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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 associeted 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 effect of gp 120 "in vivo" is more controversal. In this report, we developped 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 technic 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 ovoid 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-β1) 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 (HSV-1) *tat* gene transfer vector, dSTat, was used to transiently express HIV-1 Tat in glial cell in culture and following intracerebral inoculation in mouse brain in order to directly determine whether Tat can increase TGF-β1 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 HSV-mediated induction of the HIV-1 enhancer or the NF-κB domain. Northern blot analysis of human astrocytic glial U-87MG cells transfected by dSTat vector DNA resulted in a substantial increase in steady-state levels of TGF-β1 mRNA. Furthermore, intracerebral inoculation of dSTat followed by Northern blot analysis of whole mouse brain RNA revealed an increase in levels of TGF-β1 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 activation of TGF-β1 gene expression in brain. Tat-mediated stimulation of TGF-β1 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 know endogenous neurotoxin and agonist at excitotoxic amino acid (EAA) receptors. Kynurenic acid (KYNA), an intermediate metabolite of KYN, is a broad-spectrum antagonist at EAA receptors and able to block the neurotoxicity of QA. We examined the KYNA 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 KYNA as determined by HPLC, and the activities of enzymes synthesising KYNA, 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, KYNA 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.

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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 ^{14}C 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.

SYNCYTIUM-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 SI/NSI 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 neurotropism of such isolate since the main biological property of HIV-1 isolates infecting the central nervous system is the macrophage tropism, essentially correlated with the NSI phenotype.

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-FL 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 downtream-located proteins.

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 aminoacids 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 neuropsicological tests was performed, when possible, and anxiety and 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 (Ferrarese et al, Annals of Neurology, 33:316-319, 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.

INFECTION OF RHESUS 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 techniques. Cells are cultured with RPMI 1640 in 10% SVF, 2mM Gln, 1% PSN and 100 ui/ml GM-CSF. The medium was changed after 24h then every 4-5 days. Ameboïd 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, CD14, CD64, CD68, MHC classII have been characterized by immunochemical staining 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 disorders after one year of infection although their numbers of T4 cells were less than 500/mm³. Viral detections in the microglial cells culture supernatants were negative for the analysis of the p27 core protein antigen assay. Other monkeys are under investigation.

The HIV-1-INDUCED NEUROTOXIN, TUMOR NECROSIS FACTOR ALPHA, MEDIATES NEURONAL APOPTOSIS BY PLATELET ACTIVATING FACTOR RECEPTOR ACTIVATION

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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 monocytic 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 cPAF. These data suggest that TNFα-induced neuronal apoptosis occurs in part by PAF receptor activation. These results suggest therapeutic interventions to reduce neuronal death in HIV-1 encephalitis.

THE MOLECULAR PATHOGENESIS OF HIV-1 DEMENTIA. HOWARD E. GENDELMAN, HANS S.L.M. NOTTET, YURI PERSIDSKY, ANUJA GHORPADE, CLINT FLANAGAN, BOAZ 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/monocyte 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, quinolinic 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. An animal model system which mimics many aspects of HIV encephalitis was 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 multinucleated giant cell encephalitis, microglial nodules, astrogliosis, neuronal drop-out and local cytokine production. These works, taken together, serve as the foundation for laboratory drug testing designed to prevent HIV-1-induced neuronal dysfunction.

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

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HIV can infect human astroglial cells *in vitro*. 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* infection 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 TNF-α 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 TNF-α induced a very low viral production in astrocyte cultures latently 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 function 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 lentiviruses.

Pure primary astrocyte cultures from adult simian brains were obtained from 18 adult Rhesus (*Macaca mulatta*) and Cynomolgus (*Macaca fascicularis*) macaques. These animals has 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* gene, we detected the persistence of proviral DNA in astrocyte cultures from 8 of the 18 monkeys during 3 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.

EICOSANOIDS PRODUCTION BY HUMAN GLIAL CELLS AND ITS INTERACTION WITH NO° AND $O_2^{\circ-}$ 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 PGF2α 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 needed 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 $O_2^{\circ-}$ whereas astrocytes were poor producers of $O_2^{\circ-}$. In presence of L-NMMA, which inhibits NO° production, cytokine-induced COX 2 activity in human astrocytes was not inhibited, whereas PGF2α 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 PGF2α, while blockade of COX 2 activity by indomethacin inhibited the production of $O_2^{\circ-}$ by microglia. Finally, depletion of superoxides in astrocytes by SOD, increased the PGF2α 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 P17gag 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 p17gag 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 (VI/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.

THE SUSCEPTIBILITY OF HUMAN MICROGLIAL CELLS TO HIV1 DEPENDS ON THE STATE OF DIFFERENTION: ITS MODULATION BY IFNγ AND TNFα

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Isolated embryonic microglial cells in primary culture or in microglial cell lines, either CD4− or CD4+, were unable to support a productive HIV1 infection. To study the relationship between differentiation/activation of microglial cells and their permissivity to HIV1, we tested human embryonic microglial cells in different conditions of culture and stimulation. In unstimulated isolated CD4− microglial cells, provirus can be detected by amplification of a 338 bp of the env region but no RT activity or p24 Ag were present. Provirus detection was abolished after anti CD4 Ab treatment of the cells. A productive HIV1 infection of human embryonic microglial cells (6 times increase compare to control at day 7 pi) was evidenced in primary culture (but not in isolated microglial cells) after prestimulation (200 U/ml) by IL1β or IFNγ + TNFα for 14 days but not by TNFα alone. IFNγ induced a differentiation-like morphological change enhanced by TNFα which by itself had no effect. IFNγ did not increase the low level of endogenous TNFα but stimulated slightly the expression of TNFαR1, as judged by immunoprecipitation. Moreover, IFNγ + TNFα increased the production of PGF2α but not of LTC4 and TxB2 by microglial cells and astrocytes. Their role on IL10 production and its relationship with viral production is under study.

In conclusion: embryonic microglial cells were spontaneously able to internalise HIV1 in a CD4 dependant pathway. A productive infection was obtained after induction of an in vitro differentiation. This was induced by cytokines known to enhance viral replication and which are not spontaneously produced at an early differentiation stage.

ALTERATIONS IN AN ATF/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 macrophage/monocyte lineage (including brain microglia), there is evidence to support the existence of more tightly 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 neuroglial 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 activities 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 demonstrated that ATF/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 binding 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 ATF/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 ATF/CREB binding site. In particular, clone 2615 contains an ATF/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 multimeric ATF/CREB region forms higher order DNA-protein complexes in comparison to probes containing a single site. Analyses are underway to examine the physical variation in the ATF/CREB region and its functional significance in HIV-1 gene expression in cells of immune and nervous system origin.

EVIDENCE THAT A CELL CYCLE REGULATOR, $E2F_1$, DOWN-REGULATES TRANSCRIPTIONAL ACTIVITY OF THE HIV-1 PROMOTER. MONDIRA KUNDU, ALAGARSAMY SRINIVASAN, ROGER J. POMERANTZ, AND KAMEL KHALILI. THOMAS JEFFERSON UNIVERSITY, PHILADELPHIA, PENNSYLVANIA, USA.

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 $E2F_1$, play a critical role in G_1 and S phases by coordinately regulating expression of several important cell cycle-associated genes. Based on recent observations indicating a block in HIV-1 replication in cells arrested in G_1/S phase of the cell cycle, we sought to evaluate the regulatory action of $E2F_1$ on transcription from the HIV-1 LTR. Results from transient transfection of cells with an $E2F_1$-expression plasmid indicated that $E2F_1$ 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 $E2F_1$ repression located distally (-454 to -381), and proximally (-117 to -80) with respect to +1 transcription start site. The proximal $E2F_1$ responsive element of LTR overlaps with the binding site for NF-κB transcription factor. Results from the DNA binding studies indicated that $E2F_1$ binds to the NF-κB motif and this interaction may play an important role in the observed suppression of the viral gene expression by $E2F_1$. The $E2F_1$-mediated suppression of LTR activity was observed in a wide range of human cell lines. Expression of $E2F_1$ 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 involved in the progression of cells through the mitotic cycle could play crucial roles in determining the efficiency of HIV-1 replication during the various stages of infection. The possible role of these factors in viral latency and activation is discussed.

INTERACTION OF HIV-1 COAT PROTEIN GP120 WITH HUMAN EMBRYONIC NEURONS

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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 picomolar doses of gp120 was estimated by immunofluorescence and colorimetric assay at about 30% of neurons. Treatment of cultures with AP5 or nifedipine 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 pM gp120. In contrast, when neurons were subjected to photometric measurements using the calcium dye Indo-1 to monitor the intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$), gp120 (20-250 pM) potentiated the large rises in $[Ca^{2+}]_i$ induced by 50 µM NMDA. The potentiation of NMDA-induced Ca^{2+} responses required the presence of Ca^{2+} in the medium, and was abolished by the NMDA antagonist AP5 and the voltage-gated Ca^{2+} channel inhibitor nifedipine. Moreover, exposure of a subpopulation of spinal neurons (25 % of the cells tested) to 20-250 pM gp120 resulted in an increase of $[Ca^{2+}]_i$ that followed three patterns: fluctuations not affected by AP5, a single peak, or a progressive and irreversible rise of $[Ca^{2+}]_i$.

In conclusion, gp120 was toxic for human neurons, altered $[Ca^{2+}]_i$ homeostasis and potentiated the NMDA-induced Ca^{2+} increases without effect on NMDA activated currents nor on voltage-sensitive Ca^{2+} currents. Ca^{2+} in turn might activate a Ca^{2+} dependent signal transduction pathway such as kinases, an hypothesis under investigation.

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THE EFFECT OF GP120 ON $[Ca^{2+}]_i$ 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 ($[Ca^{2+}]_i$) in neurones.

In the present work, we have found that the effect of gp-120 on the $[Ca^{2+}]_i$ in rat hippocampal neurones is dependent on the age of neurones in culture. $[Ca^{2+}]_i$ was measured using confocal scanning microscopy with Fluo-3AM. In neurones cultivated during 5-8 days gp-120 (200-1000 pM) did not modulate $[Ca^{2+}]_i$ (n=8). In contrast, brief applications of NMDA induced transient rise of $[Ca^{2+}]_i$ in all studied neurones. The existence of responses to NMDA shows: i) that the cells which were not responding to gp 120 are neurones; ii) the initial level of $[Ca^{2+}]_i$ is low and iii) the Ca^{2+} transport systems in neurone are active. After development in culture during 9-11 days, gp-120 (200 pM) increased $[Ca^{2+}]_i$ in 7 out of 13 studied neurones. Maintenance of neurones in culture during more then 13 days resulted in an increase of $[Ca^{2+}]_i$ induced by gp-120 (200 pM) in all (n=31) experiments. To understand the mechanism of $[Ca^{2+}]_i$ 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 $[Ca^{2+}]_i$. Gp-120 modulated neither $[Ca^{2+}]_i$ 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 $[Ca^{2+}]_i$.

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 neurone with intracellular pipette solution.

EARLY NEUROPATHOGENESIS OF SIV: A COMPARISON BETWEEN VIRULENT AND ATTENUATED MOLECULAR CLONES J5 AND C8.

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Deletions of part or all of the *nef* gene of SIV have been shown to result in reduced virulence and furthermore, infection with such deleted forms of virus can protect from superinfection with more virulent strains of virus. The aims of this study were: 1) To determine whether or not infection with SIV_{mac} C8, an 'avirulent' molecular clone, leads to discernible pathology within the brain and 2) To investigate early neuropathology following infection of cynomolgus macaques with either SIV_{mac}C8 or the virulent clone SIV_{mac}J5.

Seventeen control animals and those infected with C8 or J5 for 1, 2 or 4 weeks were examined (n=2, each group), together with longer term infected C8 (n=6) and J5 (n=3) animals, using conventional light microscopy with morphometric analysis of hippocampal neuronal diameter and immunohistochemistry for virus and gliosis.

There was significant neuronal atrophy in the C8 infected animals, and in the J5 group the neurons were smaller still. No infected cells were demonstrated by immunohistochemistry for SIVgp41. In both groups there was an increase in the expression of HLA-DR on microglia, which peaked at 2 weeks and thereafter declined to close to control levels. There was a slight increase in GFAP expression in the C8 and J5 infected animals, seen initially at 4 weeks.

In conclusion we have shown that the molecular clone C8 induces pathological changes in the brain of otherwise asymptomatic cynomolgus macaques and can therefore not be considered completely avirulent. It remains possible, however, that some or all of these changes are reversible. The early peak in microglial expression of HLA-DR suggests that a component of the pathology relates to seroconversion.

DIFFERENTIAL EFFECTS OF SIV_{MAC251}, SIV_{MAC239} AND HIV-2 ON NEURONAL ATROPHY IN CYNOMOLGUS MACAQUES.

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Infection of rhesus macaques with SIV_{mac251} leads to neuronal atrophy of hippocampal pyramidal neurons. We now report a study of the effects of infection with SIV_{mac251} (a macrophage-tropic virus), SIV_{mac239} (a lymphocyte-tropic virus) and HIV-2 (a non-pathogenic virus in macaques) upon hippocampal neuronal diameter in cynomolgus macaques.

The numbers of animals infected and the range of duration of infection for each virus are as follows: SIV_{mac251}: n = 22, range = 1-31 month; SIV_{mac239} , 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. Immuno-histochemistry 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 $14.2 \pm 0.24\mu m$ (mean ± S.E.M.), to $11.80 \pm 0.22\mu m$ in SIV_{mac251} infected animals ($p < 0.0001$) and to $12.40 \pm 0.79\mu m$ in those inoculated with HIV-2 ($p = 0.001$). Macaques infected with SIV_{mac239}, however, had neuronal hypertrophy ($15.28 \pm 0.35\mu m$; $p = 0.016$). Immunohistochemistry demonstrated gliosis and increased MHC Class II expression in SIV infected animals but not in those infected with HIV-2.

In conclusion, infection of cynomolgus macaques with SIV_{mac251} or HIV-2 leads to neuronal atrophy whilst infection with SIV_{mac239}, a lymphocyte tropic virus leads to neuronal hypertrophy but comparable gliosis and MHC Class II activiation. There is little discernible gliosis or immune reaction within the brain of HIV-2 infected animals.

THE FIRST EXON OF *tat* PROTEIN OF HIV-1 CAUSES CALCIUM-DEPENDENT DIRECT NEURONAL EXCITATION AND APOPTOSIS

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We and others have shown that HIV-1 *tat* protein causes neurotoxicity when presented extracellularly. To further determine the mechanism of Tat- mediated neurotoxicity, dissociated cultures of human fetal neurons (gestational age 12-15 weeks) were treated with recombinant Tat prepared from the *tat* gene encoding 1-72 amino acids (first exon) from HIV_{BRU}. Electrophysiological recordings used whole cell and outside-out patch recordings in 2.5 mM Ca^{2+} or nominally calcium-free solutions. Tat produced dose-dependent depolarizations with fast onsets (<60 msec) and long durations (1-4 secs) in both whole cell and outside out patches. The depolarizations reversed at 0 mV. Tat responses were not affected by tetrodotoxin (TTX), but were 50% attenuated by removal of calcium. Pressure ejection of Tat produced a dose-dependent increase in intracellular calcium in neurons and astrocytes as determined by video imaging of cells loaded with the calcium indicator dye (fura-2/AM). Repetitive applications of Tat did not produce desensitization of electrophysiological or calcium responses. Electron microscopy of Tat-treated neurons showed blebbing of the cytoplasmic membrane, and clumping of nuclear chromatin with relative preservation of the cytoplasm. DNA fragmentation with a 180 base pair ladder was identified by agarose gel electrophoresis.

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SURFACE EXPRESSION OF MHC CLASS I MOLECULES ON NEURONS LIMITED BY ß2-MICROGLOBULIN AND PEPTIDE LOADING

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Major Histocompatibility Complex (MHC) class I molecules are composed of a polymorphic heavy chain, ß2-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, ß2-microglobulin and peptide transporter TAP gene transcripts in individual cultured hippocampal neurons combining patch-clamp electrophysiology with single cell RT-PCR.

While neurons lacked ß2-microglobulin and TAP mRNA, approximateley half of the neurons transcribed 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 ($H2\text{-}D^b$) were restored on the cell surface of cultured mouse hippocampal neurons by adding human ß2-microglobulin and an appropriate viral peptide (gp 33, LCMV).

Our data indicate, that ß2-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, APOPTOSIS AND ATROPHY IN RAT BRAIN. K. OLAFSON[1], M. DEL BIGIO[2], J. PEELING[3,4], M. JONES[1], A. NATH[1,5] Departments of Medical Microbiology[1], Pathology[2], Pharmacology and Therapeutics[3], Radiology[4] and Internal Medicine (Section of Neurology)[5], University of Manitoba, Winnipeg, Canada.

To determine the role of HIV-Tat in the pathogenesis of AIDS dementia complex (ADC), highly purified recombinant Tat (amino acids 1-72 representing the first exon of HIV_{BRU}) was injected intraventicularly as single (n=6 rats; 0.26-0.29 millimoles Tat/rat) or repeated injections (n=5 rats) into adult male Sprague Dawley rats. Repeated injections of 0.05 millimoles Tat were given daily for 5 days through an indwelling intraventricular cannula. Following perfusion with 2% paraformaldehyde, parafin embedded tissues were studied by using hematoxylin and eosin, and myelin stained sections, and by immunostaining for glial fibrillary acidic protein. Apoptotic cells were identified using ApoTag™. 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 immunoadsorbed (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 inflammation 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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NEURONAL CHANGES ASSOCIATED WITH FELINE IMMUNODEFICIENCY VIRUS ENCEPHALOPATHY. C POWER[1], J. PEELING[1], T. MOENCH[2], T.L. LANGELIER[1]. *University of Manitoba, Winnipeg MB[1]; Johns Hopkins University, Baltimore MD[2].*

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 neuronal 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 ^{1}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.

A ROLE FOR THE CYTOKINES TNF-α AND IFN-α IN THE PATHOGENESIS OF THE AIDS DEMENTIA COMPLEX (ADC)
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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) or IFN-α (10IU/ml, 50IU/ml) either as a mono-treatment or in combination with IFN-γ (10IU/ml,100IU/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 12,000nM/L at 48hrs) compared to that produced with IFN-γ alone (4,800nM/L at 48hrs). High levels of QUIN (up to 8,314nM/L at 72 hrs) were produced by macrophages treated with10IU/ml and 50IU/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.

Conclusion: 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[1], J.C. MCARTHUR[2], T.L. LANGELIER[1], J.D. GLASS[2], R.T. JOHNSON[2], B. CHESEBRO[3]. *University of Manitoba, Winipeg MB[1]; Johns Hopkins University, Baltimore MD[2]; Rocky Mountain Laboratories, NIAID, NIH, Hamilton MT[3].*

Background: The V1 and V2 regions (V1V2) of the HIV-1 envelope have been shown to influence HIV cytotropism and syncytia-induction.
Objectives: To determine the relationship of brain-derived V1V2 sequences to blood-derived sequences of known phenotype and to the development of HIV dementia.
Methods: Brain-derived V1V2 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, and 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 nonsyncytia-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 V1V2 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 V1V2 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 V1V2 sequences may be associated with different clinical phenotypes.

TNF APHA 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 LDH 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 bax 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. Neurotoxicity form TNF alpha was suppressed by 20% 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, neurotoxicity from TNF alpha was suppressed.

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.

INFECTION OF HUMAN FETAL ASTROCYTES BY PRIMARY HIV-1 ISOLATES

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Human immunodeficiency virus type1 (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 8 had a slow/low phenotype. No p24 Ag was detected in the supernatants of the cultures.
We also evaluated IL-1β, TNFα and a T-cell line for induction of HIV-1 expression in the latently infected cultures. The results show that either IL-1β or Jurkat-Tat 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 anti 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. STENGLEIN[1], A. Moses[1], R. Ruhl[1], B. Chesebro[2], K. Wehrly[2], D. Watry[3], T. Lane[3], H. Fox[3] and J. Nelson[1]. Oregon Health Sciences University, Portland, OR[1]; Rocky Mountain Laborotories, Hamilton, MT[2]; The Scripps Research Institute, La Jolla, CA[3].

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 (NL4-3) and nontropic (JR-CSF) strains. This region maps between positions 5785-6591 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 (SIVmac251) strain infected BMVEC in vivo and in vitro. Use of this simian 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

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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 cytopathology that was not seen with the original stock. These data support 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 are 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 conservation.
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.

^{1}H-MAGNETIC RESONANCE SPECTROSCOPY (^{1}H-MRS) INDICATES NEURONAL LOSS IN SIV MACAQUE BRAIN: POTENTIAL MODEL FOR AIDS DEMENTIA.

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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 rhesus 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 ^{1}H-MRS.

Results

Two SIV infected macaques developed encephalitis, four showed nonspecific inflammatory lesions, one developed septicemia 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 (2.94±1.37; 6.21±1.73 µmols/g wet weight, respectively ($p=0.001$) and trends between NAA content and CNS histopathological lesions were found.

Discussion

The reduced 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 had 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 immunostain assays 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.