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Basic Science Workshop 1

Cell activation and astroglia stimulation

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HTLV-I transcription during cellular differentiation and activation in bone marrow, peripheral blood, and brain

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Previous studies have demonstrated the maintenance of a high level of silent human T cell leukemia virus type I (HTLV-I) proviral DNA within the bone marrow (BM) of patients with HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP). The cell type(s) that are actually infected within the BM are not known at this time, however, CD34+ hematopoietic progenitor cells (HPCs) can be infected *in vitro* by HTLV-I and retain the intact proviral genome during terminal differentiation. If the HPC population within the BM is infected, then migration out of the BM and differentiation of HPCs down the lymphoid and myeloid lineages could result in the seeding of the PB with infected cells, thus leading to potential increases in proviral load and viral gene expression. The results described herein examine the regulation of HTLV-I gene expression in hematopoietic progenitor cells and their derivatives within the monocytic lineage. Viral gene expression from an integrated proviral genome can be regulated by several means, including promoter occupancy and methylation of proviral DNA. To begin to examine the controls governing viral latency within the BM, nuclear extracts of CD34+ HPCs from the BM of healthy donors were reacted with radiolabeled oligonucleotides corresponding to each of the TRE-1 21-bp repeats in electrophoretic mobility shift (EMS) analyses. Results have indicated that the absence of activating transcription factors such as AP-1 and ATF/CREB factors within CD34+ HPCs may be involved in preventing the initiation and maintenance of viral gene expression within this immature cell population. As HPCs differentiate down the monocytic lineage, LTR activation appears to be facilitated by the induction of differentiation stage-specific transcription factors, including AP-1, which has been shown to exhibit an enhanced LTR binding activity as TF-1 (a CD34+ progenitor cell line) and U-937 (an immature monocytic cell line) cells differentiate. These observations have been supported by transient expression studies utilizing a dominant negative AP-1 expression vector, which significantly inhibited LTR trans-activation in U-937 monocytes.

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Astrocyte Protease-Activated Receptor stimulation is associated with Gi linked release of MCP-1 and MMP-9

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Serine proteases including thrombin may be elevated in association with viral infections of the central nervous system (CNS). Such proteases may enter the CNS through a damaged blood brain barrier (BBB), and their expression within the CNS itself may be upregulated by specific pro-inflammatory stimuli. Within the CNS, serine proteases may contribute to neuropathology through the degradation of extracellular proteins such as laminin. It is becoming increasingly evident, however, that these proteases can also directly activate specific cell surface receptors. Many serine proteases activate specific protease-activated receptors (PARs), a unique set of G protein-coupled receptors which are widely expressed in the central nervous system. In the present study, we examined whether activation of PAR-1, -2, -3 and/or -4 by thrombin or specific peptide agonists might affect the release of monocyte chemoattractant-1 (MCP-1) and matrix metalloproteinase-9 (MMP-9) from astrocytes. We focused on MCP-1 and MMP-9 because these proteins may facilitate the CNS ingress of leukocytes which occurs in association with select viral infections of the CNS. Our results demonstrated that astrocytes were responsive to stimulation with both thrombin and specific PAR agonists, as determined by changes in intracellular calcium levels and/or release of MCP-1 and MMP-9. Moreover, thrombin-stimulated release of MCP-1 and MMP-9 was pertussis toxin sensitive, suggesting that Gi coupled signaling is involved in the effect. In summary, these results suggest that signaling by serine proteases such as thrombin could contribute to the pathogenesis of select inflammatory diseases of the CNS.

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CXCR4 receptor mediated HIV-1 gp120-induced intracellular calcium dysregulation in cultured rat cortical astrocytes

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The alpha-chemokine receptor CXCR4 is used as the major coreceptor for the cell entry of T-cell tropic HIV-1 isolates. Activation of this coreceptor by its natural ligand SDF1alpha is associated with an intracellular calcium increase. Since the HIV-1 glycoprotein 120 (gp120) is shedded from the surface of HIV-1 infected cells and is regarded as an injurious molecule in the pathogenesis of HIV-1 associated encephalopathy, we investigated the effects of gp120 on the intracellular calcium regulation of astrocytes and neurons. After 5 days *in vitro* SDF1alpha (50 nM) elicited a pertussis toxin sensitive intracellular calcium increase due to calcium release from internal stores which was reduced by a blocking monoclonal antibody against the CXCR4 receptor in astrocytes and neurons. Parallel with the development of the SDF1 alpha response cells became sensitive to direct application of gp120 (0.00125 mg/ml) which similarly to SDF1alpha elicited a transient intracellular calcium increase. However, short-term incubation with gp120 for 60 to 120 minutes induced a reduction of glutamate or ATP evoked intracellular calcium responses only in astrocytes and not in cortical neurons, although functional CXCR4 receptors were expressed in both cell types. Therefore, our data strongly suggest that the intracellular CXCR4 receptor-mediated signaling pathway of gp120 differs in astrocytes and neurons.

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Astrocytes and activation of the blood-brain barrier: possible role in HIV/SIV neuroinvasion

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Numerous permutations of blood-brain barrier (BBB) models have been assembled. We have constructed one using macaque microvascular brain endothelial cells (MBEC) grown to confluence on the apical surface of 3 micron pore filters before astrocytes are cultured on the basal surface. Rather than add chemokines to the lower chamber to examine leukocyte recruitment we have chosen what we think is a more physiologically relevant approach. We have added non-chemotactic proinflammatory cytokine (TNF-alpha) to the lower chamber, where it will be in contact with the astrocyte component. This has induced activation of the MBEC monolayer and the production of MCP-3 by astrocytes capable of inducing the chemotaxis of monocytes. Gene array analysis of chemokine mRNA produced by astrocytes in response to TNF-alpha showed that MCP-3 was upregulated 9-fold. In addition, there was over 1 log more MCP-3 mRNA produced by astrocytes in response to 50 ng/ml TNF-alpha than the next most highly induced chemokines tested (MCP-1 and MIP-1 beta). MCP-3 is physiologically and pathologically relevant as macaque monocytes are attracted to this chemokine and it has been shown to be upregulated within the brain of macaques with SIV encephalitis and experimental allergic encephalomyelitis. Pre-incubation of astrocytes with TNF-alpha for 24 hr induced double the chemotaxis of monocytes over control astrocytes. In addition to measuring chemotactic indices of monocytes, we analyzed the apical surface of our BBB model by confocal microscopy. We observed activation of MBEC as assessed by VCAM-1 and E-selectin staining when TNF-alpha was added to the lower well of the model. This activation from the lower (abluminal) compartment only occurred if MBEC were cocultured with astrocytes; MBEC cultures without astrocytes in coculture were not activated when TNF-alpha was added to the lower compartment. We conclude that TNF-alpha, which is not itself chemotactic for monocytes, induces the production of chemokines by astrocytes. We also conclude that astrocytes are required in order to activate MBEC in a physiologically relevant manner.