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Altered gene expression in HTLV-I-infected CD4+ T cells may promote deregulated CD8+ T cell responses associated with HTLV-I-mediated disease pathogenesis

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The human T cell leukemia virus type I (HTLV-I) is an oncogenic retrovirus which infects and transforms T lymphocytes and is the etiologic agent of adult T cell leukemia (ATL) and HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP). HTLV-I infection elicits a highly active cell-mediated immune response consisting of CD8+ T cells which recognize and eliminate cells harboring viral antigens. A perturbation of the CD8+ T cell response to HTLV-I likely contributes to the genesis of HTLV-I-associated disease. Whereas ATL is associated with an ineffective Tax-specific CD8+ T cell response, HAM-TSP is characterized by hyper-active Tax-specific CD8+ T cells which may be directly involved in the genesis of neurologic disease. The molecular mechanisms underlying the basis of these divergent CD8+ T cell responses are unknown. Activation of naïve CD8+ T cells generally requires CD4+ T cell help which enhances antigen presentation by dendritic cells (DCs). Activated CD4+ T cells express CD40 Ligand (CD40L) as well as other cell-surface molecules which provide signals mediating the functional maturation of DCs via upregulation of MHC and costimulatory molecules. The gene expression profiles of CD4+ T cells are dramatically altered by HTLV-I, and genes involved in functional modulation of DCs may possibly be deregulated. Indeed, examination of HTLV-I-infected CD4+ T cell lines revealed altered mRNA expression of CD40L and LIGHT, both tumor necrosis factor (TNF) family members which regulate DC function and CD8+ T cell activation. CD40L mRNA was absent in infected cells as compared to activated, uninfected T cells suggesting that lack of CD40L may be a potential mechanism underlying the defective DC maturation and CD8+ T cell activation associated with ATL. Conversely, LIGHT mRNA expression was up-regulated in HTLV-I-transformed cell lines. The deregulated expression of LIGHT appears to be mediated by Tax which trans-activates the LIGHT promoter and induces LIGHT expression. Deregulated expression of genes of the TNF family by HTLV-I may contribute to abnormalities in CD8+ T cell responses associated with ATL and HAM/TSP.

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HTLV-I transactivator protein Tax impacts dendritic cell maturation and immunologic function

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Human T cell leukemia virus type I (HTLV-I) is the etiologic agent of both adult T cell leukemia (ATL) and HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP). Although the genesis of HAM/TSP likely involves several steps, the generation of a highly specific and effective population of Tax-specific CD8+ cytotoxic T lymphocytes (CTLs) that migrate to the central nervous system (CNS) is of central importance in the genesis of neurologic disease. Presentation of Tax peptides by activated dendritic cells (DCs) to naïve CD8+ T cells likely plays an important role in the induction of a Tax-specific CTL response and the eventual neurologic dysfunction observed in HAM/TSP. The viral oncoprotein Tax has clearly been shown to induce the oncogenic pathway leading to T cell leukemia. Additionally, the immune response mounted during HTLV-I infection is primarily targeted against this protein, with both Tax-specific antibodies and CTLs found in HTLV-I-infected individuals, indicating that Tax must be available for immune recognition. Tax has been found to be present in cells within the serum and cerebrospinal fluid (CSF) of infected patients. Tax may potentially be secreted from HTLV-I-infected cells synthesizing viral proteins and act as an extracellular cytokine, be internalized and processed for presentation, or be transported to the nucleus where it may act as a transcriptional activator. We report herein that purified Tax may induce DC activation involving the expression of the costimulatory molecule CD86 (B7.2) and the maturation marker CD83, but not beta-actin, GAPDH, CD40, or OX40. Functionally, this may translate to an increased DC capability to process and present Tax antigenic peptides in the context of MHC class I molecules to CD8+ T cells, thus leading to an increase in the activation of Tax-specific CD8+ CTLs. The hypothesis guiding these studies is that Tax induces functional alterations in DCs causing their maturation and activation, thus increasing the efficiency of CD8+ T lymphocyte activation, ultimately leading to the generation of a highly reactive Tax-specific CTL compartment that participates in the genesis of HAM/TSP.

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Matrix metalloproteinase-1 activates a G protein-coupled receptor

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The matrix metalloproteinases (MMPs) are a family of structurally related metalloendopeptidases so named due to their propensity to target extracellular matrix (ECM) proteins. Accumulating evidence, however, suggests that these proteases cleave numerous non-ECM substrates including enzymes and cell surface receptors. MMPs may also bind to cell surface receptors, though such binding has typically been thought to mediate internalization and degradation of the bound protease. More recently, it has been shown that MMP-1 co-immunoprecipitates with alpha2beta1, a receptor for collagen. This association may serve to localize the enzymatic activity of MMP-1 so that collagen is cleaved and cell migration is facilitated. In other studies, however, it has been shown that integrin engagement may be linked to the activation of signalling cascades including those mediated by Gialpha containing heterotrimers. As an example, alpha2beta1 can form a complex with CD47 that may associate with Gialpha. In the present study we have therefore investigated the possibility that MMP-1 may affect intracellular changes that are linked to the activation of a Gi protein-coupled receptor. We show that treatment of neuroglial cells with MMP-1 is followed by a rapid reduction in cytosolic levels of cAMP. Moreover, MMP-1 is associated with a potentiation of proteinase activated receptor-1 (PAR-1) agonist linked increases in intracellular calcium, an effect which is often observed when an agonist of a Gi protein-coupled receptor is administered in association with an agonist of a Gq coupled receptor. In addition, MMP-1 stimulates pertussis toxin sensitive release of MMP-9 from cultured monocyte/macrophages and neuroglial cells. Together, these results suggest that MMP-1 signals through a Gi protein-coupled receptor.

P116

Granulocyte-macrophage colony stimulating factor increase microglial numbers and HIV viremia within brain tissue

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Background: Granulocyte-macrophage colony stimulating factor (GM-CSF) is a hematopoietic growth factor required for the growth and proliferation of macrophage and monocyte cells. Both HIV infection and antiretroviral treatment results in neutropenia in AIDS patients and this is prevented with the administration of GM-CSF. We wanted to investigate if GM-CSF has a proliferative effect on microglia, the cell type primarily infected by HIV in the brain and the implications of this on viral replication.

Method: To assess this we utilised a stationary human aggregate system, composed of all relevant cells; neurons,

astrocytes, microglia/ macrophages and oligodendrocytes, with a comprehensive neurotransmitter profile, which had already been established in our laboratory. Aggregates were cultured for four weeks and GM-CSF was added at 100, 10 and 1 ng/ml. Microglial numbers were estimated by unbiased stereological and flow activated cell sorting (FACS) methods at 1 and 2 weeks. Aggregates were infected with the macrophage tropic virus HIV-1 SF162. Viral replication was assessed by p24 ELISA.

Results: Immunocytochemical counts before the addition of GM-CSF for control aggregates were 4% microglia and 2% for HIV infected aggregates and this rose to 15% and 17% respectively (CD68 positive cells). The lowest concentration of GM-CSF had the most profound effect on microglial numbers (ANOVA, $F = 25$, $p = 0.0001$). After 2 weeks exposure to 1 ng/ml GM-CSF a six fold increase in viral replication was observed.

Conclusion: We observed a significant increase in microglial numbers and viral replication. Since GM-CSF can cross the blood brain barrier, this could have a significant effect on the viral load and lead to severe cognitive impairments in the clinical situation.

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Virus-specific CD8+ T lymphocytes to and from the central nervous system do not traffic through cervical lymph nodes

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We tracked Theiler's murine encephalomyelitis virus (TMEV)-specific CD8+ T lymphocytes in non-mutant C56BL/6 mice and mice lacking L-selectin (L-sel-/-), leukocyte-function associated antigen-1 (LFA-1-/-), or both intracellular adhesion molecule-1 and P-selectin (ICAM-1/P-sel-/-). Levels of total and virus-specific CD8+ T lymphocytes, CD4+ T lymphocytes, B lymphocytes, macrophages and NK cells in the brain, blood, cervical lymph nodes and spleen were monitored 9 days after intracerebral inoculation with TMEV. There was preferential recruitment or retention of virus-specific CD8+ T lymphocytes in the central nervous system (61-79% of all CD8+ lymphocytes), compared to the blood (11-18%) and spleen (0.9-6.3%) in mutant and non-mutant mice. Most importantly, there were no virus-specific CD8+ T lymphocytes in the cervical lymph nodes. There was a two-fold decrease in the number of total and virus-specific CD8+ T lymphocytes infiltrating the CNS in L-sel-/- and ICAM/P-sel-/- mice but an increase of a similar magnitude blood, spleen, and cervical lymph nodes (total CD8+ lymphocytes only) when compared to wildtype C56BL/6 mice. Similarly, the number of CD4+ T lymphocytes infiltrating the CNS were decreased by two-fold in L-sel-/- and ICAM/P-sel-/- mice. The ICAM-1/P-sel-/- mice allowed virus persistence in the spinal cord for up to 21 days after infection. Levels of virus-specific IgG's were consistently lower in L-sel-/- mice when compared to wildtype mice up to 28 days after infection, whereas ICAM-1/P-sel-/- mice showed a drop in virus-specific IgG levels only at 21 days post infection. These results suggest that the individual roles of L-sel, ICAM-1/P-sel and LFA-1, while not significant enough to determine virus clearance, do play a role in the length of virus persistence in the CNS.

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Endocytosis and intracellular trafficking of JC virus

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The human polyomavirus, JC causes a lytic infection of oligodendrocytes leading to the fatal demyelinating disease, progressive multifocal leukoencephalopathy (PML). In order to establish a productive infection, JC must penetrate the plasma membrane and traffic through the cytoplasm to deliver its genome to the nucleus of the host cell. We have previously reported that JC, unlike the related polyomavirus SV40, enters cells through clathrin dependent endocytosis. We now show that intracellular trafficking of JC requires intact microtubules and microfilaments as determined by treatment of glial cells with depolymerizing agents such as nocodazole and cytochalasin D. Interestingly, this is unlike SV40, which is dependent only on intact microtubules for infection. JCV infection also appears to be dependent on the acidic pH of the endosomal compartments as treatment with acidotropic weak bases such as ammonium chloride abolishes infection. This is once again in contrast to SV40 infection, which has been shown to be pH independent. We are currently examining the role of intracellular organelles such as the ER and Golgi as well as the role of microtubule minus-end directed motor proteins, in the trafficking of JC virus to the nucleus.

P119

Tracking of monocyte migration in the brain by magnetic resonance imaging of ferromagnetic cells in a murine model of HIV-1 encephalitis

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Background: The numbers of immune competent mononuclear phagocytes in brain are the best correlate of cognitive dysfunction in HIV-1-associated dementia (HAD). To date there are few ways in which the movement of monocytes can be traced in and into the brain during disease. Ferromagnetic labeling of monocytes can provide a means to trace such cell migration in mice with HIV-1 encephalitis (HIVE) when used in conjunction with magnetic resonance imaging (MRI) tests.

Methods: Human monocyte-derived macrophages (MDM) obtained by centrifugal elutriation of blood mononuclear leukocytes were incubated with superparamagnetic iron oxide particles (Ferridex). Optimal ratio and time of incubation (iron particles:MDM) was determined by cell viability, histology and *in vitro* cell migration across an artificial blood-brain barrier. Severe combined immunodeficient mice were injected with labeled MDM. A 4.7 Tesla MRI tracked cell movement using T2* weighted MRI. Brain analyses were done from 2 hours to 6 days after MDM injection.

Results: Ferromagnetic MDM were observed first at the injection site (the caudate and putamen) but migration with diffuse distribution to other regions of the brain was observed. Cells could be visualized up to 6 days and were also seen in the contralateral hemisphere. No changes in cell viability were recorded as a consequence of the labeling. Distinction between local hemorrhage and cell distribution (needle track) will require further analysis for quantitation.

Conclusions: Paramagnetic labeling of macrophages allows MRI detection of cells up to 6 days after injection. Mi-

gration studies of cells from the periphery to brain are underway. Results of co-registration of brain histology and MRI used to confirm the cellular localization will be presented.

P120

CXCL10 production from cytomegalovirus-stimulated human microglia: regulation by interleukin-10

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Microglial cells orchestrate multi-cellular immune responses to viral infections of the central nervous system and synchronize various immune functions through a regulated network of cytokines and chemokines. Recruitment of T lymphocytes to local sites of infection is critical for resolution of cytomegalovirus disease. In the present study, we investigated the production of an alpha-chemokine CXCL10 (interferon-gamma inducible protein-10, IP-10) in response to CMV infection of glial cells and the regulation of its production by interleukin-10 (IL-10). We found that human microglial cells, but not astrocytes, produced CXCL10 in response to CMV, reaching maximal levels at 48–72 post-infection. Expression of CXCL10 mRNA in CMV-stimulated microglia was not dependent on secondary protein synthesis but did require replication competent virus. Activation of NF-kappa B and p38 MAP kinase in microglial cells was observed in response to stimulation with CMV and inhibitors of this activation decreased viral-induced CXCL10 production. Exogenous CXCL10 itself had no effect on CMV replication in permissive astrocytes, however, supernatants from CMV-stimulated microglial cells increased chemotaxis of activated T-cells, which could be partially suppressed by anti-CXCL10 antibodies. Anti-inflammatory cytokines appear to play a critical role in preventing excessive CNS inflammation including T-cell accumulation. Treatment of microglial cells with IL-10 was found to inhibit CXCL10 production at the level of mRNA transcription and was associated with decreased CMV-induced NF-kappa B activation. These studies suggest that microglial cell production of CXCL10 plays a major role in recruitment of T-cells to foci of CMV infection and that IL-10 functions to prevent excess inflammation.

P121

The role of the CNS innate immune system in neurotropic coronavirus infection

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Astrocytes and microglia are the main innate immune cells within the CNS. In response to viral infections astrocytes and microglia produce a variety of cytokines, chemokines, adhesion and MHC molecules. However, the mechanism of activation of astrocytes and microglia is not fully understood. In the present study we investigated cytokine profiles in astrocytes and microglia following infection with a neurotropic (MHV-A59) and a non neurotropic (MHV-2) strain of coronaviruses. Cytokine mRNA profiles were detected by cDNA microarray at 24 hours post-infection. This was further confirmed by selected protein analysis by ELISA. In astrocytes the expression of IL-1b, TNF α , IL-6, IL-12p40 and IL-15 was

higher in MHV-A59 infection than in MHV-2 infection. IL-5 and IL-1a were up-regulated by both viruses, and TNF α , IL-10, IL-11, and IL-13 were up-regulated by MHV-2 more than MHV-A59. In microglia, the expression of IL-1b, TNF α , IL-6, IL12p40 and IL-15 was higher in MHV-A59 infection. In L2 murine fibroblasts IL-13 and TNF α were up-regulated by both viruses, while IL-1a was up-regulated by MHV-A59 infection more than MHV-2 infection. Both viruses produced similar titers in each cell type. Acute infection of mouse brains and chronic infection of mouse spinal cords exhibited up-regulation of similar cytokines as infection of tissue cultures. Thus the CNS innate immune system produces a discriminating response to viruses of different neurotropic phenotypes. The up-regulation of a set of 5 cytokines (IL-1b, TNF α , IL-6, IL12p40 and IL-15) is associated with neurotropic viral infections in both astrocytes and microglia. The activation of local immune cells in the CNS may play an important role in the pathogenesis of virus-induced neurologic disease.

P122

Age-related cell response in the adult mouse brain to inflammatory mediators circulating in the cerebrospinal fluid

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The present study was aimed at ascertaining whether the response elicited in the brain by intracerebroventricular (icv) administration of proinflammatory mediators exhibits changes in the mature brain at different ages. The role of these experiments stems from the evidence that many neurological diseases in which an inflammatory challenge has been implicated are age-dependent. For example, multiple sclerosis affects mainly young adult and middle aged individuals, while aging is known to play a crucial role in neurodegenerative diseases. We thus injected stereotactically interferon (IFN)-gamma, tumor necrosis factor (TNF)-alpha, and lipopolysaccharide (LPS) in the lateral cerebral ventricle on 10 month-old mice, and compared the data with those obtained in matched 3 month-old mice. In both groups, control experiments were based on icv injections of ovalbumin or saline. One day after icv injections of IFN-gamma + TNF-alpha in 10 month-old animals, microglia activation, indicated by hypertrophy and clustering of cells labeled by tomato lectin histochemistry, was more marked in periventricular structures than that observed in 3 month-old animals. Two days after icv injection of LPS marked induction of immunoreactivity to the anti-apoptotic protein Bcl-2 was seen in neurons and glia in the cerebral cortex and periventricular structures in both age groups. Bcl-2 induction appeared enhanced in the 10 month-old cases; quantitative differences are at present being analyzed. The data hitherto obtained indicate that microglia is implicated in age-related susceptibility of the adult brain to an inflammatory challenge and that Bcl-2 induction in neurons and glia is implicated in a protective response to such conditions.

P123

Neurotoxicity induced by HIV infection and HIV proteins in human dorsal root ganglia neurons

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Objective: To determine the effects of human immunodeficiency virus (HIV) infection and HIV proteins on human dorsal root ganglia (DRG).

Background: Patients with HIV infection frequently develop a painful distal sensory polyneuropathy the pathogenesis of which remains uncertain.

Design/Methods: Organotypic human DRG cultures were established from 58–108 days gestational age fetuses. After two weeks in culture, they were incubated with HIV3B and HIVAda-M or exposed to HIV proteins gp120 (500 pM, 1 nM) or Tat (125 nM, 250 nM) for another one or two weeks. The cultures were analyzed by light and electron microscopy, immunostaining for neuronal markers and quantitative morphological analysis. Changes in intracellular calcium and mitochondrial function by JC-1 assay were monitored in select neuronal cultures.

Results: In HIV protein treated cultures the earliest changes noted were shortening and loss of neurites followed by cell death in select neuronal populations. Ultrastructural abnormalities included loss of cristae in mitochondria, clustering of neurofilaments and microtubules, accumulation of glycogen like particles, dilation of the endoplasmic reticulum, and an increase in ribosomes. Morphological changes induced by gp120, Tat were indistinguishable. Increases in intracellular calcium occurred with gp120 and Tat treatment. Gp120 and Tat also caused significant changes in mitochondrial potential. HIV infection showed no observable toxicity. But many HIV (both HIVIII B and HIVAda-M) particles were found between microtubules and neurofilaments in an 85 days gestational age fetus DRG cultures. No HIV particles were found in neuronal cell bodies or Schwann cells. Only few immature HIV-like particles were found in neurites of a 58 days gestational age fetus DRG cultures.

Conclusions: HIV proteins cause neurotoxicity in human DRGs associated with disruption of neurites and mitochondrial toxicity. HIV cannot directly infect neuronal cell bodies and Schwann cells but can directly enter neurites without causing any obvious morphological changes. Study support by: NIH grants R01NS38428; R01NS39253; P20RR15592; R01NS39184.

P124

A placebo-controlled trial of gabapentin for painful HIV-related neuropathy

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Background: Painful HIV-related neuropathy (HIV-RN) is a common complication of HIV infection. The pathogenesis is unknown and there is no effective treatment. The anti-convulsant gabapentin (GBP) is reported to be effective in painful diabetic neuropathy and postherpetic neuralgia and its effectiveness on painful HIV-RN has been sporadically published.

Design: A multicenter, prospective, randomised, double-blind, placebo-controlled study.

Methods: The study consisted of a 1-week screening, a

4-week double-blind treatment, and a 2-week unblinded treatment phase. GBP was initiated at 400 mg/d over 2 weeks to 1200 mg/d. In cases of a sufficient improvement the dosage were retained, but if beneficial effects were insufficient, doses were titrated up to 2400 mg/d over further 2 weeks. The primary outcome was change in pain on the 10-cm Visual Analogue Scale (VAS) (0 = no symptom, 10 cm = maximal symptom intensity). Secondary efficacy parameter was the mean sleep score (VAS). Analysis of covariance was used to assess differences between treatment groups in change on pain and sleep VAS. Patients were excluded if they had concomitant medication with antidepressant, other anticonvulsant, topical capsaicin, mexiletine, alpha-lipoic acid, systemic corticosteroids or immune modulators, central analgesics or had taken nerve blocks and acupuncture.

Results: 10 patients received GBP and 8 placebo. In the placebo group one patient dropped out. In the GBP group some patients complained dizziness and somnolence but a reduction of treatment dosage was not necessary. Baseline mean pain score (GBP 4,96 ± 2; placebo 5,2 ± 2,8; p = 0,824) and baseline mean sleep score (GBP 4,3 ± 1,89; placebo 4,3 ± 2,7; p = 0,824) were not significantly different in the groups. GBP decreased the median pain VAS (GBP 2,77 ± 2,3; placebo 4,2 ± 2,6; p = 0,27) and also the median sleep VAS (GBP 3,1 ± 2,6; placebo 4,6 ± 2,3; p = 0,23) in the 4th treatment week. In the subgroup analysis (ANOVA) these results were statistically not significant.

Conclusions: In our very small study group GBP was more effective than placebo in managing pain in HIV-RN but further much more large controlled clinical trials are needed.

P125

T-lymphocytes infected by HTLV-1 increase glucose consumption and lactate release of brain astrocytes *in vitro*

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Human retroviruses induce neurologic disorders via complex interaction between infiltrating immune cells and neural cells. Our hypothesis is that astrocytes may be an important target for infiltrating infected cells, which impair critical astrocytic functions involved in the survival and operation of neurons and oligodendrocytes. We have previously shown that the uptake of extracellular glutamate by astrocytes *in vitro* is significantly altered by contact with T lymphocytes infected by HTLV-1, suggesting a less effective protection of neurons and oligodendrocytes to excitotoxic insults. Here, we studied the effects of T cells infected by HTLV-1 on glucose metabolism in astrocytes, as assessed by extracellular levels of glucose and lactate. Lactate is the main fuel for neurons and oligodendrocytes. Transient coculture (20 hours) of immature astrocytic cell line Dev with infected T cells (C8166/45 and C91PL) increased both glucose consumption and lactate release at least for 7 days after coculture when compared to the control (non-infected T-cell line CEM). The infectious C91PL T cells produced greater effects than the infected, but non-infectious C8166/45 T cells. This may result from the persistent expression of the viral transactivator

Tax in Dev cells cocultured with the infectious cell line. Stoichiometry analysis revealed an apparent shift from aerobic to anaerobic utilization of glucose in astrocytes treated with infected T cells (one molecule of glucose degraded into 1.3 and 2 molecules of lactate after coculture with non-infected and infected T-cells, respectively). The protein level per cell was significantly increased in Dev cells cocultured with infectious, but not with non-infectious T-cells. As Tax is the only viral product persistently expressed in infected Dev cells, this increased protein level represents a peculiar metabolic shutoff without cell lysis, probably due to an increased level of cellular proteins. Our data indicate that T lymphocytes infected by HTLV-1 profoundly alter the metabolic state of astrocytes, which may impair the fate and operation of neurons and oligodendrocytes.

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HIV-1 transmembrane protein gp41 induces intracellular calcium increase in cultured rat astrocytes and human neuroteratoma cells

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HIV-1 glycoprotein gp41 is the transmembrane part of the HIV-1 envelope protein and involved in HIV-1 cell entry. In order to test possible injurious properties of gp41, we incubated cultured rat cortical astrocytes and human neuroteratoma cells with gp41 (10 nM). Since gp41 is rather hydrophobic, a recombinant fusion protein of maltose binding protein (MBP) with an 82-amino acid-long extracellular region (aa565-647) of gp41 was used for the experiments (Intracel, Cambridge, Massachusetts, USA). To rule out possible side effects of MBP we used recombinant MBP (New England Biolabs, Beverly) as a negative control. Intracellular calcium concentrations were measured in fura-2 loaded cells for 60 minutes. We found an intracellular calcium increase in cells incubated with gp41 which was not found in cells incubated with an identical amount of MBP. The gp41-induced intracellular calcium increase was reduced when calcium was omitted from the extracellular solution suggesting a transmembrane calcium influx into the cells. Examining functional effects of this gp41 effect, we tested the intracellular calcium response of gp41-incubated cells to the application of glutamate and ATP which are known to physiologically induce calcium waves within the astrocytic syncytium. The calcium responses to glutamate and ATP were significantly reduced in cells preincubated with gp41. Our data suggest that HIV-1 gp41 interferes with the intracellular calcium regulation and may contribute the pathogenesis of neurological symptoms in patients suffering from HIV-1 associated encephalopathy.

P127

Role of C/EBP factors in regulating HIV-1 LTR function in the monocytic lineage *in vitro* and *in vivo*

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Cells of the monocyte/macrophage lineage serve as an important vehicle for transport of virus into the CNS and in the production of infectious virus prior to and after CNS entry. Previous studies have shown that CCAAT/enhancer binding protein (C/EBP) sites within the HIV-1 LTR are essential for efficient viral replication within cells of the monocyte/macrophage lineage. As a result of the infidelity of the retroviral reverse transcription process and subsequent selective pressures, the viral genome including the LTR can evolve with high or low affinity binding sites for C/EBP factors, resulting in viruses with different efficiencies with respect to replication in the monocyte/macrophage lineage. Many investigations have shown that sequence variation within the LTR plays an important role in HIV-1 replication within different cell types and tissues. LTR sequence variation within the C/EBP sites, as well as in other areas of the LTR, has been shown to impact basal transcription as well as Tat- and Vpr-induced trans-activation. To enhance the understanding of HIV-1 disease in humans, the importance of C/EBP binding sites within the HIV-1 LTR with respect to viral replication continues to be assessed. To this end, the activity of LTRs derived from the HIV-1 strains 89.6 (dual-tropic strain) and YU-2 (monocyte-tropic strain from the brain of a patient with AIDS dementia) LTRs will be investigated. The parental 89.6 LTR has two low affinity C/EBP sites (-107 to -118 and -172 to -180). Point mutations were introduced into the sites to make a LTR construct with the 89.6 LTR backbone and two high affinity C/EBP sites. The parental YU-2 LTR has two high affinity C/EBP sites. Point mutations were introduced into the sites to make a LTR construct with the YU-2 LTR backbone and two low affinity C/EBP sites. This series of constructs has been utilized to examine the role of C/EBP factors in regulating HIV-1 LTR activity during the differentiation of CD34+ progenitor cells and during the course of monocytic differentiation. The two LTRs and their mutated derivatives will also be used to drive the expression of a GFP reporter protein and used to generate transgenic mice.

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The role of cytokines and chemokines in neurological disease induced by polytropic retrovirus infection in mice

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Viral infection of the CNS often results in the upregulation of proinflammatory cytokines and chemokines which can lead to prominent inflammatory infiltrates of mononuclear cells in the brain. However, with immunosuppressive retroviruses, such as HIV, little CNS inflammation is observed, yet proinflammatory cytokines and chemokines are still upregulated in some patients and may mediate pathogenesis. Using polytropic murine retroviruses, which also induce non-inflammatory neurological disease, we recently found that clinical disease was associated with increased ex-

pression of proinflammatory cytokine and chemokine genes. In this mouse model, infection of the CNS with the neurovirulent retrovirus, Fr98, but not the avirulent retrovirus, Fr54, induced the upregulation of TNF-alpha, MIP-1alpha, MIP-1beta, RANTES, MCP-1 and IP-10 mRNA. The upregulation of these genes occurred prior to the development of clinical symptoms suggesting that these cytokines and chemokines might be involved in the induction of neuropathogenesis. Mapping studies indicated that the N-terminal region of the Fr98 envelope gene was responsible for the upregulation of cytokine and chemokine expression. However, in experiments using virus-infected neural stem cells to increase virus transmission to brain microglia cells, high levels of either virulent or avirulent polytropic virus correlated with upregulated cytokine and chemokine expression and clinical CNS disease. In current studies, CCR2-/- (receptor for MCP-1), CCR5-/- (receptor for MIP-1alpha, MIP-1beta, and RANTES) and TNF-alpha-/- mice are being used to determine whether upregulated cytokines and chemokines have a direct causative effect in the development of polytropic virus-induced neurological disease.

P129

Upregulation of FasL in the CNS: a mechanism of immune evasion by rabies virus

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Following its injection into the hindlimbs of mice, CVS, a highly pathogenic strain of rabies virus, invades the spinal cord and brain resulting in the death of the animal. In contrast, CNS invasion by PV, a strain of attenuated pathogenicity, is restricted to the spinal cord and mice infected with this virus survive. We have shown that T cells cannot control neuroinvasive rabies infection. Despite the production of similar amounts of virus and TNF-a mRNAs, lymphocytes display transient migration into the infected CNS in fatal rabies and sustained migration in non-fatal rabies. The transient migration of T cells in fatal rabies is associated with an increase in cell apoptosis. We found that the early production of FasL mRNAs was upregulated only in fatal rabies. FasL is produced by infected neurons. In mice lacking FasL, (gld), infection with the neuroinvasive rabies virus strain was less severe, and the number of CD3 T cells undergoing apoptosis was smaller than that in normal mice. These data provide strong evidence that fatal rabies virus infection involves the early triggering of FasL production leading to the destruction of migratory T cells by the Fas/FasL apoptosis pathway. Thus, rabies virus has selected an immunosubversive strategy which takes advantage of the immune privilege status of the CNS.

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Interferon-induced genes upregulated in SIV encephalitis are also induced in the early stages of SIV infection and are paralleled in human HIVE cases

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Studies of HIV and SIV have found many parallels in the pathology caused by viral infection in the CNS. This study utilized rhesus macaques infected with a neurovirulent SIV strain. Monkeys were sacrificed at the following time points post inoculation to investigate differences in gene expression at different stages of HIV disease: 2 weeks (n = 3), 11 weeks (4), and 18 months (3). In addition there were six monkeys that developed a simian AIDS shortly (2–4 months) after SIV inoculation due to treatment with a CD8-depleting antibody. These animals manifested SIV encephalitis (SIVE) of varying severity. These groups were compared with six Control cases, two of which were also CD8 depleted. Affymetrix Human U95Av2 GeneChips were used to examine abundance of mRNAs in the frontal cortex. Analysis was performed to find statistically significant differences in gene expression between the Control and SIVE cases. Of these several were 'interferon (IFN) inducible genes' and nine genes were found

to follow a similar pattern of expression over the five groups, relative to control: they were upregulated at two weeks post inoculation, when the virus enters the brain, with lower levels at 11 weeks and 18 months, and then upregulated in the SIVE group in parallel with high viral load in the CNS. These genes were 9–27, 1-8u, Stat1; ISGs-12, –17kDa, –54kDa and –56kDa, p44 and Mac-2 binding protein. A parallel study in human control and HIVE cases showed all but ISG54kDa were also upregulated in the HIVE cases. Immunohistochemistry and *in-situ* hybridization revealed Stat1 to be upregulated in many of the cells of the CNS whilst Mac-2 binding protein and ISG12 were confined to SIVE plaques and activated microglia/macrophages. Many of the interferon-inducible genes have been shown to be upregulated following viral infection and are known to have antiviral activities. This molecular characterization helps reveal the balance between the virus and host response within the CNS. This interplay likely contributes to CNS pathology and dysfunction shown in many people with HIV disease.

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Diagnostics of a defeat of the facial and trigeminal nerves at a respiratory virus infection

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