Changing Patterns in the Neuropathogenesis of HIV During the HAART Era

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Rapid progress in the development of highly active antiretroviral therapy has changed the observed patterns in HIV encephalitis and AIDS-related CNS opportunistic infections. Early in the AIDS epidemic, autopsy studies pointed to a high prevalence of these conditions. With the advent of nucleoside reverse transcriptase inhibitors, the prevalence at autopsy of opportunistic infections, such as toxoplasmosis and progressive multifocal leukoencephalopathy, declined while that of HIV encephalitis increased. After the introduction of protease inhibitors, a decline in both HIV encephalitis and CNS opportunistic infections was observed. However, with the increasing resistance of HIV strains to anti-retrovirals, there has been a resurgence in the frequency of HIV encephalitis and HIV leukoencephalopathy. HIV leukoencephalopathy in AIDS patients failing highly active antiretroviral therapy is characterized by massive infiltration of HIV infected monocytes/macrophages into the brain and extensive white matter destruction. This condition may be attributable to interactions of anti-retrovirals with cerebrovascular endothelium, astroglial cells and white matter of the brain. These interactions may lead to cerebral ischemia, increased blood-brain barrier permeability and demyelination. Potential mechanisms of such interactions include alterations in host cell signaling that may result in trophic factor dysregulation and mitochondrial injury. We conclude that despite the initial success of combined anti-retroviral therapy, more severe forms of HIV encephalitis appear to be emerging as the epidemic matures. Factors that may contribute to this worsening include the prolonged survival of HIV-infected patients, thereby prolonging the brain's exposure to HIV virions and proteins, the use of increasingly toxic combinations of poorly penetrating drugs in highly antiretroviral-experienced AIDS patients, and selection of more virulent HIV strains with higher replication rates and greater virulence in neural tissues.

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Introduction

Rapid progress in the development of highly active anti-retroviral therapy (HAART) has changed the patterns of human immunodeficiency virus (HIV) encephalitis (HIVE) and central nervous system (CNS) opportunistic infections (OI) in patients with the acquired immune deficiency syndrome (AIDS). Despite HAART's survival benefits, HIV neuropathogenesis continues to evolve in response to several drug-related pressures including toxicity, generally poor CNS penetrance, and drug resistance of HIV virions (Figure 1).

The introduction of antiretrovirals (ARVs) markedly altered HIV disease progression in nervous system tissues. Currently approved ARVs are listed in Table 1 and are divided into three classes, nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-NRTIs (nNRTIs), and protease inhibitors (PIs) (22). Two NRTIs and either a PI or an nNRTI are combined in most initial regimens. However, each regimen must be individualized based on multiple considerations, which include potency, tolerability, drug interactions, adherence, future treatment options, and resistance testing (9).

The use of ARVs has divided the AIDS epidemic into at least 4 stages. The first (or pre-treatment) stage, from 1982 to 1987, corresponds to the period before the introduction of antiretroviral therapy (ART). Studies performed during this period will best reflect the natural history of disease. The second (or monotherapy) stage, from 1987 to 1992, corresponds roughly to the use of the early NRTIs, ZDV and ddI, alone. During this period, HIV associated dementia (HAD) was successfully treated with high-dose ZDV, providing the first evidence that ARVs could alter HIV neuropathogenesis. The third (or early combination) stage, from 1992 to 1995, corresponds to the introduction of newer NRTIs, such as stavudine (d4T), as well as the practice of combining 2 ARVs. In small studies, such combinations of 2 NRTIs successfully suppressed HIV replication in cerebrospinal fluid (CSF) below detection (31). The fourth (or later combination) stage, from 1996 to the present, corresponds to the introduction of PIs and nNRTIs as well as the practice of combining at least 3 ARVs. This contemporary period has seen the most profound changes in AIDS and neuroAIDS. Few ARVs appear to attain effective concentrations in the CNS. Improving

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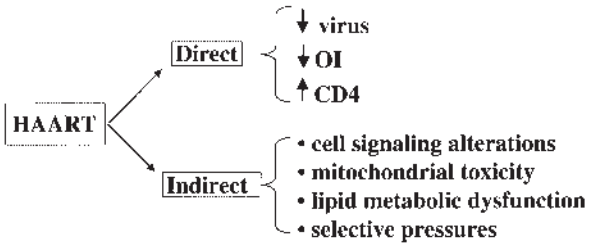

Figure 1. Potential mechanisms through which HAART influences HIV neuropathogenesis. Mechanisms include beneficial direct effects mediated by decreasing viral loads and OI and increasing CD4 counts or deleterious indirect effects.

Generic Name	Abbreviation	Commercial Name	First FDA Approval
Nucleoside/Nucleotide Reverse Transcriptase Inhibitors			
Zidovudine	ZDV, AZT	Retrovir	1987
Didanosine	ddI	Videx	1991
Zalcitabine	ddC	Hivid	1992
Stavudine	d4T	Zerit	1994
Lamivudine	3TC	Epivir	1995
Abacavir	ABC	Ziagen	1998
Tenofovir	TFV	Viread	2001
Non-Nucleoside Reverse Transcriptase Inhibitors			
Nevirapine	NVP	Viramune	1996
Delavirdine	DLV	Resriptor	1997
Efavirenz	EFV	Sustiva	1998
Protease Inhibitors			
Saquinavir	SQV	Invirase, Fortovase	1995
Ritonavir	RTV	Norvir	1996
Indinavir	IDV	Crixivan	1996
Nelfinavir	NFV	Viracept	1997
Amprenavir	APV	Agenerase	1999
Lopinavir	LPV	Kaletra	2000

Table 1. Currently approved Anti-Retrovirals. ARVs are divided into 3 classes: nucleoside/nulceotide reverse transcriptase inhibitors (NRTIs), non-NRTIs (nNRTIs), and protease inhibitors (PIs).

ARV delivery to the brain has been limited by several factors. For PIs, the most important may be the expression of P-glycoprotein (P-gp), a polarized membrane efflux transporter located on the luminal surface of endothelial cells (EC) of the blood-brain barrier (BBB). P-gp limits uptake of drugs into the brain by efficient efflux of large, hydrophobic molecules back into the blood stream (13, 15, 38, 116) thereby contributing to the poor penetrance of PIs into the brain (52, 57, 66). In vitro studies in a BBB model suggest that NVP may penetrate best, followed by ddI, d4T, ddC, and ZDV, followed then by IDV, and finally SQV (36).

The interaction of HIV-infected monocytes and brain endothelial cells initiates a complex series of events, which include upregulation of adhesion molecules on the luminal surface of brain EC, changes in tight junction integrity, and increased expression of cytokines and chemokines in the brain, all of which facilitate mononuclear cell migration into the central nervous system (CNS) (18, 63, 94, 97). After HIV-infected and activated mononuclear cells infiltrate the CNS, resting microglia are activated to produce inflammatory molecules, excitotoxins and proteases that in turn activate astrocytes and damage neurons. Neuronal loss in AIDS is likely caused by numerous factors including glial activation and subsequent oxidative stress (42).

These events lead to the development of neuroAIDS, the elements of which can include HIVE, neurodegeneration, and CNS infection with opportunistic pathogens. Prior to the introduction of ARVs, opportunistic pathogens commonly included cytomegalovirus (CMV), JC virus (JCV) and Epstein-Barr virus (EBV). The incidence of these infections has declined in the HAART era but the prevalence of HIVE has increased. In general the overall patterns of neurodegeneration have shifted from widespread damage to excitatory pyramidal neurons to more subtle injury to dendritic arbors and other neuronal populations such as interneurons (80).

HAART may influence HIV neuropathogenesis via several mechanisms. The most important mechanisms are probably attributable to the primary effects of HAART itself: suppression of HIV replication, improvement in cell-mediated immunity, and prolonged survival. A main focus of this review involves a fourth factor, related to specific interactions of ARVs with brain endothelial cells and the resulting alterations in downstream signaling events in the CNS.

Reduced bioavailability of several ARVs is due in part to first pass metabolism by the cytochrome P450 system. In some protected compartments, such as the CNS, ARV concentrations are further reduced by the MDR1 multidrug transporter P-gp located on the luminal surface of endothelial cells of the BBB (14, 41, 51, 66). For example, HAART interferes with the efflux mechanism of the P-gp in peripheral blood lymphocytes (74). Furthermore, HIV increases expression of the P-gp in monocytes and T-cells (39).

In summary, HAART may incompletely suppress HIV replication in the CNS and may alter brain endothelial signaling. Together, these effects may influence viral evolution and cellular trafficking, leading to changes in the patterns of neurodegeneration. Variations in the frequency of AIDS-related CNS pathology might be associated with the appearance of drug-resist-

ant pathogens and the severity of systemic disease, intravenous drug use, and the introduction in 1996 of new anti-retroviral treatments based on the use of protease inhibitors. In this context, the main objectives of the present review are to analyze changes in prevalence/incidence frequency of systemic AIDS pathology over time and the relationship with variations in CNS pathology.

The Neuropathology of HIV in the Pre- and Early Treatment Era

During the pre-treatment era, autopsy studies found that death was attributable to OIs affecting the respiratory tract and nervous system in the majority of patients. Sixty-three percent of all autopsied AIDS patients had evidence of nervous system involvement by either HIV or opportunistic pathogens, while 37% did not. According to our studies and those of Jellinger et al, the most common HIV-associated neuropathologies included HIVE, vacuolar myelopathy, lymphocytic meningitis, diffuse poliodystrophy, and granulomatous angiitis, followed by CMV encephalitis (CMVE) (18.0%), aspergillosis (4.9%), PML (3.4%), toxoplasmosis (2.5%), and bacterial meningitis, tuberculosis and mycobacterium avium complex (MAC) (1.6%) (Table 2) (50, 79). HIVE was manifested by formation of multinucleated giant cells (MNGC) in the parenchyma and perivascular areas, myelin pallor, astrogliosis, microgliosis and microglial nodule (MGN) formation. Most lesions were usually found in the basal ganglia, frontal cortex and white matter, but the hippocampus, brainstem, cerebellum and thalamus were also affected (7). White matter damage was characterized by focal demyelination, astrogliosis, mild infiltration by macrophages and, in rare cases, extensive destruction of the white matter tracts (Figure 2A-D).

The severity, characteristics and distribution of CNS injury were most likely associated with the viral load in the CNS, which can vary from a few thousand copies to over one million copies/mL (1). Most studies agree that in the CNS HIV productively infects macrophages and microglia primarily. Cases with abundant MNGC and/or extensive white matter damage usually display a very high viral load, while cases with lower viral loads have less severe disease, evidenced by lymphocytic meningitis, microglial proliferation, and moderate astrogliosis, but with no MNGC (78). These and other observations suggest several stages of HIV-associated disease of the CNS. First, meningeal inflammation occurs with perivascular mononuclear infiltration, leading to altered BBB permeability. Once the BBB is breached,

	BEFORE 1995	AFTER 1995
TOTAL (n=)	62	89
AGE (MEAN ±SEM)	39±2	42±2
M/F	57/5	81/8
IVDU	8	14
NO ALTER	16(25%)	26(29%)
HIVE	19(29%)	39(45%)
PML	4(7%)	5(6%)
CMV	10(16%)	15(17%)
NHL	12(19%)	8(9%)
CRYPTO	5(8%)	5(6%)
TOXO	4(7%)	0(0%)

Table 2. Comparison of the neuropathological findings in AIDS cases in the early (before 1995) and late (after 1995) Combination therapy eras. TOTAL (Total number cases), AGE (Age of patient at death), M/F (Male/Female), IVDU (Intravenous Drug User), NO ALTER (No Alterations), HIVE (HIV Encephalitis), PML (Progressive Multifocal Leukoencephalopathy), CMV (Cytomegalovirus), NHL (Non-Hodgkin's Lymphoma), CRYPTO (Cryptococcus), TOXO (Toxoplasmosis).

HIV then infects microglial cells, resulting in microglial activation, proliferation, and the formation of MGN and MNGC. Activation can lead to inflammatory events and injury of neural tissues, including myelin, and subsequent astrogliosis. This pathogenic model is consistent with studies in experimental animal models including simian immunodeficiency virus (SIV) and SCID mice grafted with HIV infected macrophages (62, 95).

In AIDS patients, studies of neurodegeneration have promoted a better understanding of the mechanisms resulting in cognitive impairment. Initial studies focused on characterizing the neuronal populations affected. These studies reported loss primarily of large pyramidal neurons and parvalbumin- and calbindin-immunoreactive interneurons in the neocortex (80). Subtle neuronal damage was described in the early phases of infection in these untreated patients, but more severe HIVE was associated with greater loss of more diverse populations of neurons (28).

The relationships among HIVE, neurodegeneration, and HAD is more complex than enumerating neuronal loss. For example, not all patients with HIVE develop dementia and not all demented AIDS patients have HIVE. In comparison, most patients with cognitive impairment do have neurodegeneration, and those with a normal neuropsychological profile show preservation of their synaptodendritic organization (28). We have shown that damage to synapses and dendrites in

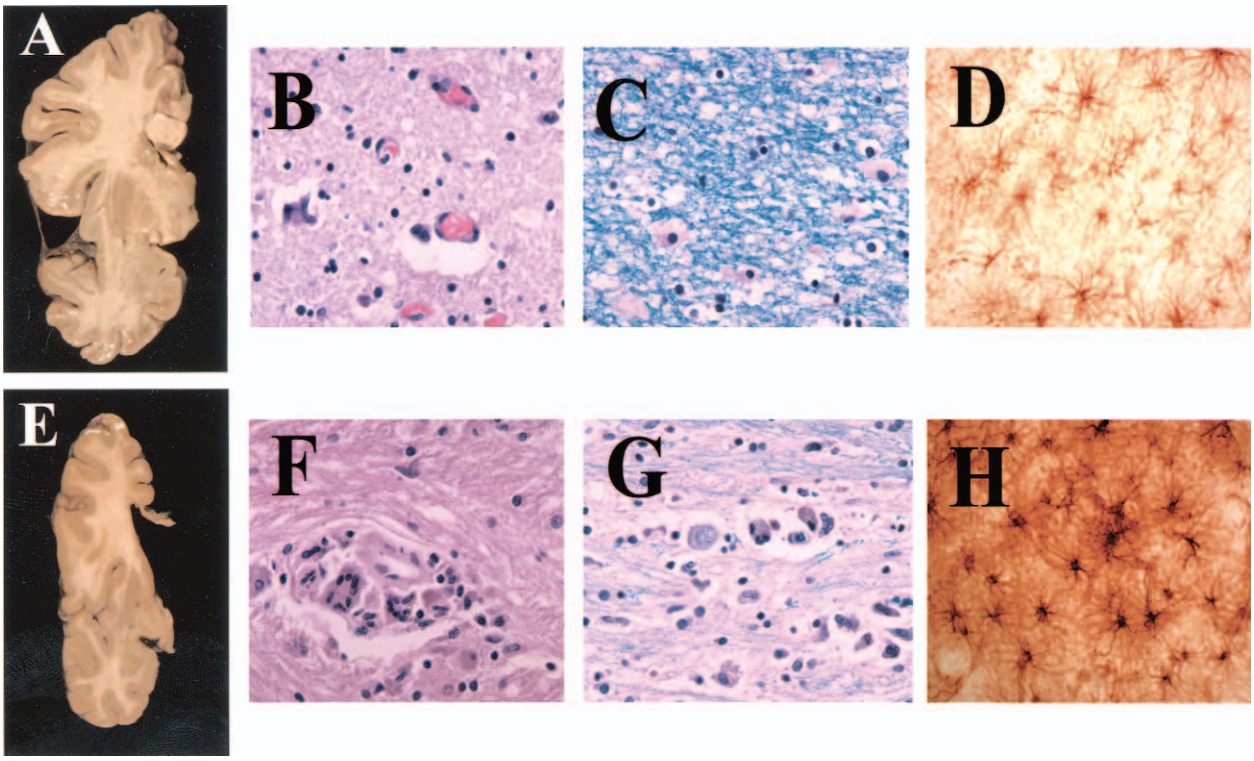

Figure 2. Comparison of white matter neuropathological alterations between the early (before 1995) and the late (after 1995) combinational treatment eras. Panels **A-D** are from a patient with HIVE that received no ARV treatment and **E-H** are from a patient that received and failed to respond to triple therapy. (**A**) Gross pathology shows mild atrophy and white matter changes. Sections in panels **B-D** and **F-H** are from the frontal cortex. (**B**) H&E demonstrates vascular involvement with mononuclear infiltration and occasional multinucleated giant cells. (**C**) Mild myelin loss by luxol fast blue staining. (**D**) Moderate white matter astrogliosis with an antibody against GFAP. (**E**) Gross pathology shows severe atrophy and white matter changes. (**F**) H&E demonstrating abundant mononuclear infiltration and multinucleated giant cells. (**C**) Severe myelin loss by luxol fast blue staining. (**D**) Severe white matter astrogliosis with an antibody against GFAP.

patients with HIVE is substantial, occurring even in early disease and correlating with brain viral load (28, 81). The heterogeneous medical and neuropsychological characteristics of patients with HIV-associated cognitive impairments further complicate analyses of the neuropathologic correlates of HAD (81). Our group classifies those with impaired performance on neuropsychological testing into one of 4 diagnostic categories: *i)* neuropsychological impairment, likely due to a cause other than HIV (NPI-O), *ii)* asymptomatic neuropsychological impairment, likely due to HIV (NPI), *iii)* minor cognitive motor disorder (MCMD), and *iv)* frank HAD. Assignment of one of the 3 sub-dementia diagnoses does not necessarily portend progression to dementia, although coexisting depression may (113). In the pretreatment era, prevalence rates for HAD ranged from 5 to 20% among patients with AIDS, while rates for those suffering from minor cognitive and motor deficits reached 30% (82, 97, 120). Without antiretrovirals, the mean survival of patients with HAD was 3 to 6 months (97).

In summary, HIV does not directly injure neurons by productive infection but via infection of macrophages and microglia and the by-products of inflammation. This indirect mechanism leads to damage of selected neuronal populations and white matter tracts and, in many cases, precedes severe and rapidly progressive cognitive impairment. ARVs have generally decreased the rate of HIV replication and the severity of the damage but, as we will discuss below, have transformed neuroAIDS to a more chronic condition (Figure 3).

The Neuropathology of HIV in the Early and Late Combination Treatment Eras

While effective prevention and treatment has helped to ameliorate the development of certain AIDS-related conditions, other illnesses quickly become the cause of

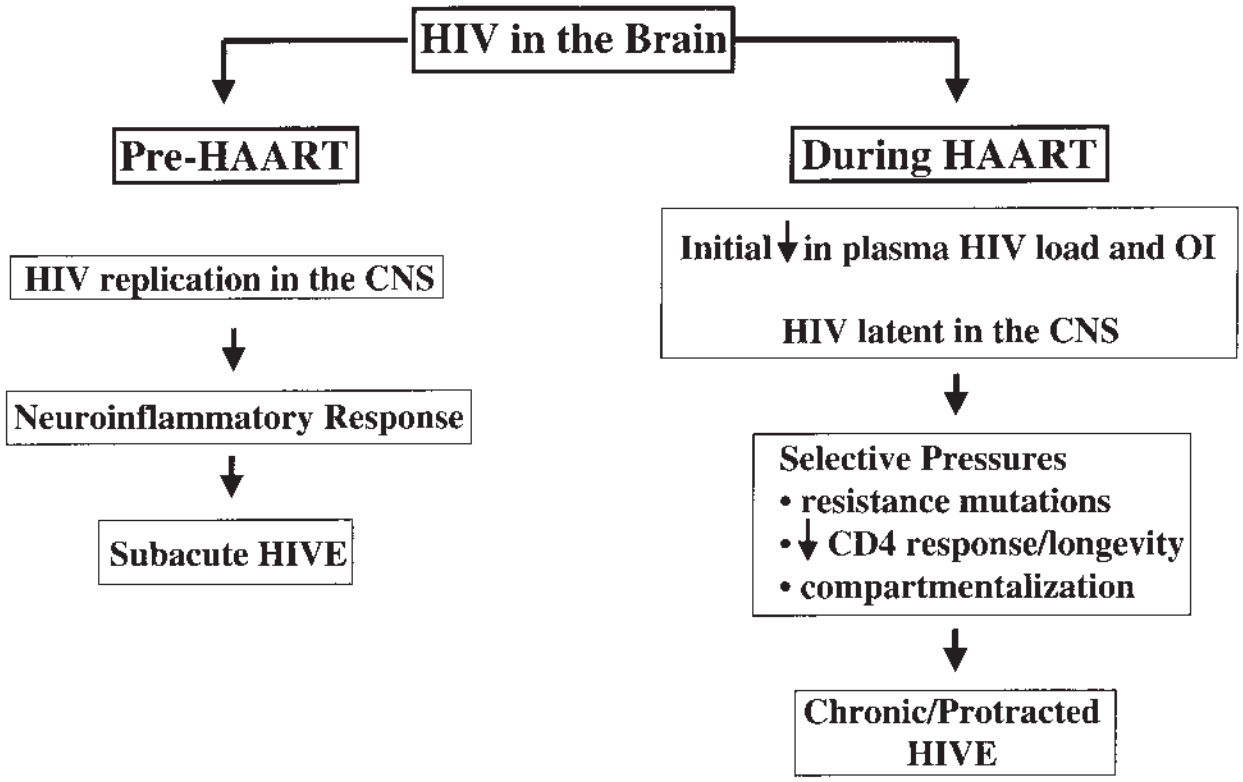

Figure 3. Comparison of the relationship between white matter disease and HIVE in the early (before 1995) and late (after 1995) combinational treatment eras. During the late treatment era, the proportion of cases with white matter damage has increased.

death. Supporting this notion, recent studies have shown increased incidence of HIV-induced brain lesions in AIDS patients with long-term survival (115). This study showed a 40% incidence of HIV encephalitis during the first years of the epidemic, however, survival was short in this period (50, 79, 115). Although the incidence of HIVE fell markedly around the time ZDV (1987) was introduced (Table 1) and remained low in patients using ZDV until death, the rate of HIVE has increased during the later years with the new cases occurring mainly in patients who had discontinued ZDV (43, 75, 115).

In autopsy studies, the frequency of HIVE has fluctuated from year to year with no clear upward or downward trend (50, 79). The frequency of CMVE and toxoplasmosis has shown a significant downward trend over time, while the frequency of fungal infection and non-Hodgkin's lymphoma (NHL) has fluctuated considerably. The frequency of herpes simplex virus (HSV), PML/JCV and MAC has remained low over time. Similarly, in a retrospective study of 450 consecutive AIDS autopsy cases in Vienna, the decrease in fungal infections was less than described in other reports (50). While bacterial organ and CNS infections (except for MAC), lymphomas, HIV-associated CNS lesions (around 30%), non HIV-associated changes (vascular, metabolic, etc.) and negative CNS findings (10-11%) remained unchanged, nonspecific CNS changes (eg, meningeal fibrosis) increased (50). Extra-cerebral pathology in subjects with advanced HIV-related CNS lesions showed more frequent but decreasing systemic bacterial and CMV infections than those with negative or nonspecific neuropathology, while other opportunistic and multiple organ infections and lymphomas showed no differences between groups. In a cohort of drug abusers, HIVE, PML, bacterial infections, hepatic encephalopathy and negative CNS findings were more frequent than in non-drug users who showed an increased incidence of CMV, toxoplasmosis, or other opportunistic CNS infections with nonspecific CNS findings (20). The frequency of lymphomas was similar in both drug abusers and non-drug users. Studies in San Diego and Vienna concluded that despite the beneficial effects of modern antiretroviral combination therapy, involvement of the brain in AIDS subjects continues to be a frequent autopsy finding (50). Other clinical series,

however, have reported a decrease in the number of cases with HIVE after HAART (102).

With the advent of prophylactic therapies such as NRTIs and later of nNRTIs, the frequency of neuro-OIs such as toxoplasmosis, and CMVE has decreased. Moreover, in the initial phases after the introduction of PI, there was a relative decrease in OI and HIVE. However, and probably due to the emergence of resistance, a surge in the frequency of HIVE and in particular of a highly destructive form of HAL has emerged (Figure 2, compare A-D with E-H). In this regard, we reported seven recent autopsy cases of leukoencephalopathy in antiretroviral-experienced patients with AIDS (64). Clinically, all 7 were severely immunosuppressed, 6 (86%) of 7 had poorly controlled HIV replication despite combination antiretroviral therapy, and 5 (71%) of 7 had HIV-associated dementia. Neuropathologically, all seven patients had intense perivascular infiltration as detected by HIV-gp41 immunoreactive monocytes/macrophages and lymphocytes, widespread myelin loss, axonal injury, microgliosis and astrogliosis. The extent of damage observed in these cases exceeds that described prior to the use of HAART. Furthermore, brain tissue demonstrated high levels of HIV RNA, but evidence of other pathogens, such as JCV, EBV, CMV, human herpes virus-8 (HHV8), and HSV1 and 2, was absent. Comparison of the stages of pathology suggests a temporal sequence of events. In this model, white matter damage begins with perivascular infiltration by HIV-infected monocytes, which may occur as a consequence of antiretroviral-associated immune restoration. Intense infiltration by immune cells injures brain endothelial cells and is followed by myelin loss, axonal damage, and finally, astrogliosis.

Diffuse damage to the white matter in the absence of OIs has been recognized for several years as a characteristic feature of the neuropathological spectrum associated with AIDS. In most cases, damage to the white matter is characterized by mild to moderate myelin loss and astrogliosis (Figure 2A-D). However and as described above, in recent years we have observed the emergence of more severe and destructive forms of leukoencephalopathy (Figure 2E-H). Although less common, the appearance of these severe cases might signal an overall increased frequency in milder forms of HAL. Consistent with this concept, recent imaging and biopsy studies have shown an increase in white matter anomalies by MRI in AIDS patients (2). During the HAART era, AIDS-related primary CNS lymphoma showed a strong decline, toxoplasmic encephalitis remained stable, and PML showed a slight increase (2). Focal white matter lesions without mass effect or contrast enhancement and non diagnostic features on histopathology became the most frequently seen focal brain lesion. To further investigate this possibility, we analyzed in our autopsy series, the frequency of white matter damage in AIDS patients in the pre-HAART and HAART eras (Figure 3). A total of 151 AIDS autopsy cases performed at the UCSD-Medical Center between 1993 and 2001 were included in this study (Table 2). Comparisons of brain pathology at autopsy between the early and late combinational treatment eras showed that the proportion of cases with toxoplasmosis and NHL decreased, while PML, CMV and cryptococcosis remained unchanged and HIVE increased (Table 2). Of the 151 cases, 62 were performed between 1993 and 1995 (corresponding to the pre-HAART era) and 89 between 1996 and 2001 (corresponding to the HAART era). Cases were divided into four groups according to the presence of white matter disease and/or HIVE. The presence of white matter disease was evaluated in the frontal cortex, cingulate cortex, corpus callosum, circle semiovale and occipital cortex by H&E, luxol fast blue and glial fibrillary associated protein (GFAP) immunocytochemistry (Figure 2). HIVE was evaluated by H&E and p24 immunocytochemistry. Cases with OIs, CNS lymphoma or ischemic lesions were not included in the final analyses. We found that in the pre-HAART era, independently of the presence or absence of HIVE, 11% of the cases displayed white matter disease (Figure 3). In contrast, in the HAART era, 26% ($p<0.05$, Chi square) of the cases presented white matter damage (Figure 3). Taken together, our findings provide evidence for the emergence of a severe form of HAL. This condition warrants further study and increased vigilance among those who provide care for HIV-infected individuals.

The impact of changes to CNS viral load and HIVE attributable to HAART has now been evaluated. Some reports indicate that although the relative frequency of HIVE has not increased since the introduction of HAART, the number of cases with persistent cognitive disorders remain (24). Poor CNS penetration of many antiretroviral agents is a possible explanation for this occurrence, but irreversible "burnt out" HIV-induced CNS changes may also play a role (42). In the Dana cohort that included 272 HIV-seropositive subjects, there were no differences in the occurrence of HAD or abnormalities below established norms for any of the neuropsychological tests conducted (103). The authors concluded that even though HAART has reduced the incidence of HAD, HIV-associated cognitive impairment continues to be a major clinical problem among

individuals with advanced infection (103). Thus, the clinical phenotype of HAD appears to be evolving from a subacute dementing disease to a more protracted disorder. Decreased CD14/CD69 levels may reflect changes in some aspects of the pathophysiology of brain injury in the HAART era. Changes in neural cell signaling, immune response and structural and functional proteins may represent more subtle neurotoxicity manifested in cell dysfunction rather than frank cell death (61). This suggests that some of the beneficial effects of HAART might be associated with substantial improvement in the immune function in many HIV-infected individuals. However, the occurrence of milder forms of neurocognitive dysfunction persists and may be caused by the poor penetration of some antiretroviral agents into the CNS. In this regard, a study on the incidence of mild neurocognitive impairment after the introduction of HAART in a group of individuals initially found to be neurocognitively normal, suggests that HAART helps to maintain intact cognitive functioning in high-risk patients with relatively unrestricted access to HAART (24). This protection is predominantly mediated by a HAART-induced improvement in immune function reflected by the CD4 cell count (24).

HAART used with non-steroidal anti-inflammatory agents leads to the suppression of inflammatory neurotoxins and can markedly improve neurologic function in HAD (34). These data, taken together, support the notion that HAD may be a partially reversible metabolic encephalopathy fueled by viral replication.

Mechanisms by which HAART may Modify the Neuropathogenesis of HIV

By far the most significant and notable changes in patterns of neuropathology in AIDS patients in the HAART era result from the dramatic decrease in OIs of the CNS and the shift in HIVE from a subacute to a chronic and protracted disease (Figure 4). As complications associated with OIs continue to decrease and HIV patients' lifespans increase, a different set of factors may contribute to neuronal damage and the changing patterns in neuropathology. HAART is both directly and indirectly responsible in part for changes observed in cellular characteristics and in patterns and distribution of neurodegeneration in the brain (Figure 1). By decreasing systemic viral load during HAART, fewer infected cells, viral particles and inflammatory factors are available to interact with cells of the BBB and the brain. Likewise, the CD4+ count increases, making OIs less frequent. In point of fact, substantial improvement was reported in patients with HAD following ZDV treatment (30) despite the low steady state levels of the drug in the CNS and a low CSF to plasma ratio of 0.5 in humans (6). On the other hand, unintended effects from the use of HAART that may interfere with CNS functioning include alterations in signaling events at the BBB and within the brain parenchyma. These deleterious effects may result from: *i)* toxic or inflammatory factors produced in response to HAART-induced systemic complications, such as hyperlipidemia and hypersensitivity; *ii)* alterations in endothelial cell P-gp expression and cavelolar changes; *iii)* direct interaction of antiretrovirals with glial cells and neurons; and *iv)* selective pressure for mutation, resistance and compartmentalization of the virus in the brain (Figure 5). In this context, changing patterns of HIVE and neurodegeneration observed in the HAART era are probably influenced in part by unintended interference of antiretroviral agents with host cell signaling (53, 117).

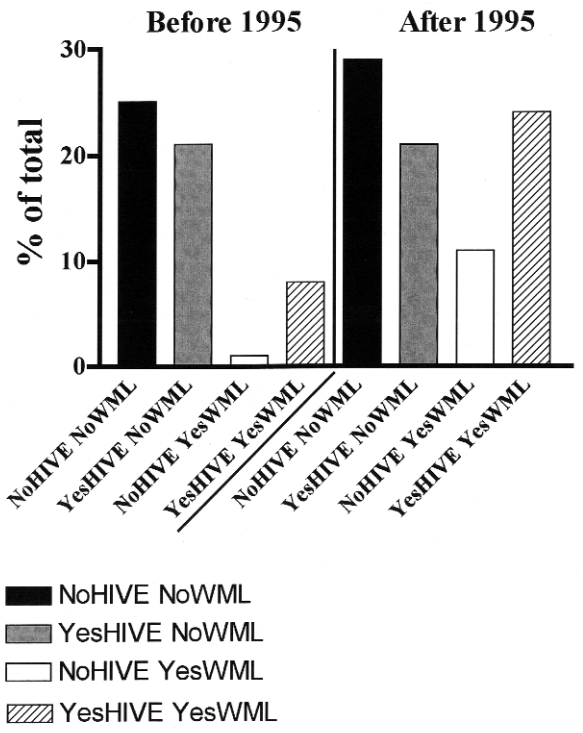

Figure 4. Progression of HIVE during the early (before 1995) and late (after 1995) combinational treatment eras. During the pre-HAART and early treatment eras, HIVE presented as a subacute condition. After HAART, HIVE changed to a chronic and more protracted disease. WML = white matter lesions.

Metabolic abnormalities associated with HAART. The success of HAART in controlling plasma and CSF viral burden may be accompanied by significant

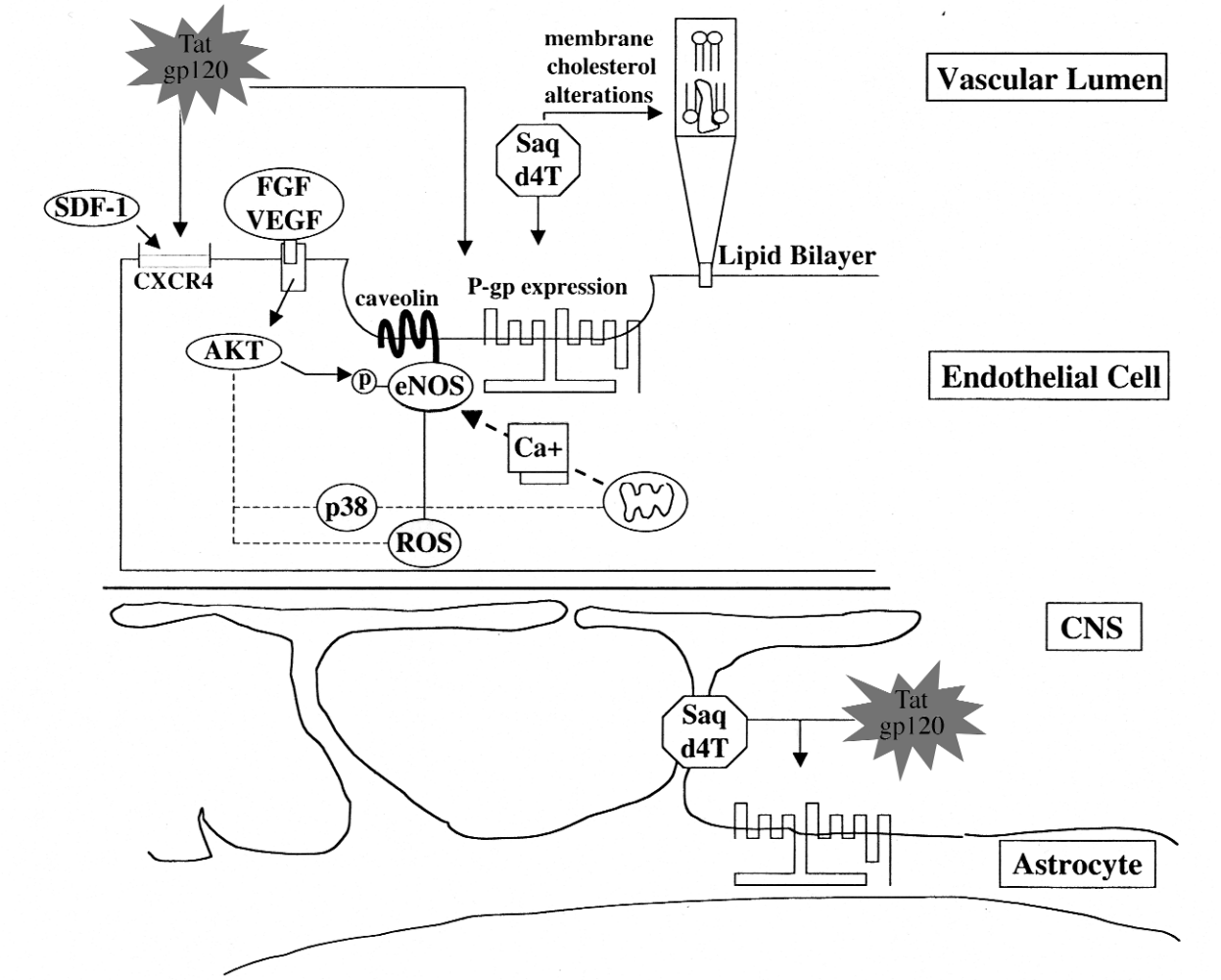

Figure 5. Diagrammatic representation of the potential molecular mechanisms through which an ARV such as SQV and d4T might interfere with host endothelial intracellular signaling pathways.tat = transactivating transcription factor; gp120 = glycoprotein 120; SDF = stromal derived factor; FGF = fibroblast growth factor; VEGF = vascular endothelial growth factor; eNOS = endothelial nitric oxide growth synthase; ROS = reactive oxygen species.

adverse side effects. Principal toxic side effects include hypersensitivity, hyperlipidemia, anemia, hyperinsulinemia, granulocytopenia, urine crystallization, increased levels of hydroxyurea, hepatitis, lactic acidosis, cytochrome p450 alterations and mitochondrial dysfunction (10, 122). Numerous studies dealing with lactic acidosis and lipodystrophy in AIDS patients demonstrate unambigously that antiretrovirals have direct metabolic effects and indicate that toxicity and cellular dysregulation may contribute to these disorders (8, 45, 70, 87, 108) and may also affect cells of the BBB and CNS. A case report by Giner et al describes reversible posterior leukoencephalopathy secondary to IDV-induced hypertensive crisis that was completely reversed once blood pressure was controlled and IDV was replaced by NFV (35). Clinical and radiological evolution excluded other diagnoses such as PML and points to IDV as a potential hypertension-inducing agent in HIV-infected predisposed patients (35).

Such deleterious side effects are of particular concern since effective treatment of HIV-1 infection usually requires long-term adherence that may be interrupted by pre-mature switching of HAART due to toxicity (25), thereby exposing the patient to numerous regimens and allowing for acquisition of resistance. On the other hand, structured treatment interruptions (STI) are proposed to allow "wild-type" drug-susceptible virus to re-emerge and overgrow resistant strains in patients expe-

riencing treatment failure or in patients maintaining HIV-1 suppression, thereby boosting antiretroviral immunologic responses to lengthen viral suppression (98). Evolution of resistance mutations of HIV has been linked to emergence of more neuro-invasive HIV strains (73). Furthermore, an SIV variant described from a neuropathogenic strain efficiently infected brain microvascular cells (114) suggesting that the development of such variants may represent significant challenges to the BBB. These findings emphasize the importance of understanding signaling interactions among antiretrovirals, HIV and neural cells.

HAART and the brain. Despite the fact that HAART extends the survival of HIV patients, the prevalence of HAD and HIVE has increased (26, 64, 79). In addition to the emergence of neuro-virulent strains, the poor penetrance of ART into CNS tissue may be responsible in part for this increase, as HIVE is observed in 40% of our AIDS autopsies (64, 79). Likewise, since most anti-retroviral agents cannot efficiently cross the BBB, the CNS is postulated to be a "reservoir" for HIV, thereby compounding the need for maintaining BBB fitness and controlling HIV infection of the brain.

Entry of HIV-1 into the brain involves the interaction of several factors such as HIV proteins, cytokines, chemokines, and adhesion molecules (33, 88, 94) and is proposed to occur early in the progression of AIDS (84, 101). Patients with HIVE show tight junction disruption (18) and increased endothelial cell DNA fragmentation (107). Thus, compromised BBB integrity results in part from exposure to HIV proteins released from infected cells in the blood and is characteristic of patients with HIVE (18, 94, 110). On the other hand, fibroblast growth factor 2 (FGF2), vascular endothelial growth factor (VEGF), stromal cell derived factor 1 α (SDF-1α) and IL-8 are host-generated proteins involved in maintaining the integrity of the endothelial cells of the BBB during oxidative, hypoxic, or mechanical stress (96). For example, VEGF synergizes with FGF2 to induce production of IL-8, SDF-1α, and CXCR4 (59, 67, 104, 105, 109). All of these factors function to support the BBB and endothelial cell fitness. FGF2 is expressed mainly by astrocytes, the glial cells that closely abut EC of the BBB and help to regulate, P-gp expression in EC (29). SDF-1α is the natural ligand for the gp120 receptor, CXCR4. Likewise HIV-1 Tat interacts with CXCR4 and regulates expression of IL-8 (76, 121). Together with IL-8, SDF-1, VEGF and FGF2 contribute to EC fitness by promoting endothelial cell replication, differentiation and migration (60, 105). Thus, synergistic interactions among these factors play a pivotal role in maintaining BBB cell fitness against angiotoxic effects of HIV-1 products such as gp120 and Tat. Adding to the complexity of interactions at the BBB among HIV-proteins, host immune response molecules, and angiogenic factors, are the anti-retroviral agents that may influence this signaling and the fitness of EC. To date, the potential role of ART on endothelial cell fitness and signaling in AIDS patients has not been addressed in great detail.

Whether the dysregulatory effects of HAART are mediated via direct or indirect mechanisms, neuropathological changes observed in HIV HAART-experienced patients are ultimately due to cellular damage to neurons, glia and cerebrovascular cells. Anti-retroviral drugs such as PIs enter the cytosol of cerebral endothelial cells on their way to the brain parenchyma, but are actively transported back into the blood stream by the multi-drug resistance-1 (MDR-1) P-gp. MRD-1 is a member of the P-gp family and is a polarized membrane efflux transporter located in the luminal surface of endothelial cells of the BBB and limits uptake of drugs into the brain by efficient back-flux into the blood stream (13, 15, 116). The P-gp system is the classical model for drug resistance in the brain (38) and is responsible for poor penetrance of ART into the CNS (52, 57, 66). The PIs, SQV, IDV, and RTV increase the activity of P-gp in rat cerebral endothelial cells in vitro (5). Blocking the P-gp or using reversing agents such as S9788 or verapamil increases endothelial transport of ART from luminal to abluminal regions of the cell (13, 29). HIV protease inhibitors are excellent substrates for P-gp (55, 57, 66) and in *mdr1a (-/-)* knockout mice lacking P-gp expression, brain levels of SQV, IDV, and NFV were 7 to 35-fold higher than in wild-type control animals (57). Protease inhibitors can also interfere with P-gp signaling in $CD4^+$ T-cells and $CD34^+$ progenitor cells (74). For example, RTV is a more potent inhibitor of P-gp than the cyclosporine analogue SDZ PSC 833 (27, 118). However, IDV concentrations in the CSF are increased when given in combination with low doses of RTV (118). Moreover, vascular targeting strategies to circumvent multidrug resistance involving P-gp have proven effective only in combination with anti-angiogenic compounds that block the VEGF receptor (58), illustrating the importance of host factor and ART interactions. Similar studies show that increased levels of VEGF in tumor-induced angiogenesis are accompanied by P-gp upregulation during hypoxic stress (119). Furthermore, HIV infection increases P-gp expression, thereby further reducing the amount of drug transported into the brain

(39). Since HIV antiretroviral agents directly affect P-gp signaling, the idea that ART may also influence HIV-specific EC signaling and maintenance of BBB integrity during HIV infection is likely. Even though significant amounts of ARTs do not pass through the BBB into the brain parenchyma, cellular signaling pathways involving membrane proteins such as P-gp and caveolin may be affected as the drugs in the blood stream are encountered by cerebral endothelial cells. Since P-gp activity mediates cholesterol redistribution in the cell membrane (32), it is likely that cell stability and trafficking are also affected by ART. Furthermore, P-gp is closely associated with caveolar domains which include signaling molecules such as tyrosine kinases, protein kinase C, growth factor receptors, GTPases, glycerolphosphatidylinositol-linked receptors and G-proteins (23, 68, 69, 72, 83, 91).

Dysregulation of BBB signaling could result in increased EC susceptibility to HIV proteins, other potential neurotoxins and inflammatory factors. Thus, insight into EC signaling in the context of interactions among P-gp, angiogenic and inflammatory factors and anti-retroviral agents is critical for the development of potential new therapeutic strategies aimed at protecting the brain from toxicities associated with HIV and anti-retroviral-mediated alterations in signaling.

Lactic acidosis and mitochondrial dysfunction. Lactic acidosis is a potentially life-threatening condition in HIV-patients on NRTI therapy and is proposed to occur via inhibition of mitochondrial DNA polymerase-γ leading to mitochondrial dysfunction (85, 108). ZDV-induced mitochondrial dysfunction with liver steatosis, myopathy, lactic acidosis and mitochondrial DNA loss in some HIV patients is well documented (11, 16). Mitochondrial DNA depletion may be detectable prior to symptoms of hyperlactatemia (124), which may indicate that changes in mitochondrial function occur early in nucleoside analogue therapy related toxicities. Supporting a role of ZDV in HAD, Hong et al report pH-dependent microglial uptake of ZDV and suggest that this system may play a significant role in the transport of weak organic cation substrates and/or metabolites within the brain (46). Deleterious effects from numerous anti-retroviral nucleoside analogues in a human hepatoma cell line include inhibition of cytochrome *c* oxidase and citrate synthase, elevated levels of lactic acid, and reduction in mitochondrially-encoded polypeptide synthesis leading to mitochondrial dysfunction (93). Likewise, nucleoside analogue treatment reduces mitochondrial DNA content in the PC-12 neuronal cell line, prevents neurite regeneration and is suggested to be, at least in part, related to peripheral neuropathy observed in HIV patients treated with ddC, ddI and d4T (17). Since nucleoside analogues seem to affect signaling in various cell types such as hepacytes, neurons and microglia, it is also possible that mitochondrial changes occur in cerebral endothelial cells.

Alterations in lipid metabolism. The increased incidence of HAART-associated hyperlipidiemia and dyslipidemia in HIV patients (108) points toward neurovascular disease as a potential risk in this group of individuals. Although hyperlipidemia has not been shown to contribute to stroke associated with the use of anti-retrovirals, it is possible that hypercholesterolemia and dyslipidemia impairs endothelial cell function since abnormal cellular function is detectable before obvious intimal lesions are established in patients with atherosclerotic risk. Dyslipidemia has been observed in patients with HIV infection in the pre-HAART era; however, the incidence appears to be increasing in the HAART era and can exist independently of abnormalities in fat distribution or other metabolic abnormalities (77, 86, 108). Excess endothelial cell derived superoxide generation occurs in hypercholesterolemia and free-oxygen radicals can inactive nitric oxide, augment oxidation of low-density lipoproteins (LDL), and lead to increased EC membrane damage through generation of peroxynitrite and hydroxy radicals (3, 19, 89, 90). Oxidation of LDL particles is important in the pathogenesis of atherosclerosis and endothelial cell dysfunction (3, 111, 112). For example, numerous lipases are bound to the endothelial cell matrix and have major effects on lipoprotein metabolism and function (99). Levels of von Willebrand factor, reflecting endothelial cell activation, are persistently elevated during HIV infection and are significantly correlated to plasma viral load (4). Furthermore, the pronounced decline in HIV RNA levels observed during HAART is accompanied by a corresponding decrease in von Willebrand factor (4). A potential role for cerebral endothelial cells in the deposition of apolipoprotein A and in the formation of atherosclerotic plaques has been described (48). Metabolic and vascular changes related to PI use for the treatment of HIV predisposes patients to atherosclerosis and are associated with atherogenic lipoprotein changes and EC dysfunction (111). Since these changes are believed to involve nitric oxide radicals (111), protease inhibitors in the blood stream may affect signaling in endothelial cells of the brain microvasculature as well, especially since PIs are substrates for P-gp located in the

luminal membrane of BBB EC. Since P-gp expression is influenced by both HIV and HAART, and P-gp activity mediates cholesterol redistribution in the cell membrane (32), it is possible that, together with serum cholesterol fluctuations due to hyperlipidemia, these affects may be compounded. Furthermore, PI-induced dysregulation of prostaglandin production by EC could be involved in angiotrophic factor expression at the BBB (100). Changes in oxidative stress levels and in lipid metabolism associated with the use of HAART are likely to participate in signaling alterations at the BBB and in the brain parenchyma, thereby adding to neural cell activation and possibly neuronal injury.

Selective Pressures Influenced by HAART

Changing patterns in the neuropathogenesis of HIV patients during the HAART era are influenced by pressures involving viral resistance mutations, selection of viral species by changing or discontinuing treatment, differences in plasma, CSF and brain viral load, and decreased CD4 lymphocyte recovery despite decreased plasma viral burden.

Resistance, compartmentalization and immune reconstitution. Resistance mutations and viral compartmentalization have contributed greatly to the changes observed in brain pathology of AIDS in the HAART era. A study on the effect of HAART on HIV-1 recovery from purified peripheral blood monocyte-derived macrophages showed that circulating monocytes constitute a significant source of replication-competent virus in HAART naive HIV patients and in HIV patients failing HAART (44). Moreover, a study by Lanier et al describing evolutionary relationships between the HIV-1 reverse transcriptase from multiple anatomical compartments of a patient enrolled in a phase III trial designed to assess the efficacy of ABC in subjects with HAD, reports compartmentalized evolution in the midfrontal gyrus of the brain and suggest that incomplete drug penetrance into this region may in part be responsible (47, 65).

Although STI have been advocated as a therapeutic strategy for HIV-infection, this approach may be accompanied by previously unrecognized changes in tissues due to differences in viral exposure and trafficking into the CNS (98). Furthermore, patients with sustained HIV suppression may benefit from STI by enhancing their immune response to the virus, however, such a response may afford opportunity for the development of resistance (123) and potential for changes in CNS viral species.

While the advent of HAART has greatly improved the outlook for AIDS patients, some patients experience clinical deterioration due to a restored ability to mount an inflammatory response (106). This condition occurs soon after HAART is initiated and is referred to as immune reconstitution or immune restoration disease. Patients suffering from this disease mount an inflammatory response to both infectious and non-infectious agents resulting in an autoimmune reaction (106).

Aging, HIV and HAART. Changes observed in the patterns of neurodegeneration in the HAART era are due, in part, to the fact that antiretroviral therapy has resulted in improved longevity for AIDS patients. Individuals over 50 years of age make up approximately 11% of the AIDS population in the United States and this population is expected to grow with improved longevity of patients on HAART (40). Prolonged survival of AIDS patients can result in multiple exposures to HIV of different strains that may carry resistance mutations. Furthermore, older AIDS patients may be at greater risk for developing HAD (12, 49) and may be more susceptible to neuro-specific conditions such as PML, stroke, PCP, or CNS lymphoma (40) due to immunosenescence that includes a disproportionate loss of naive CD4 cells and decreased T-cell proliferation (37, 56). Decreased plasma viral loads in response to HAART are usually accompanied by recovery of CD4 cells. However, in the aging HIV patient, the inability of CD4 cells to recover may lead to differences in patterns of neurodegeneration related to lower CD4 counts.

In vivo models for HIVE and HAART interaction. Although it is difficult to determine the effects of HAART in the SIVE model due to problems with oral-administration of the medication, numerous studies suggest that the neuropathological manifestation of SIVE is indistinguishable from HIVE (54, 92). Studies are underway however to characterize changes in the neuropathology of SIV-infected macques on HAART (personal communication, Dr Howard Fox, Department of Molecular and Experimental Medicine, The Scripps Research, La Jolla, Calif). This model will be a valuable asset to the understanding of disease based interactions of SIV and anti-retroviral agents in the context of host mediated response to infection and will, therefore, add considerable insight into the mechanisms involved in the neuropathogenesis observed in HAART-experienced HIVE patients. Results from efficacy studies of potent anti-retroviral drug combinations in a murine model of HIVE show that both ddl/d4T/amrenavir and

ZDV/3TC/ABC significantly decreased the numbers of infected human monocyte derived macrophages in brain (71), suggesting that once in the brain, the drugs are effective at viral suppression.

Conclusions

Accompanying the rapid progress in HAART are significant changes in the patterns of the neuropathology of AIDS. Along with the suppression of viral replication, rebound in CD4 count, decreased OI and increased longevity of AIDS patients, HAART has also introduced numerous unintended and somewhat deleterious effects on the host. Metabolic abnormalities, lactic acidosis, mitochondrial dysfunction and lipid alterations represent an added challenge to the AIDS patient during HAART. Selective pressures from HAART inducing viral mutations, compartmentalization and cell and/or tissue targeted damage add to the complex interactions among HIV proteins, anti-retroviral agents and host factors such as cytokines, chemokines and growth factors. Among the most striking changes in the neuropathogenesis of HIV during the HAART era is the shift in HIVE from a subacute disease to a chronic and protracted condition. A significant increase in white matter disease has been observed since 1995 with the emergence of resistant strains indicating that therapies targeted at this particular obstacle are needed. An ever increasing number of anti-HIV compounds have been developed targeting almost every step in the viral life cycle: adsorption, entry, fusion, uncoating, reverse transcription, integration, transactivation and maturation (for review, see 21, 22). With the advent of new anti-retroviral therapies such as T20, it is likely that the ever-changing face of HIV pathogenesis will continue to evolve and that new and more complex neuropathological entities will emerge.

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