SPEAKER ABSTRACTS

S01
MECHANISMS OF LEUKOCYTE TRAFFICKING INTO THE CNS.
Joan W. Berman.

A major complication of HIV infection, particularly in children, is encephalitis, with approximately one-third of those infected with HIV developing HIV encephalitis and/or AIDS Dementia Complex. The mechanisms that mediate CNS infection as well as the entry of inflammatory cells into the CNS are still not well understood. It is also unclear whether cellular components of the BBB are productively infected with HIV-1. We developed a tissue culture model of the human BBB in which astrocytes and endothelial cells are cocultured on opposite sides of a porous tissue culture model. We demonstrated that the chemokines monocyte chemotactic protein-1 (MCP-1) and stromal derived factor-1 (SDF-1) mediate the transmigration of specific sets of leukocytes across this culture system, thereby establishing a model for inflammatory cell infiltration of the CNS. In addition, we showed that the HIV-1 derived protein Tat facilitates leukocyte transmigration. Our data further demonstrate that astrocytes, a key cell that forms the BBB, respond to Tat by elaborating chemokines, as well as by expressing surface adhesion molecules that may further direct leukocytes within the CNS parenchyma. In addition, we demonstrated that cytokines found within the CNS of HIV infected individuals modulate the expression of the HIV-1 co-receptor, CXCR4, on astrocytes, and therefore may facilitate not only leukocyte transmigration but also direct astrocyte infection.

S02
PERSISTENT NEUROAIDS IN CHILDREN: A NEGLECTED OPPORTUNITY FOR NEUROSCIENCE.
Pam Brown.

Major advances have been made in the treatment and prevention of HIV-1 infection in infants and children. Inspection of two consecutive large scale treatment protocols spanning the last decade however suggests that the prevalence of CNS complications has remained high, particularly in the younger children. Moreover, the presence of CNS abnormalities at baseline was highly predictive of treatment outcome, indicating the importance of including CNS measures in the overall monitoring of possible HIV-1 disease progression in infants and children. We will also argue that young children, compared to adults, may be a better group to study structural brain abnormalities as a consequence of HIV-1 infection, the way these lesions are reflected in neurobehavioral abnormalities and possible treatments. In children deterioration in CNS structure and function tends to be faster and more severe, there are fewer confounding factors influencing CNS structure and function, and there are known risk factors for CNS deterioration. Finally, HIV-1 disease in infants and children may differ from that in adults due to developmental processes, also in the CNS. In the first years of life immunologic function and virologic response to HIV-1 are elevated. Neuropathological changes are noted, not seen in adults, which seems to be associated with route and time of infection, and which are predictive of poor outcome.

In summary, the persistent incidence of HIV related CNS complications in infants and young children with HIV disease requires therapeutic attention and offers an opportunity to study the effects of (HIV-1) infection on the developing nervous system.

S03
MOLECULAR ANALYSIS OF CEREBROSPINAL FLUID: POTENTIAL FOR THE STUDY OF HIV BRAIN INFECTION.
Paola Cinque.

During recent years, the analysis of cerebrospinal fluid (CSF) by means of molecular techniques has led to important improvements in the diagnosis, clinical management and understanding of HIV-associated brain infections. Using polymerase chain reaction-based techniques, it is possible to qualitatively and quantitatively detect microbial genomes in the CSF, as well as to perform genotypic analyses. Such an approach may be used for the study of HIV brain infection ‘in vivo’, with particular regard to the natural course of HIV disease, intercurrent opportunistic infections and antiretroviral treatments.

It is known that HIV-1 levels in the CSF correlate with the presence of HIV-associated dementia or HIV encephalitis at neuropathologic examination, but not necessarily with HIV-1 RNA levels in plasma, and that CSF HIV-1 RNA levels usually decline following antiretroviral therapy. The hypothesis of the brain as a protected site, in which HIV-1 replication occurs independently from the systemic events, including the effects of antiretroviral treatments, was addressed in our studies. At this purpose we studied paired CSF and plasma specimens drawn from patients with neurological disease prior to and during potent antiretroviral treatment. At a short-term follow-up (6 months), a virologic response in the CSF, as defined by a decrease of HIV-1 RNA levels <0.5 log10 copies/ml, was observed in the majority of the patients, although to a lesser extent than in plasma. The dynamics of response in the two compartments was variably dependent on a number of factors, including baseline HIV-1 RNA levels, duration of follow-up and type of neurological disease at baseline. In particular, it appeared to be influenced by the presence of genotypic resistance to reverse transcriptase (RT) inhibitors at baseline. At longer term follow-up, however, failure of virologic response in the CSF was observed in a total of 20% of patients and it was associated with duration of therapy (>1 year), but not necessarily with virologic failure in plasma or emergence of genotypic resistance to RT or protease inhibitors. When paired CSF and plasma specimens were compared, the patterns of genotypic resistance were different between the two compartments in the 27% of the patients for RT, and in 60% for the protease gene and, in general, a larger number of mutations were found in plasma than in CSF.

Overall, our studies argue for the possibility of independent HIV-1 dynamics between CSF and brain, although these two compartments appear to be highly interactive, at least in advanced stages of HIV infection. The application of sequencing techniques to the CSF for the assessment of genotypic resistance may provide a further means for studying HIV-1 response to antiretroviral drugs.

S04
THERAPEUTIC ISSUES FOR HIV BRAIN DISEASE.
David B. Clifford.

The development of therapy impacting HIV brain disease faces many challenges. The current status of testing therapy for HIV brain disease will be discussed. Evaluation of brain disease during the asymptomatic period of infection, in minor cognitive/motor disorder or for HIV-associated dementia require different goals and study designs. Piggyback studies combining careful monitoring of HIV disease, and brief but sensitive neurologic measures permit development of knowledge about treatments in the asymptomatic period of infection. The potentially crucial position of minor cognitive/motor disorder will be evaluated as a candidate for therapeutic intervention. Current status of intervention for HIV-associated dementia will be described including the challenge of testing HIV interventions at this stage of disease, and the opportunity for testing pathophysiologically based interventions.
S05

RECENT WORK ON NEURONAL DAMAGE IN HIV AND POSSIBLE STRATEGIES FOR PROTECTION.
Ian Everett.

The spectrum of structural changes in the brain associated with HIV is being clarified. Such changes include inflammatory disorders, dendritic and synaptic damage, and neuronal loss. However, certain issues are still unclear and need to be addressed. Three such issues which our research group have viewed as important and are currently addressing are:

1. The relationship of neuronal damage and loss to various clinical features such as cognitive symptoms, and risk group.

   We have observed dendritic and synaptic damage in individuals with subtle cognitive impairments. Previously we had reported loss of particular neuronal populations in the presence of mild and severe dementia. Recently, we have also observed differences in neuronal loss among different risk groups.

2. The cellular site within the brain of HIV related toxic factors.

   Our approach to clarify which is the toxic resident cell population in the HIV infected brain will be described.


   We have observed relative preservation of neurons expressing FGF and demonstrated that it ameliorates gp120 mediated neurotoxicity.

In this talk I will review these recent findings and highlight avenues of future work.

S06

HOST RESPONSE IN THE CNS OF SIV-INFECTED RHESUS MONKEYS.
Howard S. Fox.

Central nervous system damage and dysfunction are devastating consequences of HIV infection. Using the SIV/Rhesus monkey system we have obtained a reproducible neuroviral infection, resulting in both pathological and functional effects on the CNS, using virus derived from a serial passage of microglia. Alterations in circadian rhythm, general motor activity, behavior, and sensory-evoked potentials result from infection. Host factors likely play an important role in CNS pathogenesis. We have demonstrated that a cellular immune response, consisting of cytotoxic T lymphocytes (CTL) specifically reactive against SIV, are present in the cerebrospinal fluid as early as one week post-viral inoculation. SIV-specific CTL could also be isolated from brain parenchyma of SIV-infected macaques. Although crucial for the control of viral infection, the presence and activity of CTL in the CNS may contribute to neuronal dysfunction. In both HIV and SIV infection, numerous infiltrating macrophages can be found in the CNS. Expression of chemokine molecules may lead to macrophage infiltration of the CNS, and maintain a state of increased macrophage influx or turnover. Both resident cells of the CNS as well as these infiltrating immune cells can produce a variety of host defense molecules. We have found expression of a number of such molecules, including pro-inflammatory cytokines and the inducible form of nitric oxide synthase (iNOS) in the brains of SIV-infected monkeys. Again, while protective against the virus, such molecules can deleterious effects on the CNS.

S07

CHEMOKINE RECEPTORS AND MECHANISMS OF CELL DEATH IN HIV NEUROPATHOGENESIS.
Dana Gabunda, Jianbin Wang, Ass Obihagen, and Jianguin He.

Several chemokine receptors are used as receptors for HIV-1 entry in the CNS. CCR5 is the major coreceptor for HIV-1 infection of microglia. CXCR4 and CCR5 can also mediate infection of microglia by certain HIV-1 isolates. Additional chemokine receptors that can mediate HIV-1 entry in transfected cells are expressed in the brain (i.e. APL, CX3CR1/V28, and STRL33/BBZON20), but their role in CNS infection and disease pathogenesis has not been defined. Studies using autopy brain tissues showed that the frequency of CCR5-positive perivascular mononuclear cells and macrophages is increased in the brain of patients with severe HIV-1 encephalitis (HIVE). CCR5- and CXCR4-positive macrophages and microglia are also detected in inflammatory lesions in the brain of patients with HIVE. The expression of CXCR4 on subpopulations of astrocytes and microglia may contribute to mechanisms of CNS injury that are independent of direct viral infection. The regulation of CCR5 and CXCR4 on the cell surface of cultured human fetal microglia by inflammatory cytokines and other stimuli was analyzed by flow cytometry. CCR5 expression was upregulated by IL-6 and IL-10, and downregulated by LPS and PGE2. CXCR4 expression on microglia was upregulated by PGE2 and downregulated by IFN-gamma. In cultured human fetal astrocytes, CXCR4 expression was upregulated by LIF and TNF-alpha, and downregulated by IFN-beta and PGE2. These results suggest that one mechanism by which cytokines and other inflammatory stimuli can affect HIV-1 neuropathogenesis is through regulation of CCR5 and CXCR4 expression. In contrast to CCR5 and CXCR4, the expression of CCR8 was highly dependent on cell culture conditions, with optimal expression obtained when microglia were cultured with M-CSF or IFN-gamma. Diverse HIV-1 primary isolates were examined for the ability to replicate and induce neuronal and astrocyte apoptosis in primary human brain cultures. Apoptosis was induced by infection with several blood-derived viruses which use CXCR4 in addition to CCR5 or CCR3, whereas several brain-derived viruses which use CCR5 and in some cases CCR3, but not CXCR4 did not induce significant levels of apoptosis. Studies of recombinant chimeric viruses showed that replacement of the env gene was sufficient to confer the apoptosis-inducing phenotype to an otherwise non-apoptosis inducing virus. These results provide evidence that the Env is a major determinant of neurodegenerative mechanisms associated with HIV-1 infection in vitro and raise the possibility that blood-derived viruses which use CXCR4 and emerge during the late stages of disease may impact disease progression in the CNS.

S08

HIV INFECTION IN THE BRAIN: TIMING, MODE OF ENTRY AND PERSISTENCE.
Suzanne Gartner, Yiling Liu, Edward Hunter, Xiao Pei Tang, and Justin C. McArthur.

Evidence indicates that HIV enters the brain early during the course of infection. It is unclear, however, if this acute infection establishes viral persistence. If persistence is established, it must be maintained in the absence of much virus replication, since little or no HIV expression has been detected in the brains of asymptomatic individuals. Interestingly, it has been reported that intracranial inoculation of macaques with SIV does not lead to viral persistence in the brain. In general, HIV neurological disease is only seen in patients with late stage infection (AIDS). This means that either persistence has been established and virus replication within the brain is held in check during the asymptomatic stage, or that HIV reenters the brain during late stage infection, assuming a link between the presence of the virus in the brain and the development of disease. To address this issue, we have studied post-mortem brain, lymph node, bone marrow and spleen specimens from patients with and without dementia. Comparisons of gp160 sequences cloned from these tissues suggest late-stage entry of HIV into the brain, and that blood monocytes may be the vehicles of this entry. Other experiments indicated that viral nucleic acid in circulating monocytes is primarily in an unintegrated form. In addition, we observed an increase in the number of CD56+ circulating monocytes in patients with dementia, as well as increased levels of serum M-CSF, a known inducer of CD16 expression on monocytes. Since CD16 expression on monocytes is indicative of cell activation, we speculate that these cells can more readily cross the blood brain barrier, and that increased trafficking of monocytes into brain can help to explain why dementia correlates with both an increase in the number of macrophages in the brain, as well as high levels of unintegrated HIV DNA.
S10

NEURONAL APOPTOSIS IN HIV INFECTION. CORRELATION WITH STAGE OF DISEASE, DEMENTIA, MICROGLIAL ACTIVATION, AXONAL DAMAGE AND EXPRESSION OF HIV PROTEIN P24.

Françoise Gray, Homa Adle-Beissette, Fabrice Chretien, and Thierry Ereau.

In order to characterize the distribution of apoptotic neurons and their relationships with the stage of disease, a history of HIV-dementia, and the degree of productive HIV infection, microglial activation and axonal damage, we examined 20 patients with AIDS (including 3 with HIV-dementia), 10 HIV-positive asymptomatic cases and 10 seronegative controls. Neuronal apoptosis was demonstrated by in situ end labeling in 18 AIDS cases and 2 pre-AIDS cases; a single apoptotic neuron was present in the temporal cortex of a control. Semiquantitative evaluation showed that the severity of neuronal apoptosis in the cerebral cortex correlated with the presence of cerebral atrophy, but not with a history of HIV dementia. There was no global quantitative correlation between neuronal apoptosis and HIV encephalitis or microglial activation. However there was some topographical correlation between these changes. Axonal damage was identified using brain-predursor protein immunostaining in 17 AIDS and 8 pre-AIDS brains. Although no quantitative correlation could be established between axonal damage and neuronal apoptosis there were also obvious topographical correlations. Our study suggests that neuronal apoptosis and consequent neuronal loss, in HIV infected patients, are probably not related to a single cause. It seems likely that microglial activation, directly or indirectly related to HIV infection of the CNS, plays a major role in its causation possibly through the mediation of an oxidative stress. Axonal damage, either secondary to microglial activation, or to the intervention of systemic factors may also contribute to neuronal apoptosis.

S11

PATTERNS OF CHEMOKINE RECEPTOR EXPRESSION IN NEURONS.

Denis L. Kolson.

The detection of chemokine receptors and chemokines in the brain suggests a role in CNS development and function, and in the pathogenesis of HIV encephalopathy. To better define the distribution and function of these receptors on neurons, we have utilized immunohistochemical labeling, RT-PCR and ribonuclease protection analysis, and ligand-mediated signaling studies for C-C and C-X-C chemokine receptors in cultured neurons. Immunohistochemical analysis was performed in primary fetal neurons cultured from human and rodent brain, and in the human neuronal NT2.N cell line (NTera 2/A1.D1), and RNA analysis & ligand studies were performed in pure NT.2.N neuronal cultures. Antibody labeling demonstrated strong expression of CXCR4 on dendritic and axonal processes of primary fetal human, rodent and NT2.N neurons. Weak expression of CCR2 and CXCXR2 was also detected. Similar patterns of chemokine receptor expression were seen in NT2.N neurons grown as in pure neuronal cultures and in NT2.N neurons maintained a supporting layer of primary astrocytes. Calcium fluxes were induced in NT2.N neurons by SDF-1α (CXCXR4) and MIP2 (CXCXR2), but not to MCP-1 (CXCXR2) or several other β-chemokines. NT2.N neurons grown in pure cultures also released MCP-1, as did primary human fetal astrocytes. We also examined expression of APJ, a 7TM orphan receptor that can function as an HIV-1 co-receptor, since previous studies have described APJ message in CNS tissues.

Immunohistochemical labeling demonstrated abundant APJ protein expression in human and rodent fetal neurons as well as on NT2.N neurons. These data indicate that fetal and adult neurons co-express multiple functional chemokine receptors and may themselves produce chemokines which modulate cellular functions, cell migration and CNS development, and which may also participate in HIV-induced cellular dysregulation.

S12

THERAPEUTICS OF AIDS DEMENTIA WITH NMDA ANTAGONISTS.

Stuart A. Lipton.

Objective: To determine if adjunctive treatment with antagonists to the N-methyl-D-aspartate (NMDA) subtype of glutamate receptor can prevent HIV-related neuronal apoptosis in vitro and in vivo in animal models, and then in a multi-center, placebo-controlled, double-blind clinical trial.

Methods: Neuronal injury and cell death were monitored in cerebrocortical cultures in response to gp120, in gp120-transgenic mice, and in SCI mice injected with HIV-infected macrophages.

Results: Most NMDA antagonists have been found to have intolerable side effects. Our laboratory has developed a series of compounds that are clinically tolerated due to their biophysical property of blocking excessive activation of NMDA receptors while sparing physiological activation. One such drug is the open-channel blocker memantine. In preclinical studies performed in collaboration with Drs. Ulricer Maitland and Leenart Mucke, memantine ameliorated gp120-induced neuronal apoptosis and dendritic damage in vitro as well as in vivo in gp120-transgenic mice. In preliminary studies with Dr. Howard Gendelman, memantine attenuated damage in SCI mice injected intracerebrally with HIV-infected macrophages. Other tolerated NMDA antagonists, including redox-active species of NO, are also being tested. Recently, we cloned a new NMDA receptor subunit designated NR3A which, unlike other subunits, augments excessive NMDA receptor activity and may therefore be useful therapeutically.

Conclusion: One pathway to HIV-related neuronal injury involves overactivation of NMDA receptors, followed by excess Ca²⁺ influx and free radical formation. Following successful preclinical testing, clinical trials with well-tolerated NMDA antagonists are now in progress as adjunctive therapy to the best anti-retroviral regimens for AIDS dementia.
S13

THE POTENTIAL ROLE OF HIV-1 INFECTED NEURAL DERIVED CELLS IN THE PATHOGENESIS OF CNS DAMAGE.
Eugene Major.

Although the principle target cells for HIV-1 infection in the brain are the macrophages and the microglia, HIV-1 also infects astrocytes which are numerically the predominant cell type in the brain parenchyma. Studies using cell cultures from human fetal brain showed that the biology of HIV-1 infection in astrocytes differs substantially from either macrophages or microglial cells. Initiation of infection or viral entry is CD4 independent. Reports of a viral receptor have implicated surface membrane molecules of 260 and 65 kD. Multiply spliced viral mRNAs and proteins can be detected during the first weeks of infection but diminish until neither mRNA nor protein can be detected. During this stage, there is a productive but non-cytopathic infection which results in viral latency or second stage. Reactivation from latency can take place in the presence of TNF-a, IL-1, HIV-1 Tat protein, or cell-to-cell spread with CD4+ lymphocytes. In the first and second stages of infection, NF-kB is increased in astrocytes as measured by gel shift assays. NF-kB can also be stimulated by HIV-1 Tat or TNF-a. The HIV-1 Tat protein can also stimulate synthesis of the chemokine, MCP-1, in which NF-kB is increased. The molecular control of infection may be at the translational level for structural proteins during the first stage of infection and at the transcriptional level of the intact containing 9 and 4 kB mRNAs during reactivation.

S14

FACTORS ASSOCIATED WITH DURABLE CSF VIROLOGICAL SUPPRESSION IN HIV INFECTION.
McArthur JC, McClellan D, Nauze-Saposon T, Seklisky R, and Lanier LR.

Objective: To determine the relationship between cognitive status, medication adherence, and durable virological suppression in neurologically characterized individuals starting highly active antiretroviral therapy (HAART).

Background: Potent antiretroviral combinations can produce virological suppression below detectable limits within a few weeks of initiation. CSF levels of HIV RNA have been shown to correlate with the severity of HIV dementia, and decline with HAART. However, the CNS might act as a reservoir for persistent HIV replication because of limited penetration of some antiretroviral agents or poor medication adherence. The determinants, dynamics and durability of virological suppression within the CSF compartment are unknown.

Methods: Determination of HIV RNA copy number was performed on plasma and CSF using the Nuclisens (BioMerieux) assay (Guernsey Technika, Durham, NC) from individuals prior to and after initiation of HAART. 13 HIV-1 subjects with a median CD4 count of 177 and plasma HIV RNA level of 42,000 were included. Serial CSF analyses were performed with up to 12 months of follow-up.

Results: CSF HIV RNA was undetectable (<100 copies/ml) in 2/3 subjects (15%) at baseline, rising to 70% after a median of 3 months, and maintained at 66% after >6 months of HAART. The frequency of undetectable plasma HIV was 50% and 33% respectively. All individuals reporting medication adherence of >95% had durable virological suppression within the CSF. Cognitive status did not significantly influence the frequency of virological suppression.

Conclusion: With current HAART regimes, 2/3 of HIV+ individuals achieve long term suppression of HIV within the CSF compartment. The durability of virological suppression appears to be more closely linked to medication adherence than the presence of HIV-associated dementia. Genotypic resistance profiles will be presented from the observed CSF "failures" to assess the impact of specific mutations on treatment response. Additional follow-up will determine the clinical significance of failure to suppress HIV within the CSF, and whether these individuals are at higher risk of developing dementia.

S15

VIROTOXINS AND HIV DEMENTIA.
Avi Nath, M.D.

HIV infected cells release a number of viral proteins that can cause neurotoxicity. Amongst these proteins Tat is actively released into the extracellular environment and acts synergistically with gp120 or glutamate to cause changes in intracellular calcium and neurotoxicity. Both Tat and gp120 can be detected in glial cells within the brain of patients with HIV encephalitis. Further, only a transient exposure of both proteins is sufficient to cause neuronal cell death in select populations. Transient exposure of Tat is also sufficient to induce proinflammatory cytokines in glial cells and monocyte chemoattractant factor in astrocytes. Tat may also directly excite neurons by activation of the NMDA receptor at an allosteric, Zn2+-sensitive site. Further, Tat-induced depolarizations are non-desensitizing. The actions of gp120 are predominantly mediated via glial cells. Other viral proteins shown to be neurotoxic include gp41, Nef, Rev and Vif. Although these proteins have not been studied in as much detail as gp120 and Tat, they seem to have unique mechanisms of neurotoxicity. Understanding the full potential of these virotoxins and underlying mechanisms by which they cause neuronal dysfunction is essential to the development of new therapeutic approaches for the treatment of HIV dementia.

S16

LEARNING FROM NATURE'S EXPERIMENT: sMIP-II, A VIRAL CHEMOKINE AGONIST/ANTAGONIST.

The Kaposi's sarcoma-associated herpesvirus (KSHV) contains three open reading frames that encode proteins with structural features of chemokines. The presence of Kaposi's sarcoma may be associated with a decreased incidence of central nervous system involvement in AIDS and it is possible that one of the products secreted by KSHV infected cells may serve as an antagonist. The product of one of these open reading frames, sMIP-II, has been shown bind a wide spectrum of chemokine receptors and to have some anti-counterceptor activity. We have expressed sMIP-II as a recombinant protein in order gain insight into its novel repertoire of receptor binding. sMIP-II bound CCR5 and CXCR4 at high affinity, but did not induce signaling transduction through these receptors. It did, however, stimulate CCR3 signaling in stable CHO transfectants using microphysiometry. sMIP-II did not induce internalization of CCR5, but did trigger down modulation of CCR3. Whereas the MIP-1a and enroquine did not alter the binding of monoclonal antibodies to CCR5 and CCR3 transfectants following incubation at 4°C, sMIP-II blocked the binding of the respective reagents to both proteins, suggesting that it's engagement of receptor is different from that of the physiologic ligands. Pre-incubation of sMIP-II with a monoclonal antibody to the N-terminal unstructured region, mapped using a panel of sMIP-II chimeras, did not alter the binding of this viral chemokine to CCR5 and CCR3. A chimera containing the N-terminal unstructured region of MIP-1b and the three /-sheets and a-helix of sMIP-II induced signaling via CCR5 and CCR3. This suggests that unlike other known chemokines, sMIP-II function as a CCR3 agonist in not altered by replacement of the N-terminal region of sMIP-II with the corresponding region of a chemokine (MIP-1b) that lacks CCR3 activity and, thus, sequences responsible for the unique repertoire of receptor binding may reside in the three /-sheets and a-helix of sMIP-II. We demonstrate that it is possible to add modules for CCR5 signaling to this "scaffold", while maintaining some of the novel intrinsic activities.
S17

IMMUNOPATHOGENESIS OF HIV-1 ASSOCIATED DEMENTIA: A CENTRAL ROLE FOR THE BLOOD-BRAIN BARRIER.

Yuri Persidsky, M.D., Ph.D.

The neuropathogenesis of HIV-1 infection revolves around the numbers of immune activated brain macrophages and microglia. However, how monocytes gain entry into the brain during disease remains unanswered. In attempts to address this question we used co-cultivating laboratory, animal model and human tissue models of HIV-1-associated dementia (HAD) to investigate how monocytes migrate into the brain during disease. First, an artificial blood-brain barrier (BBBB) was constructed where human brain microvascular endothelial and glial cells [astrocytes, microglia and/or monocyte-derived macrophages (MDM)] are placed on opposite sides of a matrix-coated porous membrane. Measurements of monocyte migration were made utilizing this model and showed that HIV-1 infected microglia placed onto the "astrocyte" compartment induced monocyte migration (2-3.5 times) more than did similarly placed MDM. Second, a SCID mouse model of HIV-1 encephalitis (HIVE) measured "in vivo" monocyte transendothelial brain migration. Here, equal numbers of HIV-infected microglia or MDM were inoculated into the basal ganglia of recipient animals. This resulted in a marked accumulation of mouse MDM with astroglia in areas surrounding virus-infected microglia but not MDM. Third, monocyte encephalitis brain tissue analysis showed that astroglia, microglia activation and virus infection correlated with monocyte and α-1 antichymotrypsin expression. Here, microglial activation and virus infection correlated with astroglia, monocyte transendothelial migration and intense expression of b chemokines. Lastly, in laboratory experiments, infected and activated microglia and/or astrocytes exposed to microglial secretions produced large levels of b chemokines. We conclude that the interactions between virus infected/activated microglia and astrocytes play a prominent role in b chemokine-mediated monocyte migration in HAD.

S18

CURRENT APPROACHES TO TREATMENT FOR HIV-1 INFECTION.

William G. Powderly.

The last three years have seen a dramatic fall in mortality and morbidity from HIV infection. Four factors have contributed to this: an improved understanding of the pathogenesis of HIV infection; the availability of tests that could measure plasma viral burden; the development of new and more powerful drugs such as the protease and non-nucleoside reverse transcriptase inhibitors; and the completion of large clinical endpoint trials that conclusively demonstrated that potent antiretroviral combinations significantly delayed the progression of HIV disease and improved survival. Typical antiretroviral regimen must consist of at least three agents: one to two protease inhibitors or non-nucleoside reverse transcriptase inhibitors combined with two nucleoside analogs. The goal of therapy is to reduce measurable plasma viral burden to undetectable levels. Viral load testing has made it possible to individualize therapy and to more accurately determine the best time to initiate or change therapy, long before declining CD4+ counts would have given evidence of active viral replication. However, despite the impressive progress to date, there remain significant shortcomings with current treatment. Even with the most potent regimens available, there exists a proportion of patients (perhaps 20-50% of treated individuals) who fail to have complete and durable virologic responses to therapy. The shortcomings of current regimens are particularly evident in patients with high plasma HIV-1 RNA levels, extensive prior treatment, and advanced disease. Complexity, short- and long-term toxicities, cross-resistance, and drug-drug interactions all complicate current regimens. Viral resistance is increasingly encountered in clinical practice and transmission of resistant virus is well-documented. In addition, there remain concerns about the ability of the virus to evade current therapies, whether in viral reservoirs in non-lymphoid compartments or in lymphoid tissue, such as resting memory T cells. Thus there remains a need for new therapies as well as new strategies using existing drugs.

S19

THE BRAIN AS AN IMMUNE ORGAN REVISITED.

Cedric S. Raine.

The central nervous system (CNS) has traditionally been perceived as an organ sequestered from the general immune system and that all intents and purposes, it is immunologically inert. Based on a number of advances in the understanding of HIV encephalitis, a condition in which HIV-1 displays a selective neuropathy for microglial cells, multiple sclerosis, a condition of suspected viral etiology with lesions that are immune-mediated, and autoimmune demyelination in animal models, it has become abundantly clear that CNS cells are capable of orchestrating highly integrated responses involving key molecules of the immune system in an attempt to re-establish tissue homeostasis. While the neuroimmune response may be similar in character to that observed in the periphery, since the cell affected are frequently tethered, highly attenuated and involved in CNS functions, many invariably undergo irreversible structural damage. Recognizing that HIV encephalitis represents the negative outcome of a host-pathogen interaction within the CNS, the identification of the immune system molecular phenotype during CNS inflammation affords a worthwhile objective for translational therapies. In HIV encephalitis, the principal infected cell type, the microglial cell, is the resident macrophage of the CNS and a cell of monocyte lineage that is capable of considerable transformation and function. This cell type is the major immune effector cell of the CNS and is known to be capable of numerous interactions with T cells and to produce a wide variety of immune system molecules, in particular proinflammatory cytokines and chemokines. Astrocytes are also capable of considerable immunologic interaction and in the normal state, appear to do so in an immunosuppressive (Th2-type) fashion. However, during disease states, astrocytes display remarkable proinflammatory responses. Oligodendrocytes and neurons also express immune system molecules but to a lesser degree. The interaction of CNS elements with the immune system is facilitated in chronic inflammatory conditions by CNS blood vessels acquiring the properties of high endothelial venules and inflammatory infiltrates becoming organized into lymphoid-like deposits, features that in addition to the above mentioned responses, impart upon the CNS functions typical of lymphoid elements. Continued investigation of the innate and acquired immune responsiveness of the CNS will shed light on a number of issues relevant to the understanding the pathogenesis of HIV encephalitis. (Supported in part by NIH grants NS 08952, NS 11920 and NS 07098; NMSS grant M01-RR00053; the SoL Goldman Charitable Trust, and the Ettorelon Family Foundation.)

S20

NEUROLOGIC DISEASE IN INJECTION DRUG USERS: THERAPEUTIC APPROACHES.

Walter Royal, III, M.D.

Treatment of HIV-1 related neurological disease in injection drug users (IDU) can present a unique challenge as a result of effects of abused drugs on nervous system and immune function and antiretroviral drug metabolism and patient compliance. There is little evidence that drugs of abuse, such as opioid drugs and cocaine, can cause direct structural damage to adult nervous system tissue. However, clinical neurologic deficits can occur due to biochemical effects of these compounds, drug-induced inflammation and ischemia, and infections complications. In individuals treated with methadone, zidovudine levels may be increased as a result of impaired hepatic glucuronidation, and metabolism of morphine by cytochrome P450 enzymes may be impaired by co-administration of non-nucleoside reverse transcriptase and protease inhibitors. The significance of these effects on the occurrence of HIV-1 related neurologic disease have been largely unexplored. In addition, a large percentage of these medications can cause neurologic impairment with varying degrees of frequency and severity. Therefore, such potential interactions may be important for the appropriate assessment and management of neurologic symptoms and to promote treatment compliance among IDUs. New therapies will also need to be developed that target clinical and epidemiologic characteristics that are unique for individuals of this risk group.