Abstracts

P1

Correlation between cerebrospinal fluid levels of protein tau and neopterin in HIV-infection

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Elevated cerebrospinal fluid (CSF) concentrations of protein tau (tau) are associated with axonal degeneration and have been found in Alzheimer’s disease and to a lesser extent in vascular dementia and other degenerative neurological disorders. Protein tau, HIV-1 RNA, and neopterin levels were analyzed in CSF in 52 human immunodeficiency virus type 1 (HIV-1) infected patients, 37 of whom had no neurological symptoms, eight had HIV-1 associated dementia (HAD) and seven had acquired immunodeficiency syndrome (AIDS) with other neurological complications. The mean CSF tau concentration was significantly higher in patients with HAD (380 pg/ml) compared with patients with a neuroasymptomatic HIV-1 infection (120 pg/ml, p=0.01). No difference in CSF tau levels was found between patients with HAD and patients with AIDS with other neurological complications. Patients with HAD had a significantly higher mean CSF neopterin concentration (83 nmol/l), as marker of intrathecal immune activation, than HIV-1 infected individuals without neurological complications (20 nmol/l, p<0.01) and CSF tau and neopterin levels were correlated (r = -0.65, p<0.001). In contrast, there was no significant correlation between CSF tau and CSF HIV-1 RNA levels. The correlation found between CSF tau and CSF neopterin levels indicates that immune activation is a pathogenic mechanism for neural injury in HIV-1 infection, especially in HAD.

This research was supported by grants from the Swedish Medical Research Council (projects #11560 and #12103), from the Swedish Society of Medicine, and from the Göteborg Society of Medicine.

P2

Pituitary adenylate cyclase activating polypeptide (PACAP) activates HSV-1 from quiescently infected PC12 cells. R. J. Danaher, A. Sauveas-Arb, R.J. Jacob, C. S. Miller, College of Dentistry, College of Medicine, University of Kentucky, Lexington, KY.

Previously we described a novel in vitro model of a non-productive herpess simplex virus type 1 (HSV-1) infection in neurally differentiated (Nd)-PC12 cells that allows for inducible virus replication upon forskolin or heat stress treatment (Danaher et al., J NeuroVirology 5:258, 1999). In this study we determined whether a naturally occurring neuropeptide (i.e., pituitary adenylate cyclase activating polypeptide [PACAP]) is capable of inducing virus activation within this system. Nd-PC12 cells were infected with HSV-1 at an MOI of 3 and a quiescent state established using transient acylcyclonucleosine (ACV) treatment. Induction of quiescently infected, non-productive cultures on day 15 post-infection with PACAP-38 and PACAP-27 resulted in virus production in 50% of cultures as early as day 5 post-induction, whereas 0% was detected in mock-induced culture supernatants. The protein kinase A inhibitor H89 diminished the response to PACAP-38 as measured by frequency of cultures activating virus (75% less) and by slower kinetics compared with cultures not containing an inhibitor. Addition of the protein kinase C inhibitor GF had no effect on virus induction. These data indicate that PACAP, a ganglioside neuropeptide putatively associated with the transmission of nociceptive information, is capable of inducing HSV-1 activation through the protein kinase A pathway.

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Abstracts

P3

The cytokine TNFα and the HIV-1 proteins „Env“ and „Tat“ reduce the glutamate induced intracellular Ca²⁺ increase in cultured cortical astrocytes Költer, H.,1 Garrido, S.R.,1 Giesen, J.J.v.,2 Freud, M.,2 Schaak, H. and1 Jensen, G.1 Dept. of Neurology and Institute of Medical Microbiology and Virology, Heinrich-Heine-University, Düsseldorf, Germany

Locally released cytokines and virus proteins appear to contribute to the pathogenesis of HIV-1 associated encephalopathy. As immunocompetent cells, astrocytes are target cells of cytokines. Astrocytes also support the undisturbed neuronal function by maintaining local ion homeostasis and clearing extracellular neurotransmitters such as glutamate. Since astrocytes also express ionotropic glutamate receptors, they seem to modulate synaptic transmission, e.g. by propagating Ca²⁺ waves within the glial syncytium. Here, we microfluorometrically recorded the intracellular Ca²⁺ increase upon glutamate (500µM) application in cultured cortical astrocytes from newborn rats loaded with the Ca²⁺ indicator Fluo-2. We found that cell incubation with the cytokine TNFα (50-1000 U/ml) but not IL-1β (10-200 ng/ml), IL-2 (10 U/ml) and IL-6 (10-100-1000 U/ml) for 60 minutes induced a significant reduction (of about 60%) of the glutamate induced Ca²⁺ response whereas in cultured cortical neurons the glutamate induced intracellular Ca²⁺ increase was unaffected by incubation with TNFα. A similar reduction of the glutamate induced Ca²⁺ response was also observed after incubation of astrocytes with the supernatant but not with a cell extract of transiently transfected HeLa-A4 cells expressing the HIV-1 protein „Env“ or following incubation with a cell extract but not with the supernatant of transiently transfected HeLa-A4 cells expressing the HIV-1 protein „Tat“. We conclude that the cytokine TNFα and the HIV-1 proteins „Env“ and „Tat“ interfere with the electrophysiological function of astrocytes. This may contribute to the development of symptoms in patients suffering from HIV-1 associated encephalopathy.

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P4

NF-1 class D and its role in JCV replication. Monaco-Kushner, M.C.G., Frye, S., Sabath, B.F., Daruma, L.C., and Major, E.O., Laboratory of Molecular Medicine and Neuroscience, NINDS, NIH, Bethesda, Maryland, USA.

The nuclear factor-1 (NF-1) family of transcription and replication proteins is involved in the transcription of several viral and cellular genes and viral DNA replication. The regulatory sequence of JCV is characterized by several binding sites for NF-1 proteins. JCV, a small DNA virus of the polyoma family, is able to infect not only cells from the central nervous system, such as oligodendrocytes and astrocytes, but also cells in the immune system and hematopoietic progenitor cells. Recently, we have shown that when CD34 precursor cell lines were treated with PMA and differentiated into macrophage-like cells, they were no longer susceptible to JCV infection. Moreover, we demonstrated that NF-1 class D is highly expressed in the human brain cells. HeLa cells, that are not permissive to JCV infection, showed a lower level of class D RNA in comparison to primary human astrocytes, the most susceptible cells to JCV infection present in the CNS. In this study we investigated the role of NF-1 during cell differentiation and the possible correlation with the loss of susceptibility to JCV infection in hematopoietic precursor cells that differentiate into macrophages. Using a gel mobility shift assay, we detected the induction of NF-1-DNA complexes during PMA treatment of KG-1 cells but not in KG-1 control cells and human astrocytes. We also detected changes in the mobility of the NF-1-DNA complexes in comparison with the duration of PMA treatment. Preliminary data of transfection of NF-1 class D DNA into the KG-1 PMA treated cells resulted in the re-establishment of susceptibility to JCV. These data show that the expression of NF-1 class D is modulated during PMA-induced differentiation in the KG-1 hematopoietic progenitor cell line and that high level of this class reduces the susceptibility of these cells to JCV infection. These data suggest that the selective expression of NF-1 class D may correlate with successful infection of cells with JCV.

P5

The ICP0 protein of herpes simplex virus 1 does not accumulate in the nucleus of primary neurons in culture. Chen, X-F., Li, J., Goss, J., Wolfe, D., Giannoso, I.C., Mata, M., and Fink, D.I., Departments of Neurology, Molecular Genetics and Biochemistry, and GRECC, VAMC, University of Pittsburgh, Pittsburgh, PA USA.

Infected cell protein 0 (ICP0), the product of the herpes simplex virus (HSV) immediate early (IE) 0 gene, is a promiscuous transactivator of viral early (E) and late (L) gene expression. HSV mutants lacking ICP0 function are severely deficient in viral growth and protein synthesis, particularly at low multiplicities of infection. Early in the infectious process in vitro, ICP0 protein accumulates in distinct domains within the nucleus to form characteristic structures active in the transcription of viral genes. However, following infection of primary trigeminal ganglion cells in vitro with a recombinant HSV mutant that expresses only ICP0, we observed that ICP0 protein accumulated in the characteristic intranuclear distribution only in the nuclei of Schwann cells; neurons in the culture did not accumulate ICP0 despite expression of ICP0 RNA in those cells. The same phenomenon was observed in PC12 cells differentiated to assume a neuronal phenotype. In primary neurons in culture, the amount of ICP0 protein could be increased by pharmacologic inhibition of calcium activated protease (calpain) activity, or by inhibition of protein phosphatase 2B (calcineurin). The failure of ICP0 protein to accumulate in the nucleus of neurons suggests that one mechanism which may impair efficient replication of the virus in neurons, and which favor the establishment of viral latency in those cells, may be found in the cell-specific processing of that IE gene product.

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P6

A system for highly productive infection of primary human astrocytes by HIV-1. Mario Caski, Janace Ng Efong Thai, and David J. Volksky, Molecular Virology Laboratory, St. Luke’s Roosevelt Hospital Center and Columbia University, New York, New York.

Studies on the control of the HIV-1 life cycle in astrocytes have been limited by the difficulty of achieving efficient infection of these cells in vitro. We have developed a new model system that permits initial high-level infection of human fetal astrocytes with HIV-1 in vitro by exposure of the cells to virus pseudotyped with Moloney murine leukemia virus (MLV) or vesicular stomatitis virus (VSV) envelopes. Native HIV-1/LNL-3 infected only 1-2% of cells within 1 week, but pseudotyped virus infected 30-50% of cells, permitting efficient detection of HIV-1 structural and regulatory proteins and RNA transcripts. The peak of infection was Day 7 with 1700 ng/ml and 482 ng/ml p24 produced by astrocytes infected with VSV and MLV pseudotyped HIV-1, respectively. Viral proteins Nef, Gag p24 and p55, and envelope gp120 and gp160 were detected by immunoblot at Day 7 post infection. The three major HIV-1 RNA transcripts of 9kb, 4kb, and 2kb were detected at Day 2, 7, 14, and 28 post infection. Infection was long lasting but slowly declined in extent and steady-state levels of 100 ng/ml were achieved by 7 weeks after infection. Stimulation with exogenous IL-1β at Day 49 increased p24 production four-fold and increased the number of total astrocytes expressing HIV-1 antigens three-fold. After a productive phase of 28 weeks, astrocytes enter a low-level chronic phase of infection, but HIV-1 expression can be induced by IL-1β. Infection with native HIV-1/LNL-4-3 was low-productive as previously shown.

These results indicate that the major barrier to HIV-1 infection of astrocytes is at virus entry, and that once this barrier is overcome, these cells are highly permissive to HIV-1 replication.

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Journal of NeuroVirology
Impact of commonly encountered C/EFP factor binding site sequence variants found in HIV-1-infected brain tissue on LTR activation in monocyte/macrophage cell populations. Ross, I.H., Pomerance, M., Corboy, J.R., Gartner, S., McAleer, D., and Wigdahl, B., Department of Microbiology and Immunology, Penn State College of Medicine, Hershey, PA, 17033, Department of Neurology, The University of Colorado Health Sciences Center, Denver, CO, and Department of Neurology, The Johns Hopkins School of Medicine, Baltimore, MD, USA

Recent studies have shown that two C/EFP sites are critically important for efficient HIV-1 replication within monocyte cell populations, which serve as an important vehicle for transport of virus to the CNS. Given the important role that monocyte tropism plays in HIV-1 infection of the brain and the general impact of LTR sequence variation on viral replication, we examined C/EFP site sequence variation within brain-derived LTR populations. Brain-derivedLTRs commonly possess a C/EFP site I (−107 to −118) configuration (guanine substitution at position 6 of the clade B consensus sequence, designated 6G) that leads to enhanced binding of C/EFP factors over that observed with the HIV-1 clade B consensus sequence. In contrast, the 6G configuration appears infrequently within PBMC-derived LTRs. In addition, the clade B C/EFP site II (−167 to −175) consensus sequence has a very high affinity for C/EFP factors, and is conserved in over 85% of brain-derived LTRs examined, but not in PBMC-derived LTRs. Analysis of all available brain-derived LTRs, indicates that over 90% of the LTRs examined contain a high affinity C/EFP site II. Given the prevalence of C/EFP binding sites that are highly reactive with respect to binding C/EFP factors in monocyte nuclear extracts, we have investigated the relevance of each C/EFP site with respect to IL-6 stimulation, Tat trans-activation and basal LTR activity utilizing transient expression analyses. We have also investigated the relevance of these sites in different LTR backbones (LA1, YU-2, and 89.6) which are lymphotropic, monocytotropic, and dual tropic in nature. This research was supported by PHS grants to BW (NS27405 and NS32092).

Inhibitory and promoting activity of virus-induced interferon toward Borna disease virus in mouse brains

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Borna disease virus (BDV) is a non-cytopathic RNA virus that can replicate in neuronal cells of mice. We found that brains of transgenic mice expressing interferon (IFN)-α1 under control of the astrocyte-specific GFAP promoter contained strongly reduced levels of viral transcripts at 4 weeks post-infection. Immunohistochemical (IHC) analysis further showed that the number of BDV-infected cells was much lower in brains of transgenic animals than in non-transgenic littermates, indicating that transgenically expressed IFN-α1 can effectively block BDV multiplication in the CNS. To determine whether IFN induced during the course of infection might similarly help to restrict viral spread, we compared the BDV growth kinetics in cultured cells and in brains of wild-type and IFNARα mice that lack a functional type I IFN receptor. As expected if virus-induced IFN exhibited antiviral activity, BDV replicated well in embryo cells from IFNARα but not wild-type mice. However, IHC and western blot analysis showed that virus spread was not accelerated in brains of infected IFNARα mice, and that viral antigen persisted at high levels in both wild-type and mutant mice, suggesting that the IFN response after BDV infection was too weak or occurred too late for being effective. Surprisingly, Northern blot analyses with strand-specific hybridization probes showed that viral transcript levels in brains of persistently infected IFNARα mice were about 10-fold lower than in congenic wild-type 129 mice, whereas viral genomic RNA was produced in large excess in brains of IFNARα mice. Thus, IFN action in persistently infected neuronal cells seems to freeze the BDV polymease in transcription mode which results in enhanced viral mRNA synthesis and low genome replication. To our knowledge, this is the first report demonstrating that IFN can stimulate (rather than inhibit) viral mRNA synthesis.

Molecular basis of attenuation of tick-borne encephalitis virus by cell culture adaptation. Mandl, C.W., Kroschewski, H., Holzmann, H., Allison, S.L., and Heinz, F.X., Institute of Virology, University of Vienna, Vienna, Austria

Tick-borne encephalitis (TBE) virus is a human pathogenic member of the family Flaviviridae that is responsible for thousands of cases of severe neurological illnesses in Europe and Asia every year. The large envelope glycoprotein E is a major determinant of the biology and pathogenesis of this virus, mediating several fundamental functions of the viral life cycle including viral attachment to the host cell surface and membrane fusion. Structural and functional properties of the TBE virus E protein have been intensely studied during the past years, and its 3-D structure has been solved by X-ray crystallography. In this study we investigated mutations within protein E that arose spontaneously during growth of TBE virus in BHK 21 cell cultures and the influence of these mutations on the virulence of TBE virus in the mouse model. Reproducibly, single or double mutations arose within a few cell culture passages, but after that the mutant viruses remained genetically stable. In various experiments, mutations affecting more than 10 different amino acid residues of protein E were observed. According to the 3-D structure these mutations were scattered over the entire outer surface of protein E. A striking common feature was that they caused an increase in negatively charged residues (i.e. either the gain of a positively charged amino acid residue (Lys or Arg) or the loss of a negatively charged residue, and preliminary experiments suggest the involvement of cell surface glycosaminoglycans (GAG), such as heparan sulfate. To study the effects of protein E mutations independently of any unrecognized mutations that might have arisen elsewhere in the genome, three of these mutations were introduced into infectious cDNA clones of TBE virus. Analysis of the recombinant mutant viruses derived from these clones proved that the E protein mutations alone were responsible for the alterations in cell surface binding, growth properties and, most significantly, attenuation of TBE in vivo. Thus, selection of GAG binding mutants appears to be an important mechanism of TBE virus attenuation by cell culture adaptation.

JC virus as a molecular marker to distinguish neuronal and glial progenitor cells. Messam, C.A., Hou, J., and Major, E.O. Laboratory of Molecular Medicine and Neuroscience, NINDS, NIH, Bethesda MD, USA

Many neurotropic viruses are selective for subtypes of cells in the human CNS. JC virus for example, primarily infects and degrades oligodendrocytes in the human brain, resulting in the demyelinating disease, progressive multifocal leukoencephalopathy (PML). In vitro, JC virus infects human fetal brain (HFB) derived astrocytes, but does not infect neuronal cells. Although JC virus can bind to the surface of several cell types, susceptibility to infection appears to depend on the availability of cellular factors within target cells. Therefore, it is hypothesized that JC virus has the ability to distinguish between neuronal and glial cells at a molecular level. The goal of this study was to determine whether JC virus could distinguish neuronal from glial progenitor cells. HFB derived multipotent CNS progenitor cells were infected and propagated in culture using serum free defined medium containing basic fibroblast growth factor and epidermal growth factor. RT-PCR, Western blot and immunocytochemistry demonstrate that these progenitor cells express the neuroepithelial marker nestin, but does not express proteins found in differentiated neurons or glial cells. The progenitor cells can subsequently be differentiated to produce neuronal cells expressing MAP-2 and astrocytes expressing GFAP. Two weeks after infection with JC virus, viral genome could be detected by in situ hybridization and late viral protein, VP-1, detected by immunocytochemistry and Western blot. Intact virus was also produced as indicated by its ability to hemorrhaginate human type O positive red blood cells. These studies can determine when JC virus susceptible progenitor cells differentiate into neuronal or glial cells. These studies will also determine if JC virus susceptibility of neuronal or glial lineage cells is dictated at the molecular level.
P11

CCR5, CCR2 AND CXC4 EXPRESSION IN INDIVIDUALS WITH ADC

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The aim of this study was to determine whether mutations in the chemokine receptors CCR5, CCR2 and the ligand for CXC4 (SDF-1-3′A) correlate with the presence and severity of ADC. The CCR5 receptor is essential for entry of HIV into macrophages and microglia and CXC4 is thought to be important in astrocyte infection. We hypothesised that mutations in these receptors or their ligands would make it difficult for HIV to enter and infect microglia and astrocytes leading to relative protection against ADC, both in presence and severity.

PCR, PAGE and PCR-restriction fragment length polymorphism (RFLP) techniques were used to screen 36 individuals with ADC, 100 HIV-1 negative individuals and a group of 96 HIV-1 progressed. The frequency of SDF1-3′A homozygotes was significantly greater in the ADC stage 1 group compared with the ADC stage 2 group, HIV-1 progressed and the HIV-1 negative control group. In addition, there was a trend towards significant differences in CCR5A32 heterozygosity in the ADC stage 1 compared with ADC stage 2 (p=0.074).

These results suggest that there may be a genetic component in the pathogenesis of ADC. Moreover, this is the first study to show an elevation of the SDF-1-3′A polymorphism in patients with mild ADC compared with patients with more severe ADC. Further studies to look at the role of other receptors such as CCR3, Bonzo and BOB in ADC pathogenesis will need to be performed. Alternatively HIV-1 or other factors may upregulate the expression of mutated CCR5 or CXC4.

This study was supported by a NH&MRC research grant.

P12

Characterization of L-protein of Thelher's murine encephalomyelitis virus. Obara, Y., Obachl, M., and Ogadiri, T., Department of Microbiology, Kainawa Medical University, Uchinada, Ishikawa, Japan

Thelher's murine encephalomyelitis virus (TMEV) is divided into two subgroup strains, i.e., highly virulent GDII subgroup strains which do not induce neither demyelination or virus persistence, and less virulent DA subgroup strains which cause demyelination in the spinal cords of mice with virus persistence. L-protein, which is out of frame with the polyprotein and only synthesized in DA but not GDII subgroup strains, has been reported to be important for virus persistence and demyelination; however, the properties of L-protein still remain to be clarified. In the present study, we have begun to characterize L-protein in DA-infected BHK-21 cells by using a rabbit polyclonal antibody raised against synthetic peptides corresponding to L-protein amino acid residues 70 to 88. Pulse labeling experiment and Western blotting demonstrated that the synthesis of L-protein occurred 4 h post-inoculation (p.i.) and peaked 8 hr p.i. along with other viral proteins; however, the amount of L-protein was far less. Indirect immunofluorescent staining demonstrated that L-protein appeared in the cytoplasm 4 or 5 h p.i. and remained there for up to 6 h p.i. when cytopathic effect occurred. Cell fractionation analysis showed that L-protein was present in the cytosol but not in the microsomal fraction. The protein was not detected in purified virions. The present study suggests that L-protein is not packaged into virions and remains in the cytoplasm throughout infection, probably in a soluble form.

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P13


The 96-amino acid Vpr, a virion-associated protein, is essential for transcription of the HIV-1 genome. In addition, this protein regulates a number of host cellular events, including proliferation, differentiation, apoptosis, and cytokine production. It is evident that interaction of Vpr with several cellular and perhaps viral proteins is important for its activity. Here, we show that Vpr activates HIV transcription and replication through cooperative interactions with another viral regulatory protein, Tat, in primary human microglial and astrocytes. The interaction of these two viral proteins is mediated through RNA molecule. Since Tat and cyclin T1 induce the HIV-1 LTR activity through association with each other, we examined the cooperative activity of Vpr, Tat, and cyclin T1 upon LTR transcription. Results from co-transfection studies indicated that overexpression of Tat, cyclin T1, and its partner CDK9 and wild type Vpr, but not Vpr mutant R73S, increases the level of activation of the LTR. Results from protein-protein interaction studies indicated that Vpr is associated with both Tat and cyclin T1 in cells forming a ternary complex. Our data demonstrate that the binding sites for Tat and Vpr within the cyclin T1 are distinct, and that association of these viral proteins with cyclin T1 is independent from each other. The functional and structural observations from these studies provided a working model on the cooperative interaction of Vpr with viral and cellular proteins Tat and cyclin T1 and its involvement in control of viral gene transcription at immediate early stage of infection prior to the participation of other viral regulatory proteins.

P14

HIV-1 RNA Levels, Antiretroviral Therapy and Distal Sensory Polyneuropathy in Participants of ACTG 291


Background: HIV-associated distal sensory polyneuropathy (DSP) presents with numbness, paresthesia and pain affecting primarily the lower extremities. Dideoxyxymethylocule (ddN) analog drugs including didanosine (ddI), stavudine (d4T) or zalcitabine (ddC) may trigger or exacerbate DSP. The goal of HIV-1 treatment is suppression of HIV-1 plasma RNA (viral load (VL)); but symptoms of DSP may lead to ddN dose reduction or discontinuation. Design:Methods: This study was part of ACTG 291, a placebo-controlled trial of probenecid human nerve growth factor for the treatment of DSP. Viral load (VL) (Roche ampiclon 1.0, Ultrasensitive assay) was available in 236 subjects, most of whom receiving highly active antiretroviral therapy. Averag maximum measurement was measured daily by the Gracely pain scale. A global assessment of pain was also elicited. DSP was assessed by a standard neurological evaluation that included quantitative sensory testing (CASE IV). Results: VL did not differ according to age, race/ethnicity, or use of ddNs (currently, versus recent use, versus remote non-use). VL was undetectable (<200 copies/ml) in 89 subjects and detectable in 147. Subjects with detectable VL had marginally higher average and maximum neuropathic pain compared to subjects with undetectable VL (p<0.09 in both cases). In the detectable VL group, subjects with higher global assessment of pain had mean RNA levels significantly greater than those with less pain (4.51 vs 3.65 log copies/ml; p=0.001). Similarly, subjects with abnormal Toe cooling detection thresholds had higher VL compared to subjects with normal thresholds (4.15 vs 4.56 log copies/ml; p=0.005).

Conclusion: The significant correlation between severity of DSP symptoms and viral load suggests that aggressive use of antiretroviral drug regimens, including ddN, to suppress VL may reduce the incidence or severity of DSP. Therefore, virological control might outweigh ddN neurotoxicity. However, prospective studies are needed to further assess these issues.
P15

The leader protein of Thielers virus inhibits type-I interferon production and is essential for persistence of the virus in the central nervous system, but detrimental for macrophage infection.

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Thielers virus isolates are classified into two subgroups according to the disease they cause in the central nervous system (CNS) of susceptible mice. The GDVII strain causes an acute fatal encephalitis. The DA and BEAn strain provokes a chronic demyelinating disease considered as a model for multiple sclerosis. These viruses have a striking ability to persist and multiply lifelong in the CNS, in the face of a strong specific immune response.

The leader ("L") protein encoded by Thielers virus is a 76 amino acid long peptide reported to contain a zinc finger motif. On the basis of the L929 cells infection by wild-type and L mutant viruses, it was proposed that the L protein could act as an inhibitor of type-I (α/β) interferon production.

In this work, we show that the L protein indeed inhibits the production of type-I interferon by L929 cells. A point mutation in the zinc finger motif was sufficient to block this activity, outlining the importance of this motif in the protein function. Accordingly, we showed that a DA virus bearing the zinc finger mutation was dramatically impaired in its ability to persist in the CNS of SJL/J mice. This is in agreement with reports showing that the L protein is essential for neurovirulence of the GDVII strain.

Unexpectedly, we observed that the presence of the L protein is detrimental for the infection of a macrophage cell line, in spite of the fact that these cells produce type-I interferon. Thus, in these cells, the effect of interferon inhibition is counterbalanced by a yet undefined mechanism. Interestingly, macrophages appear to be a key target of the virus during the persistent phase of Thielers virus infection in the CNS.

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P16

Glia/neuronal specific activation of the JC virus (JCV) promoter in the presence of HTLV-I Tax. Okada, Y., Sawa, H., Tanaka, S., Nakashima, K., 1Laboratory of Molecular & Cellular Pathology, Hokkaido University School of Medicine, Department of Pathology, Sapporo Municipal Hospital, Department of Pathology, National Institute of Infectious Disease, Japan and Department of Medical Microbiology, Conway Institute of Bionucleolar and Biomedical Research, University College Dublin, Ireland

It has been previously reported that the cell-specific activation of the JCV promoter is regulated by multiple cellular proteins present in glial cells. We have already demonstrated that HTLV-I Tax activates the transcriptional potential of the JCV promoters in human neuronal/gliai cells via the NF-κB pathway, and that transactivation of the virus promoter by Tax was not detected in non-neuronal cells. To investigate the mechanisms of the neuronal/gliai cell specific activation of the JCV promoters by Tax, we have first analyzed the role of Tax-1/Oct6, which is specifically expressed in brain and known to be a JCV transactivator. Immunoprecipitation assays showed that while overexpressed Tax-1/Oct6 bound to Tax, co-transfection of Tax-1/Oct6 and Tax failed to transactivate the JCV promoter in non-neuronal cells. Then, we have begun to investigate the cellular factors which participate in the neuronal cell-specific transactivation of JCV using EMSA, and we could demonstrate the existence of a non-neuronal cell-specific protein complex, which interacts with NF-κB and Tax, and may be associated with the inhibition of the transactivation by Tax. This study is the first demonstration of the mechanism of neuronal cell-specific activation of JCV promoters regulated by an inhibitory protein in non-neuronal cells in the presence of Tax.

This research has been supported by CREST of JST (Japan Science and Technology).

P17

CD4+ lymphocyte-mediated suppression of cytomegalovirus expression in human astrocytes. Yager, S.L., Cho, C.J., Gokser, G., D., Hu, S., Peterson, P.K., and Lokeshwar, J.R., Institute for Brain and Immune Disorders, Minneapolis Medical Research Foundation and the University of Minnesota Medical School, Minneapolis, MN

Human cytomegalovirus (CMV) encephalitis is associated with decreased T-lymphocyte counts in advanced AIDS. Although activated T cells, recruited across the blood-brain-barrier, have limited effector functions, they do produce cytokines that have antiviral properties. To test the hypothesis that T lymphocytes mediate anti-CMV activity in brain cells through the production of soluble factors, highly purified primary human astrocyte and stimulated T-cell co-culture systems were employed. Three clinical isolates and a laboratory strain (AD169) of CMV were used to demonstrate that DNA replication increased exponentially in astrocytes but not in microglia. Both CD4+ and CD8+ lymphocytes confer an antiviral state in human astrocytes, without accompanying cytotoxic damage. CMV-stimulated CD4+ lymphocytes from seropositive but not seronegative donors suppressed viral gene expression in astrocytes. This suppressive activity was mediated through soluble factors. Supernatants from astrocyte/CD4+ lymphocyte co-cultures confer this antiviral state to fresh astrocytes. Although CMV-stimulated CD8+ lymphocytes from seropositive donors suppressed viral gene expression in astrocytes, they may require additional cellular signals to mediate these antiviral effects. Neutralizing antibodies to TNF-α and IFN-γ did not abrogate the CD4+ lymphocyte-mediated antiviral properties. These findings suggest that multiple factors may be responsible for the CD4+ lymphocytes-mediated non-cytotoxic antiviral function. These mechanisms may have potential host defense implications in CNS infections.

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P18

Perforin is not essential for development of T cell-mediated neurological disease after Borna disease virus infection of susceptible mice. Haarmann, L., Schramek, K. and Staebell, P., Dept. of Virology, Institute for Medical Microbiology, University of Freiburg, Hermann-Heider-Str. 11, 79104 Freiburg, Germany

Borna disease virus (BDV) infection can induce severe neurological disorder in young MRL/Mp (H-2b) mice which is mediated by CD8+ T cells. We backcrossed the functionally inactivated perforin gene to the MRL genetic background and tested these mice (designated MRL/PKO) for susceptibility to BDV-induced disease. MRL mice lacking a functional perforin gene showed similar susceptibility to neurological disease as wildtype MRL/Mp mice. Rates of severe neurological disorder varied from 20% to 70% depending on age at time of infection and type of BDV variant used. Lymphocytes prepared from brains of diseased MRL mice have previously been shown to exhibit a highly activated phenotype and strong MHC-restricted CD8+ T cell-dependent cytotoxic activity specific for the BDV nucleoprotein. When brain lymphocytes from heavily diseased MRL/PKO mice were used for cytotoxicity assays, no specific lytic activity was observed, although activated CD8+ T cells predominated in brain lymphocyte preparations like in the wt situation. Thus, lytic activity of BDV-specific brain lymphocytes did not reflect the pathogenic potential of this T cell subset. Immunohistological analysis showed that the distribution of T cell subsets was similar in both MRL/Mp and MRL/PKO mice. Like in wildtype mice, CD8+ T cells of MRL/PKO mice were predominantly found in the brain parenchyma, whereas CD4+ T cells were mainly located in the perivascular cuffs. Virus load in MRL/PKO mice was comparable to that in wildtype mice, but interestingly, MRL/PKO mice were also able to limit virus spread in the CNS after peripheral immunization prior to BDV infection with a recombinant vaccinia virus expressing the BDV nucleoprotein. These data suggest that perforin-mediated lysis is not operative as an effector mechanism of CD8+ T cells in BDV induced neurological disorder nor in control of neuronal virus spread.

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**P19**

Vpr-mediated HIV-1 LTR Transactivation: Implications relevant to CNS Infection. Hogan, T.H., McAllister, J.J., Steiniec, K.E., Ross, H.L., and Widgahl, B., Department of Microbiology and Immunology, Penn State College of Medicine, Hershey, Pennsylvania USA

HIV-1 replication is regulated, in part, by interactions between cellular transcription factors and cis-acting eukaryotic promoter elements within the long terminal repeat (LTR) and the viral transactivator, Vpr. HIV-1 Vpr has been shown to activate basal transcription through interactions with members of the Sp transcription factor family and the G/C box array of the HIV-1 LTR as well as by mediating cell cycle arrest at the G2/M interface.

In addition, sequence heterogeneity within the LTR influences both basal and activated LTR activity. Previous studies have shown C/EBP site 1 (--120 to --109) and site II (--178 to --159) are required for viral replication in monocyties, but not CD4+ T lymphocytes. We have previously demonstrated that C/EBP site I often contains a guanine substitution at position 6 (6G) leading to a high affinity configuration in 43% of brain-derived LTRs. A well-conserved high affinity C/EBP site II is found in 90% of brain-derived LTRs. Utilizing electrophoretic mobility shift (EMS) analysis, we have shown a direct association between C/EBP site I and a Vpr-GST fusion protein (derived from 89.6 dual tropic HIV-1 strain). This association was enhanced by the use of a lower affinity C/EBP site I sequence variant. In addition, we examined the effect of Vpr on a panel of recombinant HIV-1 LTR-luciferase constructs containing C/EBP site I and II elements of low and/or high affinities. These studies indicate that a low affinity C/EBP element at site I enhances both the magnitude and fold-activation of a LTR-based LTR by Vpr and is dependent on a high affinity site II. Studies are now underway to determine whether this effect is primarily mediated through the absence of C/EBP factors or by an enhanced ability of this sequence variant to directly recruit Vpr. To extend these observations, we have cloned additional Vpr constructs from a brain-derived M-tropic YU-2 and a blood-derived T-tropic LAI molecular clones and are currently investigating their interaction with C/EBP site configurations that are preferentially encountered in HIV-1-infected brain tissue in the context of a brain-derived (YU-2) and a blood-derived (LAI) LTR.

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**P21**

Therapy resistant Borna disease virus infection-related psychosis improved by CSF filtration

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In extant seropidemiological and clinical studies evidence was found, that mild BDV encephalitis can underlie some cases of schizophrenic or affective psychoses (Bechter et al 1995 + 1998). Possible mild BDV encephalitis was assumed, when BDV specific immunoglobulin was found produced within CSF spaces of BDV seropositive patients during psychosis. BDV encephalitis in animals is an immune pathological disorder, a possible pathogenic similarity to human neurological disorder, Guillain-Barré syndrome (GBS) was hypothesized. GBS can be treated by plasmapheresis or therapy resistant GBS by cerebrospinal fluid filtration (CSFF) (Wollinsky et al 1994). We introduced CSFF in psychiatric treatment. 4 BDV seropositive patients suffering from therapy resistant schizophrenic or affective psychoses were treated by CSFF by a lumbar CSF catheter 250-300 ml CSF was filtered within about 3 hours daily, over 5 days. Clinical status was assessed by tests and ratings (Stroop, ZVT, GSP, HAMD, SCL-90 a.s.o.). Patients improved impressively under CSFF, 3 patients relapsed partially 1 to 3 weeks later and were again filtrated. After 1 or 2 filtrations in all cases a lasting therapeutic effect of CSFF even to fall asleep was observed (observation time 2 ½ years/ 10/102 months). Within the CSFs of all patients an antineurotropic peptide was detected, earlier described in various CNS inflammatory disorders (Brinkmeier et al 1996, Aulkenemeyer et al, in press; Weber et al 1999). Supported by Thedorea & Vada Stanley Foundation

**P20**

Intrathecal immunomodulation, but not cerebrospinal fluid (CSF) viral load, correlates with impairment of blood-brain barrier function in HIV-1 infection. 1Gisslen, M., 2Swennerholm, B., 3Fuchs, D., 4Hagberg, L. Department of Infectious Diseases and 5Virology, Sahlgrenska University Hospital, Göteborg, Sweden, and 6Institute of Medical Chemistry and Biochemistry and Ludwig Boltzmann-Institute for AIDS Research, University of Innsbruck, Austria

HIV-1 infects the central nervous system (CNS) and impairment of the blood-brain barrier is a common finding in HIV-1 infection. The pathogenesis behind this impairment is not fully settled. Blood-brain barrier function (i.e. albumin ratio; CSF-albumin/serum-albumin), intrathecal immunomodulation (CSF neopterin), and CSF HIV-1 RNA levels were measured in 112 HIV-1-infected patients without antiretroviral treatment. All patients were neuro-asymptomatic, and their median CD4+cell count was 259 (range 9-1664) x10⁴/μl. Twenty-two of them had AIDS (CDC stage C), 25 had minor symptoms (CDC stage B), and 35 were asymptomatic (CDC stage A). The median albumin ratio was 5.1 (reference value <7.0) and 29 (26%) of the patients had albumin ratios >7.0 as sign of impaired blood-brain barrier functions. The median CSF neopterin concentration was 16.7 (reference value <6.2) and the median HIV-1 RNA level was 3.4 log₁₀ copies/ml CSF and 4.3 log₁₀ copies/ml plasma. A significant correlation was found between albumin ratios and CSF neopterin concentrations (r=70.35, p<0.001), but not between albumin ratios and CSF HIV-1 RNA levels.

The results suggest that impairment of the blood-brain barrier could be found in about 1/3 of HIV-1-infected individuals, and that intrathecal immunomodulation rather than CSF viral load is associated with blood-brain barrier dysfunction.

**P22**

Borna disease virus infection possibly underlying psychiatric disorders - clinical and epidemiological evidences

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Serpodiemological studies on psychiatric neurological and surgical patients of the clinics of Gunzburg (n=5000) showed significantly increased BDV seroprevalence in psychiatric patients (RR=6.8, quartile 17-30 years; Bechter et al 1998), suggesting possible causality for psychiatric disorders. BDV seroprevalence increased with age in surgical controls and neurological patients from Gunzburg and in blood donors from different region, indicating life long risk of infection.

BDV seropositive neurological patients showed more frequently acute and chronic meningoencephalitis. BDV seropositive psychiatric patients showed increased psychiatric comorbidity (Bechter et al 1998) more frequently brain atrophy (Bechter et al 1994; Waltrip et al 1995), conesthesias (Bechter 1998), more severe schizophrenic courses (Walthrip et al 1997; Bechter et al 1998). During acute affective or schizophrenic psychoses BDV specific immunoglobulin G was found intrathecal produced, suggesting mild BDV encephalitis underlying related psychoses (Bechter et al 1995 and 1996). This view was recently corroborated by detection of an antiallergic peptide within CSF of such patients (Bechter et al 1999), earlier described in various CNS inflammatory disorders (Brinkmeier et al 1996, Köller et al 1997).
**Abstracts**

**P23**

Mutation of Ser257 of glycoprotein E gene in clinical isolates of herpes simplex virus type 1 of encephalitic origin. Styrenius, M.*, Studahl, M.*, Conrad, N., Bergström, T. Departments of 1Clinical Virology, 2Infectious Diseases and 3Pathology, Göteborg University, Göteborg, Sweden.

Herpes simplex virus type 1 (HSV-1) is a neurotrophic herpesvirus that occasionally may cause a severe, focal encephalitis (HSE). Earlier work in our laboratory has shown that isolates from brains of HSE patients display a neuroinvasive phenotype, and the current project aims at defining the molecular basis for this virulence property. We have analyzed the DNA sequence of the glycoprotein E (gE) gene in HSE isolates as well as in control strains from patients with oral herpes. Two genotypic variants of the gE gene were found, of which one was prevalent in all (9/9) of the CNS isolates but in only half (5/10) of the oral strains. This variant contained a number of mutations as compared to the published sequence though the other variant was identical to the reference. Among the minority mutations of the CNS-associated genotype, one (Ser257) was of special interest since three different nucleotide alterations of this codon was detected. Furthermore, this mutation was also detected in 3/3 CSF samples derived from HSE patients. The resulting amino acid replacement was localized within a functional domain of gE responsible for complex-forming with another envelope glycoprotein (gF) and subsequent binding of monomeric IgG. Further studies are aimed at defining eventual functional consequences of the genotypic differences of the gE gene with special emphasis on Fc-binding and cell-cell spread. We suggest that alteration of Ser257 in HSV-1 gE is a prerequisite for CNS invasion of the virus.

This research was supported by Swedish Medical Research Council. . .

**P24**

NEURONAL CHEMOKINE RECEPTOR RESPONSES ARE ENHANCED BY HIV ENVELOPE AND GLUTAMATE.

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Chemokine receptors have been implicated in the pathogenesis of neuroAIDS via their activation by both chemokines and viral envelope. Increased levels of chemokines are found in brains of patients with HIV-induced encephalitis and neurological manifestations of HIV-1 infection appear to correlate with the amount of virus in the central nervous system. We have previously reported the presence of functional HIV coreceptors CCR3, CCR5 and CXCR4 on fetal human neurons, immediately ex vivo and after several weeks in culture. These chemokine receptors were shown to respond to their appropriate chemokine ligands with increases in intracellular calcium. Here we show that human fetal neurons can also flux calcium in response to monomeric HIV envelope proteins. Both of these responses require pretreatment with KCl, suggesting that chemokine receptor activation is only possible when there is membrane activity. To further probe this finding, we examined chemokine receptor responses after treatment with depolarizing vs. osmotic agents and found that other depolarizing agents such as osmahan can also enhance chemokine receptor responses. In addition, we demonstrate that glutamate, an excitatory amino acid neurotransmitter can enhance chemokine receptor responses while GABA, an inhibitory neurotransmitter, does not. These results suggest that chemokine receptor signaling can be modulated by other neurotransmitter receptors and suggest potential targets for therapeutic interventions.

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**P25**


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Using soluble divalent HLA-Ig fusion proteins (HLA-A2/Ig, HTLV-I Tax11-19-specific CD8+ cells from PBL of HLA-A*0201 HAM/TSP patients were found to represent a high proportion of the total CD8+ population. To discern the T cell subtype associated with T-cell differentiation, the expression of CD45RA and CD27 was measured from total CD8+ cells and HTLV-I Tax11-19 peptide-specific CD8+ cells in PBL of patients with HAM/TSP. The phenotypically defined memory and/or effector cells (CD45RA-CD27+, CD45RA+CD27- and CD45RA-CD27-) were increased in CD8+ cells of HAM/TSP (70.8%) compared to that of HTLV-I seronegative healthy donors (49%). The cells with a memory and/or effector phenotype dominated (82.5%) in Tax-A2/Ig positive CD8+ cells in HAM/TSP patients. The effector type cells (CD27-CD4+) abundantly contained perforin and functionally demonstrated high cytolytic activity. The percentage of HLA-DR positive cells was also increased in CD8+ cells (28.5%) and Tax-A2/Ig positive CD8+ cells (53.9%) of HAM/TSP compared to HLA-DR+CD8+ cells of healthy donors (94%). The proportion of cells with a memory and/or effector phenotype in Tax-A2/Ig positive CD8+ cells of HAM/TSP correlated with that of Tax-A2/Ig positive HLA-DR+CD8+ cells. In addition, HTLV-I proviral load correlated with the frequency of Tax-A2/Ig CD8+ cells. The high frequency of memory and/or effector type HTLV-I Tax11-19-specific CD8+ cells suggest that continuous restimulation driven by HTLV-I antigens in vivo and help to define the role of HTLV-I specific T cell populations in the pathogenesis of HAM/TSP.

**P26**

Confounding factors in the HHV-6S MS connection. Blumberg, B.M.†, Mock, D.J.‡, Powers, J.M.‡, Baker, J.V.‡, Goodman, A.D. †VA-Bio-Medical Research Institute, East Orange, NJ, and University of Rochester Medical Center, Rochester, NY, USA

HHV6 has been proposed as an etiologic agent in MS. Perilesional cells containing HHV6 genome were prevalent in PML, a primary demyelinating disease of the human CNS, and HHV6 antigen was expressed along with JC virus antigen in swolen oligodendrocytes. This suggests that PML, thought to be caused exclusively by JC virus, may involve co-infection by JCV and HHV6, and opens the question whether HHV6 may act as a co-agent for demyelination in MS. To investigate this possibility, a unique and sensitive In-Situ PCR (iSPCR) procedure was used to detect the presence of HHV6, JCV and also HHV-1 genomic DNA in archived PML, MS, AIDS and control CNS tissues. The prevalence of viral genome was taken as a marker for the pathogenic potential of these viruses. Immunocytocchemistry (ICC) for HHV6 p41/ig101 was used in conjuction with iSPCR to determine viral antigen expression and cellular localization. We found that HHV6 genome was present at similarly high frequency (>50 infected cells/20x field) in both MS and PML perilesional regions, in larger amounts than JCV genome in PML lesions, in lesser amounts than HHV-1 genome in AIDS encephalopathy, and in similar amounts in PML lesions in the presence or absence of HHV1. Much lower amounts of HHV6 genome were detected in most normal, non-PML AIDS, and other control brains. HHV6 genome but not antigen was found mainly in oligodendrocytes in chronic MS plaques, and numerous bodies identified as corpora amylacea by PAS counterstaining were positive by JCV but not by iSPCR for HHV6. The high pathogenic potential of HHV6 in PML lesions, suggests that HHV-6-JCV interaction due to co-infection may be critical in the etiology of this demyelinating disease. In contrast, HHV6 does not appear to interact with HHV1 in non-PML AIDS encephalopathy. In chronic MS, HHV6 has equally high pathogenic potential as in PML and by analogy may act as a co-agent for oligodendrocyte death and demyelination, but HHV6 antigen expression is cryptic and staining artifacts are likely to cause confusion.
P27

Transfections of L929 cells with plasmid cassettes encoding type I IFN subtypes confers resistance to herpes simplex virus type 2

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It is well known that various subtypes of type I interferon (IFN) show different activities with respect to anti-viral, anti-proliferative effects as well as enhancing natural killer (NK) cell activity and stimulating cytokine production. Recent studies have compared proteins of purified, recombinant, or hybrid subtypes of type I interferon for their activity in various biological tests. In the present study, a plasmid delivery system (pCMV) encoding for interferon IFN-α, -α1, -α2, -α6, -α5, -β, or -β was employed to assess the anti-viral efficacy against HSV-2 in murine L929 fibroblast cells. L cells transfected with any of the type I IFN plasmid constructs neutralized HSV-2 replication as determined by plaque assay of supernatants from the transfected cells 24 hr post infection. The inhibition ranged from 15-55% over plasmid vector controls with IFN-α1, -α5, -α6, and -β displaying the greatest effect and IFN-α5 exhibiting the weakest effect. Supernatants from transfected, non-infected L cells were also evaluated for biologically active IFN levels using vesicular stomatitis virus and Vero cells as well as augmentation of NK activity. The IFN bio-assay revealed that L cells transfected with the IFN-6 transgene showed the greatest production of IFN ranging from 400-500 units/ml compared to other type I IFN transgenics with ranges from 100-200 units/ml. Supernatants from L cells transfected with the IFN-α5 or -α6 transgenes showed the capacity to significantly elevate NK cell activity compared to vector, whereas other IFNs showed no enhancement. Collectively, these results illustrate the disparity of biological effects of the subtypes of type I IFN measuring anti-viral and immune parameters.

P28

Assessment of excitotoxic induced necrotical neuronal loss after feline immunodeficiency virus infection in the cat.

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HIV-1 associated motor-cognitive impairment is a progressive neurologic disturbance leading to dementia prior to the onset of AIDS in select infected individuals. Similar to HIV-1, feline immunodeficiency virus (FIV) infection of the cat results in alterations of behavior and neurophysiological function due to indirect neuronal injury and/or loss via glutamate induced excitotoxicity. One potential mechanism is that excitotoxicity is associated with altered astrocyte glutamate uptake. The purpose of this study was to determine if neocortical neuronal loss after FIV-Maryland isolate infection was associated with decreased astrocyte glutamate transporter (GLT-1) expression and subsequent excitotoxicity. Neocortical tissue was collected 6 (n=6) and 18 (n=6) months after peripheral inoculation at 3 days of age with 1000 TCID50 FIV-MD from cats. Age-matched, uninfected cats served as controls. Stereotaxical estimation of hemispheric neocortical neuronal number yielded an estimate of 222 X10⁶ neurons (coefficient of error = 0.679). Determination of GLT-1 expression and the presence of excitotoxicity by estimation of spectrin degradation products will be accomplished from ex vivo brain tissue.

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P29

Genotype distribution of the human JC virus (JCV) in Iceland.


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Iceland was probably first discovered by Irish (Celtic) explorers in 800 A.D. Officially colonized by Norse settlers in 874 A.D., Iceland has maintained a relatively small population throughout most of its history. Prior to Norse migration to Iceland, many of the early Norse settlers spent time in Scotland and Ireland and many kept Irish slaves who were later transported to Iceland. While the Nordic genetic influence is substantial, it seems likely that the present day genetic makeup of the Icelandic peoples may contain residual genetic contributions from Celtic settlers as well. To test this hypothesis, we utilized the human polyomavirus JCV to look at the viral genome distribution in a group of 50 Iceland people. JCV is a population and geographic-specific virus that co-evolves and co-migrates with the human host. Of the 8 known JCV genotypes (and multiple subtypes), Types 1 and 4 are of European origin, Types 2 and 7 are Asian, Types 3 and 6 occur in individuals of African descent, and Type 8 occurs in Papua New Guinea and the Southwest Pacific. A single Type 5 from an individual of mixed heritage (Irish and Italian) has also been identified and may make up a portion of the Irish JCV genotypes. From 50 Icelandic samples, 22 were JCV positive (44%) and 15 of these (82%) had a Type 1 (European) genotype, 1 was of Type 4 (5%), and 2 samples were Type 5 (10%). Therefore, despite the small population size and potential founder effects in Iceland, at least 10% of the JCV genetic input is not of traditional mainland European origin and may represent a background of JCV genotypes from early transient Irish explorers and/or slaves.

P30

Viral infection of the central nervous system alters expression of extracellular matrix-degrading metalloproteinases, MMPs and endogenous inhibitors, TIMPs.

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Neurotropic viruses can trigger transient or irreversible neurological disorders by altering neural cell connectivity, synaptic plasticity and even cell death. To study these alterations, we have developed a mouse model of central nervous system infection with a morbillivirus, the Canine Distemper Virus (CDV). Viral replication is transient and occurs predominantly in neurons of selectively targeted brain structures: cortex, spinal cord, monoaminergic and hypothalamic nuclei, resulting in inhibition of catecholamine synthesis, dopaminergic cell loss and alteration of the leptin network. These disorders could be linked to late pathologies (paralysis, obesity) developed by mice during the persistent phase of infection. Since remodeling of extracellular matrix (ECM) by MMPs has a crucial role in CNS operation and brain tissue integrity, we hypothesized that aberrant ECM degradation by miscontrolled MMPs and TIMPs, could be involved in CDV induced neuronal dysfunctions. This hypothesis is supported by our previous observations showing that virus and inflammatory mediators perturb the MMP/TIMP balance in neural cells. Expression of MMP-2, 3, 7, 9, 14 and TIMP-1, 2, 3 was therefore analyzed in infected mice brain, using zymography, in situ zymography, immunodetection and semi quantitative RT-PCR. MMP-9, -14, TIMP-1, -3 expression was upregulated in the viral rostral brain targets, i.e. hypothalamus, cortex, hippocampus and spinal cord. MMP's expression and gelatinolytic activity were detected mainly in neurons. In the brainstem and cerebellum (caudal part of CNS), MMP and TIMP expression was not modified. Interestingly, upregulated MMP and TIMP expression significantly correlated the presence of proinflammatory cytokines (TNFα, IFNα, IL6), latter being specifically linked to viral expression. Alteration in MMP/TIMP was associated with neovasculity and increased fibronectin deposit, supporting the idea of altered ECM. These data give new clues to understand the pathomechanisms of degenerative or inflammatory disorders that may follow CNS viral infection. This work was supported by INSERM / INRA grant 4M-401D
P31


JC virus (JCV) is the etiologic agent of progressive multifocal leukoencephalopathy (PML), a demyelinating disease of the central nervous system. It affects immunosuppressed patients and is consistently associated with disorders of cell-mediated immunity in which defects in humoral antibody response may exist. The high incidence of PML among individuals with AIDS in comparison to other immunocompromised patients suggests that there is an interaction between the regulatory proteins of JCV and HIV. Earlier studies have indicated an intercommunication between HIV-1 and JCV through the HIV-1 encoded regulatory protein Tat. In this report we provide evidence that Agno-protein of JCV, a 7-amino-acid basic protein that can bind DNA, can interact with the HIV-1 Tat protein. In order to examine the direct interaction between Agno and Tat, GST pull-down assays were performed. 15S-methionine-labeled in vitro synthesized Agno was mixed with GST or GST-Tat beads and after elution, the bound proteins were analyzed. While no signal corresponding to Agno was detected upon elution of the GST beads, a noticeable band which co-migrates with Agno was observed in eluates from GST-Tat. In a reciprocal study, in vitro synthesized Tat was used in GST pull-down assays with GST or GST-Agno beads. In vitro synthesized Tat was able to bind to GST-Agno, but not to GST beads. These results suggest that Agno can directly bind to Tat and form an Agno-Tat complex in vitro. In the next series of experiments we evaluated the functional interaction of Agno and Tat. Primary human fetal astrocytes were transfected with a DNA plasmid containing the luciferase reporter gene under the control of the HIV-LTR promoter alone or together with plasmids pCMV-Agno and pCMV-Tat. Thirty-six hours post-transfection protein extracts were examined for luciferase gene activity. As expected, the expression of Tat caused a dramatic increase in the activity of LTR promoter. Agno slightly decreased LTR promoter activity, while co-expression of Tat and Agno significantly decreased the activity of the LTR promoter. Co-transfection of various cells with Tat and Agno showed that while Tat is found exclusively in nuclei, Agno is localized around nuclei, where it may interact with Tat. The importance of these findings in pathogenesis PML in AIDS patients will be discussed.

This research was supported by grants awarded by NIH to K. Khalili.

P32

POTENTIAL MOLECULAR DETERMINANTS THAT MAINTAIN BRAIN TROPISM DURING HIV-1 INFECTION

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The determinants for HIV-1 brain tropism remain elusive. Sequences encoding reverse transcriptase (RT) for brain-tropic HIV-1 isolates are phylogenetically distinct from those derived from spleen, lymph node, and peripheral blood mononuclear cells. Furthermore, reverse transcription of certain HIV-1 strains can be blocked within specific cell types, such as T cells and macrophages, indicating a potential role for reverse transcriptase in tropism. In the present study, computer and mathematical analyses were used to test the hypothesis that specific changes in brain-tropic RT sequences result from positive selection. Computer-assisted sequence alignments performed at both the amino acid and nucleotide levels were used to identify specific substitutions that occurred with significant frequency. The Ks/Ka ratios were calculated from nucleotide sequence alignments of brain-tropic and non-brain-tropic RT sequences. The Ks/Ka ratio represents the number of synonymous substitutions observed per synonymous site divided by the number of nonsynonymous substitutions observed per nonsynonymous site. Positive selection is indicated by Ks/Ka < 1. Amino acid alignments and statistical analyses revealed specific changes unique to brain-tropic variants of HIV-1, specifically amino acids 357(TV→GL/I/L), 41(M→L/L), 82(K→R), 179(V→A), and 357(M→T). Results on the nucleotide sequence analyses are forthcoming. Further study of HIV-1 brain tropism is needed for developing novel therapeutic strategies, understanding pathological manifestation within the central nervous system, and designing gene therapy strategies that rely on lentiviral vectors.

P33


PurC is a 322 amino acid single-stranded DNA- and RNA-binding protein involved in regulating several viruses which infect the central nervous system including JC virus (JCV), the etiologic agent of the fatal human demyelinating disease progressive multifocal leukoencephalopathy. Previous studies have demonstrated that PurC controls several aspects of JCV including gene transcription and viral replication. To gain further insight into the mechanisms underlying the molecular functions of PurC, we investigated the DNA unwinding activity of this single-stranded nucleic acid binding protein. Here we demonstrate that PurC possesses helix destabilizing activity and this activity is dose- time- and temperature-dependent. To identify the region(s) of PurC involved in this activity, DNA unwinding assays were performed with a series of amino- and carboxy-terminal deletion mutants. Results from these mapping studies demonstrated that the central region of PurC, spanning amino acids 55-274, was involved in its helix destabilizing activity. This region contains the DNA binding domain of PurC. Since the region of PurC which was involved in its helix destabilizing activity mapped to its DNA binding domain, DNA unwinding assays and electrophoretic mobility shift assays were performed after preincubation of PurC at various temperatures. Results from these experiments demonstrated that the unwinding activity of PurC correlated with its capacity to interact with DNA. Taken together, these studies demonstrate that PurC possesses helix destabilizing activity and that this activity maps to the associated with its ability to interact with DNA. The importance of these observations with respect to the role of PurC in the control of DNA transcription and replication of human viruses will be discussed.

This research was supported by grants awarded by NIH to K. Khalili.

P34

Increased neuronal CXCR4 chemokine co-receptors and parenchymal CD4 T lymphocytes in AIDS hippocampus. Petito, C.K., Roberts, B., Duncan R. Department of Pathology and Epidemiology, University of Miami, Miami, FL USA

We found hippocampal injury in AIDS patients that included significant increases in reactive astrocytes and activated microglia, CD4 neuronal cell loss, and focal lesions of HIV encephalitis (HIVe) within the hippocampus itself. To see if there is a relationship between altered chemokine co-receptor expression and inflammation, we examined these parameters in immunohistochemistry in AIDS patients with HIVe, without HIVe (HIVve), and normal controls. CXCR4 and CCR5 immunoreactivity were significantly higher in CA3 and CA4 neurons than CA1 neurons. CXCR4 increased with AIDS and with HIVe and CCR5 decreased (*: p<0.05). CD4 lymphocytes were present in AIDS white matter (53/400x field) and averaged 9 cells per CA4 region. This study shows that CD4 T cells infiltrate brains of end stage AIDS patients in hippocampus and white matter. If infected, they represent a means by which T-tropic HIV can interact with its increased chemokine co-receptor on hippocampal neurons and mediate brain damage in this region.

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P35

Characterization of infection by Herpes Simplex Virus-I in human corneal epithelial cells in vitro. Heggstrom, Carol D., Parra, Adri5, and LaVaile, Jennifer H.5, 7. Department of Anatomy5, and Department of Ophthalmology7, University of California, San Francisco, California USA.

Recurrent herpetic infection of the cornea is a leading cause of corneal blindness in humans. Herpes simplex virus type I (HSV-1) enters neuronal processes in the corneal epithelium and stroma and is transported retrogradely along the trigeminal nerve axons to their cell bodies in the ophthalmic division of the trigeminal ganglion. Virus can remain latent or become reactivated, upon which HSV-1 is transported axonally in an anterograde direction to the cornea. In the corneal epithelium there is a polarized spread of virus from cell-to-cell. We used an immortalized human corneal epithelial cell line (HCE cells; Anuki-Sasaki et al., 1995) to study the infectivity, growth and spread of HSV-1 in corneal cells in vitro. Vero cells were used for comparison. We found that HCE cells are more susceptible infection by HSV-1 than Vero cells. When confluent HCE and Vero cells are infected with an equal number of viral particles, there are more plaques formed in HCE cells than Vero. In contrast, the amount of virus produced in infected cells is higher in Vero than HCE cells. The slopes of the viral growth curves are similar in both cell types, but a greater absolute number of virions is released from Vero cells.

Cell junctions may mediate the spread of HSV-1 through the cornea. We grew HCE cells on different substrates (laminin, polylysine and collagen) and observed cell junctions. We also measured the spread of virus. The substrates affected both plaque size (spread of virus) and the relative number of zona cuneolus and macula adherens in the HCE cells. These results suggest that the processes of infection and growth of virus within HCE and Vero cells may involve slightly different mechanisms.

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P36


Perivascular macrophages and not parenchymal microglia are a major target of infection in rhesus macaques at peak viremia and terminal AIDS with SIV encephalitis (SIVE). Perivascular macrophages are distinct from parenchymal microglia; they differ in their expression of immune molecules and turnover in the CNS. CNS tissue from three groups of macaques: SIVE, peak viremia and uninfected were assessed for expression of proliferating cell nuclear antigen (PCNA). PCNA plays an essential role in nucleic acid metabolism as a component of the replication and repair machinery. Combined SIV in situ hybridization and PCNA immunohistochemistry demonstrated a predilection of both SIV infected cells and PCNA positive cells to SIVE lesions. Numerous infected perivascular macrophages were PCNA positive. However, there did not appear to be a direct correlation between SIV and PCNA. Distribution of other cellular proliferation markers, including BRDU, Ki-67 and topoisomerase IIα indicate that cells within the lesion were not proliferating. This observation is consistent with perivascular macrophages being terminally differentiated, nondividing cells. In vitro studies demonstrate that SIV infection of peripheral blood derived monocyte/macrophages is required for PCNA expression. These data support the observation of the preferential infection of perivascular macrophages and underscore the biological differences between these cells and parenchymal microglia that may define mechanisms of infection.

P37

Analysis of the function and subcellular localization of JC virus (JCV) agnoprotein (Agno). Sawa, H., Endo, S., Okada, Y., Orba, Y., Komagome, R., Tanaka, S., Nagashima, K. Laboratory of Molecular & Cellular Pathology, Hokkaido University School of Medicine, Japan.

JC virus (JCV), the causative agent of progressive multifocal leukoencephalopathy (PML), encodes the oncoregulatory proteins, large T and small t antigen, viral coat proteins VP1, VP2/3, and an 8 kDa protein of unknown function, the agnoprotein (Agno). To examine the role of the Agno in viral replication and infection, we generated a polyclonal antibody to Agno using a polypeptide containing its hydrophilic residues. The specificity of the antibody was confirmed by Western blotting. Immunohistochemistry of the JCV carrier cell culture (JCI cells) showed that Agno was localized in the cytoplasm. Immunohistochemistry of JCV infected PML brain demonstrated diffuse fibrillary and partly punctuate staining in the demyelinated areas, and double staining with large T antigen and VP1 showed dendritic staining of Agno around the cells which had nuclei positive for large T and VP1. An Agno deleted JCV mutant could replicate in a susceptible cells. Pulldown assays using GST-Agno showed that the Agno bound to 50, 75 and 100 kDa proteins, which is similar to that reported for BK virus. The present studies demonstrated that JCV Agno was expressed in dendritic form in the infected cells, and the infected cells corresponded to oligodendroglia because they were negative for GFAP, NSE or CD68. It could also be demonstrated that Agno may bind to cellular proteins, but that this may not be associated with JCV replication.

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P38

Infrequent incidence of SV40 large T antigen in medulloblastomas and other primitive neuroepithelial tumors. UJ Koralnik JY, Encosta D, Pifer I, Garcea R, Pomroy W, Department of Neurology and Medicine, Beth Israel Deaconess Medical Center, Department of Neurology, Children's Hospital, Department of Pediatric Oncology, Dana-Farber Cancer Institute, Boston. Division of Pediatric Oncology, University of Colorado, Denver.

Simian vacuolating virus 40 (SV40), JC virus and BK virus are double stranded DNA virus of the polyomavirus subfamily of the Papovaviridae. SV40 was first discovered as a contaminant of polio vaccines, prepared from rhesus monkey kidney cell cultures. SV40 can transform a variety of non-host mammalian cell types that are not permissive for viral replication. JC virus induces medulloblastomas in hamsters, and SV40 DNA sequences have been detected in a variety of human tumors, including epidymal and choroid plexus tumors. Neoplastic transformation is regulated by the T antigen, which binds two host tumor suppressor gene products, RB and p53.

We examined genomic DNA from two medulloblastoma cell lines D283Med and Dasy from the ATCC, as well as fifteen fresh frozen primary medulloblastoma and five supratentorial primitive neuroectodermal tumors (PNET) for the presence of SV40, JCV or BKV DNA. In addition, immunocytochemical studies were performed for the identification of SV40 T antigen. PCR amplification was performed using primers common for SV40, JCV and BKV, followed by hybridization with virus specific probes. Four different pairs of JCV specific primers and probes were used and one nested pair of primers specific for SV40 DNA. Sequences for the SV40 T antigen could be amplified only in the D283Med medulloblastoma cell line. All other samples were negative for SV40, JCV or BKV DNA. Immunocytochemistry performed on D283Med cell line using the Pab549 antibody showed nuclear staining for the T antigen protein of SV40. The T antigen was not identified in the patient tumor sections. The tumor was removed from the patient, or whether it subsequently has been introduced into the cell line. These studies indicate that SV40 can only be found rarely in human medulloblastomas.
Abstracts

P40

Persistent SIV infection of a model BBB. Struelow, L., and Nelson, J. A., Department of Molecular Microbiology and Immunology, Oregon Health Sciences University, Portland, Oregon USA

Neurological deficits are a common sequela of human immunodeficiency virus (HIV) infection of the central nervous system (CNS). Although HIV may enter the CNS early during the disease course, overt neurological impairment may not manifest until later stages, when immunological impairment is also present. One of the susceptible cell types within the CNS is the macrophage/microglial cell. With the advent of more sensitive detection methods, it has become clear that cell types relevant to brain disease, such as astrocytes and microvascular endothelial cells (MVEC), are also susceptible to HIV infection. Importantly, astrocytes and MVEC constitute the blood-brain barrier (BBB), which has been shown to be disrupted in a subset of HIV-infected patients both with and without CNS deficits. We have developed a dynamic, in vitro model of BBB, the DIV-BBB, in order to model aspects of HIV infection of the BBB. In the DIV-BBB, brain MVEC are cocultured under flow with astrocytes. Additional MVEC types such as macrophages or T cells can be added to this system as a greater approximation of the in situ BBB. The DIV-BBB replicates the reduced permeability to sucrose seen for the in situ BBB. Cells cultured in the context of the DIV-BBB can be maintained for over a year without a significant loss of viability. This feature of the DIV-BBB allowed us to determine whether a persistent infection of BBB in vitro was possible. Simian immunodeficiency virus (SIV) strain SIVmac251 was inoculated into two separate DIV-BBB cartriidges. A persistent infection of brain MVEC, but not astrocytes, was reproducibly observed. Parallel experiments in static culture demonstrated that several different sources of primary simian brain MVEC were susceptible to persistent SIV infection with a variety of SIV strains, suggesting that SIV readily infects MVEC in vitro. Persistent SIV infection of a brain cell type may be critical for the continued dissemination of virus into the brain, as a mechanism of BBB disruption or for the persistence of virus in the face of ongoing antiretroviral therapy (as a viral reservoir).

P41

The T cell Chemotactic Chemokines IP-10 and Mig are Essential in Host Defense Following Viral Infection of the Central Nervous System. Lin, M., T., Chen, B.P., Orentel, P., Hamilton, T., A.2, and Lane, T.E.1, 1Department of Molecular Biology and Biochemistry, University of California at Irvine, Irvine, California, USA. 2Department of Immunology, The Lerner Research Institute, Cleveland, Ohio, USA.

The roles of the T cell chemotactic CXC chemokines interferon gamma inducible protein 10 (IP-10) and monokine induced by interferon gamma (Mig) in host defense against mouse hepatitis virus (MHV) infection of the central nervous system (CNS) was examined. Rabbit polyclonal antibodies specific for both IP-10 and Mig were generated and administered by intraperitoneal injection to MHV infected mice at select times post-infection. Treatment with either anti-IP-10 or anti-Mig resulted in increased clinical disease and a corresponding increase in mortality as compared to mice treated with normal rabbit serum (NRS). In addition, mice treated with either anti-IP-10 or anti-Mig displayed delayed clearance of virus from the CNS as compared to control animals. Analysis of T cell infiltration by flow cytometry revealed that either anti-IP-10 or anti-Mig treatment resulted in a significant reduction (P<0.005) in CD4+ and CD8+ T cell entry into the CNS as compared to NRS treated mice. Investigation into the interaction of positive and negative effects that selective inhibition of chemokines have on cytokine production revealed that IFN-γ protein levels are significantly decreased (P<0.01) in mice treated with either anti-IP-10 or anti-Mig as compared to NRS treated animals. These data show that both IP-10 and Mig are important factors contributing to host defense against viral infection of the CNS by attracting T cells into the CNS compartment.

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P42

Cytomegalovirus induces cytoktine and chemokine production differentially in microglia and astrocytes: antiviral implications in the central nervous system. Chee, T., Chen, M.C.O., Hu, S., Vager, S.L., Gekker, G., Peterson, P.K., and Lokengard, J.R., Institute for Brain and Immune Disorders, Minneapolis Medical Research Foundation, and the University of Minnesota Medical School, Minneapolis, MN.

Glia cells function as sensors for infection within the brain and produce cytokines to limit viral replication and spread. We examined both cytokine (TNF-α, IL-1β, IL-6) and chemokine (MCP-1, MIP-1α, RANTES and IL-8) production by primary human glial cells in response to cytomegalovirus (CMV). Although CMV-infected astrocytes did not produce antiviral cytokines, they generated significant quantities of the chemokines MCP-1 and IL-8 in response to viral infection. On the other hand, supernatants from CMV-stimulated purified microglial cell cultures showed a marked increase in the production of TNF-α and IL-6, as well as chemokines. Supernatants from CMV-infected astrocyte cultures induced the migration of microglia towards chemotactic signals generated from infected astrocytes. Antibodies to MCP-1, but not to MIP-1α, RANTES or IL-8, inhibited this migratory activity. These findings suggest that infected astrocytes may use MCP-1 to recruit antiviral cytokine-producing microglial cells to foci of infection. To test this hypothesis, co-cultures of astrocytes and microglial cells were infected with CMV. Viral gene expression was determined. No virus production was detected in these cultures. These results support the hypothesis that microglia play an important antiviral role in defense of the brain against CMV. The host defense function of microglial cells may be directed in part by chemokines, such as MCP-1, produced by infected astrocytes.

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