSPINAL FLUID LEVEL OF GLUTAMATE IN AIDS-DEMENTIA COMPLEX

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A possible role of excitotoxicity in the pathogenesis of AIDS-dementia complex has been hypothesized. Direct stimulation of glutamate receptors by HIV-induced substances, such as quinolinic acid, or modifications of glutamate release or uptake could be responsible for excitotoxic cell death. In order to evaluate the role of endogenous glutamate in this pathology, we investigated CSF concentration of aminonacids in HIV positive patients with AIDS-dementia complex.

CSF was collected from 20 AIDS patients and 10 age-matched controls suffering from peripheral neuropathies, without signs of central nervous system (CNS) involvement. AIDS patients were classified according to the Center for Disease Control (CDC) and to the AIDS-dementia complex scales; opportunistic infections of the CNS were ruled out by biological and imaging studies. The degree of CNS pathology was assessed by measurements of cerebral atrophy by CT and NMR scans; moreover, a battery of neuropsychological tests was performed, when possible, and anxiety depression were evaluated by Hamilton’s and Zung’s rating scales.

CSF samples were collected, immediately treated with perchloric acid to inactivate enzymes, and stored at -80°C. Glutamate, GABA and glutamine levels were analyzed by reverse-phase HPLC, according to the method previously described (Ferraresi et al., Annals of Neurology, 36:316-319, 1993).

Glutamate concentration was increased two folds in CSF of AIDS patients, respect to controls. The increase was highly significant (p < 0.02) in the group of patients with brain atrophy, while no change was observed in patients with normal CT scan. A positive correlation (R = 0.6; p < 0.05) was observed between the increase in glutamate level and the time from seroconversion. No change in GABA and glutamine levels was observed in AIDS patients.

These data may support a role of excitotoxicity in neuronal apoptosis, which lead to brain atrophy in patients with AIDS-dementia complex.

REGULATION OF HIV-1 JR-CSF GENE EXPRESSION BY THE TRANSCRIPTION FACTOR AP-1 IN HUMAN BRAIN CELLS

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We have recently analyzed the interactions of the transcription factor AP-1 present in human neuronal, glial, HeLa and Jurkat T cells, with the long terminal repeat (LTR) from the neurotropic JR-FI, and JR-CSF HIV-1 strains, compared with the LAI strain. AP-1 is unable to recognize the -352/-324 and the -306/-285 AP-1 putative binding sites, but interacts only with the -247/-222 region of both neurotropic strains. These interactions are cell-type specific, since they are detected only with extracts from glial and HeLa cells, and not from neuronal or Jurkat cells. Moreover the -247/-222 sequence mediates AP-1-induced transcriptional activation in glial and not in neuronal cells.

By Western analysis and gel supershift assays, we have investigated the composition of the jun and fos components of AP-1 in the various cell types, either non stimulated or stimulated with phorbol esters or with various cytokines. Transient expression experiments revealed the functional contribution of the c-jun, junB, junD and fos components on HIV JR-CSF gene transcription in glial cells. Our results indicate that some AP-1 components stimulate HIV-1 LTR-mediated gene transcription. Moreover our data suggest that this activation is mediated both by direct interactions with the LTR binding site and by cross-coupling interactions with upstream and downstream-located proteins.

INFECTION OF RHEUS MACAQUES WITH NEUROPATHOGENIC SIV INDUCES COGNITIVE AND ELECTROPHYSIOLOGIC ABNORMALITIES: VIRAL AND HOST FACTORS LEAD TO CNS DYSFUNCTION


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We have recently reported that serial transfer of microglia-associated SIV selected for neuropathogenic viral variants. We now find that infection with this virus induces functional changes in the brain.

Juvenile rhesus macaques were infected with virus from the second microglial passage. Behavioral testing of animals revealed that cognitive deficiencies developed in attentional set-shifting. Electrophysiological measurements indicated that brainstem auditory evoked potentials showed a characteristic delay in certain latencies.

In the brains of infected animals, macrophage and T cell infiltrates were present. Cytokine expression was detected in the brains, as was the inducible form of nitric oxide synthase. SIV-specific cytotoxic T lymphocytes were also identified in the brains of infected animals. Furthermore, the serial passage expanded the tropism of the virus, allowing the infection of cerebrovascular endothelial cells. Thus both viral and host (including cellular and soluble toxic) factors may play a role in neuropathogenesis.

This SIV/rhesus monkey system provides an excellent model to question the mechanism and causation of CNS dysfunction induced by HIV. We are currently correlating the functional changes in the CNS with the analysis of viral and host components of the infection in the monkeys to address the pathogenic role of these factors.
IN VITRO CULTURE OF MICROGLIAL CELLS FROM ADULT SIMIAN BRAIN INFECTED BY HIV-2 AND/OR SIV.

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Productive HIV-infection in human brain has been shown to involve predominantly perivascular microglia and macrophage-like cells, although other brain cells may be infected. We have developed a protocol allowing isolation and purification of microglial cells from adult rhesus monkey brain. This protocol is based on Percoll gradient allowing cells to be cultured with RPMI 1640 in 10% FCS, 2 mM Gln, 1% PSN and 100 u/ml GM-CSF. The culture was changed 24 h then every 4-5 days. Ameoboid shape cells could proliferate more than one month in culture with GM-CSF. The number of cells could triple during the first week then between the 8th and the 12th day. The expression of the cell surface markers CD4, CD8, CD64, CD68, MHC class II have been characterized by immunostaining from 10-day-old microglial cells. Three rhesus monkeys (M. mulatta) infected by SIVmac251 or HIV-2 then superinfected by SIVmac251 have been investigated. They did not show any neurological disorder after one year of infection although their numbers of T4 cells were less than 500/mm³. Viral detection in the microglial cells culture supernatants are negative for the analysis of the p27 core protein antigen assay. Other monkeys are under investigation.

THE MOLECULAR PATHOGENESIS OF HIV-1 DEMENTIA. RONALD P. GENDelman, HANS S. L.M. NOTTERT, TUKI PERSIADIE, ANGELA GRANADAS, CLINT PLANTAGAN, ROAZ GRANOT, University of Nebraska Medical Center, Omaha, NE, USA.

Our laboratory has investigated the molecular and cellular event leading to HIV dementia. HIV-1 inoculation of primary human brain cell [macrophages, microglia, astrocytes and microvascular endothelial cells (MVEC)] demonstrated that cells only of macrophage/microglial lineage are productively infected with lentivirus. Studies of virus-macrophage-neural cell interactions showed cellular, endogenous control mechanisms for neurotoxic production. Mechanisms for the penetration of the virus through the blood-brain barrier were uncovered. Using macrophages as viral target cells and human autopsy brain tissue (for substantiation of test results), we discovered a number of "putative neurotoxins" induced by HIV-1 that include: nitric oxide, platelet-activating factor, eicosanoids, glutamic acid and pro-inflammatory cytokines. To determine the biologically relevant constitution of HIV-induced neurotoxins, high performance liquid chromatography (HPLC) quantitatively analyzed culture fluids/cells at various stages of virus-macrophage infection. Accumulation of arachidonic acid precursors and specific HIV-induced eicosanoids were identified. Moreover, we showed the selective upregulation of vascular cell adhesion molecule 1 (VCAM-1) and E-selectin in MVEC within and brain tissue. This correlated with similar findings during cocultivation of HIV-infected monocytes MVEC in laboratory experiments. Binding of monocytes to encephalitic brain endothelium was inhibited with antibodies to E-selectin and VCAM-1. In an animal model system which mimics many aspects of HIV encephalitis, we developed. Here stereotactic injection of HIV-1-infected monocytes resulted in "human cell" placement into the basal ganglia of severe combined immunodeficiency virus (SCID) mice. Resulting histopathological observations included multiloculated giant cell encephalitis, microglial nodules, astrogliosis, neuronal dropout and local cytokine production. These works, taken together, serve as the foundation for laboratory drug testing designed to prevent HIV-1-induced neuronal dysfunction.

THE HIV-1-INDUCED NEUROTOXIN, TUMOR NECROSIS FACTOR ALPHA, MEDIATES NEURONAL APOPTOSIS BY PLATELET ACTIVATING FACTOR RECEPTOR ACTIVATION. HARRIS A. GELBA, SETH PERRY, KIRK A. DZENKO, LEON G. EPSTEIN. The University of Rochester Medical Center, Rochester, NY USA.

We have previously demonstrated that tumor necrosis factor alpha (TNFα) and platelet activating factor (PAF), secreted by HIV-1-infected macrophages, are able to induce neuronal apoptosis in a dose-dependent fashion. Because PAF can regulate TNFα synthesis in monocyte cells, we wondered whether a reciprocal mechanism might mediate TNFα-induced neurotoxicity. We now demonstrate that TNFα (10 ng/ml) can induce a 4.2-fold increase (p<0.01 vs. control) in apoptotic neurons as measured by TUNEL immunostaining in primary human fetal cortical neurons. Ceramide (10 μM), a second messenger for TNFα, also induces a 4.3-fold (p<0.01 vs. control) increase in neuronal apoptosis. Neuronal apoptosis mediated by TNFα (10 ng/ml) is reversed in large part (1.9-fold increase vs. control; p<0.01 vs. TNFα) by co-incubation with a competitive PAF receptor antagonist. In contrast, incubation with carbamyl PAF (250 ng/ml), resistant to metabolism, induces a 3.4-fold increase in neuronal apoptosis (p<0.01 vs. control); but co-incubation with a monoclonal antibody to TNFα has no significant effect on the amount of neuronal apoptosis induced by ePAF. These data suggest that TNFα-induced neuronal apoptosis occurs in part through PAF receptor activation. These results suggest therapeutic interventions to reduce neuronal death in HIV-1 encephalitis.

IN VITRO INFECTION OF ASTROCYTES DERIVED FROM SIMIAN MATURE BRAINS WITH SIVmac 251.

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HIV can infect human astroglial cells in vivo. Furthermore, recent studies demonstrated infection in the brain of AIDS patients. These infected astrocytes may contribute to the neuropathological mechanisms involved in AIDS encephalopathy.

In this study, we performed in vitro infections with SIVmac 251 of astrocytes in primary cultures from adult macaque brains. The first week post-infection, we detected a low viral production using p27 antigen capture assay. Then, we observed a viral persistence in astrocyte cultures using specific PCR amplifications during 60 days post-infection. Immunocytochemical staining demonstrated that, 45 days post-infection, few astrocytes expressed viral regulatory protein Nef but no structural proteins. Preliminary treatments of astroglial cultures with TNFα or IL-6 or GM-CSF before viral infections, did not modify the kinetic of viral production. However, stimulation at 42 days post-infection with TNFα induced a very low viral production in astrocytes cultures laterly infected by SIVmac 251.

These results demonstrated the capacity of SIVmac 251 to infect persistently simian astrocytes with over-expression of the Nef protein. This study tends to confirm the potential functional role of viral reservoir of astrocytes in the CNS.
INFECTION OF ASTROCYTES IN ADULT MACAQUE BRAINS INFECTED WITH SIVmac 251 AND/OR HIV-2

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Several recent studies demonstrated infection by HIV-1 of astrocytes in the brain of adult and pediatric AIDS patients. Thus, astrocytes appeared to play important functions in neuropathological mechanisms involved in CNS invasion by leukocytes.

Pure primary astrocyte cultures from adult simian brains were obtained from 18 adult Rhesus (Macaca mulatta) and Cynomolgus (Macaca fascicularis) macaques. These animals have been infected by intravenous or vaginal or rectal tracts with SIVmac 251 and/or HIV-2 for an average of one year before sacrifice. They did not develop any neurological and immunological symptoms. At one month of culture, purity of astrocyte cultures was confirmed by immunocytochemical stainings which demonstrated the lack of potential contaminant cellular types present in the CNS such as microglial cells or oligodendrocytes.

Using specific PCR amplifications in pol and env genes, we detected the persistence of proviral DNA in astrocyte cultures from 8 of the 18 monkeys during 6 months of culture. However, no viral production was detected in these culture supernatants using p27 antigen capture assay. No integrated virus could be detected in astrocyte cultures from the 10 remaining monkeys.

These results suggest that astrocytes are infected in brains of asymptomatic experimentally infected macaques and serve as potential viral reservoir.

EICOSANOID PRODUCTION BY HUMAN GLIAL CELLS AND ITS INTERACTION WITH NO AND O2^- REGULATORY PATHWAYS.

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We demonstrated the presence of the inducible form of cyclooxygenase (COX 2) in cultured human embryonic astrocytes and microglia since: (i) large amounts of PGE2 were released in response to IFNγ and IL1β, (ii) the response, after stimulation, was delayed and led to a progressive accumulation of product and (iii) the secretion reached a protein synthesis and was blocked in presence of indomethacin. In the same culture conditions and using the same inducers, human embryonic astrocytes but not microglial cells produced NO and possessed an inducible form of NOS (iNOS). Conversely, microglial cells were induced by cytokines to generate O2^- whereas astrocytes were poor producers of O2^-.

In presence of L-NMMA, which inhibits NO production, cycloxygen-induced COX 2 activity in human astrocytes was not inhibited, whereas PGE2 secretion by human microglia was reduced by 37% suggesting an endogenous production of NO in these cells. The presence of a constitutive NOS activity was also supported by the detection in double staining experiments of a NOS protein, in CD68 positive cells. In the tested human astrocytes, iNOS activity was slightly increased in presence of indomethacin which blocks the production of PGE2, while blockade of COX 2 activity by indomethacin inhibited the production of O2^- by microglia. Finally, depletion of superoxide in astrocytes by SOD, increased the PGE2 production in these cells. In conclusion: interactions between eicosanoids and ROS synthesis pathways in astrocytes and microglial cells have a major role in the regulation of the inflammatory intra parenchymatous response and in the induced neurotoxicity.

INVESTIGATION OF THE DYNAMICS OF HIV SPREAD TO THE BRAIN BY PHYLOGENETIC ANALYSIS OF SEQUENCES IN P17/gag AND V1/V2

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A proportion of HIV-infected individuals develop a progressive dementia or neurological deficits attributable to HIV infection of the brain. It is not known whether this spread to the brain is dependent upon the evolution of neurotropic variants of HIV, nor is it known when this event occurs in the course of disease.

We carried out sequence comparisons of variants in lymphoid and non-lymphoid tissues (including brain) from 3 patients with giant cell encephalitis at time of death. We sequenced the p17 gag region as previous studies have accurately established its rate of sequence change in vivo. From the p17 gag sequences we were able to estimate a maximum time of divergence of 2 years between variants in brain and lymph node before death in each patient, closely coinciding with the onset of AIDS. These data were confirmed by a separate analysis of sequences from the env gene (V1/V2). Phylogenetic analysis of sequences in both regions indicated that variants infecting the brain were found on several lineages that also contained variants from other lymphoid and non-lymphoid tissues. There is therefore no evidence that the appearance of AIDS dementia in an individual is dependent of the evolution of a uniquely neurotropic virus.
ALTERATIONS IN AN ATFC/CREB BINDING SITE ADJACENT TO THE NF-κB ENHANCER REGION IN HIV-1 LTR QUASISPECIES DERIVED FROM THE IMMUNE AND CENTRAL NERVOUS SYSTEMS.

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Although the principal cell types infected by human immunodeficiency virus type 1 (HIV-1) within the central nervous system (CNS) of individuals with progressive neurologic disease are cells of the microglage/monocyte lineage (including brain microglia), there is evidence to support the existence of more highly regulated infections in other CNS cell types, including astrocytes.

The regulation of viral expression by the HIV-1 long terminal repeat (LTR) is affected not only by the sequence of the LTR quasispecies and their transcription factor binding sites, but also by the cell type-specific array of transcription factors that may regulate transcription from the LTR. To better understand the regulation of HIV-1 expression in cells within the CNS, we have initiated investigations into the relationship between HIV-1 LTR diversity and the regulation of viral expression in neuronal cell populations. LTRs amplified from HIV-1-infected peripheral blood and post-mortem brain tissue were cloned and sequenced.

Transient expression analyses demonstrated that LTR variants varied significantly in U-373 MG cells as well as in Jurkat cells. Activities varied among LTRs isolated from different individuals, as well as between LTRs isolated from the same individual. While LTRs which demonstrated the highest activities in U-373 MG cells also yielded high activities in Jurkat cells, the LTRs were generally more active in Jurkat cells when compared to the LAI LTR. To investigate the relationship between nucleotide changes within selected cloned LTRs and their activities during transient expression, electrophoretic mobility shift (EMS) assays were performed. Using DNA probes specific to the -130 to -104 region of two functionally divergent LTRs, we have determined that ATFC/CREB transcription factor family members in U-373 MG nuclear extracts form DNA-protein complexes with a previously unidentified binding site located between the LEF-1 and distal NF-κB-inducible sites. Furthermore, the number and abundance of these complexes differ from those formed using Jurkat nuclear extracts, suggesting cell type-specific LTR regulation involving the ATFC/CREB site.

Although this binding site is relatively well conserved in HIV-1 clinical isolates and laboratory strains, some variations have been identified. We have obtained several LTRs from HIV-1-infected brain tissue which contain alterations in the ATFC/CREB binding site. In particular, clone 2615 contains an ATFC/CREB site and a 24 bp insertion with two additional imperfect repeats of this site. EMS analyses using the brain-derived LTR clone 2615 indicate that the ATFC/CREB region forms higher order DNA-protein complexes in comparison to promoters containing a single site.

Analyses of nucleotide sequence data will examine the physical variation in the ATFC/CREB region and its functional significance in HIV-1 gene expression in cells of immune and nervous system origin.

INTERACTION OF HIV-1 COAT PROTEIN GP120 WITH HUMAN EMBRYONIC NEUROBLASTS.

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We evaluated the neurotoxic effect of the envelope glycoprotein of the human immunodeficiency virus (HIV-1) gp120 on cultured human embryonic cerebral and spinal neurons from 8 to 10-week old embryos. The neurotoxicity of pliomolecular doses of gp120 was estimated by immunofluorescence and colormetric assay at about 30% of neurons. Treatment of cultures with APS or nefidipine reduced gp120-induced toxicity by 70 and 100 %, respectively. Electrophysiological properties as well as N-methyl-D-aspartate (NMDA)-induced currents were recorded from neurons maintained in culture for 10-30 days. Neither voltage-activated sodium or calcium currents nor NMDA-induced currents were affected by exposure of neurons to 250 μM gp120. In contrast, when neurons were subjected to photometric measurements using the calcium dye Indo-1 to monitor the intracellular free Ca²⁺ concentration (Ca²⁺), gp120 (20-250 μM) potentiated the large rises in [Ca²⁺] by 50 μM NMDA. The potentiation of NMDA-induced Ca²⁺ responses required the presence of Ca²⁺ in the medium, and was abolished by the NMDA antagonist AP5 and the voltage-gated Ca²⁺ channel inhibitor nifedipine. Moreover, exposure of a subpopulation of spinal neurons (25 % of the population) to 20-250 μM gp120 resulted in an increase of [Ca²⁺]; that followed three patterns: fluctuations not affected by APS, a single peak, or a progressive and irreversible rise of [Ca²⁺].

In conclusion, gp120 was toxic for human neurons, altered [Ca²⁺] homeostasis and potentiated the NMDA-induced Ca²⁺ increases without effect on NMDA activated currents nor on voltage-sensitive Ca²⁺ currents. Ca²⁺ in turn might activate a Ca²⁺ dependent signal transduction pathway such as kinases, an hypothesis under investigation.

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EVIDENCE THAT A CELL CYCLE REGULATOR, E2F1, DOWN-REGULATES TRANSCRIPTIONAL ACTIVITY OF THE HIV-1 PROMOTER:

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Proliferation of eukaryotic cells is orchestrated by a series of cellular proteins which participate in various stages of the cell cycle to guide the cell through mitosis. Some of these proteins, including E2F1, play a critical role in G1 and S phases by coordinate regulating expression of several important cell cycle-associated genes. Based on recent observations indicating a link between HIV-1 replication in cells arrested in G1/S phase of the cell cycle, we sought to evaluate the regulatory action of E2F2 on transcription from the HIV-1 LTR. Results from transient transfection of cells with an E2F2 expression plasmid indicated that E2F2 has the ability to suppress basal transcriptional activity of the long terminal repeat (LTR) and to diminish the extent of the Tat-induced activation of the viral promoter. Deletion analysis of the HIV-1 LTR in transfection studies revealed the presence of two major elements responsive to E2F2 repression located distally (-454 to -381), and proximally (-117 to -80) with respect to +1 transcription start site. The proximal E2F2 responsive element of LTR overlaps with the binding site for NF-κB transcription factor. Results from the DNA binding studies indicated that E2F2 binds to the NF-κB motif and this interaction may play an important role in the observed suppression of the viral gene expression by E2F2. The E2F2-mediated suppression of LTR activity was observed in a wide range of human cell lines. Expression of E2F2 by a transgene showed an inhibitory effect on the levels of reverse transcriptase activity obtained upon introduction of proviral genome into cells.

The data presented in this study suggest that cellular regulatory proteins that suppress the progression of cells through the cell cycle could play crucial roles in determining the efficiency of HIV-1 replication during the various stages of infection. The role of these factors in viral latency and activation is discussed.
THE EFFECT OF GP120 ON (Ca²⁺), DEPENDS ON THE AGE OF THE CULTURE OF RAT HIPPOCAMPAL NEURONES

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HIV-1 envelope glycoprotein gp-120 is known to induce cell death in cultured postnatal rat ganglion neurones (Lipton, 1990, 1991). One of the earliest effects of GP120 is an increase of intracellular calcium concentration ([Ca²⁺]i) in neurones.

In the present work, we have found that the effect of gp-120 on the [Ca²⁺]i in rat hippocampal neurones is dependent on the age of neurones in culture. [Ca²⁺], was measured using confocal scanning microscopy with Fluo-3AM. In neurones cultivated during 3-8 days gp-120 (200-1000 pM) did not modulate [Ca²⁺]i (n=8). In contrast, brief applications of NMDA induced transient rise of [Ca²⁺]i in all studied neurones. The existence of responses to NMDA shows: 1) that the cells which were not responding to gp-120 are neurones; ii) the initial level of [Ca²⁺]i is low and iii) the Ca²⁺ transport systems in neurone are active. After development in culture during 9-11 days, gp-120 (200 pM) increased [Ca²⁺]i in 7 out of 13 studied neurones. Maintenance of neurones in culture during more then 13 days resulted in an increase of [Ca²⁺]i induced by gp-120 (200 pM) in all (n=31) experiments. To understand the mechanism of [Ca²⁺], increase we have studied the effect of gp-120 on neurones developed in culture during 14-18 days using simultaneous whole-cell patch clamp recording and measurement of [Ca²⁺]i. Gp-120 mediated neither [Ca²⁺], nor transmembrane ionic current in neurones which were recorded in voltage-clamp mode (n=7) out of 7 studied neurones. In contrast, in these cells application of NMDA or activation of voltage-gated calcium currents induced reversible increase of [Ca²⁺].

Lack of effect of the protein on neurones recorded in whole-cell mode suggests that the action of gp-120 is mediated by an intracellular factor whose activity is modulated during wash-out of neurones with intracellular pipette solution.

DIFFERENTIAL EFFECTS OF SIVmac251, SIVmac239 AND HIV-2 ON NEUROANAL ATROPHY IN CYTOMEGALOVIRUS MAQUAECS.

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Infection of rhesus macaques with SIVmac251 leads to neuronal atrophy of hippocampal pyramidal neurones. We now report a study of the effects of infection with SIVmac239 (a macrophage-tropic virus), SIVmac251 (a lymphocyte-tropic virus) and HIV-2 (a non-pathogenic virus in macaques) upon hippocampal neuronal diameter in cytomegalovirus macaques.

The numbers of animals infected and the range of duration of infection for each virus are as follows: SIVmac251: n = 22, range 1-31 months; SIVmac239, n = 7, range 3-4m and HIV-2, n = 6, range 7-10m. Paraffin sections were stained with cresyl violet and the average diameter of 20 adjacent pyramidal cells from each CA sub-field calculated. Two-tailed T-tests were used to compare size data from control (n = 17) and infected groups. Immunohistochemistry for glial fibrillary acidic protein (GFAP) and HLA-DR (MHC Class II) was also performed.

Hippocampal neuronal diameter was reduced, in comparison to control values of 11.2 ± 0.24µm (mean ± S.E.M), to 11.80 ± 0.22µm in SIVmac251 infected animals (p < 0.001) and to 12.49 ± 0.70µm in those inoculated with HIV-2 (p = 0.001). Macaques infected with SIVmac251, however, had neuronal hypertrophy (15.28 ± 0.35µm; p = 0.016). Immunohistochemistry demonstrated glialis and increased MHC Class II expression in SIV infected animals but not in those infected with HIV-2.

In conclusion, infection of cytomegalovirus macaques with SIVmac251 or HIV-2 leads to neuronal atrophy whilst infection with SIVmac239, a lymphocyte-tropic virus leads to neuronal hypertrophy but comparable glialis and MHC Class II activation. There is little discernible gliosis or immune reaction within the brain of HIV-2 infected animals.
SURFACE EXPRESSION OF MHC CLASS I MOLECULES ON NEURONS LIMITED BY β2-MICROGLOBULIN AND PEPTIDE LOADING

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Major Histocompatibility Complex (MHC) class I molecules are composed of a polymorphic heavy chain, β2-microglobulin and a short antigenic peptide. In contrast to most other cell lineages, neurons of the healthy central nervous system do not express MHC class I molecules on their surface. Lack of MHC class I molecules enables neurons to escape immune surveillance, but favors viral persistence in neurons.

We analyzed expression of MHC class I heavy chain, β2-microglobulin and peptide transporter TAP gene transcripts in individual cultured hippocampal neurons combining patch-clamp electrophysiology with single cell RT-PCR.

While neurons lacked β2-microglobulin and TAP mRNA, approximately half of the neurons expressed MHC class I heavy chain gene transcripts. MHC class I molecules were not detectable on the cell surface by confocal laser scanning microscopy. However, MHC class I molecules (H2-Dp) were restored on the cell surface of cultured mouse hippocampal neurons by adding human β2-microglobulin and an appropriate viral peptide (gp33, LCMV).

Our data indicate, that β2-microglobulin and the peptide loading transporter are limiting factors for expression of MHC class I molecules on the neuronal cell surface.

HUMAN IMMUNODEFICIENCY VIRUS PROTEIN, Tat, CAUSES INFLAMMATION, APOTOPSIS AND ATROPHY IN RAT BRAIN. K. OLAFSON, M. DEL RIGO, J. PEELING, M. JONES, A. NAHL.
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To determine the role of HIV-Tat in the pathogenesis of AIDS dementia complex (ADC), highly purified recombinant Tat (amino acids 1-79 representing the first exon of HIVm) was injected intraventricularly as single (n=6 rats; 0.26-0.29 millilitres Tat/rat) or repeated injections (n=5 rats) into adult male Sprague Dawley rats. Repeated injections of 0.05 millilitres Tat were given daily for 5 days through an indwelling intraventricular cannula. Following perfusion with 2% paraformaldehyde, parafernent embedded tissues were studied by using hematoxylin and eosin, and myelin stained sections, and by immunosasting for glutaryl fibrillary acidic protein. Apoptotic cells were identified using ApoTaq™. Tat injected brains showed inflammation, reactive astrocytosis, apoptosis and ventricular enlargement at 7 days following the last injection. Brains injected with equivalent moles of bovine serum albumin (n=5, single injection; n=5, repeated injection) or solutions from which Tat had been immunoabsorbed (n=2) did not show any pathological changes. Inflammation of the brain was neutrophil predominant 1 day after injection, followed by a mixed macrophage and neutrophil inflammatory at 3 days and finally, a macrophage and lymphocyte infiltration at 7 days. No abnormality in myelin staining was noted. The pathological changes of macrophage infiltration, astrocytosis, apoptosis and cerebral atrophy are similar to those seen in patients with ADC.

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NEUROANOMALIES ASSOCIATED WITH FELINE IMMUNODEFICIENCY VIRUS ENCEPHALOPATHY. C. POWER, J. PEELING, T. MOENCH, T.L. LANGEIERR. University of Manitoba, Winnipeg MB; Johns Hopkins University, Baltimore MD.

Background: Feline Immunodeficiency Virus (FIV) is a lentivirus like Human Immunodeficiency Virus and is associated with immune suppression and encephalopathy in cats.

Objectives: To determine the neurological abnormalities associated with FIV encephalopathy.

Methods: Adult and neonatal cats were experimentally infected (EI) [N=12] with a CSF-derived strain of FIV and compared to uninfected (UI) [N=8] cats by prospective behavioral testing. Brains from necropsied EI and UI animals were analyzed by H-NMR spectroscopy, histopathology, Western blot analysis and immunocytochemistry.

Results: EI animals developed neurological abnormalities including ataxia (p=0.01) and psychomotor slowing (p=0.001) compared to UI animals. Histopathological and immunocytochemical studies indicated that neuronal loss, gliosis and FIV p24 antigen were present in the brains of EI animals but not in UI animals. NMR spectroscopic studies of frontal cortex revealed significantly elevated glutamate/GABA ratios (p=0.01) and glutamate levels (p=0.05) in EI animals but NAA levels did not differ between groups. Glutamate decarboxylase (GAD) expression in frontal cortex was markedly reduced in the EI group compared to the UI group by Western blot and immunocytochemistry.

Conclusions: FIV encephalopathy is accompanied by neuronal loss, increased glutamate levels, and diminished GAD expression in frontal cortex suggesting that altered glutamate metabolism may be involved in the pathogenesis of lentivirus-induced encephalopathy.

Centre for Immunology, Department of Neurology, St Vincent's Hospital and the National Centre in HIV Epidemiology and Clinical Research, Darlinghurst, Sydney, Australia.

Objectives: We sought to determine the role of the macrophage mediated cytokines IFN-α and TNF-α in Quinolinic acid (QUIN) production as the macrophage is considered central to the pathogenesis of ADC and QUIN has been correlated with ADC.

Methods: Primary human monocytes were isolated by plastic adherence and cultured. TNF-α (1ng/ml, 10ng/ml) IFN-α (10U/ml, 50U/ml) either as a mono-treatment or in combination with IFN-γ (10U/ml, 50U/ml) were added to the cells. Triplicate samples were then taken at 24, 48 and 72 hours and assayed for QUIN production by mass spectrometry.

Results: No significant levels of QUIN were detected in the cells treated with TNF-α. However, TNF-α combined with IFN-γ, led to a marked increase in QUIN (up to 12,000ng/ml at 48hrs) compared to that produced with IFN-γ alone (5,000ng/ml at 48hrs). High levels of QUIN (up to 8,314ng/ml at 72 hrs) were produced by macrophages treated with 100U/ml and 500U/ml IFN-α. However, there was no significant increase in QUIN production in the cells treated with a combination of IFN-α and IFN-γ.

Conclusions: These data support a positive feedback model for QUIN production by macrophages initiated by cytokines and HIV-1 infection. This would explain excessive QUIN production with low levels of productive HIV-1 infection in the brain.
BRAIN-DERIVED HIV-1 ENVELOPE V1 AND V2 REGION POLYMORPHISM C. POWER, J.C. MCARTHUR, T.L. GANELIER, J.D. GLASS, H.T. JOHNSON, B. CHESEBRO: University of Manitoba, Winnipeg MB; Johns Hopkins University, Baltimore MD; Rocky Mountain Laboratories, NIAD, HIV, Hamilton MT.

Background: The V1 and V2 regions (V1/V2) of the HIV-1 envelope have been shown to influence HIV cytokotropism and syncytia-induction.

Objectives: To determine the relationship of brain-derived V1/V2 sequences to blood-derived sequences of known phenotype and to the development of HIV dementia.

Methods: Brain-derived V1/V2 was amplified, cloned and sequenced from 10 AIDS patients with (HIVD; N=5) and without (ND; N=5) HIV dementia. Patients did not differ in CD4 count, age, or antiretroviral use.

Results: Extensive length polymorphism, especially in regions of hypervariability, were observed in all sequences. Comparison of HIVD and ND sequences to previously reported blood-derived norepinephrine-inducing (NSI) and syncytia-inducing (SI) sequences revealed that the V2 region of the brain-derived sequences had fewer N-linked glycosylation sites (p=0.0001) than the NSI and SI V2 sequences and fewer positively charged amino acids (p=0.02) than the NSI group. HIVD and ND V1/V2 sequences did not differ from each other in total mutation frequency when compared to the brain-derived consensus sequence but the rate of insertion was higher among ND (p=0.002) compared to HIVD sequences resulting in significantly longer V1/V2 amino acid fragments (p=0.002) and more N-linked glycosylation sites (p=0.05) in the V2 region among the ND sequences.

Conclusions: Reduced N-linked glycosylation within the V2 region may be important in the development of HIV neurotropism. Similar to studies of blood-derived sequences, diversity in brain-derived V1/V2 sequences may be associated with different clinical phenotypes.

REGULATION OF HIV-1 GENE EXPRESSION BY COUP-TF AND RETINOIC ACID RECEPTORS IN HUMAN BRAIN CELLS.

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Transcription factors belonging to the steroid/thyroid/retinoic acid receptor family have previously been shown to affect HIV-1 gene expression in non-central nervous system-derived cells. We have investigated the presence and the role of the retinoic acid receptors (RAR and RXR) and of the orphan nuclear receptor COUP-TF in human glial, neuronal and microglial cell lines. Cotransfection experiments revealed that these nuclear receptors regulate HIV-1 gene transcription directed by the long terminal repeat (LTR) region in a cell type-specific manner. In oligodendroglioma, but not in astrocytoma cells, COUP-TF, RAR and RXR function as potent transcriptional activators. In neuronal cells, LTR-driven transcription is enhanced by COUP-TF in the presence of dopamine, a catecholamine neurotransmitter. In microglial cells, COUP-TF also stimulates HIV-1 gene transcription. These results identify COUP-TF and the dopamine signaling pathway as important activators of HIV-1 gene expression in different brain cells. Moreover our results reveal a novel mechanism of retinoic acid-induced repression of the transcriptional activation mediated by the retinoic acid receptors. Our data further reveal the complexity of the molecular mechanisms which govern the activation mediated by the LTR of the lymphotropic LAI and the neurotropic JR-CSF HIV-1 strains. Depending on the LTR sequence and the nuclear receptor, transcription is activated either via direct interactions with the -356/-320 LTR binding site or via cross-coupling interactions with downstream-located proteins.

TNF ALPHA TOXICITY IN PRIMARY HUMAN BRAIN CELLS IS ALTERED BY OVEREXPRESSION OF BCL-2.

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Objective: To determine if apoptosis from TNF alpha treatment of primary human neural cells in vitro can be altered by gene manipulation.

Methods: TNF alpha-induced apoptosis in primary human brain cell aggregates was determined by ELISA assay for DNA Fragmentation; necrosis was determined by ELISA assay for DNA release. The level of bax mRNA in TNF alpha-treated brain aggregates was analyzed. Since bcl-2 has been shown to reduce apoptosis, primary brain aggregates were transfected with bcl-2 to overexpress this gene.

Results: After 24 hours of TNF alpha treatment, there was an increase in DNA expression in brain aggregates which preceded apoptosis followed by a decrease in bax after 48 hours. Brain aggregates were successfully transfected with bcl-2 and a control vector with resulting high viability. Neurototoxicity form TNF alpha was suppressed in 80% which correlated with the number of neural cells transfected.

Conclusions: We have successfully shown alterations in bax expression in a primary human brain aggregate system after treatment with TNF alpha. By overexpressing bcl-2, neurototoxicity from TNF alpha was suppressed.

INFECTION OF HUMAN FETAL ASTROCYTES BY PRIMARY HIV-1 ISOLATES.

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Human immunodeficiency virus type 1 (HIV-1) infection in the brain has been associated with neurological and neuropathological manifestations in both adults and children. Microglial cells are the predominantly infected cell type in vivo. Although astrocytes appear to be infected by HIV-1, it is yet unknown whether primary isolates of HIV-1 can replicate in fetal astrocytes. We selected 26 HIV-1 isolates with different phenotypes to infect astrocytes. The infected cells were monitored by PCR and detection of p24 antigen in the supernatants. Eight of the 26 isolates infected astrocytes. Interestingly 5 of the 9 had a slow/low phenotype. No p24 Ag was detected in the supernatants of the cultures. We also evaluated IL-1 beta production by a T-cell line for induction of HIV-1 expression in the latently infected cultures. The results show that either IL-1 beta or Jurkat-T cell line induce HIV-1 active replication in fetal astrocytes. Analysis of the V3 loop sequences of the isolates able to infect astrocytes did not reveal the presence of any particular amino acid position crucial for the infection of astrocytes. The V3 loop does not appear to play a role in the uptake of the virus in astrocytes, since V3 loop antibodies did not prevent the HIV-1 infection. In this study, we present evidence that fetal astrocytes can be infected by primary HIV-1 isolates which results in viral latency which can be reactivated using cytokines and/or a susceptible T-cell line.
HIV AND SIV BRAIN ENDOTHELIAL CELL TROPISM IN AIDS DEMENTIA COMPLEX (ADC). S. STENGLEIN1, A. Mose1, R. Ruhl1, B. Grosskreutz1, K. Wemyss2, D. Watry2, T. Lane3, H. Foxe3 and J. Nelson1. Oregon Health Sciences University, Portland, OR1; Rocky Mountain Laboratories, Hamilton, MT2; The Scripps Research Institute, La Jolla, CA3.

The development of ADC is dependent on HIV entry into the CNS which is contingent on the ability of HIV to cross the blood-brain barrier (BBB). Brain microvascular endothelial cells (BMVEC) which are a primary component of the BBB and constitute a primary obstacle for viral entry into the CNS are infected by both HIV and SIV in vivo and in vitro. We have mapped HIV genetic determinants which determine BMVEC tropism utilizing recombinant virus derived from BMVEC tropic (NL-A-3) and non-tropic (JR-CSF) strains. This region maps between positions 5786-6091 and includes HIV open reading frames for vpr, vpu, rev, tat, and the C1 portion of env. This region was unique for BMVEC tropism and did not co-segregate with either macrophage or T-cell tropism. To extend these observations in vivo, we have also established an SIV/macaque model in which a highly neuroinvasive and neuropathogenic SIV variant (SIVmac182) was isolated by serial peripheral injection of SIVmac251-infected microglia into naive macaques. Interestingly, the neuroinvasive (SIVmac182) but not the parental (SIIVmac251) strain infected BMVEC in vivo and in vitro. Use of this similar model will elucidate the role of BMVEC in the development of ADC.

DIFFERENTIAL TROPISM OF ACUTE HIV-1 ISOLATES FOR MONOCYTE DERIVED MACROPHAGES (MDM) AND BRAIN MACROPHAGES/MICROGLIA.


The factors responsible for the neurovirulence of HIV are poorly understood, but virus is present in the CNS at all stages, and brain macrophages/microglia are the most commonly infected cell type.

To determine if acute isolates could be neurotropic, twenty-seven HIV-1 isolates obtained from acutely infected individuals were tested for replication in adult microglial cultures and compared with replication in MDM. Most isolates displayed parallel tropism, but several replicated preferentially in one of the 2 cell types, suggesting that although MDM- and microglial tropism overlap, some isolates manifest more discrete phenotypes. Of four isolates that replicated preferentially in microglia, two came from the only individuals who had neurological manifestations at the time of primary infection.

One isolate was adapted to microglia by 14 sequential passages, at which point it demonstrated a 100-1000 fold increase in p24 production in comparison with the original unpassaged virus stock. Cultures infected with the passaged virus showed marked cytopathy that was not seen with the original stock. These data suggest the hypothesis that HIV-1 infection can be established in the CNS by viruses present early in HIV infection, and that once established, adaptation can result in the selection of a pool of predominantly neurotropic viruses.

DNA SEQUENCE ANALYSIS OF 1-LTR CIRCLES OF HIV PROVIRAL DNA FOUND IN AIDS BRAINS WITH MULTINUCLEATED GIANT CELLS.

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Background: HIV encephalitis is histologically characterised by the presence of multinucleated giant cells. These cells of monocyte/macrophage origin. They contain high levels of unintegrated HIV proviral DNA.

Methods: 13 frontal lobes with multinucleated giant cells and no other pathology were compared with 13 frontal lobes without multinucleated giant cells but with other histopathological evidence of secondary pathology. The studies performed were (a) PCR and sequencing of the 1-LTR region of the circular forms of HIV DNA found, (b) immunohistochemistry for gp41 and p24.

Results: In 9 of 13 brains with multinucleated giant cells, circular forms of HIV DNA were found. They were present predominantly as 1-LTR circles. Immunohistochemical staining for gp41 and p24 was strongly positive. In brains without these cells but with other secondary pathology, low MWT forms (ie: 2-8 kb) of HIV proviral DNA were found but their presence was not due to 1-LTR or 2-LTR circles of HIV proviral DNA. Immunohistochemistry for gp41/p24 was usually negative. Data from a 500 bp region of the LTR of both integrated proviral DNA and unintegrated 1-LTR circular proviral DNA showed sequence conservation. Analysis of the 1-LTR circles in 6 brains and of HIV-LTRs in lymphoid organs from the same patients also showed considerable sequence conservatism.

Conclusions: Only AIDS brains with multinucleated giant cells are associated with the presence of 1-LTR circles of HIV proviral DNA. The conservation of sequence in both the LTR from integrated provirus and unintegrated 1-LTR circles suggests that the 1-LTR circles have the correct control elements for transcriptional activity. LTR sequences of brains from different patients showed high sequence conservation which was also reflected in the lymphoid organs.

'H-MAGNETIC RESONANCE SPECTROSCOPY (H-MRS) INDICATES NEURONAL LOSS IN SIV MACAQUE BRAIN: POTENTIAL MODEL FOR AIDS DEMENTIA.


Introduction

Infection with either Human or Simian Immunodeficiency Virus (HIV or SIV, respectively) often results in mental abnormalities (AIDS Dementia Complex (ADC) in humans). As SIVmac affects tissue macaques similarly to HIV in humans, it is considered an animal model for AIDS and ADC. Neuronal loss has not been shown in SIV brain. We quantified, therefore, N-Acetyl Aspartate (NAA), a recognised neuronal marker, in extracts of SIV infected and control macaque brain to determine whether neuronal damage occurs.

Methods

Nine animals infected with isolates of SIVmac were compared to six serologically SIV-negative rhesus monkeys. Brain tissues were collected at necropsy for histopathology and H-MRS.

Results

Two SIV infected macaques developed encephalitis, four showed nonspecific inflammatory lesions, one developed septicaemia and two had no inflammatory CNS lesions. No histologic abnormalities were observed in controls. A significantly reduced NAA in the SIV group compared to controls (2.9±1.7; 6.2±1.7) µmol/g wet weight, respectively (p<0.001) and trends between NAA content and CNS histopathological lesions were found.

Discussion

The decreased NAA content in SIV brain indicates extensive neuronal damage in SIV brain, as seen in ADC patients. This is the first report of neuronal damage in SIV brain and confirms its suitability as an animal model of ADC.
MOLECULAR MIMICRY IN HIV-1 NEUROPATHOGENESIS: HUMAN ANTIBODIES THAT BIND TO GP120 V3 LOOP AND HUMAN BRAIN PROTEINS (HBP)

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Background and Objective: AIDS Dementia Complex (ADC) is a frequent neurological complication associated with HIV-1 infection. The precise mechanisms for ADC pathogenesis are unknown, but could include induction of autoimmune responses. For example, retroviral infections may involve molecular mimicry. We have described molecular mimicry between the HIV-1 gp120 V3 loop and HBP. Importantly, patients with ADC have anti-gp120 antibodies including anti-V3 loop antibodies. In the present study we sought to characterize: (1) human antibodies that bind to V3 loop and HBP, and (2) V3-like epitopes in HBP.

Methods and Results: Using homogenized tissues from normal brains and sera from 26 ADC patients, the presence of cross-reactive antibodies to V3 loop and HBP were analyzed. Western blot analysis of the brain proteins revealed that 7 from 26 ADC patient sera recognized the V3-like HBP. Furthermore, using paraffin-embedded brain tissue, we determined the anatomic distribution of HBP that react to anti-V3 antibodies. Both immunohistochemistry and laser confocal scanning microscopy showed that primarily neurons expressed the V3-like domain.

Conclusions: The results indicate that the V3 loop of HIV-1 gp120 shares an epitope with HBP. An immune response to the V3 loop that generates antibodies that cross-react with neuronal proteins may be an autoimmune mechanism by which HIV-1 infection damages the central nervous system.

INDUCTION OF IMMUNOLOGIC NITRIC OXIDE SYNTHASE IN AIDS DEMENTIA CORRELATES WITH MACROPHAGE ACTIVATION, HIV-1 VIRAL GLYCOPROTEIN EXPRESSION, AND NEUROTOXICITY

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Neurocognitive deficits are common in HIV-1 infection and 20-30% of patients with AIDS develop progressive dementia during the course of their illness. The pathogenesis of AIDS dementia remains elusive. Tissue injury most likely results from indirect mechanisms mediated via macrophage-astrocyte interaction. We have shown that activation of immunologic nitric oxide synthase (iNOS) in in vitro culture systems can cause neuronal injury. We therefore tested the hypothesis that activation of iNOS may be linked to the development or progression of HIV dementia. Using semi-quantitative reverse transcriptase polymerase chain reaction (RT-PCR) for human iNOS mRNA expression we analyzed cortical brain tissue from HIV positive (AIDS) demented and non-demented patients and seronegative controls. Human iNOS is markedly elevated in AIDS with severe dementia and modestly elevated in AIDS with mild dementia and in AIDS without dementia compared to seronegative controls. The clinical severity of dementia correlates significantly with macrophage activation as assessed by HAM56 immunostaining. Also, gp41 immunostaining trends higher in the most severely demented cases. Application of gp41 to mixed neuronal/glial cultures results in neuronal cell death that is reversible with NOS inhibitor L-NAME. These data indicate that iNOS activation and subsequent NO production may play a role in severe AIDS dementia.