Journal of NeuroVirology, 8(suppl. 1): 71–78, 2002
 ISSN 1355–0284/02 $12.00+.00
DOI: 10.1080/13550280290050064

Neuropathology

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Neuron loss and neuropathology in the basal ganglia and hippocampus of SIV-infected Rhesus macaques

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Infection of macaques with neurovirulent strains of simian immunodeficiency virus (SIV) has proven to be a useful model for investigating the neurological manifestations of HIV-1 infection. In previous reports, we have documented significant performance deficits in reaction time tasks and in a motor skill task (Marcario *et al*, 1999a,b), as well as significant neurophysiological changes (Raymond *et al*, 1998, 1999, 2000) in a cohort of monkeys infected with neurovirulent SIVmac. In order to determine whether neuron loss may correlate with behavioral and neurophysiological deficits, stereological techniques were used to assess neuron number, volume and neuronal density for all neurons in the globus pallidus (GP), the hippocampus and for dopamine-containing (DA) neurons in the substantia nigra (SN) of 8 SIV-infected and 5 control animals. Wilcoxon tests showed a significant difference between rapid progressors and controls for both neuron number ($p < 0.01$) and neuronal density ($p < 0.05$) in the GP, and a significant difference between rapid progressors and controls for neuron number ($p < 0.05$) in the SN. Neuron loss ranged from 6% to 70% in the GP and from 10% to 50% in the SN. Stereological analysis of the CA1, CA2, CA3 and CA4 regions of the hippocampus is progressing. In all structures examined, neuropathological analyses revealed microglial nodules, extensive perivascular cuffing and/or the presence of multinucleated giant cells in the majority of the rapid progressors. Alterations in neuronal morphology were also evident in some rapid progressors. Slow progressors showed very little, if any, neuropathology. The loss of SN dopamine neurons and GP neurons and the possible 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. Neuron loss and neuropathological changes in the hippocampus may contribute to the development of cognitive impairment, as assessed by the working memory task, in the same SIV-infected macaques. (Supported by NIH grants NDA12827 and HD02528.)

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Effects of drug use and HIV infection in microglial activation

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Introduction: The pathogenesis of HIV associated dementia (HAD) remains elusive. The morphological substrate of HAD is HIV encephalitis (HIVE), and it is accepted that the number of activated microglial cells plays an important role in the development of HAD. In the Edinburgh HIV cohort, a differential frequency of HIVE and HAD has been shown among two groups of cases with different risk factor, namely drug users (DU) and men who have sex with men (MSM).

Aims: The main objective of this work was to evaluate the effect of drug use in the activation of microglial cells in the white and grey matter of three different brain areas.

Cases and methods: Thirty-six cases were studied and they were divided in five groups namely: normal controls (Group I, $n = 4$), HIV negative drug users (Group II, $n = 10$), HIV positive pre-symptomatic drug users (Group III, $n = 7$), HIVE drug users (Group IV, $n = 9$) and MSM with HIVE (Group V, $n = 6$). CD68 (DAKO) immunostained sections of frontal, temporal hippocampus and thalamus were used for quantification. This was carried out using a Leika Q 500 IW analysis system. Nine consecutive high power ($\times 20$) fields of both white and grey matter were analyzed. Analysis of variance (ANOVA) and Tukey HSD post-Hoc test were used to assess differences among groups.

Results: Significant differences in all areas studied were obtained when Groups IV and V were compared with groups I and II. Group III did not differ significantly from Groups I and II in any of the studied brain areas, although a higher value of positivity was observed in Group III than in Group II, and these cases in turn showed higher values than Group I. Group III differs significantly from Group V in the grey matter of temporal hippocampus only ($p < 0.004$), suggesting that this area may be a specific target of advanced HIV infection of the brain. Group III differs significantly from Group IV in the white and grey matter of the thalamus ($p < 0.02$ and $p < 0.004$ respectively) and grey matter of temporal hippocampus ($p < 0.0005$).

Conclusion: These findings suggest a synergistic interaction between drug use and advanced HIV infection in microglial activation in thalamus and hippocampus.

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Expression of JC virus T-antigen in a patient with multiple sclerosis and a glioblastoma multiforme

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The post-mortem examination of an immunocompetent patient with a neurological disorder revealed the concurrence of multiple sclerosis plaques in the subcortical white matter of the brain and a glioblastoma multiforme in the region of the thalamus with caudal extension into the brainstem. Histological examination revealed the presence of severe myelin loss with axonal spearing in the MS plaques. The tumor consisted of a highly cellular neoplasm with atypical pleomorphic cells, mitotic figures and areas of necrosis and pseudopalisades.

PCR analysis of DNA from the demyelinated plaques and the tumor area using primers derived from specific regions of the JC virus genome revealed the presence of viral DNA corresponding to the viral early and late genes. Further examination of the samples for the JCV regulatory region identified the presence of sequences identical to JCV Mad-4 and JCV W1 viral isolates in the tumor and the demyelinated regions. Results from immunohistochemistry showed the detection of the viral early protein, T-antigen, and the cellular tumor suppressor protein, p53, in the nuclei of neoplastic cells. Interestingly, expression of T-antigen, but not p53, was observed in neurofilament positive cells with neuronal morphology and in glial fibrillary acidic protein positive astrocytes in the cortex juxtaposed to the MS plaques. Examination of viral late gene expression by immunohistochemistry showed no evidence for viral capsid proteins thus ruling out productive replication of JCV in the tumor and MS demyelinated plaques.

These observations provide the first molecular and clinical evidence of the association of JCV in the brain of a patient with concurrent glioblastoma multiforme and multiple sclerosis.

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Production and detection of vascular endothelial growth factor in CSF of AIDS patients with cryptococcal meningitis

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Meningoencephalitis caused by Cryptococcus neoformans (Cn-M) is a severe opportunistic infection associated with the development of hydrocephalus and cryptococcomas. Hydrocephalus in this setting is generally imputed to interference of cryptococci with CSF re-absorption in the arachnoid villi. Clinical studies demonstrated disruption of the blood brain barrier (BBB) and revealed that patients' CSF typically contained few leukocytes with a mononuclear predominance. Here, we hypothesized that mediators involved in BBB disruption might contribute to the disturbed CSF re-absorption. Vascular endothelial growth factor (VEGF)—as a potent regulator of endothelial permeability involved in diseases associated with inflammation and edema—would be a likely candidate to fulfill this role. Cn or its purified capsular antigens dose-dependently induced VEGF secretion by PMN, monocytes and PBMC in vitro. The CSF of patients with Cn-M showed significantly ($p < 0.05$) elevated VEGF levels (up to 220 pg/ml vs. <20 pg/ml in controls). Assessment of this clinical correlation suggests that VEGF release during Cn infection might mediate BBB disruption, as well as the development of hydrocephalus and increased intracranial pressure characterizing Cn-M.

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Isolation and HIV infection of microglia derived from autopsy brain specimens

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Microglial cells are an important reservoir of HIV-1 infection in the central nervous system (CNS). Adult human microglia are difficult to obtain because surgically removed temporal lobes are scarce. Fetal microglia are more easily obtained but may be functionally different from adult microglia. We report results on studies to refine the technique of isolating human microglia from autopsy brain specimens and infecting them with HIV-1. Microglia were successfully isolated from autopsy brain as much as 24 hours post-mortem, both from HIV-infected and uninfected individuals. Contamination of cultured cells with microorganisms was negligible. We observed no strong correlation between the number of viable cells recovered and numerous factors, including post-mortem time, HIV status, age, or gender. Yields of mixed glial cells were on average $0.5 \times 10e6$ cells per gram of wet tissue. Yields of viable microglia varied consistently with brain region in the following order: frontal lobe > temporal lobe > occipital lobe > cerebellum. Frontal lobe microglia were successfully infected with an M-tropic strain of HIV-1. A "rapid autopsy" protocol was not required to obtain these results, as post-mortem delays of 24 hours prior to the autopsy often produced acceptable yields of viable cells. Most factors that influence microglial cell viability and yields after death remain unclear. Our results show that brain specimens obtained at autopsy can be a practical and steady source of viable microglial cells to study HIV-1 infection of the CNS.

Supported by NIH grants MH 59656 and DC 04970.

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Molecular gene analysis identifies Borrelia burgdorferi as one of the spirochetes involved in the pathogenesis of Alzheimer's disease

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There is increasing evidence supporting the possibility of an infectious etiology of Alzheimer's disease (AD). In 1907 Fischer already suggested that senile plaques might correspond to colonies of microorganisms. From the brain of 3 AD cases and from the blood of a healthy forester, spirochetes

were cultivated in BSK medium. In order to characterize the cultured spirochetes an ultrastructural study, Western blotting and 16S rRNA gene analysis were made. In addition, serological tests for Lyme disease, detection of specific spirochetal antigens and genes in the brain of the same AD patients were performed. Electron microscopy revealed that the microorganisms have ultrastructural characteristics of Borrelia species with 10-15 periplasmic flagella. Phylogenetic analysis of 16S rRNA gene sequences of the cultured spirochetes from 2 AD cases and from the healthy forester were definitively identified as Borrelia burgdorferi sensu stricto. A positive serology for Lyme disease was found in 2 AD cases and in the case of the healthy forester. The Borrelia burgdorferi antigens, detected in the brain of the 3 AD cases, were co-localized with bacterial peptidoglycan and beta-amyloid. The accumulated masses of spirochetes, forming colony like structures in the cerebral cortex were identical to those described in stationary paralysis in syphilis. These results indicate that Borrelia burgdorferi is present in the cerebral cortex in the parenchymatous, long standing form of Lyme neuroborreliosis associated with dementia but also, that Borrelia burgdorferi may survive in clinically asymptomatic high risk individuals in a latent stage. The results also indicate that Borrelia burgdorferi is implicated in the pathogenesis in few AD cases. Several other spirochetes (of oral cavity and intestinal tract) may lead to chronic neurospirochetoses and may be involved in the pathogenesis of AD. Amyloidogenic bacterial remnants may persist in host tissue, inducing inflammatory cytokines, free radicals, proteoglycan synthesis and apoptosis, all reported to be present in AD. The implication of other pathogenic infectious particles including viruses in AD's pathogenesis will be discussed.

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Beta amyloid and Alzheimer's type changes induced in vitro by Borrelia burgdorferi

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The possibility that a slow acting unconventional infectious agent, acquired at an early age and requiring decades to become active may be involved in AD has been proposed by several authors. In more recent studies an association of several bacterial but also viral infection (Herpes Simplex virus) with AD has been suggested. Chronic infectious diseases, including leprosy, tuberculosis and syphilis all lead to or are associated with amyloid deposition. Spirochetes were repeatedly cultivated from the brains of patients with AD suggesting that AD might correspond to end stages of neurospirochetoses. It was proposed that spirochetes might contain amyloidogenic protein. Indeed, the beta-hairpin peptide of OspA, a surface protein of Borrelia burgdorferi forms amyloid fibrils in vitro similar to human amyloidosis.

Rat and human primary glial cells, neurons from rat and chicken embryo as well as rat CNS cell aggregates were infected with the B31 strain of Borrelia burgdorferi and with spirochetes cultivated from the brain of an AD patient. After 1, 2, and 4 weeks of incubation, the detection of beta-amyloid was made using histochemistry, immunohistochemistry and Western blotting. The in situ secondary structure of the beta-amyloid peptide was also analyzed by infrared microspectroscopy (IRMS) using a Synchrotron Infrared Source.

After 2 weeks of incubation beta-amyloid was detected in the co-cultures. Senile plaque, neurofibrillary tangle and granulovacuolar degeneration like formations similar to those of AD were also observed. The results reinforce previous observations that bacteria or amyloidogenic bacterial remnants may well trigger a cascade of events leading to chronic inflammation and amyloid deposition in AD. The results further indicate that AD may well correspond to end stages of neurospirochetoses. The putative association of several infectious agents, including Herpes Simplex virus and other neurotropic viruses will be discussed.

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Progressive panencephalitis with elevated rubella antibodies

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We report the case of a 65 year old female patient, who presented with optical hallucinations and rapid mental deterioration one year after heart transplantation, progressing within 7 months to a vegetative state and death. EEG revealed diffuse slowing with short groups of periodic complexes. Cranial MRI displayed generalised brain atrophy and subtle white matter signal abnormalities. Opportunistic CNS infections and toxic encephalopathy due to Tacrolimus (FK506) could be excluded. A positive 14-3-3 protein and elevated tau-protein of 3625 pg/ml indicated considerable neuronal damage. CSF analysis showed an intrathecal IgG-synthesis with positive oligoclonal bands, partially specific for rubella antigen (6/25). A markedly increased rubella virus-specific antibody index of 265.4 was the most prominent parameter, whereas antibody indices against measles virus (4.6) and CMV (2.5) were only slightly elevated or normal for HSV and VZV. Rubella virus RT-PCR was negative from CSF and snap frozen brain tissue obtained at early autopsy. Besides generalised atrophy there was a massive neuronal loss, astrocytic proliferation, microglial activation and marked infiltration of CD8 positive T-lymphocytes. Spongiform encephalopathy was ruled out histopathologically.

The clinical and histopathological features of this unique case show evidence of a rapidly progressive encephalitis in an immunocompromised patient. Whether the remarkable anti-rubella CSF-antibody-index is indicative of a rubella panencephalitis or whether it is part of a polyspecific immune response associated with an encephalitis of a yet unknown cause has to be discussed.

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Cytokine and chemokine gene expression profile in HTLV-I Tax transfected astrocytes

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Human T-cell lymphotropic virus type I (HTLV-I) is associated with a chronic neurological disease termed HTLV-I-associated myelopathy/tropical spastic paraperesis (HAM/TSP). Although the pathogenesis of this disease remains to be elucidated, evidence suggests that immunopathological mechanisms are involved. It has been shown previously that in the affected lesions within spinal cords of HAM/TSP patients, HTLV-I mRNA colocalized with glial fibrillary acidic protein (GFAP)-producing cells. Therefore, we have developed an in vitro HTLV-I Tax expression model using astrocytic cell lines, to determine the effect of HTLV-I infection in these cells on the induction of cytokine/chemokine expression or antigenic molecules which may be involved in a pathogenetic process in the CNS. Using human glioma cell line U251 and primary astrocyte derived from CNS precursor cells, we established stable or transient transfectants that expressed high levels of HTLV-I tax mRNA under the control of GFAP promoter coupled to the green fluorescence protein (GFP). In order to determine the immunological mechanisms involved by HTLV-I in CNS, we studied the gene expression profile of transfected astrocytes, specifically focusing on cytokine/chemokine expression, by using quantitative RT-PCR, cDNA microarray and enzyme-linked immunosorbent assays (ELISA). In GFAP-HTLV-I Tax transfected cells, proinflammatory cytokine levels were increased (TNF-alpha, IFN-gamma, IL-6, IL-15, IL-18) and chemokine receptor expressions were also up-regulated (CXCR4, CCR3, CCR7). Several auto-antigenic molecules (PLP, PMP, S100-beta) were also up-regulated in transfected astrocytes. In addition, immune functional assays with these GFAP-HTLV-I Tax transfected U251 cell lines also demonstrated that these cells could serve as immunological targets for HTLV-I-specific CD8 + CTL. This system can be used to characterize molecular and immunological events associated with HTLV-I Tax-specific expression in glial cells. These results are consistent with a model whereby HTLV-I can induce the destruction of glial elements and/or the release of a cascade of proinflammatory cytokines and chemokines which result in CNS damage.

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Transactivation in human neuroblastoma cell lines of the human endogenous retrovirus HERV-W that encodes a human superantigen

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The newly discovered human endogenous retrovirus family HERV-W family comprises at least one member associated with extracellular retroviral particle production, named MSRV, for Multiple Sclerosis associated Retrovirus Virus. These virions are produced by cultured choroid plexus cells, macrophages and EBV-transformed B-Lymphocytes from multiple sclerosis (MS) patients, but not from the same cultured phenotypes from healthy controls. We recently brought evidence that superantigen activity is associated with HERV-W members that can encode and express Env protein homologous to MSRV-Env (Perron *et al*, 2001, Virology 287:321–332). The unique ability of superantigen to cause antigen-independent polyclonal activation of any T lymphocyte with a specific variable b chain (TCR Vb) can result in abnormal autoreactive T-cell activation.

In an attempt to reconcile an aetiopathogenic hypothesis for MS comprising a CNS infection with a common virus, and the expression of a latent retrovirus member of the HERV-W family, we tested the possibility that an encephalitic virus infection can stimulate HERV-W protein expression. This has been tested by cytofluorimetry in several human neuroblastoma and astrocytoma cell lines. Our results indicate that herpesvirus type 1 (HSV-1) and cytokines reactivates the expression of Env protein in the human neuronal cell, the genome of which contains HERV-W sequences. This supports the possibility that MS immunopathology can be caused by an endogenous retrovirally encoded superantigen transactivated by an herpesvirus. Superantigen reactivation by unrelated viruses of the Herpesviridae family may be the missing link to explain the role of viral infection in the aetiology of autoimmune diseases, such as multiple sclerosis.

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Management of the viral infection associated with peripherial neuropathy: tradition and innovation

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Human neurotrophic Herpes Viruses (VZV-Varicella Zoster Viruses and HSV-Herpes Simplex Viruses) are characterized by latent infection in nervous tissue, especially in spinal ganglia. VZV remains latent in spinal and cranial ganglias of any localization, also in vestibular and spiral ganglias, which aren't innervated from skin.

The study included 195 patients with various neurological manifestations. Among them, 132 cases (67%) had sensory ganglias injury of different localization and type, out of which in 43 patients (17–54 age group) immune status basic criteria was studied. Study data revealed that: CD4 was 24.72 ± 2.09 (normal value −34–60); CD8 was 25.04 ± 3.77 (normal value −16−30); CD4/CD8 was decreased in 24 cases (mean value 1.35 ± 0.26); IgG was 12.06 ± 3.85 g/e (normal value −8.4−14.5 g/e); IgM 1.42 ± 0.74 g/e (normal value −0.8−1.9 g/e); IgA -1.9 ± 0.85 g/e (normal value −1.5−4.2 g/e).

Based on the data, patients were divided into 2 groups: 1. Immunocompetent and 2. Immunocompromised. This division was based on CD4/CD8 value decrease. First group involved 19 cases and second 34 cases. In the first group, 19 patients were treated by Aciclovir 3000 mg daily dose and Tactivin-1 ml subcutaneosly for 10-14 days. In the second group, 20 patients were treated by the same scheme and the remaining 14 patients were treated by Aciclovir increased dose up to 5000 mg/daily, Tactivin with the same dose and in addition by Actovegin and Curantil.

Thus, the study concludes that the dynamics of neurological manifestation and clinical symptoms restoration despite the performed etiological, pathogenetic and immunomodulative treatment are connected with the condition of human immune system and are lengthened in immunocopromised patients more than in immunocompetent ones. Therefore, increase of Aciclovir daily dose accompanied by Actovegin and Curantil, resulted in the effectiveness of the treatment in the Immunocopromised group of patients.

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HTLV-I infected cells can sensitize non-infected targets by release of immunodominant viral peptides: role in the immunopathogenesis of viral associated neurologic disease

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Human T-cell lymphotropic virus type I (HTLV-I)-associated myelopathy/tropical spastic paraparesis (HAM/TSP) is a chronic, progressive neurological disease characterized by marked degeneration of the spinal cord and the presence of infiltrating T cells and macrophages. HAM/TSP patients generally harbor very high frequencies of HTLV-I infected T cells and HTLV-I specific CD8+ cytotoxic T lymphocyte (CTL). HTLV-I infected T cells and HTLV-I specific CTL have been demonstrated to accumulate in central nervous system (CNS), in which high cellular immune response continuously driven by this virus may contribute to the inflammatory process within CNS lesions in HAM/TSP patients. However, it is still controversial whether HTLV- I infects resident CNS parenchymal cells. To investigate the possibility that uninfected CNS parenchymal cells can be the target of virus-specific CTL, a novel antigen presenting cell/effector system was devised whereby target cells were transfected with the HLA A201 molecule coupled to the green fluorescence protein (GFP). Upon recognition of an immunodominant HTLV-I Tax peptide, HLA A201 specific CD8+ cells have been shown to specifically incorporate GFP which can be assessed by FACS analysis. Using this assay, we can demonstrate that peripheral blood mononuclear cells (PBMC) of HAM/TSP patients can sensitize these GFP-HLA-A201 transfected B cell line. This sensitization was achieved from supernatants of HAM/TSP PBMC and across a transwell chamber separated by a filter and was not associated with infection of the target cell. Sensitization was also proportional to the amount of HTLV-I proviral DNA and mRNA load in HAM/TSP PBMC. These data suggest that virus infected cells from peripheral blood can sensitize uninfected cells, possibly through the release of small immunodominant peptides. These "bystander sensitized" cells can then become targets for antigen-specific immune responses and may represent a novel immunopathogenic mechanism for virus associated neurologic disease.

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Putamen but not pre-frontal cortex displays a glutamate increase in the SIVmac251 infected macaque model: an *ex vivo* 1H-MRS study

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Background: Neurological impairment is a major complication of HIV infection with clinical and pathological abnormalities associated with brain structural damages, inflammation, dendritic and synaptic damages, and neuronal loss. Neuronal injury is also suggested by magnetic resonance spectroscopy (MRS) that demonstrated a decrease in N-acetylaspartate (NAA). However, most MRS studies were performed in vivo and did not allow absolute quantification of various metabolites. Moreover, as regional differences in brain viral loads were reported, the relevance of brain region to the development of cognitive/motor impairment needs to be clarified. The main goal of this study was to evaluate, in the model of SIV infected macaques, metabolic modifications in distinct regions of the CNS, and to seek for correlates with virology and histopathology.

Methods: Fourteen cynomolgus macaques infected by SIVmac251 for 233 to 2352 days, all asymptomatic, were compared with 8 healthy animals. Each had virological and immunological follow-up. One hemisphere was used for 1H-MRS and absolute concentrations of acetate, alanine, aspartate, creatine, gamma-aminobutyric acid, glutamate, glutamine, lactate, myo-inositol, NAA, taurine, valine were measured from the putamen and pre-frontal cortex, separately. In parallel, histological studies for astrogliosis and monocyte/macrophage infiltration were performed on the other hemisphere.

Results: In putamen, a 21% increase of glutamate concentration was observed in 233–314 days post-infection group of macaques ($n = 6$) compared to controls ($n = 8$; $p = 0.025$; t-test) along with general increase in glial fibrillary acidic protein (GFAP) and more pronounced monocytes/macrophages infiltration. By contrast, we did not observe any change in glutamate concentration in the pre-frontal cortex and others metabolites, including NAA, in the prefrontal cortex.

Conclusions: These results show that a regional increase of glutamate in CNS may be detected during SIV infection and bring attention on glutamate metabolism perturbations that could be involved in neuronal injury and neurological impairment.

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Selection of lentiviral variants by ramified microglial cells during acute central nervous FIV infection

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There is consensus of opinion that HIV enters the central nervous system shortly after peripheral infection to establish a long lasting persistence in brain resident microglial cells. Although a causative relationship between this early CNS infection and neuropathogenicity remains to be clarified several lines of evidence indicate emergence of distinct viral variants during persistent CNS infection. These viruses seem to be mainly characterized by specific sequences or mutations in the V3-loop of the env gene. So far, however, experimental data were assessed using either crude CNS material or cerebrospinal fluid (CSF) samples that do not appropriately reflect composition of viral population in brain tissue. Therefore, we used the animal model of feline immunodeficiency virus (FIV) infection of cats to analyse the dynamics of lentiviral evolution in highly purified microglial cells from acutely infected animals. Following amplification of the variable V3 region of the FIV gp95, PCR products were cloned and at least 10 serum- and microglia-derived recombinants, respectively, were sequenced. Compared to the inoculated virus, nucleotide sequence alterations in serum samples were rarely detectable, if at all. In contrast, up to 19 nucleotide exchanges could be identified within the 186 basepair-sized FIV-V3 from microglia.

Even though not all of these basepair substitutions resulted in amino acid shifts, mutation frequency of the deduced protein sequences reached 14,5 % in maximum. Also, phylogenetic reconstruction of V3 fragments revealed clustering of serum and microglial samples in distinct clades. These findings suggest lentiviral adaptation to brain-resident target cells that might form an important reservoir of drug-resistan viruses.

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The role of corticosteroid and beta-adrenergic receptors in stress-mediated suppression of cytotoxic T lymphocyte (CTL)-mediated protection against mucosal herpes simplex virus (HSV) infection

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Psychological stress-induced, neuroendocrine-mediated modulation of immune function has been well documented using both in vivo and in vitro experimental approaches. However, the mechanisms underlying this modulation and its contribution to the development and progression of viral pathogenesis has not been well determined.

Early studies from our laboratory examined the effects of stress-associated activation of the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system on cytotoxic T lymphocyte (CTL) generation in vivo and function in vitro. More recently, we used a murine model to demonstrate that stress suppresses the protective ability of HSV-specific memory CTL (CTLm) in vivo. We have recently extended these findings by determining the role of both glucocorticoid and beta-adrenergic receptor agonists and antagonists on the ability of HSV-specific CTLm to protect against a lethal, mucosal HSV-2 infection. Both corticosterone alone and restraint stress effectively suppressed the protective ability of CTLm in vivo. However, in the stress model, the administration of RU486 was unable to block this suppression suggesting that the type II glucocorticoid receptor is not involved. In contrast, administration of the type I receptor antagonist, spironolactone, significantly delayed the rate of HSV-induced mortality. Beta-adrenergic receptor activation does not appear to play a role in CTLm function since (1) terbutaline (a beta adrenergic receptor agonist) did not alter the rate and extent of HSV-induced mortality and (2) administration of b-adrenergic receptor antagonist, nadolol, did not block the stress-induced suppression of CTLm function. Studies are in progress to determine the combined role of the type I and type II glucocorticoid receptors and the impact of adrenalectomy on HSV-specific CTLm function. We are also examining the effects of stress on the distribution and activation state of these HSV-specific CTLm at both the site of primary HSV infection and in the central nervous system of HSV-infected mice.

Supported by National Institutes of Health Research Grant AI49719.

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Human CDW150 (SLAM) transgenic mice: a model for measles virus neurological infection

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Measles infection is a childhood disease, which can be associated with severe neurological complications including acute encephalitis, subacute sclerosing panencephalitis (SSPE) and measles inclusion body encephalitis (MIBE). The human molecule SLAM (signaling lymphocyte activation molecule) has been recently identified as the receptor for measles virus (MV). In order to create a murine model of infection by wild type strains of MV, we generated transgenic mice expressing human SLAM (hSLAM). The use of a ubiquitous promoter allowed us to obtain a wide expression of SLAM . In the CNS, hSLAM RNA and protein were detected by RT-PCR and FACS analysis in several regions including spinal cord, hippocampus, cortex and cerebellum.

The transgenic mice have been challenged for their susceptibility to MV infection using an intracerebral route of inoculation. New-born and eight day old hSLAM mice, infected with a wild type strain of MV, developed a neuropathological syndrome characterized by weight lost and ataxia leading to death. This acute CNS disease is associated with virus production in the mouse brain, astrogliosis and neuronal expression of viral proteins.

Our transgenic mouse is the first murine model of infection by a wild type strain of MV, which will be a suitable tool to study the neuropathogenesis of MV-induced encephalitis.

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Induction of indoleamine-2,3-dioxygenase during neuro-AIDS in the SIV-infected Rhesus monkey in macrophages and multinucleated giant cells is susceptible to anti-retroviral therapy

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Objective: The aim of this study was to investigate the still open issue of the cellular origin of indoleamine-2,3-dioxygenase (IDO) biosynthesis—the first and rate limiting enzyme of the kynurenine pathway—in the brain of rhesus macaques infected with the simian immunodeficiency virus (SIV) and to determine the influence of brain selective antiretroviral treatment on IDO biosynthesis.

Methods: IDO protein and IDO mRNA expression were analyzed on brain tissue sections of uninfected control, asymptomatic SIV-infected and symptomatic SIV-infected monkeys with and without anti-retroviral treatment with the CNS-permeant 6-chloro-2′,3′-dideoxyguanosine (6-Cl-ddG) by qualitative and quantitative immunohistochemistry (IHC) and in situ hybridization (ISH). Confocal double fluorescence laser scanning analysis and combination of ISH with IHC were carried out to identify the cell types synthesizing IDO protein or IDO transcript. Virus burden,

microglial/macrophage reactions and treatment effectiveness were monitored by SIV glycoprotein gp120 and Iba1 staining.

Results: IDO protein and mRNA were not detectable in the brain of normal and SIV-infected rhesus monkeys without clinical manifestation of AIDS. IDO biosynthesis was induced in the brain of monkeys exhibiting AIDS which was restricted to nodule and syncitium forming macrophages. In contrast to previous suggestions neither resident nor activated microglial cells appeared to express IDO. Anti-retroviral treatment with 6-Cl-ddG suppressed IDO induction and blocked virus burden as well as the SIV-induced appearence of monocytic infiltrates, microglial nodules and multinucleated giant cells in the CNS of AIDS-diseased animals.

Conclusion: Our data indicate that cerebral biosynthesis of IDO during SIV infection is restricted to monocyte/macrophage cells, dependent on viral burden and fully susceptible to anti-retroviral treatment. Thus IDO is a reliable indicator of reversible overt inflammatory events in the SIV-infected monkey brain.

(Supported by the Volkswagen Foundation).

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Human cytotoxic T cell responses generated against virus-infected macrophages in a murine model of HIV-1 encephalitis

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HIV-1 infection of brain mononuclear phagocytes (MP; microglia and perivascular macrophages) is associated with progressive motor and cognitive abnormalities. During subclinical disease such cells are cleared rapidly from the brain by HIV-1-specific cytotoxic CD8+ T cells (CTL). To study the role of CTL in the pathogenesis of HIV-1 encephalitis (HIVE), we developed a small animal model of disease where peripheral blood lymphocytes (PBL) from HLA-A2-positive donors reconstitute NOD/SCID mice. HIVE was created by stereotactic injection of syngeneic HIV-1-infected human monocyte-derived macrophages (MDM) into the putamen. Control groups of mice included HIV-1-infected and uninfected MDM infected in the putamen with or without PBL and sham-operated animals. Mice were sacrificed at days 4, 7, 14 and 21 for neuropathological analysis (quantitation of leukocyte infiltration, astrogliosis, microgliosis, neuronal loss, neurotrophins and neurogenesis). The numbers of HIV-1-reactive human cells in spleen were determined by tetramer staining and IFN-g ELISPOT. Profile of CD4, CD8, CD56 cells and levels of HIV-1p24 antigens in plasma were analyzed for each animal. A significant and sustained infiltration of CD8+/granzyme B+(GrB) cells with elimination of HIV-1 infected MDM (85–100%) were observed in hu-PBL-HIVE animals at day 14 after MDM injection. The neuropathology of the hu-PBL-HIVE animals paralleled those commonly observed during human HIVE and SIV encephalitis. Destruction of infected MDM led to restoration of brain tissue morphology and abrogation of lymphocyte migration. When elimination of HIV-1-infected MDM was not achieved; lymphocytes continued migration and pathologic changes were sustained. An upregulation of neurotrophins and changes in neurogenesis were also observed. This murine model of HIVE may be used effectively to study the mechanisms underlying immunoregulation of viral infection in brain with direct applicability for vaccine development.

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Role of the subclass of anti-lactate dehydrogenase-elevating virus IgG antibodies in the protection against polioencephalomyelitis

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Neuropathogenic isolates of lactate dehydrogenase-elevating virus (LDV) trigger in mice with the appropriate genetic background and co-infected with endogenous retrovirus a polioencephalomyelitis leading to paralysis and death of the infected animals. For its development, the disease requires also some immunosuppression, indicating that it can be controlled by the immune system. Various cell populations, including CD4+ cells, have been shown to participate in this immune protection. Anti-LDV antibodies can also prevent the development of the disease. Because infection with LDV triggers the selective production of IgG2a antiviral antibodies, we analyzed the role of this subclass in the prevention of polioencephalomyelitis with switch variants derived from an IgG3 anti-LDV monoclonal antibody. Our results indicated that indeed IgG2a delays the onset and the progression of the disease more than the other anti-LDV subclasses. This was in contrast with in vitro LDV neutralization by the same antibodies. This suggests that the IgG2a predominance observed in many IgG antibody responses elicited by live viruses could, at least in some circumstances, correspond to the selection of the best protection for the infected host.

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Murine cytomegalovirus infection in the brains of immunocompetent and immunodeficient mice

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Previous *in vitro* studies from our laboratory have demonstrated that glial cells produce chemokines in response to cytomegalovirus infection and mounting evidence suggests that lymphocytes responding to these factors play a major role in the blockade of intracerebral viral spread. To examine the role of functional immune responses in resolving viral brain infection in vivo, we compared spread of murine cytomegalovirus (MCMV) in the brains of immunocompetent and immunodeficient mice. Following intracerebroventricular (icv) infection of immunocompetent C.B-17 mice, viral spread was limited to the ventricular walls and these animals resolved the infection by day 10. In contrast, icv infection of C.B-17 SCID-Beige mice resulted in viral spread from the ventricles throughout the brain parenchyma. These immunodeficient mice were unable to resolve MCMV infection and succumbed to the virus by day 10. Adoptive transfer of CMV-stimulated total spleen cells into immunodeficient mice appeared to limit viral replication and spread. Experiments are currently underway to examine chemokine production in the brains of these mice and to determine if adoptive transfer of specific immune cell components will control viral spread into the brain parenchyma.

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Pathogenic and nef-interrupted simian-human immunodeficiency viruses (SHIV) traffic to the macaque CNS and cause astrocytosis early after inoculation

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In this study, we investigated the co-localization of pathogenic and vpu negative, nef-interrupted SHIVs at early stages following inoculation. The first virus designated SHIV50OLNV was isolated from the lymph node of a pig-tailed macaque which developed severe CD4+ T cell loss and neurological disease. The second virus was a molecularly cloned virus in which the vpu gene was deleted and the gene for the enhanced green fluorescent protein (EGFP) has been inserted in-frame within the nef gene of the pathogenic SHIVKU-1bMC33 (designated as SHIVKU-1bEGFP). Three pig-tailed macaques were inoculated intravenously with equivalent amounts of two viruses, two macaques inoculated with SHIVKU-1bEGFP and two macaques with SHIV50OLNV. The PBMCs were isolated from bleeds obtained 3, 7, 10, and 14 days post-inoculation and monitored for syncytia-inducing virus and for fluorescent cells. Virus was detected in the PBMCs as early as 3 days post-inoculation and was present throughout the course of this short-term study. At 14 days post-inoculation, the macaques were sacrificed and examined for virus in lymphoid tissues and different regions of the CNS following necropsy. Our results revealed the presence of both viruses in lymphoid and CNS tissues, although SHIV50OLNV was present to a much greater extent. Histological examination revealed one macaque displayed signs of meningitis and all three macaques developed massive cortical astrocyte activation as demonstrated by immunostaining for glial fibrillary acidic protein (GFAP), but only limited microglial activation. In the two macaques inoculated with SHIV50OLNV, astrocyte activation similar to the macaques inoculated with both viruses was observed while no astrocyte activation was observed macaques inoculated with SHIVKU-1bEGFP. Thus, this study demonstrates that SHIV viruses with an intact nef (SHIV50OLNV) as well as those lacking a vpu gene and with a non-functional nef gene (SHIVKU-1bEGFP) are capable of invading the CNS and that pathogenic SHIVs are capable of causing reactive astrocytosis early after inoculation.