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Gonadal steroids' effects on synergistic neurotoxicity of cocaine with HIV-proteins

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HIV-Associated Dementia (HAD) is more prevalent among HIV infected IV drug users than non-users. The HIV infection rate is growing faster among women than men, and IV drug use plays a larger role in HIV transmission to women than it does to men. We have developed an *in vitro* model of human neurotoxicity to investigate cellular mechanisms that may contribute to HAD. Our previous work has shown that the HIV-proteins, Tat and gp 120, are dose-dependently neurotoxic and that physiological levels of cocaine (Coc)(1.6 uM), in conjunction with subtoxic levels of gp 120 and Tat, produces synergistic neurotoxicity.

The goal of this work was to determine whether Coc or its longer-lived metabolite, benzoylecgonine (BE), promotes toxicity and to identify gonadal steroids' effects in this model. After 15-hour drug treatment, cell death was assayed by trypan blue exclusion. We found that BE is not neurotoxic, and unlike Coc, does not synergize with HIV-proteins. This is true even at the supraphysiological levels that may accumulate with repeated Coc use (100uM). Additionally, Coc and HIV-protein synergistic neurotoxicity is reversible by both 17beta-estradiol(E2) (10 nM) and 5alpha-testosterone(Test) (10 nM). Neither the non-aromatizible testosterone analogue, dihydrotestosterone (DHT) (1-100 nM), nor progesterone (1-100 nM) reverses this synergistic toxicity. However, the anti-oxidant, vitamin E (1-100 nM), is potently neuroprotective.

In sum: 1) Neurotoxicity is not due to the Coc metabolite, BE; 2) Coc, not BE, produces synergistic neurotoxicity; 3) E2, Test and vitamin E have neuroprotective effects, but DHT and progesterone do not. This is evidence for a direct interaction of Coc with HIV-protein mediated neurotoxicity. Furthermore, while an estrogen receptor-mediated neuroprotective mechanism is not proven, the current data strongly support such a process. Importantly, our results suggest that investigation of receptor-mediated, steroid-specific treatment of HAD is warranted.

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HIV protein Tat potentiation of the neurotoxic effects of methamphetamine in the striatum of the rat

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HIV infection of the brain can lead to the development of clinical syndromes reminiscent of Parkinson's disease, suggesting that HIV infection may damage nigrostriatal DA neurons. Although the responsible mechanisms have not been well defined, neurotoxic viral proteins, such as Tat, released from infected cells may be involved. Drug abuse is a major risk factor for contracting HIV infection. Methamphetamine (METH), a psychostimulant with high abuse potential, may also be toxic to brain DA neurons. Thus, the combination of METH abuse and HIV infection may lead to substantial alterations in DA neuron functioning. The present experiments examined how Tat, alone and with METH, affects DA release in the striatum. Male rats were given an intrastriatal injection of Tat (25 ug) or vehicle 24 hours before treatment with METH (5 mg/kg, s.c., 4 injections at 2-hr intervals) or saline. Seven days later microdialysis studies were carried out to measure potassium and amphetamine evoked overflow of DA from the striatum. The Tat treatment alone led to no change in evoked overflow of DA, but there was a 16% decrease in striatal DA content. The METH alone led to a 38% decrease in striatal DA overflow and content. The combined treatment with Tat and METH led to significantly greater 68–78% decreases in striatal DA overflow and content. These results indicate that Tat enhances METH-induced striatal damage, possibly in a synergistic manner, and suggest that METH abusers infected with HIV may be at increased risk for basal ganglia dysfunction. Supported in part by USPHS grants DA10115, DA13144 and NS39253.

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Effect of toxic interaction between FIV and methamphetamine on the auditory brainstem response in cats

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It has been shown that methamphetamine (METH) and HIV-1 both induce neurotoxicity in the CNS. However, their potential neurotoxic interaction remains unstudied. Employing the well-established feline model of neuro-AIDS (FIV-PPR), we investigated herein the sensory alterations induced by the

co-administration of FIV and METH. Twenty four cats were divided into 4 groups and housed with ad libitum access to food and water, and maintained on a 12:12-h light-dark cycle. The first group received, during 6 weeks, a weekly (5 days) single daily-administration of METH (1 mg/kg orally) with 2 weeks drug-free between bouts of METH administration. The second group was treated with an identical schedule but, in addition received an intravenous injection (104 TCID50) of FIV-PPR 5 days after the first METH treatment bout. The third group only received an intravenous injection of FIV-PPR. The last group received a placebo ip injection of sterile saline. Auditory brain stem evoked potentials were recorded every 3 weeks in isofluorane anesthetized cats. Results were analyzed using one way ANOVA. METH treated cats infected with FIV showed a delay in the latency of P5 and P6 ($p < 0.05$, compared to control), 4 and 25 days after FIV infection, respectively. Whereas the FIV group showed a significant delay in the latency of P5 and P6 ($p < 0.01$, compared to control), 25 and 88 days post-FIV infection, respectively. Our results indicate that co-administration of METH and FIV induce earlier impairments in the conduction of the electrical potentials upward through the brainstem, indicating a synergistic toxic effect between METH and FIV.

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Premorbid neuropsychiatric status and HIV/AIDS: effects on eye movement control and neuropsychological test performance

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Antisocial Personality Disorder (ASPD) is a severe behavioral disorder, with a childhood onset, that is defined by repeated acts of rules violations, aggression, deceitfulness and theft, or destructiveness. ASPD is a significant risk factor for substance dependence and, thereby, for HIV/AIDS. In previous studies, it has been associated with neuropsychological and neuroimaging abnormalities suggestive of frontal brain dysfunction. The present study endeavored to examine the contribution of ASPD to eye movement and neuropsychological findings in 90 HIV/AIDS patients and 25 seronegative controls. A series of 2 (ASPD− vs. ASPD+) by 3 (HIV- vs. HIV+/viral load < 10,000 copies per ml vs. HIV+/viral load > = 10,000 copies per ml) ANOVAs were performed. Among subjects without ASPD, the gain (i.e., accuracy) and magnitude of smooth pursuit eye tracking declined with HIV seropositivity and viral load. However, among subjects with ASPD, eye movement gain and magnitude were generally impaired and showed no further impairment with the presence of HIV/AIDS. In comparison to subjects without ASPD, ASPD+ subjects also showed significant performance impairments on the Vocabulary and Matrices Subtests of the KBIT (a brief intelligence test). These findings indicate that a premorbid risk factor for HIV/AIDS is itself associated with neurophysiological impairments and can thereby interact with, or add to, the neurophysiological effects of HIV/AIDS.

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Usutu virus, an African mosquito-borne flavivirus of the Japanese encephalitis virus group causes avian mortality in Central Europe

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Surveillance of avian mortality is a powerful tool for the prediction of human cases of certain mosquito-borne viral infections. In late summer 2001 an episode of avian mortality was observed in several species of birds in Austria, reminiscent of the begin of the West Nile Virus (WNV) epidemic in the United States. Dead birds were necropsied and examined by various methods: histology; immunohistochemistry (IHC) using antibodies to WNV; virus isolation in Vero cell cultures; RT-PCR employing specific as well as universal flavivirus primers; nucleotide sequencing and phylogenetic analysis; and *in situ* hybridization (ISH). Pathological and immunohistological investigations suggested a WNV virus infection, a finding which could not be confirmed by RT-PCR and ISH with WNV specific primers and probes, respectively. Subsequently, the virus was isolated, identified by RT- PCR, partially sequenced and subjected to phylogenetic analysis. Interestingly, the isolates exhibited 97% identity to Usutu virus; also, Usutu virus specific ISH was clearly positive. Usutu virus is a mosquito-borne flavivirus of the Japanese encephalitis virus group, which had never been observed outside tropical or subtropical Africa and had never been associated with fatal disease in animals or man. If established in Central Europe, this virus will have considerable effects on avian populations. Usutu virus can be considered a human pathogen since it was isolated from a man with fever and rash. It is, however, unknown, whether this virus has the potential to cause severe or even fatal human disease, as many viruses of this group do.

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Varicella zoster encephalitis in two immunocompetent adults mimicking variant Creutzfeld Jacob disease

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Introduction: Varicella zoster virus (VZV) is a neurotropic human herpesvirus that establishes persistence in dorsal root ganglia subsequent to primary infection. Rarely, reactivation may be complicated by infection of the central nervous system producing encephalitis and / or meningitis.

Methods and results: We describe the clinical presentation of two women aged 18 and 20 years who presented to different centres with subacute encephalopathy characterised principally by psychiatric symptoms. Neither patient was reported to have suffered systemic symptoms or rash prior or during the period of encephalopathy. Neither patient was immunosuppressed. Due to the phenotype of their presentation, variant Creutzfeld Jacob Disease was suspected and subsequently excluded. Although the cases had markedly abnormal EEGs, both had normal MR imaging of their heads.

Cerebrospinal fluid (CSF) obtained at lumbar puncture was tested for a variety of viruses by polymerase chain reaction (PCR) and was positive only for VZV on one occasion in the second of the cases. However, both the cases had evidence of intrathecal synthesis of oligoclonal IgG.

The CSF was screened against a panel of antigens from neurotropic microbes using a dot blot assay. CSFs from both patients were strongly reactive to VZV antigen preparation. Furthermore, the second patient's CSF in addition showed weak reactivity to Herpes simplex virus antigen. Subsequently we used a novel VZV and HSV antigen mediated immunoblotting assay to identify virus specific oligoclonal IgG. This assay confirmed that the intrathecal production of oligoclonal IgG was specific for VZV.

Conclusion: Although PCR detected VZV nucleic acids in the CSF of only one case on one occasion, intrathecal synthesis of VZV-specific oligoclonal IgG was demonstrated using our novel assay in both cases on all occasions. We believe that these cases illustrate the expanding clinical phenotype of VZV encephalitis. Furthermore, they reiterate the need to seek evidence of CNS viral infection not only by PCR, but also through demonstration of microbe-specific CNS humoral response.

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Molecular epidemiology of JC virus genotypes in Italian regions

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Sequencing of human Polyomavirus JC (JCV) genome indicates the existence of at least eight major genotypes and various subtypes. These different viral strains have been primarily detected in specific geographic regions of the world and more recently it has been suggested that some of the identified JCV genotypes could posses a more pronounced neurotropism and/or neurovirulence.

This study reports the results of the first molecular characterization of JCV genotypes and subtypes, defined by a distinctive pattern of nucleotide polymorphism in the viral protein 1 (VP1) coding region, circulating among healthy individuals living in various Italian regions.

JCV DNA was searched in the urine samples collected from 212 immunocompetent subjects of twenty years of age or more, born and living in Northern, Central, Southern and in the main islands Sicily and Sardinia, by means of a nested-PCR that amplify the large T (LT) antigen coding region.

LT DNA was amplified in 97 urine samples with an overall excretion rate of 45.7%, without significant differences between the different studied geographic areas. JCV viruria did not differ between males and females, but it seems related to the age with a low viral excretion in young adults and a significant increase in those older than forty years.

The nucleotide sequence analysis of the VP1 coding region, performed on the amplified viral strains, indicated the presence of JCV genotypes 1-4, with interesting significant differences among the various regions. JCV genotype 1 and 4 are respectively the most (57.7%) and the second more frequent (27.8%) genotype in Italy, followed by genotype 2 (13.4%), whereas genotype 3 seems to be very rare (1.1%). Of particular interest is the recovery of a peculiar point mutation in a representative number of JCV genotype 4 strains (nt 1851: C G) and in a consistent fraction of JCV subtype 1B amplified among some individuals resident in Sicily and Sardinia islands (nt 1787: CA).

The data of this study indicate that the epidemiology of JCV genotypes in Italy is more complex than expected and suggest the possible existence of new viral subtypes that need to be further defined.

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A prospective study on Neuro-AIDS with emphasis on cerebrospinal fluid analysis in an Ugandan rural hospital

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Objective: To define the Neuro-AIDS patterns among HIV-infected patients in North Uganda for designing a diagnostic flow chart.

Patients and methods: 244 HIV-infected patients with neurological impairment admitted to the Internal Medicine Unit of St Mary's Hospital in the period May-December 2001 were prospectively studied. All of the patients underwent clinical neurological evaluation and cerebrospinal fluid (CSF) samples were collected from most of them. CSF was analysed for glucose, Pandy test, differential cell count, and Gram, Zeehl-Nielsen and Indian Ink stains. Frozen aliquots from 44 patients were tested by polymerase chain reaction (PCR) for the detection of HSV-1/–2, VZV, CMV, EBV, JCV, Mycobacterium tuberculosis and T. gondii, and for quantitation of HIV-1 RNA. No patient underwent neuroradiological examination or CD4+ cell counts. Antiretroviral treatment was not available.

Results: 26/44 (59%) patients had symptoms and signs suggestive of meningitis: a definite diagnosis was achieved in 15 patients, including 6 cryptococcal, 6 bacterial, one CMV and two tuberculous meningitis. 11/44 (25%) patients showed a diffuse encephalopathy, that was consistent with AIDS dementia complex in 7 cases. 4/44 (9%) patients presented focal signs suggestive of a focal encephalopathy, including one case of progressive multifocal leukoencephalopathy and one of neurosyphilis. 3/44 (7%) patients had numbness of lower limbs consistent with peripheral neuropathy. CSF analysis enabled to achieve a diagnosis of etiology in 17/44 (39%) cases. The median CSF HIV-RNA load in CSF was 110,000 copies/ml (range <400- > 10^6), with the highest values found in the patients with cryptococcal meningitis or presumptive ADC.

Conclusion: Monitoring of Neuro-AIDS in Africa is an important element of AIDS control program in order to design the best prophylactic and therapeutic protocols for patients with neurological impairments. Diagnosis of neurological complications may benefit from CSF analysis. High CSF HIV RNA levels suggest that CNS infection by HIV may account for substantial morbidity among neuro-AIDS cases.

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Evidence for involvement of pur-alpha in neuronal cell differentiation in a murine model

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Pur-alpha has been implicated in a diverse series of biological events including transcription, replication, and control of cell proliferation and differentiation. Mice with targeted disruption of the Pur-alpha gene in both alleles were apparently normal at birth, but at two weeks of age developed neurological problems manifested by severe tremor and later, spontaneous seizures. The Pur-alpha −/− mice failed to grow normally after two weeks of age and died by four weeks thereafter. Neuorpathological evaluation of brain showed defects in cortical lamination as evidenced by a decrease in the number of neurons and alterations in cortical layers. Immunohistochemical studies revealed a lack of phosphorylation of neurofilaments in large numbers of neurons. Results from Western blot analysis showed a severe decrease in the phosphorylation of the large subunit of neurofilament (NF200) in Pur-alpha −/− mice. Examination of cdk5 and its known activator, p35, a protein which is responsible for phosphorylation of neurofilaments showed no significant changes in the level of these proteins in Pur-alpha −/− mice. However, results from kinase assay revealed a noticeable decrease in the kinase activity of p35 from Pur-alpha −/−. Further study showed the ability of Pur-alpha to physically interact with cdk5 suggesting that the association of Pur-alpha with cdk5 enhances the activity of p35 in the brain. Thus, the cooperative association of Pur-alpha with cdk5 is an important event in neuronal differentiation and corticogenesis.

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Neuroprotective effects of IGF-I against TNF-alpha-induced neuronal damage in HIV-associated dementia

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Human immunodeficiency virus type 1 (HIV-1) infection often results in disorders of the central nervous system including HIV associated dementia (HAD). Clinical manifestations of HAD include cognitive motor disorders as well as behavioral and cognitive abnormalities, which are observed in at least two thirds of AIDS patients. The brains at autopsy often exhibit microglial nodules containing multinucleated giant cells, increased numbers of perivascular macrophages and activated macrophages/microglia in brain parenchyma. Among multiple factors considered to play a role in the pathogenesis of HAD, release of tumor necrosis factor-alpha (TNF-alpha) by activated and/or infected macrophages/microglia cells may participate in the process of neuronal damage. Conversely, activation of the insulin-like growth factor I receptor (IGF-IR) represents a strong neuroprotective mechanism against a wide variety of insults. Here we investigate the ability of IGF-I to protect neuronal cells against the deleterious effects of conditioned medium derived from HIV infected macrophages (HIV/CM).

Our results demonstrate that HIV/CM causes loss of neuronal processes in differentiated PC12 and P19 neurons and that these neurodegenerative effects are associated with the presence of TNF-alpha. Furthermore, we demonstrate that IGF-I rescues differentiated neurons from both HIV/CM and TNF-alpha-induced damage. The neuroprotective effect of IGF-I is due, at least in part, to IGF-IR-mediated activation anti-apoptotic pathways, which are functional in differentiated neurons independently from the presence or absence of HIV/CM. These observations suggest that the neuronal response to injury may depend on the balance between TNF-alpha and IGF-I signaling pathways, and show that ectopic expression of the IGF-IR exerts a strong neuroprotective effect in this HIV-related experimental setting.

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Immunophilins as markers of disease in HIV encephalitis

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Immunophilins (IP) are receptors for immunosuppressive drugs (e.g. FK506) and also aid protein folding, assembly and trafficking. The IP ligand FK506 can inhibit the growth of HIV-1 infected cells *in vitro*. Immunophilins like FK506-binding protein (FKBP-12) can interact with the human immunodeficiency virus (HIV) proteins gag and gp120 suggesting a role in the pathogenesis of HIV infection.

Our preliminary studies showed that FKBP12 is expressed in the normal human brain, especially in basal ganglia, predominantly in neurons and occasionally in glia. The levels are elevated in the substantia nigra, hippocampus and deep gray matter of patients with Parkinson's disease, Alzheimer's disease and dementia with Lewy bodies. Our current immunocytochemical study analyses the alterations in FKBP levels in the basal ganglia and cortex of patients with HIV encephalitis (HIVE), compared to HIV positive patients free of neurologic symptoms and non-HIV controls. Compared to controls, HIVE patients display increased cortical levels of FKBP12, especially in cortical layers III and IV and putaminal neurons, white matter tracts, astrocytes and microglia. Substantia nigra also exhibits increased IP levels in fiber tracts and astrocytes.

The significance of the altered FKBP12 expression in these patients is still to be established. The increased levels might be linked to the abnormal protein folding and axonal transport that characterize the neurodegenerative process and also explain the selective vulnerability of the basal ganglia to HIV infection. Altered expression of FKBP12, a growth- associated protein, in brain regions affected by the disease process might also reflect a compensatory regenerative phenomenon.

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HIV-1 gp120-induced neuroblastoma cell death implicates abnormal expression of COX-2: protection by NS-398 and inhibitors of caspase-1

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Recently, we have reported that gp120 causes activation of the arachidonate metabolizing enzymes, cyclooxygenase (COX) and lipoxygenase (LOX), and this is involved in the mechanism of cell death because inhibitors of COX or LOX activities, i.e indomethacin and flufenamic acid or MK-886 and caffeic acid (1,2), afforded protection. Here we report that human CHP100 neuroblastoma cells express the inducible isoform of COX protein, e.g., COX-2, under normal culture condition and that 10 pM gp120 increases COX-2 expression. To further implicate abnormal COX-2 expression in gp120-caused death, CHP100 cell cultures were incubated with the viral protein in the presence of various concentrations of the selective COX-2 inhibitor, NS-398 (3). Exposure of cultures to 1.0 and 10 microM (n = 5 experiments per concentration) NS-398 prevented neuroblastoma cell death whereas a lower concentration (0.1 microM; n = 4 experiments) resulted ineffective against gp120 citotoxicity. Quite importantly, Ac-YVAD (100 microM) and Boc-Asp-(OBzl)-CMK (2.5 microM), two inhibitors of caspase-1 (i.e. ICE), abolished COX-2 expression enhanced by gp120. Interestingly, the latter effect was seen at concentrations known to prevent enhancement of IL-1beta levels and death of CHP100 cells caused by gp120 (4) thus suggesting that IL-1beta may underlie the enhancement of COX-2 expression observed here.

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HIV-1 infected macrophages and gp120 induce quantitative alterations in neuronal dendrites and axons: relevance to HIV-1 associated dementia

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Damage in dendritic arbor and a reduction in synaptic density are important neuropathological signatures of HIV-1 associated dementia (HAD). This type of damage to the neuritic network is thought to be an early event in the pathway leading to neuronal dropout via apoptosis. While HIV-1 infected and activated brain mononuclear phagocytes (MP) (macrophages and microglia) are believed to cause neuronal injury, the mechanism by which this occurs remains unresolved. To study this process we developed a method to quantitatively assess how specific secretory products from HIV-1 infected and activated MP injure individual neurons. Changes in multiple aspects of individual neurons including the number of neurites, arbors, and branch nodes, the total neuron area, and the maximum and average arbor lengths were quantitatively determined by computer based image analysis. ELISAs for measuring neuronal antigens and apoptosis were also utilized. Our results showed that the crude secretory products from HIV-1 infected and/or activated human monocyte derived macrophages (MDM), purified HIV-1 whole virion (IIIB and ADA), viral protein gp120, and glutamate all produced a significant decrease in the average neurite number and the amount of branching. TNF-alpha only had a minimal effect on the same parameters. Neuronal apoptosis, however, was observed by whole virion, gp120, glutamate and TNF-alpha. Neuronal apoptosis, mediated by gp120, could be blocked by inhibitors to caspases 3, 8, and 9 suggesting a role for these caspases in HIV-1 induced neuronal demise. These observations suggest that HIV-1 infected and activated MP secretory products can cause neuronal damage by different mechanisms. Early stage damage to neurons, such as that seen in the dendritic arbor or branch nodes, has the potential to be reversed. By studying neuronal damage at the level of individual neuron, we hope to better understand this early stage and the process that eventually leads to neuronal demise. Ultimately, this could aid in the search for appropriate targets for therapeutic intervention and the treatment of HAD.

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Striatal neuronal apoptotic cell death induced by HIV-1 Tat/gp120: differential activation of mitochondrial endonuclease G and caspase-3

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HIV infection affects the striatum resulting in gliosis and neuronal losses. To determine whether HIV-1 viral proteins induce striatal neurotoxicity through an apoptotic mechanism, the effects of HIV-1 Tat(1-72) (100 nM) and gp120 (500 pM) were assessed on isolated embryonic day 15 (E15) mouse striatal neurons maintained *in vitro* by using time-lapse photography. To assess the role of particular proapoptotic events, c-jun-N-terminal kinase (JNK1,2) phosphorylation, mitochondrial release of cytochrome c, caspase 3 activation and neuron viability, as well as an alternative apoptotic path involving endonuclease G (endo G), were assessed at 4 and/or 72 h by using enzyme assays and immunoblotting. The results show that Tat and gp120 induced significant neuronal losses at 24, 48, and/or 72 h. Tat increased JNK phosphorylation, cytochrome c release, caspase 3 and endo G activation at 4 and/or 72 h. In contrast, gp120 failed to activate JNK or trigger endo G release from mitochondria. Interestingly, although Tat and gp120 induced significant neuronal losses in striatum, the nature of the apoptotic events preceding death differed. Collectively, our findings suggest that HIV-1 viral proteins are intrinsically toxic to striatal neurons, and Tat and gp120 toxicity is mediated through separate actions involving both caspase 3 and/or endo G. Supported by NIH DA 13559, DA13728 and NS39253.

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Rapid enhancement of IL-1beta expression in discrete intracellular compartments and neuronal apoptosis induced by HIV-1 gp120 in the neocortex of rat are prevented by SDF-1alfa

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Intracerebroventricular (i.c.v.) injection of a recombinant HIV-1 gp120 coat protein causes apoptosis of brain neocortical cells in rat [see 1]. The mechanism of apoptosis involves enhanced expression of the pro-inflammatory cytokine, IL-1beta [see 1], that occurs in discrete subcellular compartments (e.g. mitochondrial, cytosolic and nuclear cell fractions) [2]. Here we report that enhancement of IL-1beta expression by gp120 is a rapid event minimized by prior i.c.v. administration of SDF-1alfa, the natural ligand for the CXCR4 chemokine receptors; the latter chemokine also afforded neuroprotection. For neuropathology, male Wistar rats (250–280 g) were administered with a single dose of gp120 or SDF-1alfa given daily for up to seven consecutive days to each individual rat (1–2 microl volume; 1microl/min rate) and 24 hours after the last injection the rats were perfused-fixed and the brain processed for *in situ* analysis of DNA fragmentation [see 1]; for electron microscopy, brain tissue samples were processed as previously reported [1]. Immunoreactive IL-1beta levels in subcellular fractions from individual brain cortical tissue were assayed using an established, rat specific, ELISA technique (see methods in [2]). Under these experimental conditions, administration of a single dose (100 ng i.c.v.) of gp120 yielded a rapid enhancement IL-1beta expression in the cytosolic, mitochondrial and nuclear fractions of neocortical cells obtained 6 hours after treatment; this effect was minimized by prior administraion of SDF-1alfa (0.25 pmoles given i.c.v. 1 hour beforehand). Interestingly, this dose of SDF-1alfa (0.25 pmoles given i.c.v. 1 hour before gp120) reduced apoptosis typically elicited by subchronic administration of gp120 (100 ng given i.c.v. for seven consecutive days) in the neocortex of rats [1].

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Apoptotic and anti-apoptotic pathways coupled to neuronal chemokine receptors

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Chemokines have been implicated in the neuropathogenesis of AIDS and neuro-inflammatory disorders. However, as both neuronal and non-neuronal cells express chemokine receptors in the CNS, it is unclear to what extent cell type-specific receptors mediate the effects of chemokines on neurons. There is also controversy about the possibility that chemokines may be beneficial or toxic for neurons in various physiological and pathological conditions, their action being affected by the activity of other endogenous systems. Our research aims to determine the direct effects of chemokines on neurons independently of their action on inflammatory and glial cells, and identify the molecular mechanisms that regulate the activity of neuronal chemokine receptors including their interaction with major neurotransmitter and neuropeptide systems. Existing evidence suggest that chemokines may be coupled to both neurotrophic and neurotoxic pathways. Thus, we hypothesized that differences in activation/inactivation of individual neuronal chemokine receptors (i.e. CXCR4) account for the recruitment of apoptotic rather than anti-apoptotic pathways. To test this possibility the interaction of neuronal CXCR4 with SDF-1alpha (its natural ligand) and the neurotoxic HIV-1 protein gp120 has been studied in different models of neuronal apoptosis as well in human cell lines expressing recombinant CXCR4. Biochemical, molecular biological, pharmacological and imaging approaches have been employed to study the activation of chemokine receptors by the two ligands and test their effects on the intracellular pathways involved in neuronal survival and apoptosis. Our data so far suggest that receptor down-regulation/desensitization might not occur at the same rate for the two ligands and that gp120 does not properly activate the receptor and may couple to different downstream pathways. For instance, in neurons as well as in cell lines SDF-1alpha and gp120 differ in their ability to evoke Ca2+ responses and recruit neurotrophic mediators, such as the Akt kinase and the transcription factor NFkB. (Supported by NIH/NIDA 15014-01, and amfAR 02-816-30RG grants to O.M.)

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Neurotoxic properties of HIV+ CSF and macrophage-derived products: looking beyond glutamate receptors

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Neurotoxins of microglial and macrophage origin are thought to underlie the development of neural damage associated with AIDS-related CNS disease. To assess the development of toxic activity and the cellular response to macrophage toxin(s), we examined the kinetics of intracellular calcium changes in response to toxins secreted from infected choroid plexus macrophages as well as toxins in CSF collected from humans infected with HIV or cats infected with the feline immunodeficiency virus (FIV). A similar profile of toxic activity was found from each source, due to the presence of hydrophobic/basic substances of less than 30 kDa. Toxicity of human HIV+ CSF collected within the last 2 years was substantially reduced relative to samples collected prior to the year 2000. Toxic activity from macrophages infected *in vitro* appeared prior to a detectable productive infection suggesting that virus replication was not necessary. When HIV+ CSF was applied to neurons *in vitro*, greater toxicity was seen in mature relative to immature cells. The toxic profile was highlighted by a late calcium deregulation in the neurons as well as large, temporally-linked bursts of activity in microglia. Changes in intracellular calcium were not consistent with a

mechanism involving direct activation of glutamate receptors. Instead, an inhibition of calcium recovery was seen that could be mimicked by inhibition of the plasma membrane calcium pump and possibly other transporters. The ability of the toxins to destabilize intracellular calcium homeostasis by inhibiting calcium recovery processes produces a general deficit that would serve to facilitate neuronal dysfunction in response to any excitatory challenge that relies on increases in intracellular calcium for signal transduction.

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Microarray analyses of genes in JCV infected primary human fetal astrocytes

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The human polyoma JC virus (JCV) is the well established etiological agent for the neurological disorder progressive multifocal leukoencephalopathy (PML), the incidence of which is on the rise with the advent of AIDS. By adulthood about 80% of the population have been exposed to the virus as indicated by anti-JCV antibodies. However, PML is seen to occur as a result of reactivation of the latent virus in the elderly and in immunocompromised individuals with defects in cell mediated immunity. PML is seen in nearly 5% of HIV infected patients and is fatal within a year of diagnosis due to the absence of any effective treatment. In addition, JCV has been found to be associated with brain tumors in animal models and more recently in humans. The present study was undertaken to gain a better understanding of the host cell-virus interactions using microarray technology. Primary human fetal astrocyte cultures were infected with JCV and RNA was extracted 15 days after infection. We then compared the gene expression profiles of JCV infected cells with that of uninfected primary human astrocytes by means of a cRNA microarray system. Approximately 12,000 sequences were examined by the Affymetrix U95A array and among them expression was found to be stimulated or repressed in more than 400 genes in varying fold changes due to the viral infection. We report that the expression was found to be altered in several gene families including cell cycle regulators, transcriptional activators, cytokines, genes involved in signal transduction pathways and a number of genes of unknown function. Information from our microarray analyses should help direct future research in investigating the lacunae in knowledge of JCV pathogenesis and in designing treatment strategies for AIDS patients with PML.

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Leukemogenicity of neuropathogenic Friend murine leukemia virus A8

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A8 virus is a neuropathogenic variant of Friend murine leukemia virus, which induces spongiform degeneration in the central nervous system (CNS) of infected rats. A8 virus induces more aggressive leukemia/lymphoma with rapid progression than clone 57. Studies using chimeric viruses between A8 and 57 demonstrated that the determinant responsible for inducing aggressive leukemia exists in the fragment containing the LTR and 5′ half of the 5′ leader sequence of A8. The chimeric virus Rec2, which contains the pol and env genes of 57 virus on the background of A8, induced leukemic cell infiltration of the CNS. In contrast, after infection by A8 and other chimeric viruses containing the LTR and the 5′ half of 5′ leader sequence of A8, little or no infiltration of the CNS was observed. Therefore, the determinants for CNS infiltration and induction of aggressive leukemia seemed to be located at different sites. So, we first focused on determining the sequences responsible for aggressive leukemogenicity. We constructed mutants with different enhancer motifs in the U3 region of LTR, since major differences between A8 and 57 viruses in the LTR were found in the U3 region, especially in the enhancer motifs. After one of the three FVa motifs in A8-U3 was changed to the NF1/FVb2 motif, the incidence of thymoma in rats infected by the variant virus was decreased. Furthermore, after changing of the NF1/FVb2 motif of 57-U3 to the FVa motif, the variant virus induced thymoma at 7 weeks postinfection. In the thymus, the production of the virus containing three FVa motifs was higher than that when the virus contained two FVa motifs. The transcriptional strength of the LTR containing three FVa motifs was higher than that of the LTR containing two FVa motifs. These findings indicate that the enhancer element FVa contributes to efficient replication of the virus in the thymus and accelerates the manifestation of leukemia/lymphoma.

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Association of JC virus genotype 1 and HLA A3 with human brain tumors

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To verify the possible involvement of JC virus in the pathogenesis of human brain tumors, we performed a study to evaluate the frequency of JCV detection in the tumor tissue of a group of HLA typed patients suffering of high grade brain tumors. JCV DNA was searched using a nested PCR designed to amplify the LT coding region in brain tumor fresh tissue collected from 22 histologically different cases of neuroepithelial-derived brain tumors (16 males, 6 females, mean age 55 years). The presence of LT-antigen transcripts (LT-mRNA) was also searched in the LT DNA-positive samples. Moreover, since it was suggested that a particular viral genomic organization could be associated with a pronounced neurotropism and neurovirulence, the JCV genotypes distribution, as well as the rearrangements

of the transcriptional control region (TCR), have been studied by nucleotide sequence analysis. In 17 of the 22 tumor patients and 46 ethnically homogeneous healthy controls, HLA A, B, C, DQB1 and DRB1 were typed using the SSP method.

JCV DNA was amplified in the tumor tissue of 9 (40.9%) of the 22 patients, and in particular in 8 out of 14 glioblastomas (57.1%) and in one of three astrocytomas (grade III) (33.3%). Due to the limited amount of available tissue, the search of LT-mRNA was performed only in 4 glioblastomas, and 3 samples (75%) resulted positive. The genotype analysis showed the presence of JCV type 1a in 4 and of JCV type 1b in 3 tumor tissues, while the evaluation of TCR rearrangements revealed the presence of 1 archetype-derived (type II) and 2 Mad-4 organizations. The genetic evaluation showed a significant different distribution frequency of HLA A locus ($p < 0.01$, $df = 8$), and in particular of HLA A3 ($p < 0.001$) in tumor patients in comparison to the healthy controls.

On the whole, the data obtained suggest that HLA A3 could be a possible marker of genetic predisposition to develop brain tumors. Moreover, the detection of JCV DNA and mRNA supports the idea that JCV, and in particular genotype 1, could play a crucial role pushing a neoplastic genetic predisposition to glioblastoma.

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Involvement of Wnt signaling pathway in murine medulloblastoma induced by human neurotropic JC virus

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By using the early genome of the human neurotropic polyomavirus, JCV, we have created transgenic animals that develop cerebellar primitive neuroectodermal tumors which model human medulloblastoma. Expression of T-antigen was found in some, but not all, tumor cells, and examination of the clonal cell lines derived from the tumor population showed enhanced tumorigenicity of cells expressing T-antigen in comparison to T-antigen negative cells. Considering the earlier notion on the potential involvement of beta-catenin with human medulloblastoma, we investigated various components of the Wnt signaling pathway including beta-catenin, its partner transcription factor, Lef-1, and their downstream target gene c-myc in these two cell populations. Immunohistochemical staining of the cells revealed enhanced nuclear appearance of beta-catenin in T-antigen positive cells. Results from Western blot showed higher levels of beta-catenin and Lef-1 in T-antigen positive cells in comparison to those in T-antigen negative cells. The enhanced level of Lef-1 expression correlated with the increase in DNA binding activity of this protein in nuclear extracts of T-antigen posotive cells. Results from Northern and Western blot analysis revealed that the level of c-myc expression is augmented both at the RNA and protein levels in T-antigen posotive cells. These observations corroborated results from transfection studies indicating the ability of JCV T antigen to stimulate c-myc promoter activity. Further, co-transfection experiments revealed that the amount of c-myc and T-antigen protein in tumor cells may dictate the activity of JCV early promoter in these cells. Results from the recent studies revealed physical interaction of T-antigen with Wnt factors providing a mechanism for T-antigen induce tumor formation in brain. These observations are interesting in light of recent discoveries on the association of JCV with human medulloblastoma and suggest that communication between JCV and the Wnt pathway may be an important event in the genesis of these tumors.

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Detection of JC virus DNA sequences and expression of viral oncoproteins in human colon adenocarcinomas

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Colon cancer is one of the most prevalent malignant tumors, being the second cause of death due to cancer in the United States. In recent years a great amount of evidence concerning the mechanisms involved in the development of this neoplasia has been accumulated, pointing to a key role for the members of the Wnt signaling pathway in the early stages of malignant transformation. However the etiology of colon carcinomas remains unknown.

In light of earlier observations on the detection of the JCV genome in several colon cancer and the interaction of JCV T-antigen and Wnt signaling pathway, we performed a study on 28 surgically excised human colon adenocarcinomas, utilizing gene amplification and immunohistochemical techniques to further investigate the role of JCV, p53 and beta-catenin in the pathogenesis on human colon carcinomas.

Immunohistochemistry against viral proteins demonstrated the presence of T-antigen in 17 tumors (60.7%). 19 samples were positive for p53 (67.8%), 13 of them also expressing T-antigen (46.4%). The JCV accessory protein Agno was positive in 6 samples (21.4%), and five tumors express both Agno and T-antigen (17.8%). VP-1 was negative in all samples, ruling out productive infection of the virus in the neoplastic cells. In addition, of the total of tumors, 9 were immunoreactive for beta- catenin, and 8 of them positive for both T-antigen and beta-catenin. LEF-1 was positive in 15 samples, TCF-1 in 11 and TCF-4 in 18 tumors.

Results from the present study, Further supports a possible association between JC virus and human colon adenocarcinomas.

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JCV T-antigen interacts with the Neurofibromatosis Type 2 gene product in a transgenic mouse model of malignant peripheral nerve sheath tumors

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The human polyomavirus, JC virus, has recently been associated with several human CNS tumors, including medulloblastomas and a broad range of glial-origin tumors. This ubiquitous virus is the causative agent of the rare demyelinating disease, Progressive Multifocal Leukoencephalopath y in immuocompromised individuals. Expression of the viral protein, T-antigen, which possesses the ability to transform

cells of neural origin, has been detected in human CNS tumors. In an effort to further understand the transforming potential of JCV T-antigen, transgenic mice expressing JCV T-antigen under the control of the Mad-4 promoter were generated. As described previously, approximately 50% of the animals developed pituitary adenomas by one year of age (Gordon, *et al.* 2000 Oncogene 19(42):4840-6). However, a small subset of the animals developed solid masses arising from the soft tissues surrounding the salivary gland, the sciatic nerve, and along the extremeties which histologically resemble malignant peripheral nerve sheath tumors, rare neoplasms that occur in individuals with neurofibromatosis. The tumors were further characterized by immunohistochemistry and Western blot analysis which detected expression of the transgene, T-antigen, in tumor, but not normal tissues. Neurofibromatosis types 1 and 2 are autosomal dominant disorders characterized by multiple neurofibromas and other associated lesions. In both disorders, loss of the gene products of NF1 and 2, termed neurofibromin and merlin, respectively, have been implicated and their roles as tumor suppressor proteins have been suggested. As T-antigen is known to inactivate tumor suppressor proteins such as Rb, we examined the ability of T-antigen to physically interact with NF1 and NF2 in extract prepared from tumor tissue. Results from immunoprecipitation/Western blotting revealed that T-antigen has the ability to bind NF2, but does not associate with the NF1 gene product. Work is in progress to determine the functional consequences of this interaction which may suggest new mechanisms of T-antigen mediated tumorigenesis.

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Physical and functional interaction between the viral protein R and p21waf1

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Human immunodeficiency virus type 1 infection is often associated with the central nervous system (CNS) dysfunction. The viral protein R (Vpr), one of the proteins encoded by HIV-1 genome, is composed of 96 amino acids. Among its many functions, Vpr is involved in the arrest of cell cycle progression at G2 phase. Also, it has been shown by several laboratories including ours that Vpr binds to a number of viral (Tat, p17, p7, and p6 of Gag) as well as cellular proteins (Sp1, p300 and p53). The physical interaction between Tat and Vpr may contribute to increased viral replication through HIV-LTR activation. In this study we identified a novel interaction of Vpr with p21, a cell cycle modulator that controls cell progression throughout G1/S phase. We also found that Vpr and p21 contribute to up-regulation of the HIV-1 LTR in astrocytic cells. The physical interaction between Vpr and p21 was shown by GST-pull down assays and Western blot analysis. This interaction leads to the inhibition of p21 during cell cycle and its degradation in astrocytic cells. Furthermore, overexpression of Vpr overcomes p21-mediated cell cycle arrest, suggesting that Vpr interferes with the inhibitory function of p21. Based on these studies, we propose that the activity of p21 in cells, during HIV-1 infection of astrocyte, can be perturbed through its interaction with Vpr. The implication of these findings in apoptosis will be discussed.