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Nuclear export and cellular localization of human T cell leukemia virus type 1 transactivator protein Tax in immune and nervous system cell targets. A. J. T. Altermatt, H. T. K. Grant, C., and W. Dähne, Department of Microbiology and Immunology, Penn State College of Medicine, Hershey, Pennsylvania USA

Human T cell leukemia virus type 1 (HTLV-I) is the etiologic agent of adult T cell leukemia (ATL) and tropical spastic paraparesis (TSP). During infection, Tax is known to specifically interact with cellular transcription factors, including members of the ATF/CREB family, to enhance viral gene expression. Historically, Tax expression constructs have indicated that Tax normally traffics to the nucleus utilizing the characterized nuclear localization sequence (NLS) at amino acids 2-58. Tax has also been detected in the serum of HTLV-I-infected patients suggesting that it is secreted from infected cells via a specific pathway or cell lysis. To evaluate this hypothesis, we have performed confocal microscopic analyses of cell lines previously utilized for in vitro localization studies (HeLa and CV-1) and cells modeling HTLV-I-Host cell targets (U-373 MG, SK-N-MC, Jurkat, and U-937) subsequent to transfection with full-length and mutant expression constructs encoding Tax proteins conjugated to, via the C-terminus, green fluorescent protein (GFP). As previously described, delivery of full-length Tax-GFP to U-373 MG, SK-N-MC, HeLa, and Jurkat cells led to nuclear Tax localization. In contrast, results using the TaxΔ215-GFP and TaxΔ245-GFP constructs were unexpected. In all cell types except U-373 MG, TaxΔ215-GFP and TaxΔ245-GFP were retained in the cytoplasm with no detectable nuclear localization whereas the TaxΔ58-GFP construct was localized to the nucleus. This is interesting considering that TaxΔ215-GFP and TaxΔ245-GFP still contain the putative NLS. U-373 MG cell transfection results were also of interest. In this cell population, TaxΔ215-GFP and TaxΔ245-GFP were detected in both the cytoplasm and nucleus in a punctate manner while all other constructs the majority of the Tax-GFP fusion protein was localized in the nucleus with several small particles located in the cytoplasm. Further examination of the Tax amino acid sequence reveals a putative nuclear export signal (NES), IEELKYSLI. To determine its functionality, we linked the Tax-NES to GFP and constructed two truncation mutants, TaxΔ100-GFP and TaxΔ60-GFP, which border the NES. An inhibitor of nuclear export (leptomycin B) was included in transfections to halt nuclear export via the CRM-1/exportin pathway. These results suggest that small amounts of Tax do exit the nucleus via the NES during normal infection and could be a prerequisite for a Tax cellular export pathway.

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Detection of infectious agents from brain of patients with acute haemorrhagic leukoencephalitis. M. S. F. Groves, M., S. Scaravelli, F., Department of Neuropathology, Institute of Neurology, UCL, London UK

Acute haemorrhagic leukoencephalitis (AHL) was first described by Hurst in 1947. It is rare, usually fatal, although survival of patients has been confirmed by biopsy. The brains of 6 patients with this disorder and who died between 1963 and 1994 were chosen from the files of the Department. Clinical signals included: recent flu-like illness (1 case); early changes on brain CT (4 cases); presence of red cells in the CSF (1 case); deterioration despite broad spectrum antibiotics and antiviral therapy (2 cases). However, diagnosis was established only by brain biopsy/autoptay.

Except for the classical neuropathological features, histology did not give any clue as to the possible aetiology of the disorder. Using the polymerase chain reaction (PCR) in paraffin embedded brain specimens we could detect varicella zoster virus (VZV) in 2 cases, herpes simplex virus (HSV) and human herpes virus-6 (HHV-6) in one case each. No more than one virus was found in any single case. On the other hand Cytomegalovirus (CMV), Epstein-Barr Virus (EBV) and mycoplasma bacteria could not be found.

AHL is a hyperacute disease. The finding of virus in the brains supports the hypothesis that a viral infection may be implicated in its pathogenesis through an autoimmune mechanism. It is hoped that results of the study of this acute demyelinating disorder may help our understanding of the chronic demyelinating form, multiple sclerosis.

This research was supported by Hugo James Ross Research Fund.

P45

Presence and variable expression of Human Herpesvirus 6 in the lesions of Acute Multiple Sclerosis compared with Progressive Multifocal Leuкоencephalopathy. M. C. O. Goodwin, A. D. Powers, J. M., Baker, J. V., and Blumberg, B. M., Department of Neuroimmunology, University of Rochester, Rochester, Rochester, N. Y.

MS and PML are distinct demyelinating diseases of the central nervous system which nonetheless share pathological features of inflammatory focal demyelination. Demyelination in PML is widely believed to result from cytology of oligodendrocytes as a consequence of JC virus infection. The pathogenesis of MS is unknown but is thought to be immune-mediated, perhaps in response to viral infection. Recent work from our laboratory has demonstrated extremely high frequencies of HHV6 DNA containing cells (primarily oligodendrocytes) within and surrounding the demylinating lesions of PML. Immunocytochemical studies have confirmed dual infection of lesional oligodendrocytes with JCV and HHV6 as assayed by antibody to the JCV large T antigen and to HHV6 p41 and p101 antigens. In the present study, we used a unique two-step in situ polymerase chain reaction (ISPCR) technique together with immunocytochemistry to HHV6 p41, p101, and gp116 antigens in fifteen stereotactic biopsy specimens from six patients presenting clinically with acute CNS mass lesions. However, their subsequent histopathology, MRI imaging studies, and clinical course confirmed the diagnosis of MS. Very high frequencies of oligodendrocytes, microglia, and lymphocytes containing HHV6 DNA were found within and surrounding the lesions. In addition, immunostaining with antibody specific for the HHV6 gp16 demonstrated staining of cells that appeared, morphologically, to consist mainly of reactive astrocytes not some microglia. In contrast to PML, no immunostaining to HHV6 p41 or p101 antigens was detected. Control cases, including a variety of cases with other neurological diseases, demonstrated consistently lower frequencies of HHV6 DNA and no immunostaining to these HHV6 antibodies. The consistent presence of high frequencies of cells containing HHV6 DNA by ISPCR together with expression of the HHV6 gp116 in these earliest available acute MS biopsy specimens supports a potential role for HHV6 in the pathogenesis of Multiple Sclerosis.

P46

Viruses can silently prime for autoimmune CNS disease. T. Thei, D., 1, T. Trojcm, I., 1, Rodriguez, F., 2, Whitten, J. L., and Fujimori, R., 1, Department of Medicine, University of Utah School of Medicine, Salt Lake City, Utah USA and 5, Department of Neuroimmunology, Scripps Research Institute, La Jolla, California USA.

The etiology of multiple sclerosis (MS) is not known. While viruses have been implicated in the pathogenesis and many viruses have been isolated from MS patients, no single agent has been identified as the causative agent. However, epidemiological data suggests that infections early in life can set the stage for MS. We propose a model where infections having molecular mimicry with central nervous system (CNS) proteins early in life could silently prime an animal for experimental allergic encephalomyelitis (EAE), which is triggered later by a non-specific challenge. Three-week-old SJL/J mice were injected with a DNA encoding ubiquitinated myelin proteolipid protein (PLP), pCMV/pLp. The ubiquitination of PLP favors presentation through the MHC class I pathway, similar to viral proteins during infection. Mice were injected 3 times with pCMV/PLP. This procedure does not by itself induce EAE. When mice were subjected to a non-specific challenge of complete Freund’s adjuvant (CFA), 20% of animals developed clinical signs and CNS lesions. Lymphocytes proliferated in response to the encephalitogenic PLP peptide PLP139-151. Next, instead of priming mice with pCMV/PLP, mice were infected with recombinant vaccinia viruses encoding myelin associated glycoprotein or glial fibrillary acidic protein, and later given a CFA challenge. Five of 5 mice in each group developed inflammatory lesions in myelinated regions of the CNS. In the last set of experiments, mice were sensitized with pCMV/PLP and challenged later with a recombinant vaccinia virus encoding g-halocollidase instead of CFA. Twenty percent of mice developed inflammatory lesions in the CNS. These data indicate that viruses having cross-reacting epitopes with self-CNS proteins can silently prime for autoimmune CNS disease, which can be triggered by a nonrelated challenge at a later time.

This research was supported by NIH grant AI42525.
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Herpes simplex virus induces cytokines and chemokines in nonproductively infected primary human microglial cells but not during viral replication in astrocytes or neurons. Lokenesgard, J.R.; Hu, S.; Sheng, W.; vanOjen, M.; Cheeman, M.C.-J.; Gekeer, G.; and Peterson, P.K., Institute for Brain and Immune Disorders, Minneapolis Medical Research Foundation, and University of Minnesota Medical School, Minneapolis, MN

Local expression of cytokines has been demonstrated to correlate with control of viral replication in the brain. Cytokines likely play an important role in regulating inflammation during viral encephalitis. We examined cytokine (TNF-α, IL-1β, IL-6, and IFN-α, β, and γ) and chemokine (IL-8, IP-10, MCP-1, MIP-1α, and RANTES) production by herpes simplex virus 1 (HSV) infected primary human brain cells. Primary astrocytes as well as neurons were found to support viral replication but neither of these permissve cell types produced cytokines or chemokines in response to HSV infection. On the other hand, microglial cells did not support HSV replication and no immediate-early (ICP4) or late antigens were detected by immunochemical staining. However, through the use of a virus containing a cellular promoter driving reporter gene expression, microglia were found to be nonproductively infected with HSV. Microglial cells responded to this nonpermissive viral infection by producing considerable amounts of TNF-α, IL-1β, IP-10, and RANTES, together with smaller amounts of IL-6, IL-8, and MIP-1α, as detected by RNase protection assay and sandwich-ELISA.

Surprisingly, no interferons (α, β, or γ) were produced by microglial cells in response to viral infection. These results support a role for microglial cells in antiviral defense of the brain and the amplification of the immune response during inflammation.

This research was supported by PHS grants NS-38836 and DA-04381.

P48

E2F1 and hyperphosphorylated Rb exhibit altered subcellular localization in models of HIV. K.L. Jordan-Schettino, S. Brown, B. Murray, G. Wang, C.A. Wiley and C.L. Achin, Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA

Neurodegeneration associated with HIV encephalitis (HIVE) has been associated with factors produced by infiltrating, HIV-infected or activated macrophages, including chemokines and neurotrophins. We hypothesized that the plethora of signals present in the extracellular milieu of the HIVE brain causes inappropriate activation of the neuronal cell cycle machinery. To test this hypothesis we examined cell cycle regulators: Rb, and E2F1 in an in vitro and an in vivo model of HIVE. Our in vitro system is based on second trimester human fetal neuronal cultures treated with neurotrophins, chemokines, or HIV-infected macrophage conditioned media. In parallel, we analyzed the basal ganglia and frontal cortex from SIV infected macaques that have developed encephalitis. We have consistently observed changes in E2F1 and hyperphosphorylated Rb (pRb) in both models. In controls (untreated neuronal cultures or non-encephalitis brain regions) we see E2F1 and pRb in the nuclei of neurons and astrocytes. However, after treatment of cultures with chemokines or neurotrophins or in cases with encephalitis, both E2F1 and pRb are observed in the cytoplasm of neurons. E2F1 is seen also in the cytoplasm and processes of astrocytes, while pRb remains nuclear in these cells in both models. We are currently assessing the changes in E2F1 and Rb subcellular localization in response to treatments with supernatants from HIV infected macrophages and in the brains of patients with HIVE. Our results suggest that cell cycle regulators are responding to extracellular signaling in HIV and may contribute to neuronal death. MH 58528, NS 35731, NS10572

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THE TOPOGRAPHY OF LYMPHOCYTIC CHORIOMENINIGITIS VIRUS (LCMV) AND OF ITS PUTATIVE RECEPTOR IN THE DEVELOPING RAT BRAIN. Bonthius, D.J., Taggour, D., Mahoney, J., and Assouline, J., Departments of Pediatrics, Neurosurgery and Pharmacology, College of Medicine, University of Iowa, Iowa City, IA

LCMV is a well-known pathogen that can cause severe damage to the developing human brain when the infection occurs during pregnancy. Although the pathogenesis of the virus is known to be immune-mediated, little is known regarding the mechanism of its neurotropic pattern. Recently, the dystrophin-associated glycoprotein, alpha-dystroglycan (αDG) was identified as a putative receptor for LCMV. In this study, the developing rat brain model was used to compare the topography of LCMV infection with the spatial expression αDG. Following a single intracerebral injection of 1000 pfu of LCMV on postnatal day 4 (P4), brains were examined at a variety of time points post-inoculation (PD6 to PD120) using immunolabeling for LCMV and αDG. Within 4 days of inoculation (PD8), the leptomeninges, choroid plexus, ependymal lining, as well as astrocytes contain large quantities of viral antigen. Furthermore, each of these cell types expresses αDG. By PD12, the infection begins to spread from astrocytes to neurons. Purkinje cells, mitral cells of the olfactory bulb, periventricular neurons and granule cells throughout the brain are selectively infected. Interestingly, αDG is present on the somatic surfaces of each of these cell types, with the exception of the dentate granule cells, which express αDG only on their dendritic trees. However, some neuronal populations express αDG but do not label for the virus. Beginning at PD21, viral antigen is no longer detectable in astrocytes, but is restricted to neurons. By PD90, the virus is cleared from all granule cell populations, but viral antigen persists in Purkinje cells, mitral cells and periventricular neurons. Viral antigen is no longer detectable by PD120. We conclude that inoculation of the neonatal rat with LCMV results in a highly stereotyped natural history and topography of infection and that expression of αDG on cell surfaces may be a necessary, but not sufficient, factor to explain LCMV infectivity. Supported by NIH grants NS02007 and P30 HD227748.

P50

Potential degradation of prions by murine bone marrow-derived dendritic cells. Lin, K., Wallin, B., Ljunggren H.G., and Kristensson K., Department of Neuroscience, and Microbiology and Tumor biology Center, Karolinska Institute, Stockholm, Sweden

Prior to involvement of the central nervous system, infectious prion particles can be detected in lymphoid tissue, in which they can reach high titers. The cellular type, which in these tissues is responsible for the ensuing spread of prions to the central nervous system, is under debate. However, a number of reports have associated prion replication with prion protein-expressing follicular dendritic cells. In order to examine if dendritic cells, in general, are involved in the pathogenesis of prion infections, we analyzed the occurrence of prion protein in a most commonly used system for studies on dendritic cell functions; primary cultures of mouse dendritic cells originating from bone marrow precursor cells. In our experiments the precursor was differentiated into post-mitotic dendritic cells with the granulocyte macrophage colony stimulating factor. Immunoblot analyses using the recombinant antibody D13, failed to reveal any cellular prion protein in these cells, but showed marked positivity in GT1-1 cells, which is a hypothalamic cell-derived cell line that favors prion replication. When prion-infected GT1-1 cells were co-cultured with the dendritic cell, a rapid killing of the GT1-1 cells and loss of prions were seen. Thus, subpopulations of dendritic cells may serve an important role in degradation of a prion proteins.

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A viremic threshold is required for neuroinvasion by Venezuelan equine encephalitis virus, Bernard, K.A. and Johnston, R.E., Department of Microbiology and Immunology, University of North Carolina, Chapel Hill, North Carolina USA

Venezuelan equine encephalitis virus (VEE), an alphavirus, causes a fatal CNS disease in mice. After subcutaneous (SC) inoculation of mice, neuroinvasion occurs via the olfactory and trigeminal cranial nerves. It was hypothesized that a viremia was required for neuroinvasion via this neural route. In this study, molecularly cloned VEE viruses were used: a virulent, parental virus and eight attenuated, site-specific glycoprotein mutants derived from the parent virus. Eight mice per group were inoculated SC with \(10^3\) PFU of virus. A serum sample was obtained at peak viremia, 24 hours post-inoculation. At 72 hours, brains were harvested for virus titration. The parental virus and three of the mutants invaded the brain in 100% and 50-100% of the mice, respectively. These mice had a viremia of \(10^2\) to \(10^4\) PFU/ml for the parental virus and \(10^4\) to \(10^5\) PFU/ml for the three mutants. In contrast, the other five mutants produced lower viremia (<250 to \(10^4\) PFU/ml) and did not invade the brain in any of the mice. Logistic regression analysis revealed a strong correlation between the level of viremia and neuroinvasion (p=0.0001), and the data support a viremic threshold of \(5 \times 10^4\) for neuroinvasion. Furthermore, when three "low viremic" viruses were inoculated intravenously, neuroinvasion occurred in 15 out of 18 mice. These attenuated viruses, therefore, are not innately defective for neuroinvasion, but are unable to produce an adequate viremia for neuroinvasion to proceed. Previous results suggested that one mechanism for low viremia was rapid clearance from the blood, which correlated with heparan sulfate interaction in vitro. This work was supported by NIH AI-01432-02.

P52

Role of Systemic Events in the Development of HIV Encephalitis and Dementia. Suzanne Gartner, Yifeng Liu, Edward Hunter, Ned Sacktor, Katherine Conant, Carlos A. Paolo and Justin C. McArthur, Dept of Neurology, Johns Hopkins University; Baltimore, MD, USA

We have recently proposed a model suggesting that HIV dementia is linked to increased activation and subsequent enhanced trafficking of blood monocytes into the brain parenchyma (Science 287:602-604, 2000). To further explore this possibility, we performed immunohistochemistry sequentially collected specimens from patients with and without HIV-associated cognitive impairment. We also performed phylogenetic analyses of HIV gp160 sequences from uncultured anti-mortem blood specimens and post-mortem tissues from patients with AIDS. In addition, we used immunohistochemistry to characterize brain macrophage populations. Cognitive impairment was associated with increased numbers of CD16+ monocytes, which persisted in some patients. CD16 expression on monocytes, a marker of activation, was often associated with downregulation of CD14 expression. In some cases, we also detected a considerable number of CD130(CD14)+ cells, a phenotype which could reflect egress of more immature monocytes from the bone marrow (BM). HIV gp160 sequences from the deep white matter (DWM), or subcortical regions of brain, clustered with sequences from BM, but not with those from lymphoid tissues. In some cases, high homology between BM and brain sequences was observed. Blood monocyte and T-lymphocyte populations were found to harbor different gp160 sequences and moreover, the monocyte, but not the T-lymphocyte sequences, clustered with sequences recovered from brain and BM tissues collected subsequently, following death. To attempt to distinguish perivascular macrophages from resident microglia, we used anti-CD14 immunostaining. In sections of DWM from HIV-negative individuals and HIV-infected patients without dementia, we observed occasional CD14+ cells situated exclusively adjacent to blood vessels. In contrast, sections of DWM from patients with HIV dementia contained large numbers of CD14+ cells both at perivascular locations, as well as within focal areas of the parenchyma. Some CD14+ cells exhibited a ramified morphology, whereas others were amoeboid in shape. This increase in CD14+ cells could reflect increased trafficking of blood monocytes into the brain, or alternatively, the induction of CD14-16 expression on resident microglia. These findings indicate that bone marrow-derived monocytes can serve as a mode of HIV entry into the brain. Taken together, they also provide further support for a causal association between enhanced migration of blood monocytes into the brain, and the development of HIV dementia.

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P53

California Encephalitis Project

Carol Glaser, Sabrina Gilliam, Dave Schmurr, Bagher Forghani and Duc Vugia, Division of Communicable Disease Control, California Department of Health Services and Jordan Tappero, CDC, Atlanta

The goal of the California Encephalitis Project is to better understand encephalitis by comprehensive diagnostic testing and collection of detailed epidemiologic data. Physicians in California refer cases to the project. A case is defined as an immunocompetent individual hospitalized with encephalopathy with one or more of the following: fever, seizures, focal neurological findings, CSF pleocytosis, EEG findings consistent with encephalitis or abnormal neuroimaging. Demographic, exposure, clinical and laboratory information is collected on all cases. Spinal fluid, serum, throat and rectal swabs are submitted and a core battery of tests is performed by various methods, including PCR, serology, and viral isolation. Core testing for 14 different agents includes herpesviruses, enteroviruses, arboviruses, Bartonella spp, and Mycoplasma pneumoniae. Individualized testing for other agents is done as needed. Diagnoses are classified as confirmed, probable, possible or unknown based on test results, clinical features of the case. From June 1998 through March 2000, 225 cases were enrolled. Of these, 21 (9%) were later found to have a non-infectious cause of their CNS illness. Of the 204 remaining cases, a confirmed agent was found in 29 (14%) cases; HSV-1 (9), Bartonella henselae (5), encephalitis virus (3), Mycobacterium tuberculosis (3), Yersinia pestis (2), Mycoplasma pneumoniae (2), SSPE (1), Balamuthia macrocra (1) and 3 others. A probable agent was identified in an additional 14 (7%) and a possible agent in 22 (11%) and included rotavirus, adenovirus, influenza and chlamydia. None of the cases had evidence of acute infection with CMV, SLE, WEE or HSV-2. Despite extensive testing using state-of-the-art techniques, a large percentage of cases remained unexplained.

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P54

Comparison of the VH repertoire in MS plaques and corresponding blood reveals a CNS restricted antigen-driven response. Owens, G.P., Burgess, J., M.P., Smith-Jones, P., T., and Gilden, D.D., Departments of Neurology and Microbiology, University of Colorado Health Sciences Center, Denver, Colorado USA

The specificities of oligoclonal IgG in MS CSF and plaques and their relevance to the pathogenesis of MS is unknown. To better understand the nature of B cell activation in MS, we used a nested PCR strategy to obtain a representative sampling of rearranged IgG heavy chain sequences (VH) from MS brain and blood. For comparison, we also analyzed the brain V1 response in individuals with subacute sclerosing panencephalitis (SSPE), an encephalitis caused by measles virus (MV) and characterized by a strong antigen-driven oligoclonal IgG response against MV proteins. Alignment of MS and SSPE V1 sequences to the 51 functional human germline segments revealed the closest VH germline segment, and the extent and location of somatic mutations for each IgG.

When compared to the closest germline segment, VH sequences from both SSPE and MS brains were heavily mutated with a preferential accumulation of replacement mutations in complementarity determining regions (CDRs) relative to framework regions. Although the use of particular family germline segments was biased in individual brains, overall, each SSPE and MS brain had its own unique IgG response. In each SSPE and MS brain studied, specific VH sequences were overrepresented. Within some of the overexpressed populations, distinct sequence differences were detected (clonal variants) indicative of clonal expansion. In one MS brain (MS 98-1), we further compared the VH repertoire in plaques to that in blood. VH sequences found in plaque regions were not detected in a representative sampling of blood. Together, our results indicate that the clonal repertoire found in MS plaques shares the same features as the antigen-driven response in SSPE brain and appears to be CNS restricted.

This research was supported by PHS grant NS-32623.
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JC Virus in the South Pacific: New Variants with an Unusual Regulatory Region Rearrangement in Papua New Guinea. Stoner, G. L.1, Friedlander, J. S., Jobes, D. V., Agostini, H. T., China, S. C., Mgone, C. S., Yanagihara, R., and Ryschawy, C. F. 1NIH, Bethesda, MD; 2Dept. of Anthropology, Temple University, Philadelphia, PA; 3Inst. of Medical Research, Goroka, PNG; 4Univ. of Hawaii, Honolulu, HI

We have studied JCV from the highlands of Papua New Guinea (PNG) (20 samples) and from two ethnic groups on the nearly island of New Britain: the non-Austroenis-speaking Baining (14 samples) and the Austroenis-speaking Tolai (21 samples). Twenty samples were also studied from the Chamorros of Guam. All PNG strains fell into the broad Asian group, but they were distinct from both Type 2 and Type 7. In the Chamorro JCV samples, 8 strains were like Type 7 found in S China and SE Asia (Guam-1), while 9 were different (Guam-2). In PNG we identified three JCV variants in the VP1 gene typing region (PNG-1, PNG-2 and PNG-3). PNG-1 strains were present in all three populations (Highlanders, the Baining and the Tolai), but PNG-2 strains were restricted to the Highlanders. The single PNG-3 strain, identified in a Tolai individual, was closely related to the Guam-2 strains. As the Tolai and Chamorro both speak Austroenis languages, these strains may have originated in a common ancestor. The regulatory regions of PNG-2 variants have a unique deletion with a nested duplication reminiscent of the changes seen in brains of progressive multifocal leukoencephalopathy (PML) patients. This is the first complex rearrangement in JCV (deletion with duplication) found to be excreted widely in a human population. A subtype of the PNG-2 group (PNG-2B) has a deletion in the agnoprotein gene that removes amino acids 57-63 of this 71-kDa protein. We suggest that both the regulatory region rearrangement and the agnoprotein deletion may have given these variants a selective advantage promoting spread within the Highlands. AIDS is now increasing in PNG, and study of PML there may reveal whether variants such as PNG-2B have increased neurotropism or neurovirulence.

P56

Transduction with recombinant polyomavirus protein, VP1, explores mechanisms of cell susceptibility. Jensen, P. N. and Major, E. O., Laboratory of Molecular Medicine and Neuroscience, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland USA

Polyomaviruses are nonenveloped, closed-shell particles with aspects of conserved isoschadal capsid structure. Human polyomaviruses, BKV and JCV, are associated with diseases. BKV is linked with several urinary tract disorders, where JCV is the etiologic agent of CNS demyelination responsible for progressive multifocal leukoencephalopathy (PML). Both of these polyomaviruses elicit specific immunologic responses in humans. The major structural protein (BKVP1, or JCP1) accounts for greater than 70% of the total protein content of each virion and governs capsid assembly. We determined that recombinantly produced VP1, rBKVP1 and rJCVP1, share properties with the native counterparts: the recombinants appear antigenically identical to the native forms, both sets of proteins agglutinate human type O erythrocytes, and the recombinants independently assemble into virus-like particles (VLP) in buffer containing free calcium ions. The rBKVP1 and rJCVP1, expressed with baculovirus systems, were purified by ultracentrifugation techniques due to this propensity for VLP assembly. Chelating calcium with ethylenebis(oxymethylene)tetraacetate acid (EDTA) dissociated VLP into assembly units analogous to viral capsomeres. These pentamers were reconstituted into pseudovirions by the addition of a marker-gene plasmid followed by excess calcium. When pseudovirions were added to tissue culture media, transduction of cells was observed by the subsequent expression of the green fluorescent marker protein (GFP). In the case of rBKVP1, pseudovirions efficiently transferred the marker gene. The rJCVP1 pseudovirions, however, behaved unstably and demonstrated less transduction ability, analogous to native JCV infection efficiencies. Electron microscopy was used to show the level of VP1 structure sufficient for transfer of plasmids into cells. The pseudovirions were also used for transduction experiments that may clarify the extent to which cellular susceptibility to native viral infection is dependent on nuclear transcriptional control proteins and/or membrane receptors.

P57

Sp and AP-1 modulate human T cell lymphotropic virus type I LTR activity during monocytic differentiation. Grant, C., Milhouse, S., Phillips, D., Steinman, K., Afzalani, T., and Wigdahl, B., Department of Microbiology and Immunology, Penn State College of Medicine, Hershey, Pennsylvania USA

Human T cell lymphotropic virus type I (HTLV-I) is the etiologic agent of adult T cell leukemia (ATL), a neoplasia within the T lymphocyte population, and tropical spastic paraparesis (TSP), a progressive neurologic disorder. Although CD4+ T cells are a major target for HTLV-I infection, additional cell types may also become infected, including cells of the myeloid lineage. Previous studies have demonstrated that the bone marrow of TSP patients contains large numbers of HTLV-I proviral DNA+ cells with little, if any, detectable expression of viral RNA and protein. These observations suggest that bone marrow progenitor cells may contain integrated proviral DNA that may be subject to differential gene regulation during differentiation of the progenitor cells down the monocytic pathway prior to or after leaving the bone marrow. To examine this possibility, we are utilizing chemical differentiation models of the monocytic lineage, including the PMA-treated U-937 monoblastic cell line, to determine if the course of basal and/or Tax-mediated LTR activity is modulated during monocytic differentiation. Transient transfection studies utilizing isolated 21-bp repeats attached to a minimal HTLV-I promoter suggest that PMA treatment of U-937 cells alters transcriptional activity in a repeat-specific manner. These observations are consistent with electrophoretic mobility shift (EMS) studies, which have indicated that PMA-induced differentiation of U-937 cells coincides with differential expression and/or modification of cellular transcription factors that bind to the 21-bp repeats. These factors include the Sp family, which interacts with the 21-bp repeat III, and the AP-1 family, which interacts with 21-bp repeat II. These factors may enable HTLV-I to remain latent in a cell population that may serve as a virus reservoir while allowing for transcription of viral genes when the cells mature and/or become activated. The trafficking of these cells to brain may play a role in TSP pathogenesis. This research was supported by a PHS grant (NS27405) awarded to BW.

P58


JC virus capsid is composed of the three capsid proteins, VP1, VP2 and VP3. We have shown that JCV capsid proteins are synthesized from alternatively spliced mRNAs, and then are cooperatively transported from the cytoplasm to the nucleus (J. Virol. 2000 74:1840-1853). In this study, we focused on distribution of VP1 within the nucleus. COS-7 cells were transfected with the expression vector encoding VP1, VP2, and VP3. VP1 was detected by using the peptide antibody against the potential BC loop structure that presents the highly antigenic site on the capsid surface. In cells, VP1 was efficiently transported to the nucleus in the presence of VP2 and VP3, and was localized to the distinct subnuclear regions. By confocal microscopy, foci of condensed VP1 signals were identified near the nuclear membrane, and the signals were spread into the center of the nucleus potentially along subnuclear structures. By electron microscopy, recombinant virus particles were identified near the nuclear membrane, and this observation is consistent with the JCV-infected patient’s brain. This suggests the presence of the distinctive subnuclear regions, which support virus capsid formation. This research was, in part, supported by Science and Technology Agency of the Japanese Government.
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Specific phenotypic restoration of an attenuated virus by knockout of a host resistance gene.

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Defining the mechanism of action of neurovirulence factors in vivo has, to date, proved difficult. To produce disease, viruses must enter the host, replicate, spread, and overcome or evade host immune responses. At each stage in this infectious process, specific microbial and host genes determine the ultimate virulence of the virus. Genetic approaches have identified many viral genes that play critical roles in virulence and are presumed to target specific components of the host innate and acquired immune response. However, formal proof that a virulence gene targets a specific process in a host response in vivo has not been obtained. Based on cell culture studies, it has been proposed that the herpes simplex virus type 1 (HSV-1) gene ICP54.5 enhances neurovirulence by regulating antiviral functions of the interferon (IFN)-inducible double-stranded RNA-dependent protein kinase or PKR. (Chou, J., Chen, J.J., Gross, M., and Roizman, B. (1995) Proc. Natl. Acad. Sci USA 92, 10516-10520). Here we show that a herpesvirus neuroattenuated by deletion of ICP54.5 exhibits wild-type replication and virulence in a host from which the interferon receptor (IFNAR) or PKR gene has been deleted. We show that restoration of virulence is specific to ICP54.5 and PKR using additional host IFNB dependent RNAseL, mutant mice and viral HSV rtk mutants. This demonstrates a central role for PKR in defense of the nervous system against virus infection, and defines PKR as a key target for the 4.5 gene in vivo. The use of recombinant viruses to infect animals with null mutations in host defense genes provides a formal genetic test for identifying in vivo mechanisms and targets of virulence genes.

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MCP-1 Expression in CSF Predicts the Development of SIV Encephalitis

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Macques infected with SIV frequently develop encephalitis characterized by perivascular and diffuse infiltrates of macrophages and lymphocytes. These cells infiltrate the CNS in response to a gradient of chemotactic factors. In this study, the SIV/macaque model was used to examine the relationship between MCP-1 expression in the CSF and plasma and the severity of SIV encephalitis. Fitted macaques (Mac nemerusa) were co-inoculated with an immunosuppressive virus strain (SIV/DeltaB70) and a neurovirulent molecularly cloned virus (SIV17E-Fr) and euthanized after the development of AIDS. MCP-1 and viral RNA in plasma and CSF were measured throughout infection by ELISA and real time RT-PCR, respectively. The degree of activation and inflammation in the brain (quantitated by image analysis on brain sections immunohistochemically stained for GFAP and for markers on macrophages, CD4+ T cells, cytotoxic lymphocytes, and NK cells) were compared with MCP-1 expression in CSF and plasma at the time of death and with the severity of encephalitis. The ratio of MCP-1 in CSF vs. plasma (the gradient of MCP-1 expression) increased during acute infection, peaking at 10 days p.i., and then declined to pre-infection levels by 28 days p.i. After 28 days p.i., there was a significantly higher MCP-1 ratio (p<0.05) in macaques that eventually developed neurepnea to severe encephalitis. In contrast, MCP-1 ratios remained constant at pre-infection levels in macaques that had mild or no encephalitis. Macaques with higher MCP-1 ratios had significantly higher GFAP expression in gray matter, higher numbers of macrophages and cytotoxic lymphocytes, and higher expression of viral antigen in the brain than macaques with low MCP-1 ratios. The results indicate that a gradient of MCP-1 expression between the CNS and blood is important for the development of inflammatory changes in the brain during SIV infection and suggest that the ratio of MCP-1 in CSF vs. plasma can be used as a predictor for lentiviral encephalitis.

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Effects of HIV-1 and inflammatory cytokines on chemokine production and transcriptional activity of human astrocytes.

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Astrocytes play a critical role in brain homeostasis, response to injury, and signal transmission, but little is known about their function during HIV-1 disease. Here we tested the induction of chemokines and cellular transcription factors NF-kB, AP-1, and Oct-1 in human fetal astrocytes infected with HIV-1 or after exposure of the cells to gp120 envelope glycoprotein or inflammatory cytokines TNF-α and IL-1β. Exposure of astrocytes to TNF-α and IL-1β for 4 hours induced massive secretion of the chemokine MCP-1 that reached 500 ng/ml of culture supernatant on Day 7. Recombinant, glycosylated gp120 (MN strain, 1pM) or BSA (1 pM) used as control had no effect. A small increase in MCP-1 secretion was observed in astrocytes exposed to uninfected HIV-1, which infects only a small fraction of these cells. HIV-1 pseudotyped with MuLV or VSV-G envelopes infected 30-50% of astrocytes and induced high levels of MCP-1, up to 500 pg/ml of MCP-1 per ml of culture supernatant at the peak of infection, and high MCP-1 levels were still detectable after 10 days. Electrophoretic mobility shift assays demonstrated that treatment of astrocytes with TNF-α dramatically increased nuclear levels of transcription factors NF-kB and AP-1 and induced a novel, high-molecular weight form of Oct-1. Neither gp120 treatment nor infection by intact or pseudotyped HIV-1 had a marked effect on NF-kB, AP-1, or Oct-1 expression, but TNF-α induced a higher level of these factors in HIV-1 infected than in uninfected cells.

These results indicate that both HIV-1 infection and exposure to inflammatory cytokines may have significant, lasting, and in certain responses, cooperative effects on astrocyte biology that may contribute to astrocyte dysfunction during HIV-1 dementia.

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ADENO-ASSOCIATED VIRUS MEDIATED DELIVERY OF GLIAL CELL LINE DERIVED NEUROTROPHIC PROTEINS TO MOTOR NEURON LIKE CELLS FROM APOPTOSIS.

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Motor neuron disorders including amyotrophic lateral sclerosis may benefit from the induction of neurotrophic factors such as glial cell line derived neurotrophic factor (GDNF) which are known to be trophic and protective for motor neurons. However, the application of such factors is limited by an inability to successfully target their expression in the nervous system. In this study we investigate using the defective parvovirus adeno-associated virus (AAV) as a vector for gene delivery into motor neurons. Using a recombinant AAV vector expressing the reporter gene β-galactosidase (AAV-LaZ) we successfully demonstrated the utility of AAV for gene transfer in two separate motor neuron cell lines. In addition, motor neuron cell lines infected with an AAV-GDNF vector express and secrete high levels of GDNF which are maintained throughout the time course of the study. The AAV-GDNF vector is demonstrated to be both neurotrophic and neuroprotective for motor neurons. Following infection with AAV-GDNF a significant upregulation in levels of choline acetyl transferase (ChAT) is demonstrated as compared to AAV-LaZ or uninfected controls. Following withdrawal of trophic support through serum deprivation a reduction in ChAT levels and an increase in the number of cells entering apoptosis is observed. The reduction in ChAT levels and the percentage of apoptotic cells are however significantly reduced in cells infected with the AAV-GDNF vector, but not the AAV-LaZ vector or uninfected controls.

This work demonstrates the potential of using AAV as a vector in motor neuron like cells and should prove important in devising future gene therapy strategies for the treatment of in vivo motor neuron disorders.

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Abstracts

P63
Physiopathogenesis of TSP/HAM: multiple processes involving brain astrocytes.
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HTLV-1-infected T-lymphocytes infiltrating the CNS are suspected to play a prominent role in the neurologic disease associated with this virus (TSP/HAM) via inflammatory cytokines and the viral protein Tax-1. Given the primary role of astrocytes in CNS homeostasis, we hypothesized that infected T-lymphocytes may perturb astrocytes to alter neuronal or oligodendroglial functions. Indeed, astrocytes manage the extracellular level of glutamate in brain parenchyma and provide metabolism precursors to neurons and oligodendrocytes. Transient coculture of HTLV-1-infected T-cells with astrocytes was used as a model to study the effect of HTLV-1 infection on astrocytic functions. After contact with T-cells, astrocytes acquire a phenotype typical of gliosis: production of proinflammatory cytokines (TNFalpha, IL1alpha, IL6) and matrix metalloproteinases (MMP-9, MMP-3). The critical balance between MMPs and their endogenous inhibitors (TIMP-1 and TIMP-3) was affected as shown by their transcripts or proteins expression. This may alter extracellular matrix, hence cell microenvironment. HTLV-1 infected T-lymphocytes also impaired the interaction of astrocytes to eliminate glutamate from extracellular space (transport and catabolism). A decrease in glutamate uptake was associated with a downregulation of glutamate transporters GLAST and GLT1, while the expression of astrocytic enzymes catabolizing glutamate was altered (upregulation for glutamine synthetase and downregulation for glutamate dehydrogenase). These changes results from direct or indirect effects of Tax-1 via TNFalpha. Such modification in glutamate uptake/catabolism and in cell connectivity via MMP/TIMP imbalance could compromise the operation of neurons and oligodendrocytes, eventually affecting the overall integrity of the CNS.

P64
High frequency of VZV DNA detection in the cerebrospinal fluid of multiple sclerosis patients. Ferrante P, Mancuso R, Pagani E, Caputo D ’Calvi MG
Chair of Virology, Dept. of Preclinical Sciences, University of Milan; Laboratory of Biology and Multiple Sclerosis Unit, Don C.Gnocchi Foundation ONLUS, IRCCS, Milan, Italy.

In order to verify the possible role of viruses in the pathogenesis of multiple sclerosis (MS) we studied cerebrospinal fluid (CSF) samples collected from 38 clinically defined MS patients (23 relapsing-remitting and 15 chronic progressive), 28 patients with other neurological diseases (OND) and 19 with other non neurological diseases (NONND). The OND cases had meningitis, encephalitis and other acute inflammatory CNS diseases including neurological disorders in AIDS. All the CSF were analyzed, using different specific nested polymerase chain reactions (PCR), for the presence of genomic sequences belonging to a group of viruses well known for their neuropatia, including herpes simplex virus 1 (HSV-1) and 2 (HSV-2), Epstein-Barr virus (EBV), varicella zoster virus (VZV), human cytomegalovirus (HCMV), human herpes virus 6 (HHV-6) and JC virus (JCV).
The CSF collected from the 19 ONND cases resulted negative for all the studied viruses

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Don Gnocchi Foundation, IRCCS, Milan; St. Mary’s Lacor Hospital, Gulu, Uganda; Laboratory of Epidemiology and Biotestistics, ISS, Rome; Infectious Diseases Dept, Sacro Hospital, Milano; and Chair of Virology, Dept. of Preclinical Sciences, University of Milan, Italy.

Cerebrospinal fluid (CSF), serum and urine samples have been collected from 35 patients suffering of acute inflammatory neurological diseases admitted to the St. Mary’s Lacor Hospital in Gulu in northern Uganda. After transfer in Italy the samples have been examined to define the etiology of the neurological disorders that had been diagnosed, on the basis of clinical symptoms, as meningoencephalitis (8 cases), cerebral malaria (3 cases), senile dementia (1 case) and unspecified (3 cases). Seven of the patients had AIDS with concurrent neurological disorders.

Using serum and CSF, blood-brain barrier (BBB) integrity, IgG intrathecal synthesis indices and the presence of IgG oligoclonal bands were evaluated using standard procedures. DNA belonging to selected specific regions of herpes simplex virus 1 (HSV-1) and 2 (HSV-2), Epstein-Barr virus (EBV), varicella zoster virus (VZV), human cytomegalovirus (HCMV), human herpes virus 6 (HHV-6), JC virus (JCV), HTLV-1, and Mycobacterium tuberculosis (MT) were searched in CSF using nested polymerase chain reactions (PCR).

A decrease of CSF glucose level was observed in 4 cases, suggesting the bacterial etiology of the neurological disorder. Microbial DNA search in CSF samples showed that a total of 6 (40%) cases resulted positives for one or more of the studied infectious agents with a single infection in 2 cases, a dual infection in 3 cases and a multiple infection in one case, who had HSV-1, HCMV, EBV and MT DNA in the CSF. EBV DNA was found in 4 (27%) patients (3 meningoencephalitis and 1 cerebral malaria), HSV-1 and HCMV had been found in 2 (14%) patients, and only one had JCV DNA in the CSF. MT DNA was detected in 3 (20%) cases (2 meningoencephalitis and 1 senile dementia).

This is the first etiological study performed, using molecular methods, in neurological patients from north Uganda, and indicates that the frequency of single or multiple infectious agents in the CSF is higher in Ugandan than in Italian patients.

P66
Regulation of matrix metalloproteinases in human leukocytes and astrocytes: Implications for HIV-1-associated dementia. Ghorpade, A., Lonze, C., Persidskaia, R., Wu, L. and Gondelman, H.E. Center for Neurovirology and Neurodegenerative Disorders, University of Nebraska Medical Center, Omaha, NE USA

The production of matrix metalloproteinases (MMPs), by circulating leukocytes and/or glial cells, can influence monocyte migration into brain and thereby affect HIV-1 associated neurological disorders. MMPs when produced by microglia and astrocytes degrade extracellular matrix proteins of the brain (collagen, laminin, fibronectin, neurotrophins) at the infected HIV-1-expressing cells (neuropil) and affect neuronal function. To decipher how HIV-1 infection of mononuclear phagocytes (MMP) (macrophages and microglia) and astrocytes influence MMP secretory activities and affect HIV-1-associated dementia (HAD) we studied the production of MMP-1, -2, -3, and -9 from HIV-1-infected and immune-activated monocyte-derived macrophages (MDM), primary human microglia and astrocytes. MMP levels were determined by ELISA, gelatin zymography and/or by RT-PCR. These were performed to provide cross validation for all experimental findings and to differentiate the pro- and the active- forms of MMPs. Cell differentiation of monocytes and microglia induced a significant and sustained increase in MMPs.

Microglia constitutively produced MMPs but activation with CD40 ligand (CD40L) enhanced all enzyme levels early during differentiation. HIV-1 infection downregulated MMP-9 and MMP-2 levels in infected Mφ. MMP-2, but not MMP-9, was expressed constitutively by human astroglia. Interestingly, human astrocytes propagated in vitro led to an increase in MMP-2 expression. In addition, MMP-9 expression was induced in astroglia exposed to pro-inflammatory cytokines (IL-1β and TNFα) and culture supernatants derived from human-activated MDM. All astrocytes produced MMP-2, but the exception of MMP-9, microglia generated higher enzyme levels than MDM. MDM did not produce stromelysin (MMP-3). Our data suggest that cell differentiation and activation of MP (as would occur during advanced HIV-1 disease) result in high levels of MMP production. These immune effects may affect both monocyte transendothelial migration and/or ECM degradation influencing the course of HAD.

This research was supported by Pediatric AIDS Foundation Grant 77324-23; NIH grants R01-N34239 and P01-N331492. Journal of NeuroVirology
C/EBP binding site variants commonly encountered in brain-derived HIV-1 LTRs have also been identified in the simian immunodeficiency virus (SIV) transcriptional regulatory unit. Nonnemacher, M.R., Hogan, T.J., Ross, H.L., and Wigdahl, B., Department of Microbiology and Immunology, Penn State College of Medicine, Hershey, Pennsylvania USA. Human immunodeficiency virus type 1 (HIV-1) infection causes severe immunologic dysfunction and abnormalities in a number of organ systems including the central nervous system (CNS). We have previously identified two CCAAT/enhancer binding protein (C/EBP) sites within the HIV-1 long terminal repeat (LTR) that are critically important for efficient viral replication within monocytes/cells, which serve as an important vehicle for transport of virus to the CNS. To enhance understanding of HIV-1 disease in humans, several model systems have been utilized to address basic questions concerning retrovirus pathogenesis. As one approach to assess the importance of C/EBP binding sites in retroviral pathogenesis, we proceeded to examine the role of C/EBP binding sites in the context of members of the C/EBP transcription factor family. Utilizing electrophoretic mobility shift (EMS) analyses, we have identified four SIV LTR sequences with affinity for human monocytic C/EBP α and β. C/EBP upstream site 1 (US 1), upstream site 2 (US 2), downstream site 1 (DS 1), and downstream site 2 (DS 2) are located at nucleotides -102 to -88, -386 to -373, +131 to +144, and +265 to +280, respectively. We examined these C/EBP binding sites using competition and supershift EMS analyses and have demonstrated that C/EBP DS 1 and DS 2 exhibit high affinity for both C/EBP α and β. By comparison, both US 1 and US 2 exhibit lower affinity for C/EBP α, and we potentially interact with other C/EBP transcription factors. C/EBP US 1 lies immediately upstream of a series of Sp1 cis-acting elements and may be itself bind members of the Sp transcription factor family. In addition, C/EBP US 1 partially overlaps a NF-kB binding site suggesting that this site may be involved in responding to two families of regulatory proteins important in differentiation and activation of cells of monocyte/macrophage lineage. Future studies will examine the physical and functional properties of the SIV C/EBP binding sites in viral gene expression and replication.

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TWO POTENTIAL MECHANISMS FOR THE DELIVERY OF HSV FROM INFECTED TRIGEMINAL GANGLION CELL ROSETS TO THE CORNEA. LaVail, Jennifer H.1, Taucher, Andrew N.2, and Ohara, Peter T.3, Department of Anatomy1 and Department of Ophthalmology2, University of California San Francisco, San Francisco, CA 94143 USA. Recently we have developed an in vivo animal model in which Herpes simplex virus type 1 (HSV) can be introduced directly into the mouse trigeminal ganglion where the virus replicates. Three days after inoculation the viral infection is evident. Using this model we investigated the direct spread of virus from infected trigeminal cells to corneal epithelial cells using EM immunochemistry. This study extends those findings to present information about the form in which the viral particle is transported to axon endings and the spread of virus within the trigeminal nerve endoneurium.

We identified many HSV immunostained axon profiles located in the trigeminal nerve or in the submucosal layer of the cornea. The nerve fibers were identified by their small caliber, the presence of vesicles, and immunoreactive viral particles. The viral particles were uniformly nucleocapsid (mean diameter = 99.37 ± 12.20 nm, n=52). We conclude that in vivo HSV is intraxonally transported in the anterograde direction at least in part as a nucleocapsid, possibly with tegument proteins, but without a viral envelope or additional cellular membranes. The processing and delivery by transport of the envelope components remain to be clarified in this whole animal model.

We also identified virally infected Schwann cells that envelop the sensory ganglion axons in the trigeminal nerve. Viral capsids were found in the nucleus of the cells and mature enveloped and capsids were found in the cytoplasm. The surface and neuromus of the Schwann cells were also immunostained with HSV. Nucleocapsid and virus of HSV were first detectable by immunofluorescent labeling 4 to 6 days after HSV-1 infection through life the host is exposed to multiple viral strains. LATs function may be to protect the viral reservoir from constant cytotoxic infections. Since latency is established in neurons that receive sensory afferents from the peripheral site of infection, the cells that already express LATs may serve as a barrier that prevents recurrent herpes infection to penetrate the same ganglion, and eventually spread into the brain leading to herpes encephalitis. Thus, LATs may explain both local and neuronal immunity against recurrent and eventually fatal HSV-1 infections.

This research was supported by a NIH grant (NS 32092) awarded to BW.

P70

The latency-associated gene of herpes simplex virus type 1 might protect the human host from herpes encephalitis. Israel Steiner, Nurih Mador and Amos Panet, Laboratory of Neurovirology, Department of Neurology, Hadassah University Hospital, Jerusalem. Herpes simplex virus type 1 (HSV-1) establishes latent infection in human peripheral sensory ganglia (PGS) followed by periodic reactivations. During latency, only the viral latency-associated transcripts (LATs) take place and has been linked to the viral reactivation ability, but its action mechanisms are unknown. We have shown (J. Virol. 72:5067-5075, 1998) that the LATs repress viral replication in neurons, thus facilitating establishment of HSV-1 latent infection in these cells. The present study was aimed to look at the specificity of this action and to identify at what stage during viral replication it occurs. Stably transfected neurons containing: 1) HSV-1 entire latency transcriptionally-active DNA fragment; 2) the same sequence deleted of its regulatory elements; and 3) the coding sequence of the LATs; were infected with each one of the following: two HSV-1 mutants; HSV-2; vaccinia virus; adenovirus; or an MuLV-based vector. All viruses contained the β-gal reporter gene and X-gal staining and QNPG analysis enabled to quantitate viral replication. Neurons stably expressing the LATs were protected from HSV-1 infection, but not from infection by other viruses including HSV-2. LATs interfered with HSV-1 infection at a very early stage of the replication cycle and irrespective of expression of the viral immediate early genes. Deletion of the LATs promoters inactivated this function. Our findings explain the observation that human PGS are resistant to HSV-1 spread even through life the host is exposed to multiple viral strains. LATs function may be to protect the viral reservoir from constant cytotoxic infections. Since latency is established in neurons that receive sensory afferents from the peripheral site of infection, the cells that already express LATs may serve as a barrier that prevents recurrent herpes infection to penetrate the same ganglion, and eventually spread into the brain leading to herpes encephalitis. Thus, the LATs seems to endow local and neuronal "immunity" against recurrent and eventually fatal HSV-1 infections.
The *gpr* gene is not required for neuropathogenesis of SHIV. Stephens, E.B.1, McCormick-Davis, C.,2 Pinson, D.M.1, Wong, S.W.3, and Berman, N. E. J.1 Departments of Microbiology and Anatomy and Cell Biology2, University of Kansas Medical Center, Kansas City, Kansas1 Oregon Regional Primate Center, Beaverton, Oregon, USA.

We have used a molecular clone of simian-human immunodeficiency virus (SHIV) known as SHIV3218dc32 to analyze the role of the *gpr* gene product in the CD4+ T cell loss caused by this virus in pig-tailed macaques. A mutant was constructed (M3218dc32) in which 42 of the 82 amino acids of Vpr were deleted, including the first α-helical domain and the first phosphorylation site. This virus was used to inoculate four macaques. One macaque developed a severe decline in CD4+ T cell numbers within one month and by 35 weeks post-inoculation had developed signs of neurological disease (blindness and tremors). At necropsy, histological examination of the lymphoid tissues revealed lymphoid depletion characteristic of pathogenic SHIV. Examination of the brain and spinal cord revealed perivascular cuffing, microglial nodules and neuronal loss. Microarray analysis revealed the up-regulation of several host genes in the macaque with neuroAIDS that were not found in the macaques without neuroAIDS. PCR analysis revealed high levels of M3218dc32 in all 14 regions of the brain and spinal cord examined. Sequence analysis of the virus from the brain of this macaque revealed significant amino acid changes in Env and nef-frame deletions in Nef. The other macaques inoculated with this virus developed a moderate or no loss of CD4+ T cells and no neurological disease. The virus sequences isolated from these macaques did not show any consensus amino acid changes in Env or Nef. These results indicate that Vpr is not required for the CD4+ T cell loss and neurological disease in pig-tailed macaques and its absence can be compensated for by changes in other viral genes.

This research was supported by PHS grant MH-61230.

**P73**

Modulation of apoptosis associated with the induction of a persistent infection of neural cells by Vescular Stomatitis Virus mutants. Destefano, Marc 1,2, Talbot, Pierre J.,1 and Poliquin, Laurent. Human Health Research Center, INRS-Institut Armand Frappier, Université du Québec, 1 Department of Biological Sciences, Université du Québec à Montréal, Québec, Canada.

Neurodegenerative diseases may be linked to persistent viral infections of the central nervous system (CNS). Vascular Stomatitis Virus (VSV) provides a good model to characterize the mechanisms involved in initiation and maintenance of viral persistence. Wild type (wt) virus replicates in mouse CNS after intranasal infection and, unlike the highly cytopathic wt virus, several VSV mutants were shown to persist in CNS cells after subcutaneous injection into hamsters. To understand the relationship between persistent viral infection and neuronal tissue degeneration that may result, we first characterized the outcome of VSV infections of neural cells. Viral strains varying in host shut-off characteristics, all infected and caused cytopathic effects (CPE) in either primary or immortalized neural cells. However, the rate of CPE induction inversely correlated with the ability to persist. Even though all viruses replicated well, only the Indiana matrix (M) protein mutants and the wild type New Jersey strain were able to establish persistent infections. We showed that the various virus variants could persist for up to one hundred days in the H4 human neural cell line. Further characterization suggest that virus-mediated modulation of apoptosis could be associated with this outcome. Modulation of the expression of the proapoptotic protein p53 could be related to this phenomenon since the H4 and TP6 variants, both unable to persist, induced apoptosis more strongly and led to an accumulation of this protein in the first day of infection. The expression of Bax, another proapoptotic protein, was also modulated by several virus variants during the first 2 days of the infection. Further characterization of the modulation of the apoptotic pathway involved during infection of neural cells by various VSV variants will lead to a better understanding of how this usually cytopathic virus can persist in CNS cells.

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**P72**

Apoptosis in cerebrovascular endothelial cells infected with Théler's virus. Nayak, M.1, Dean, D.D.1, Smith, R.2, and Welsh, C.J.R.1,2. 1Department of Veterinary Anatomy and Public Health; 2Department of Veterinary Pathobiology, Texas A&M University, College Station, Texas USA.

Théler's virus is classified as a Cardiovirus in the family Picornaviridae. The neuroviral GDVII strain of Théler's virus causes fatal encephalitis following intracranial injection in all strains of mice, whereas BeAn strain of Théler's virus persists in the central nervous system of susceptible strains of mice and causes a demyelinating disease similar to multiple sclerosis (Théler's virus induced demyelination-TVID). Théler's virus has been shown to induce apoptosis in vivo and in non-permissive cells in vitro. This current study investigates the relative abilities of the neuroviral GDVII and persistent BeAn strain of Théler's virus to induce apoptosis in cloned mouse cerebrovascular endothelial cells (CVE). CVE derived from TVID-susceptible (SLL/J) and TVID-resistant (Balb/c) were persistently infected in vitro with either BeAn or GDVII. Acridine orange/ethidium bromide staining and electronmicroscopic studies revealed evidence of increased apoptosis in GDVII infected CVE. Annexin V staining confirmed these results. Therefore, there appears to be a correlation between neurovirulence of Théler's virus and the ability to induce apoptosis in cerebrovascular endothelial cells in vitro.

This research was supported by NSF grant IBN-9973640.

**P74**

Non-pathogenic poliovirus recombinants for the treatment of malignant glioma. M. Groeneveld, P.H. Gatin, and E. Wimmer. Dept. of Microbiology, Duke University, Durham, NC; Dept. of Mol. Genetics & Microbiology, SUNY @ Stony Brook, NY; 2Neural, Surgery Service, Mem Sloan Kettering Cancer Ctr., New York, NY.

Poliovirus neurovirulence depends on its internal glusulat cysteine residue (IRES) located within the 5′ untranslated region of its RNA genome. Exchange of the cognate IRES of poliovirus with its counterpart from human rhinovirus type 2 yielded a chimeric virus (PVr/RIPO) that had lost its neuropathogenic potential when inoculated intraspinally into non-human primates. PVr(RIPO), although unable to replicate within neuronal cells, grew with wild type kinetics in cell lines established from human astrocytomas.

While normal glial resist infection, malignant glioma cells are highly susceptible to poliovirus. Permissivity toward poliovirus infection may be mediated by the expression of the human poliovirus receptor CD155 in these tumors. We reported expression of CD155 to be developmentally regulated. Similar to other members of the immunoglobulin superfamily, CD155 is ectopically expressed in neuroectodermal malignancies. An association of CD155 with neuroectodermal tumors has recently been confirmed by microarray analysis of differential gene expression in medulloblastomas.

Poliovirus chimeras with obtained neuropathogenicity exhibit natural tropism for gliomas expressing the poliovirus receptor, CD155. We have tested the oncolytic activity of PVr(RIPO) against intracerebral glioma xenografts in athymic mice. Single intratratal infection into advanced hemispheric tumor xenografts were sufficient to produce remission of neurological symptoms and tumor regression. Treated xenografts receded dramatically and, eventually, disappeared altogether. We propose highly attenuated oncolytic poiovirus variants as a novel class of oncolytic viruses with highly specific tumor tropism and anti-tumor activity against malignant gliomas.

M.G. is a recipient of a 1999 Barroughs Wellcome Career Award.
**P75**

THE CHOROID PLEXUS AND AIDS-RELATED CNS DISEASE: 
Brasse DC, Childers T, Boles J, Tompkins M, Tompkins W, Meeker R. 
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Neuropathological studies indicate that choroid plexus (cpx) macrophages may be infected by lentiviruses such as HIV-1, although the nature of this infection and its significance for AIDS-related neuropathogenesis remain unclear. In the present study we established primary cultures of feline cpx and examined the response to the feline immunodeficiency virus (FIV), a lentivirus frequently used as a model for AIDS-related CNS disease. DNA extracted from cpx cultures inoculated with FIV was amplified with primers against the viral gag gene, indicating the presence of provirus up to 25 days post-inoculation. Cell-free supernatants collected from cultures inoculated with different viral isolates were assayed for the FIV p26 core antigen. Although cpx cells did not typically support a high level of viral replication, they were highly efficient at transferring infection to feline CD4+ lymphocytes. Expression of chemokines that could recruit peripheral immune cells to the cpx was determined by RT-PCR, indicating that FIV induces expression of MIP-1α, MIP-1β, MCP-1, and RANTES within the cpx. Cpx cultures inoculated with FIV showed an increased matrix metalloproteinase activity, which correlated with an increased toxic activity in the culture supernatants. Medium collected over time from cpx cultures exposed to FIV produced increasing levels of toxicity in feline neural cultures, whereas medium from control cultures inoculated with vehicle produced little cell death. Neurons exposed to medium from FIV-infected cpx cultures exhibited a prolonged, toxic accumulation of intracellular calcium. These data suggest that FIV may induce cpx cells to release a soluble factor(s) that may produce toxicity by disrupting neuronal calcium homeostasis. Thus the choroid plexus may represent an important reservoir for lentiviruses within the CNS. It may be capable of seeding the CNS with viral and soluble factors that promote neurotoxicity within peripheral immune cells across the blood-CSF barrier.

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**P76**

Acute encephalomyocarditis associated to HTLV-I infection. 
Federal University of Rio de Janeiro, RJ, Brazil, New York Blood Center, New York, NY, USA.

HTLV-I associated myelopathy, also called tropical spastic paraparesis (HAM/TSP), is a chronic neurological disease. Acute cases have been rarely described. Here, we report a case of acute myelopathies in a 41-years old female infected with HTLV-I. Her history included loss of 10 kg in the previous last 5 months. Neurological symptoms started with numbness of the left bulbar that ascended within 2 weeks to the left leg and right hand. It was associated with weakness of the lower extremities and constipation. General physical examination revealed cauesia, angular quetlulis, xeroderma. There was no adenopathy or organomegaly. Neurologic examination showed weakness, hypotonus, hyporeactive deep tendon reflexes and decreased sense of vibration in the lower limbs. Antibodies to HTLV-I were detected in both serum and CSF. Serological analysis showed lymphocytic pleocytosis, high levels of protein, a persistent hypoglycorrhachia and high IgG index. There was no intrathecal synthesis of antibodies to HTLV-I. The patient died of bronchopneumonia. At autopsy, the most important pathological findings included perivascular mononuclear cell infiltrates in the spinal cord, dorsal root ganglia, brainstem, cerebellum, deep gray nuclei and cerebral white matter. In addition, there was lymphocytic infiltration in skeletal muscle, salivary, adenal and pituitary glands. Conclusions. This is an uncommon presentation of a neurological disorder associated to HTLV-I infection occurring in a patient with a consumptive state of unknown etiology. In a previous study, we found that patients with HAM/TSP with high titers of HTLV-I in CSF and no intrathecal synthesis of specific antibodies had serious and rapidly progressing clinical disease.

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**P77**

Isolation of a novel human gene which binds to NF-kB and is important in apoptosis. 
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Transcription of human neurotropic viruses including JCV and HIV-1 is modulated by a series of ubiquitous and inducible DNA binding transcription factors. NF-κB represents a family of inducible transcription factors which are important in the regulation of JCV and HIV-1 in CNS cells. Further, infection of the CNS with HIV-1 results in upregulation of cytokines, such as TNFα, which has the ability to stimulate NF-κB expression and activity in CNS cells. In their active form, NF-κB family members are dimers, the best-known combination being the p50/p65 dimer. Results from our earlier studies show that the subunits of NF-κB such as p50/p65 may interact with other cellular proteins and that interaction modifies their activities. In order to examine new potential NF-κB binding proteins, we utilized a Yeast Two-Hybrid system. This method revealed a novel gene, which was isolated using a cDNA library from the human astrocytic cell line, U-87MG. The cDNA bound to the p50 subunit of NF-κB. Northern blot analysis has shown that this transcript is approximately 5.8 kb and is expressed in the human astrocytic cell lines, 198Q and U-87MG and in the murine astrocytic cell line C6. Computer analysis of the cDNA has revealed that it has high homology to the mouse gene ALC-1, which induces activation of NF-κB and upregulates Fasl, both of which are important activators of apoptosis. The importance of the gene in NF-κB mediated transcription and apoptosis is discussed.

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**P78**

SIV encephalitis (SIVE) in rhesus macaques is associated with overexpression of interferon-gamma and tumor necrosis factor-alpha. M. O'Conel, X. Alvarez, K. Williams, A. Luckner, 
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Cytokines are believed to play multiple roles in the pathogenesis of HIV-1-associated neurologic disease. To examine this in the SIV-infected macaque model, cytokine gene expression (IL-1β, 2, 4, 6, 10, TNA-α and IFNγ) was evaluated by RT-PCR in brains of macaques infected with SIVmac251. Ten animals were evaluated during the acute phase of infection and 10 during the terminal stages of AIDS. TNF-α and IFN-γ transcripts were upregulated at 7 and 14 days post inoculation (PI), coincident with peak viremia, and appeared to resolve by one month PI. Terminally, brains from SIV-infected animals without encephalitis expressed lower TNF-α and IFN-γ (USF) transcripts, but not both. In comparison, brains from 4 out of 5 animals with SIVE were found to express both TNF-α and IFN-γ transcripts. Within an encephalitic brain, TNF-α and IFN-γ transcripts were detected in 6 out of 7 regions with histologic evidence of SIVE, suggesting a direct relationship between neuropathology and altered cytokine gene expression. Using combined fluorescent in situ hybridization and immunohistochemistry, TNA-α mRNA-expressing cells were frequently identified as CD68-positive macrophages within perivascular lesions. These observations provide evidence that cytokines produced by activated inflammatory macrophages are an important element in the pathogenesis of SIVE.
Neuronal loss in the basal ganglia of SIV-infected rhesus macaques. Marcario, J.K. 1, Manuye, K. 2, Mittal, S. 1, Karra, S. 1, Raymond, L.A.M. 1, Chetry, P.D., and Berman, N.E.J. 1. University of Kansas Medical Center, Kansas City, KS and Howard University, Washington, DC

Infection of macaques with neurovirulent strains of simian immunodeficiency virus (SIV) has proven to be a useful model for investigating the neurological manifestations of AIDS. In previous reports, we have documented behavioral task performance deficits (Marcario et al., 1999a; 1999b) and neuropathological changes (Raymond et al., 1998; 1999; in press) in a cohort of 9 monkeys infected with neurovirulent strains of SIVmac. We have also shown a loss of neurons in the parvicellular layer of the lateral geniculate nucleus in macaques inoculated with the same neurovirulent strains of SIV (Berman et al., 1998). Neuronal loss associated with HIV infection has been demonstrated in the putamen (Everall et al., 1995) and the substantia nigra (Reyes et al., 1991), which are both movement-related structures. In an effort to determine whether neuronal loss might contribute to the motor system deficits observed in SIV-infected macaques, we used unbiased stereological techniques to assess neuron number and neuronal density in the globus pallidus (GP) and the substantia nigra (SN) of 8 SIV-infected and 5 control animals. Analysis of the GP shows that, compared to controls, most SIV infected animals showed neuron losses of greater than 25%, with some exceeding 50%. In addition, there was a mean decrease in neuronal density of approximately 15%. Large, disseminated microneuronal rosettes and extensive perivascular cuffing were found. Inspection of thin-in-stained sections suggests a relationship between the occurrence of microneurial nodules and the severity of ataxia. Alterations in neuronal morphology (i.e., loss of dendrites) may also be associated with the presence or absence of tremor. Analysis of the SN has just begun. Thus, neuronal loss and alteration of neuronal function due to morphological changes caused by SIV infection may be important factors contributing to the motor impairments observed in this cohort of monkeys.

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Abstracts

Prostaglandins (PGs) are potent immunomodulators among which PGF2α is a main factor released in high amounts in inflamed tissues. Cyclooxygenase-2 (COX-2) is the inducible enzyme responsible for high output of PG. Overexpression of COX-2 has emerged as an important determinant of the cytotoxicity associated with inflammatory brain pathologies and tumors. Elevated levels of PGs were also detected in spinal fluid of Human Immunodeficiency Virus type 1 (HIV-1)-infected patients with neurological disorders. Proinflammatory cytokines such as IFNγ, TNFα and IL-1β have also been detected at increased levels in brain parenchyma of these patients. Although several cytokines and bacterial products have been described as modulators of COX-2 in human and rodent glial cells, the combined effect of the network of cytokines within the inflammation loci on COX-2 is not completely understood. We investigated the effect of IL-1β and TNFα, together with IFNγ, on the COX-2 expression in human astrocytes, microglia and an astrocytoma cell line (CCF-STTG1). No COX-2 protein expression is detected in untreated astrocytes and microglia, whereas COX-2 expression is upregulated in glioma cell line. A time-dependent increase in COX-2 protein and PGE2 expression is induced by IL-1β in human astrocytes, microglia and CCF-STTG1 cells. TNFα has no effect on COX-2 and PGE2 expression in primary cells, whereas it increases COX-2 expression in CCF-STTG1 cells both alone and in synergy with IL-1β. Interestingly, IFNγ induces a marked decrease in COX-2 protein expression after IL-1β treatment. Furthermore, the time-dependent induction of COX-2 by IL-1β and the down-regulatory effect of IFN γ on IL-1β-induced COX-2 expression seem to take place at transcriptional level. Further studies are under investigation to determine whether IL-1β and IFNγ affect the stability of COX-2 mRNA or act on the promoter of the COX-2 gene.

Cutaneous silent period (SP) in HIV related peripheral neuropathy M. Osio1, F. Massal1, J. Zampini1, A. Casteglia1, A. Carugatti2, Dept. Neurolog 1 2nd Dept. Internal Medicine, Sacco Hosp Milan, Italy.

Background. Small-calibered fibers loss has found in HIV-related, painful, axonal peripheral neuropathy (pPNS). In clinical practice, measurement of the cutaneous SPs after finger stimulation may provide information in patients with damaged small-calibered nerve fibers. Our objective was to evaluate if SPs alteration predicts upper limbs involvement in HIV-related axonal sensory pPNS. Method. 38 HIV-positive patients (pts) (mean age 38.7±9.2 yrs), with clinical signs of pPNS and 8 normal subjects (mean age 34.7±8.5 yrs) were studied. All the subjects underwent motor conduction (MC) and F-wave study of peroneal, tibial and ulnar nerves, sensory conduction (SC) study of sural and ulnar nerves, needle EMG examination of 4th dorsal interosseous (1stD1) of the hand and tibialis anterior muscles. Pts were divided in two groups: either with or without upper limbs MC and SC and 1stD1 needle examination was normal. Cutaneous SP was elicited stimulating 4th and 5th fingers of the hand and was recorded from activated 1st D1. T-test for independent samples was applied to compare SP differences between groups. Results. SP parameters in normal subjects were: latency 68.1±13.3 ms, duration 130.2±32.6 ms, amplitude 79.7±28.3 µV and area 667.2±3277.8 mV.s. In pts with altered upper limb neurophysiological parameters, SP latency was 85.2±9.7 ms and was significantly longer than in normal subjects (p<0.05). Even the patients with normal upper limbs SCs and/or MCs and normal upper limbs assessment, SP latency values were significantly prolonged (80.7±10.5 ms; p<0.05). Other SP parameters (duration, amplitude and area) were not different from those recorded in normal subjects. Conclusion. Upper limbs cutaneous SP latency is prolonged in HIV-related pPNS even if motor and sensory nerve conduction studies of the upper limbs were normal. Further studies are ongoing to evaluate if cutaneous SP is an early predictor of HIV-related pPNS of the lower limbs.

Protease Inhibitors and male impotence S. Solimena1, M. Osio1, A. Alcomani2, T. Maga3, L. Testa1, A. d'Aquino Monforte1, T. Bini2, F. Muesca4, 1 Inst. Inf. Trdp Diva Univ Milan, 2 Dept. Neurolog 1 3 Dept. Psychol L. Sacco Hosp Milan, 4 Sleep Dist Ctr and 4 Dept. Urol Sano Raffaele Hosp Milan, Italy.

Background. HIV+ve men receiving HAART including one or more protease inhibitors (PIs) often complain of impotence. Our objective was to evaluate if erectile dysfunction (ED) could be related to PIs. Methods. 115 male patients (pts) aged 25-45 years, receiving PIs from at least 6 months, with no history of mental disorders, liver failure, diabetes or current alcohol and iv drug abuse, were questioned about ED according to the International Index of Erectile Function. HIV risk factors were: unknown 7%, previous iv drug use 53%, homosexual 13%, heterosexual 29%. Pts with ED undertook neurophysiological (np) (bulbocavernous reflex, pudendal and tibial nerve SSEP, tibial and sural nerve conduction study, nocturnal penile tumescence monitoring), psychiatric (SCC 90-R and BD1), hormonal (serum FSH, LH, testosterone and prolactin), and vascular (peripheral doppler US (PDU) after intracavernous injection of pg-E1) assessments. Results. 25 pts out of 115 reported ED. Median (MD) age was 37 years (25-45). MD CD4 cell count was 493/mL (32-864), pVL was <400 copies/mL in 56% of pts while in the remaining MD pVL was 18,000 copies/mL (140-1,200,000). MD time on PIs was 28 months (10-37) and MD time of onset of ED was 13 months (3-32). Up to now, 8/25 pts completed all diagnostic procedures. No one showed psychiatric or hormonal abnormalities. As referred to np assessment, 3 pts had all tests abnormal, the others showed at least one pathological finding 6/8 pts revealed a sensory axonal peripheral neuropathy (nPN). PDU was normal in 7/8 pts. Conclusion. 22% of pts on PIs has ED. Preliminary results show that neuropsychic factors seem to be related to ED. Several antiretroviral drugs can cause ED but no one has been yet related to ED, nor ED has been related to np. Most pts report ED after being on PIs, but the small number of our present cohort does not allow to correlate PIs to this disorders. Further studies are ongoing on naive and PI-unexperienced subjects.

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