Journal of NeuroVirology, 10: 223–232, 2004

ISSN: 1355-0284 print / 1538-2443 online
DOI: 10.1080/13550280490463532

Taylor & Francis
Taylor & Francis Group

Inflammatory changes and breakdown of microvascular integrity in early human immunodeficiency virus dementia

Malcolm J Avison,[1,5] Avindra Nath,[1,4] Robin Greene-Avison,[3] Frederick A Schmitt,[1] Rodney A Bales,[1] As'ad Ethisham,[1] Richard N Greenberg,[2] and Joseph R Berger[1]

Departments of [1]Neurology, [2]Internal Medicine, and [3]Magnetic Resonance Imaging and Spectroscopy Center, University of Kentucky Medical Center, Lexington, Kentucky, USA; [4]Department of Neurology, Johns-Hopkins Medical School, Baltimore, Maryland, USA; [5]Departments of Radiology and Radiological Sciences and Neurology, Vanderbilt University Medical Center, Nashville, Tennessee, USA

Increased postcontrast enhancement in contrast-enhanced magnetic resonance imaging (CE-MRI) of the central nervous system (CNS) is a predictor of human immunodeficiency virus (HIV) dementia severity in HIV-infected subjects. The present study confirms this earlier finding in a mildly impaired patient cohort, and demonstrates that the increased postcontrast enhancement is correlated with increased cerebrospinal fluid (CSF) levels of monocyte chemoattractant protein (MCP)-1, an inflammatory chemokine, and increased CNS levels of mI, a microglial marker. These results suggest that early CNS inflammation may underlie the microvascular changes observed, and may be a factor in the development of HIV dementia. *Journal of NeuroVirology* (2004) **10,** 223–232.

Keywords: AIDS; blood-brain barrier (BBB); CNS; HIV dementia; inflammation; magnetic resonance; magnetic resonance imaging (MRI); magnetic resonance spectroscopy (MRS)

Introduction

Neurocognitive impairment (NCI), ranging in severity from subclinical, through so-called minor cognitive-motor disorder (MCMD), to human immunodeficiency virus (HIV) dementia (HIVD), remains a common complication of HIV infection. Highly active antiretroviral therapy (HAART) has had a major impact on many of the neurological complications of HIV infection (Dore *et al*, 1999; Sacktor *et al*, 2001); however, its effect on NCI remains uncertain (Kandanearatchi *et al*, 2003). Although the incidence of HIVD in the era of HAART appears to be declining (Sacktor *et al*, 2002), the prevalence of HIV encephalitis (HIVE) may be increasing (Neuenburg *et al*, 2002), presumably due to longer survival of HIV-infected persons. Also, despite the reduction in the incidence of HIVD, the cognitive impairments associated with HIV infection, chiefly, but not exclusively, deficits in psychomotor/motor speed and verbal and visual memory, remain significant problems and appear to have been only minimally impacted by the introduction of HAART (Sacktor *et al*, 2002). Furthermore, the finding that the presence of NCI is a significant predictor of HIVE at autopsy (Cherner *et al*, 2002) suggests that the increased incidence of HIVE (Neuenburg *et al*, 2002) may underlie, at least in part, the persistence of NCI in the era of HAART (Sacktor *et al*, 2002). Despite HIV's early penetration of the central nervous system (CNS), clinically significant NCI generally occurs relatively late in infection, providing a relatively large window of opportunity to intervene. Unfortunately, the development of appropriate interventional therapies to prevent or treat NCI has been hampered on the one hand by the difficulty of identifying those patients at risk for developing NCI, and on the other by our relatively

Address correspondence to Malcolm J. Avison, PhD, Vanderbilt University Institute of Imaging Science, R-1302, Medical Center North, 1161 21st Avenue South, Nashville, TN 37232-2675, USA. E-mail: calum.avison@vanderbilt.edu

The authors are grateful for the assistance of the physicians of the University of Kentucky Bluegrass HIV Clinic, and the University of Kentucky GCRC. The studies were supported by grants from NIMH, NIDCD, NINDS, and NCRR.

Received 17 December 2003; revised 12 February 2004; accepted 15 February 2004.

poor understanding of the complex sequence of early neuropathogenic events that ultimately lead to NCI, and in particular, the way in which these events are modulated by the array of antiretroviral agents available in the era of HAART (Langford *et al*, 2003).

HIV's early, and usually asymptomatic, penetration of the CNS may involve an initial infection of the brain capillary endothelial cells (Bagasra *et al*, 1996; Levy, 1997; Moses *et al*, 1993) and/or choroid plexus (Falangola *et al*, 1995); however, CNS infection is believed to occur principally by trafficking of infected macrophages across the blood-brain barrier (BBB). Our understanding of the mechanisms by which macrophages cross the BBB remains incomplete (Hickey, 1999), but the endothelial adhesion molecules have been implicated as a critical mediator of such trafficking, and may be subject to up-regulation by perivascular inflammatory processes (Hurwitz *et al*, 1994; Nottet, 1999) as well as HIV proteins (Hofman *et al*, 1994; Woodman *et al*, 1999). Indeed endothelial adhesion molecules are up-regulated in HIV and particularly HIVD patients (Seilhean *et al*, 1997). Simple exposure of endothelial cells to HIV-infected monocytes has been demonstrated to result in a disruption of the endothelial cell monolayer that would permit inflammatory cell infiltration (Dhawan *et al*, 1995). Neuropathological series demonstrating the presence of infected perivascular macrophages and multinucleated giant cells of macrophage lineage (Budka *et al*, 1987; Jones *et al*, 2000; Pumarola Sune *et al*, 1987; Smith *et al*, 1990) are consistent with this model of early HIV entry. Furthermore, the demonstration of early microglial infection (Bagasra *et al*, 1996; Jones *et al*, 2000; Pumarola Sune *et al*, 1987; Smith *et al*, 1990) and proliferation (Gray *et al*, 1992) suggests that infection and activation of perivascular microglia represents the next step in HIV invasion of the CNS.

Increased CNS *myo*-inositol (mI), a marker of microglial proliferation, is a hallmark of MCMD and HIVD (Chang *et al*, 1999a, 1999b; Ernst *et al*, 2000; Laubenberger *et al*, 1996; Salvan *et al*, 1997b). Microvascular abnormalities, including increased regional blood volume and BBB compromise, are evident in neuropathologic series (Petito and Cash, 1992; Power *et al*, 1993; Weis *et al*, 1996) as well as *in vivo* (Berger *et al*, 2000; Tracey *et al*, 1998). Both increased CNS mI and microvascular abnormalities have been demonstrated in the basal ganglia of patients with HIVD. The extent to which these observations are related is unclear, but activation of perivascular macrophages and microglia, and the associated elaboration of proinflammatory cytokines (Tyor *et al*, 1992) and matrix metalloproteinases (MMPs) might be expected to lead to significant BBB compromise (Conant *et al*, 1999; Liuzzi *et al*, 2000). The observation of a correlation between cerebrospinal fluid (CSF) levels of neopterin, a marker of CNS inflammation, and CSF/serum albumin ratio, a marker of BBB disruption (Andersson *et al*, 2001), lends support to this idea. Thus perivascular inflammation and subsequent BBB breakdown may, by accelerating the rate of viral entry into CNS and permitting the entry of potentially toxic serum proteins, represent a key step in the pathogenesis of NCI. The present study directly examines the relationship between microglial activation and BBB compromise, and their respective roles in the pathogenesis of NCI in HIV-infected patients.

Results

Subject demographics

Of 28 patients initially enrolled, 2 patients chose not to continue before collection of any data, and 1 patient was excluded due to the recent (<3 months) initiation of HAART, which may have led to transient and/or incomplete metabolic and neurocognitive variations. Contrast-enhanced magnetic resonance imaging (CE-MRI) data were unavailable for an additional 2 patients (1 technical failure, 1 patient unable to complete MRI study), and proton magnetic resonance spectroscopy (^{1}H-MRS) data were unavailable for 4 patients (all technical failures).

Table 1 summarizes the demographics and clinical characteristics of the 25 patients included in the study. These patients ranged from cognitively intact (MSK = 0, HDS = 16)[1] to moderately neurocognitively impaired (MSK = 2, HDS = 3), although the majority of patients with NCI exhibited only mild impairment (MSK $\leq$ 1.0, HDS $\geq$ 6). The severity of NCI determined by HDS and by MACS NPZ8 were correlated ($R^2 = .40$, $P = .0009$). Furthermore, both HDS and MACS NPZ8 were correlated with the MSK rating (HDS versus MSK, $R^2 = .41$, $P = .0007$; MACS NPZ8 versus MSK, $R^2 = .28$, $P = .0076$).

Microvascular and metabolic predictors of NCI

Basal ganglia and frontal white matter have been identified as early targets of HIV neuropathology, and were therefore the regions investigated in the present study. We have previously reported a significant dependence of severity of neurological impairment in HIV patients on the degree of microvascular disruption in the basal ganglia. The present study confirms our earlier result, again demonstrating a significant dependence of the severity of NCI on the severity of BBB disruption in the basal ganglia (Figure 1: HDS versus FE30, $R^2 = .45$, $P = .0006$; MACS NPZ8 versus FE30, $R^2 = .37$, $P = .0023$). There was no significant dependence of NCI severity on any CE-MRI marker of microvascular or BBB disruption in the areas of frontal white matter analyzed.

Severity of NCI was also predicted by the mI/creatine ratio (mI/Cr) in the basal ganglia (Figure 2: HDS versus mI/Cr, $R^2 = .43$, $P = .0022$; NPZ8 versus

[1] See methods for definitions of cognitive scales.

Table 1 Subject demographics

Subject ID	*Age*	*Gender*	*MSK*	*HDS*	*NPZ8*	*Viral load*	*CD4*	*Art*
A001c	38	M	0.5	16	−0.10	<400	200	Lamivudine, zidovudine, abacavir
A002a	38	M	0.5	13	−0.76	<400	1361	Lamivudine, zidovudine, efavirenz
A005a	49	M	0	15	−0.39	5020	449	Naïve
A006a	36	M	0.5	14	−0.86	6745	252	Failure
A007a	30	M	0	10	−1.00	24106	458	Naïve
A008a	47	M	2	10	−1.30	926	106	Zalcitabine, zidovudine, nelfinavir
A010a	42	M	0	14	−0.50	783	189	Lamivudine, zidovudine, efavirenz
A011a	40	F	0	165	1.14	28222	45	Failure
A012a	44	F	0	13	−0.37	<400	706	Lamuvidine, zidovudine, nevirapine
A013a	29	M	0.5	13.5	−3.21	3588	267	Failure
A014a	30	M	0	14	−0.25	<400	24	Lamivudine, zidovudine, efavirenz
A015a	30	M	0.5	12	−2.17	<400	1299	Lamivudine, zidovudine, abacavir
B001a	48	M	1	8	−2.59	<400	283	Lamivudine, zidovudine, abacavir
B002a2	38	M	0.5	13	−1.07	2831	561	Failure
B003a	34	M	0.5	12	−0.85	1273	984	Failure
B004a	33	M	2	3	−2.23	725956	115	Failure
B005a	37	M	0	16	−0.51	18603	400	Failure
B006a,ss	43	M	0	13	−0.29	<400	359	Lamivudine, zidovudine, nevirapine
B007a	57	M	1	12	−1.82	<400	158	Lamivudine, abacavir, efavirenz
B008a	36	M	0.5	13.5	−1.14	8193	641	Lamivudine, didanosine, nevirapine
B009a	41	M	0	13.5	0.77	22152	610	Failure
B011a	41	M	0.5	6.5	−0.42	<400	840	Lamivudine, zidovudine, nevirapine
B012a	44	F	0	15	0.55	702	1012	Lamivudine, amprenavir, nevirapine
B013a	31	M	N/A	N /A	−1.61	9616	306	Lamivudine, zidovudine, abacavir
B015a	46	M	0.5	4	−3.54	<400	331	N/A

MSK = Memorial Sloan Kettering AIDS Dementia Scale (Price and Brew, 1988).
HDS = HIV Dementia Rating Scale (Power *et al*, 1995).
NPZ8 = MACS NPZ8 (van Gorp *et al*, 1994).

mI/Cr, $R^2 = .40$, $P = .0026$), but not in frontal white matter.

Finally, severity of NCI was predicted by CSF levels of the inflammatory chemokine monocyte chemoattractant protein-1 (MCP-1) (Figure 3: HDS versus MCP-1, $R^2 = .704$, $P < .0001$; NPZ8 versus MCP-1, $R^2 = .40$, $P = .011$).

CNS inflammatory markers and microvascular compromise

Because perivascular macrophages, multinucleated giant cells of macrophage lineage, and perivascular microglial nodules are neuropathologic hallmarks in HIV patients (Budka *et al*, 1987; Jones *et al*, 2000; Pumarola Sune *et al*, 1987; Smith *et al*, 1990), and these perivascular sources of proinflammatory cytokines may promote BBB breakdown (Andersson *et al*, 2001; Conant *et al*, 1999; Liuzzi *et al*, 2000), we next examined the dependence of BBB breakdown (measured by postcontrast enhancement) on the degree of microglial activation. We first examined the relationship between CSF levels of MCP-1, a marker of CNS inflammation, and microvascular disruption in basal ganglia and frontal white matter. The CSF levels of MCP-1 were significantly correlated with BBB compromise in basal ganglia (BG) (MCP-1 versus BG FE30, $R^2 = .32$, $P = .035$), but not in frontal white matter.

The severity of BBB breakdown was also correlated with mI/Cr in the basal ganglia (Figure 4: BG FE30 versus BG mI/Cr, $R^2 = .25$, $P = .034$), but not in frontal white matter.

Discussion

The interval between the early entry of HIV into the CNS and the much later emergence of neurologic symptoms remains poorly understood, but is clearly a critical period in the pathogenesis of NCI, and represents a window of opportunity for therapeutic intervention. HIVE, which is believed to be the neuropathological substrate of HIVD, is characterized by a distinctive constellation of inflammatory features, including activated macrophage infiltrates, multinucleated giant cells of macrophage lineage, and microglial nodules (Budka *et al*, 1987; Jones *et al*, 2000; Pumarola Sune *et al*, 1987; Smith *et al*, 1990). These inflammatory infiltrates are also found in many neurologically normal HIV patients (Bell, 1998; Masliah *et al*, 2000; Soontornniyomkij *et al*, 1998), suggesting that they play an important early role in NCI pathogenesis. Support for such a view can be found in the growing number of ^{1}H-MRS studies that have demonstrated increased mI (Chang *et al*, 1999a, 1999b; Ernst *et al*, 2000; Laubenberger *et al*, 1996; Salvan *et al*, 1997b), a marker of microglial proliferation, in subcortical grey and white matter not only in HIVD patients, but also in patients with MCMD (Chang *et al*, 1999a). Similarly, elevated subcortical cholines, characteristic of macrophage

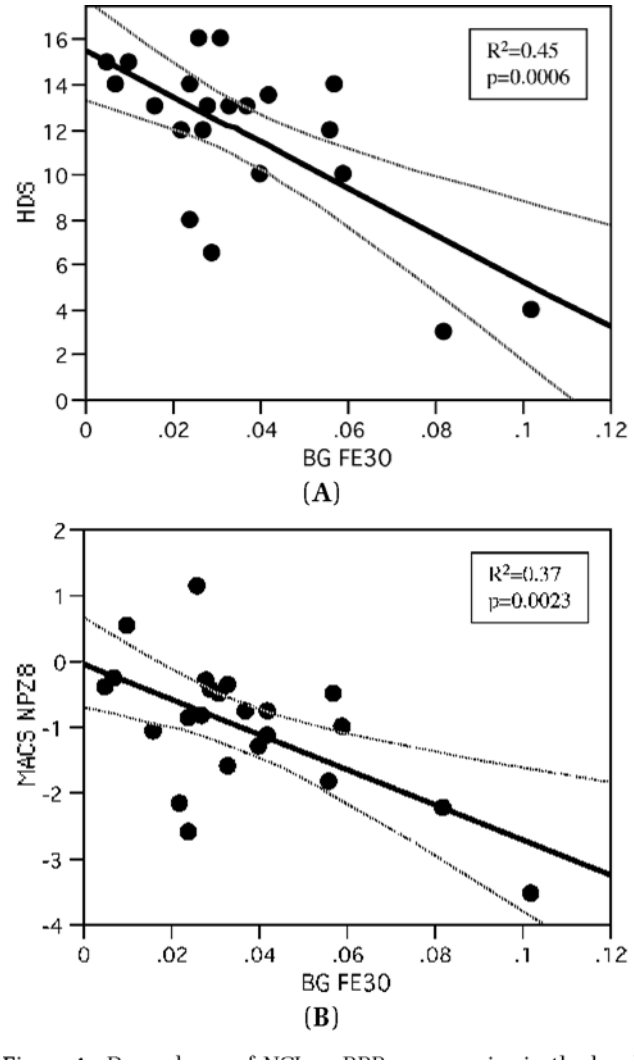

Figure 1 Dependence of NCI on BBB compromise in the basal ganglia. NCI was assessed using the HIV dementia scale (HDS; **A**), and the MACS NPZ8 (**B**). BBB compromise was measured by the fractional T1-weighted MRI signal enhancement in the basal ganglia 30 min after contrast administration (BG FE30).

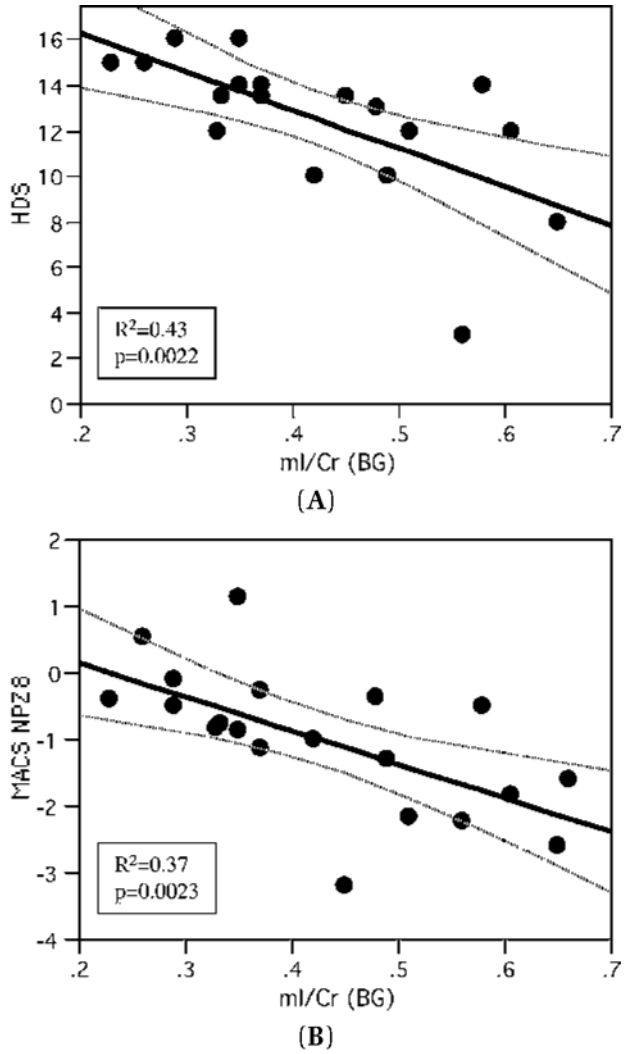

Figure 2 Dependence of NCI on degree of microglial activation in the basal ganglia. NCI was assessed using the HIV dementia scale (HDS; **A**), and the MACS NPZ8 (**B**). Degree of microglial activation was determined from the mI/Cr ratio in the basal ganglia.

infiltration and/or glial activation, were one of the earliest metabolic abnormalities reported in the CNS of HIV patients (Barker *et al*, 1995; Meyerhoff *et al*, 1996; Salvan *et al*, 1997a, 1997b; Tracey *et al*, 1996). Furthermore, the severity of NCI is correlated with the levels of these ^{1}H-MRS inflammatory markers, and neurological improvement following initiation of antiretroviral therapy (ART) is correlated with normalization of mI (Chang *et al*, 1999b).

The present study confirms these earlier reports, again finding that NCI was strongly correlated with basal ganglia mI/Cr (Figure 2). No such relationships were found in the frontal white matter; however, a secondary analysis indicated that NPZ8 was significantly correlated with frontal white mI/Cr when those patients with basal ganglia mI/Cr in the upper quartile were excluded from the analysis (NPZ8 versus mI/Cr, $R^2 = .65$, $P = .0048$; data not shown). Thus early effects of frontal white mI/Cr on NCI in this mildly impaired population may be obscured by the greater early influence of basal ganglia changes. Such an observation is not surprising, given HIV's predilection for the basal ganglia (Brew *et al*, 1995; Fujimura *et al*, 1997; Kure *et al*, 1991; Neuen-Jacob *et al*, 1993; Pumarola Sune *et al*, 1987; Wiley *et al*, 1998).

The degree of basal ganglia BBB disruption was determined in this study using the fractional enhancement 30 min post contrast (FE30). Gadolinium-DTPA does not traverse the vasculature in health and late contrast enhancement has been used as an indicator of BBB disruption (Silver *et al*, 2001a, 2001b). Our observation that the severity of NCI is correlated with BBB disruption (Figure 1) is consistent with immunohistochemical studies demonstrating BBB breakdown in HIV patients with and without HIVE (Petito and Cash, 1992; Power *et al*, 1993), as well as more recent *in vivo* studies demonstrating

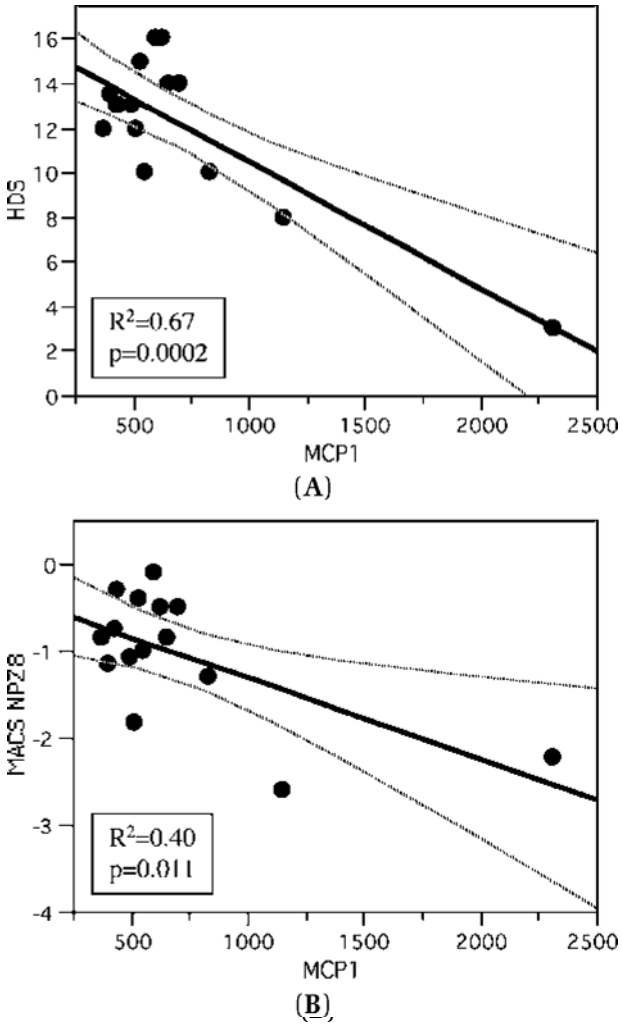

Figure 3 Dependence of NCI on CSF level of monocyte chemoattractant protein-1 (MCP-1; pg/ml). NCI was assessed using the HIV dementia scale (HDS; **A**), and the MACS NPZ8 (**B**).

BBB disruption and microvascular changes in the basal ganglia of HIVD patients (Berger *et al*, 2000; Tracey *et al*, 1998). These abnormalities may be a direct consequence of HIV infection of brain microvascular endothelial cells (BMEC) (Bagasra *et al*, 1996; Levy, 1997; Moses *et al*, 1993), but the characteristic perivascular location of inflammatory cells in HIVE (Budka *et al*, 1987; Jones *et al*, 2000; Pumarola Sune *et al*, 1987; Smith *et al*, 1990) suggests that BBB compromise may result from increased perivascular levels of proinflammatory cytokines such as tumor necrosis factor alpha (TNFα), which have been shown to up-regulate MMPs and increase BBB permeability *in vitro* and *in vivo* (Conant *et al*, 1999; Liuzzi *et al*, 2000). The finding that BBB permeability is correlated with mI/Cr in the basal ganglia (Figure 4) is consistent with this idea, suggesting as it does that BBB compromise in the basal ganglia is related to microglial activation.

The dependence of NCI on CSF levels of MCP-1 (Figure 3) observed in this and previous studies

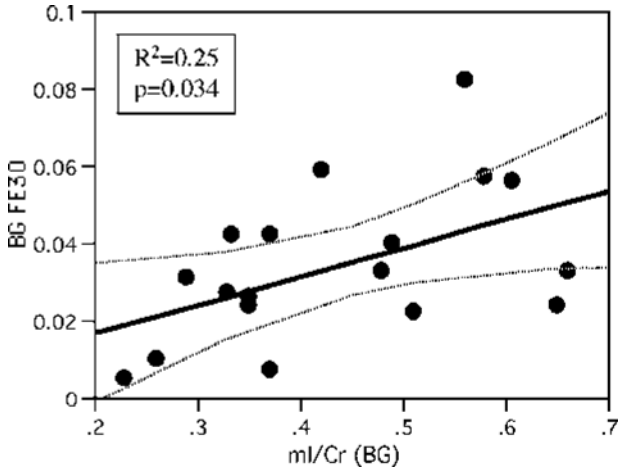

Figure 4 Dependence of basal ganglia BBB disruption (BG FE30) on degree of basal ganglia microglial activation (mI/Cr).

(Kelder *et al*, 1998) further supports an inflammatory mechanism in the pathogenesis of NCI. In contrast to earlier studies, however, we found no dependence of NCI on CSF levels of TNFα (data not shown). Indeed CSF levels of TNFα were generally undetectable. This discrepancy may simply reflect the fact that the majority of our patients were only minimally impaired, because consistent increases in TNFα are generally observed only in patients with quite advanced HIVD. Thus the increases in basal ganglia mI/Cr, and the associated BBB disruption, appear to be more sensitive indicators of early inflammatory changes than are CSF cytokine and chemokine levels, although an expansion of the analysis of the repertoire of markers of neuroinflammation, e.g., β2 microglobulin and neopterin, in future studies may help address this issue. Most importantly, stepwise and multiple regression analysis indicate that microglial activation and BBB breakdown are not independent predictors of HDS or NPZ8, suggesting that NCI and BBB breakdown are both consequences, at least in part, of microglial activation. It thus appears that late postcontrast enhancement may be a useful surrogate for early inflammatory changes following HIV infection, with greater spatial resolution than is currently available with ^{1}H-MRS.

BBB compromise may be an important determinant of rate of progression and/or response of NCI to ART. Previous studies have demonstrated a significant relationship between NCI and CNS viral burden as measured by CSF viral load (Cinque *et al*, 1998; McArthur *et al*, 1997; Robertson *et al*, 1998). Thus mechanisms that increase the rate of viral penetration of the CNS, or limit HIV elimination, are likely to accelerate the development and/or progression of NCI. BBB compromise may increase the rate of viral entry by allowing movement of free virions from blood into the CNS in those patients with significant viremia. The observation of a correlation between CSF HIV viral load and BBB damage supports this view (Burger *et al*, 1997).

HAART can delay the development of NCI in HIV-infected individuals (Deutsch *et al*, 2001), and initiation of ART in HIVD patients can slow or even reverse HIVD progression (Chang *et al*, 1999b; Schmitt *et al*, 1988). This reversal of clinical symptoms appears to be correlated with reversal of both microvascular (Tracey *et al*, 1998) and some metabolic abnormalities (Chang *et al*, 1999b). Interestingly, in many patients the salutary effects of ART are only temporary, despite continued control of peripheral viral load. This short-term efficacy, with longer term rebound, may reflect the initial effective penetration of antiretrovirals across a compromised BBB, but later resealing of the BBB may prevent effective continued control of virus within the CNS, because most antiretrovirals have poor BBB penetration. Alternatively, long term use of ARTs may lead to dysregulation of BBB maintenance (Langford *et al*, 2003).

The influence of the nature and duration of HAART on BBB integrity is beyond the scope of the present paper, because our study patients have quite variable treatment histories. Nonetheless, the possibility that BBB function may be modulated directly or indirectly by the nature and duration of ART is an important question that remains to be addressed. Interestingly, as we note below, some of the longer duration HAART-responsive patients in this study had significant BBB compromise despite effective peripheral viral suppression. The possibility that long-term HAART may itself lead to BBB compromise in some patients cannot, therefore, be ruled out.

It has been suggested that poor CNS penetration by antiretrovirals may allow viral CNS escape, with continued neurological progression despite peripheral control. As several of the patients in this study exhibited significant BBB compromise despite being well controlled on HAART, it seems likely that isolation of the CNS compartment, if reestablished by ART, may only be temporary, however. These findings are consistent with several proposals that CNS inflammatory mechanisms of neurodegeneration, once established, may become self-sustaining despite eradication of the virus (Avison *et al*, 2002), and the observation of sustained increases in CSF proinflammatory cytokines and chemokines despite control of CSF viral load following initiation of HAART (Gisolf *et al*, 2000). If this is in fact the case, it argues for early and effective control of the rate of viral entry into CNS, as well as initiation of effective anti-inflammatory therapy, to prevent neurocognitive degeneration.

Conclusions

In summary, this study demonstrates a correlation in HIV patients between severity of NCI and both CNS microglial activation measured by mI/Cr, and BBB compromise measured by postcontrast MR enhancement. Furthermore, the severity of BBB compromise is correlated with the degree of CNS inflammation. This observation, taken together with the consistent neuropathologic finding of perivascular microglial activation in HIV patients, suggests that BBB compromise is mediated, at least in part, by activation of perivascular inflammatory processes.

Methods

Subjects

HIV-infected patients, ≥18 years old and ranging from neurologically intact (MSK = 0) to moderately impaired (MSK = 2), were recruited from the HIV clinics of the University of Kentucky, independent of their viral load and ART status, provided that it was stable for at least 3 months prior to study. Patients were excluded from the study if they had evidence of CNS opportunistic infections or space-occupying lesion, a history of CNS disorder unrelated to HIV infection such as trauma, congenital malformations or genetic disorders, systemic illnesses such as diabetes, hepatic or renal dysfunction that may affect cerebral function, or active substance abuse. Patients with remote history of substance abuse (>6 months) were not excluded.

Written informed consent was obtained from each patient following a detailed explanation of the study, according to protocols approved by the University of Kentucky Medical Institutional Review Board.

Neurological and neuropsychological assessments

Detailed general physical examinations and neurological examinations were performed on all study subjects. The latter included the HIVD scale described by Power and colleagues (1995). A score on the Memorial Sloan Kettering AIDS Dementia Scale (MSK) (Price and Brew, 1988) was assigned at the time of each visit.

Neurocognitive functioning of the study participants was evaluated with a battery of neuropsychological measures that assessed attention, verbal memory and fluency, processing speed, reaction time, and motor performance. Procedures reflected those used in the Multi-Center AIDS Cohort Study (MACS; van Gorp *et al*, 1994), the AIDS Clinical Trials Group's Neurologic AIDS Research Consortium (NARC) studies (Clifford *et al*, 2002), and other studies of cognitive functioning in response to antiretroviral therapies (Schmitt *et al*, 1988, 1997). For the purpose of the present analyses, a subset of tests from the MACS battery was used as a composite measure. Using Z-score transformations of the raw scores from the following tests derived this composite score from: Controlled Oral Word Association (COWA), Grooved Pegboard (GP), Rey Auditory Verbal Learning Test (RAVLT), Symbol Digit Modalities Test (SDMT), and Trailmaking A and B (TMT). The resulting raw scores from these measures were transformed to Z-scores using age- and education-adjusted normative data from HIV control subjects provided by the MACS

study (courtesy of Drs. Ola Selnes and Eric Miller). Z-scores were then averaged across tests to provide the final composite score. This composite score is defined as the MACS NPZ8 (COWA, RAVLT-total, RAVLT-delayed recall, TMTA, TMTB, GP-dominant, GP-nondominant, SDMT).

CSF

CSF was collected by lumbar puncture using a 20-gauge needle. Lumbar puncture was performed on all study subjects at the time of study entry. Standard CSF analysis included cell count, protein, glucose, microbiological studies, HIV viral titers TNFα, and MCP-1. CSF was also banked for possible future analysis. HIV-1 viral loads were determined by Amplicor HIV-1 monitor test version1.5 (Roche Diagnostics Corporation, Indianapolis, IN) according to manufacturer's protocol.

Immunoassay determinations of MCP-1 (catalog no. DCP00) and TNFα (catalog no. HSTA00C) were performed following the procedures from the manufacturer (R&D Systems, Minneapolis, MN). Briefly, the assays employ the quantitative sandwich enzyme immunoassay technique in which a monoclonal antibody specific for either MCP-1 or TNFα has been precoated onto a microplate. Standards and CSF samples were pipetted into the wells and any target factor present was bound by the immobilized antibody. After washing away any unbound substances using the ELX-50 AutoStrip Washer (Bio-Tek Instruments), an enzyme-linked polyclonal antibody specific for either MCP-1 or TNFα were added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, substrate solution was added to the wells and color developed in proportion to the amount of target factor bound in the initial step. The color development was stopped and the intensity of the color was measured using a μQuant Spectrophotometer plate reader (Bio-Tek Instruments). Intra- and interassay coefficients of variance (CVs) were both below 10%. Values are reported as pg/ml for both MCP-1 and TNFα.

MRI

Patients were scanned on a 1.5-T Siemens Magnetom Vision MR system (Siemens Medical Systems, Iselin, NJ) using a standard, circularly polarized head coil. The following sets of MR images were collected initially to rule out the presence of neurologically significant structural lesions:

T1-weighted spin echo (sagittal): TR/TE = 500/14 ms, FA = 70°, THK = 5 mm, 3% interslice gap, FOV = 230 mm, MA = 192 × 256.

PD/T2-weighted spin echo (axial): TR/TE1/TE2 = 2000/20/80 ms, FA = 65°, FOV = 256 × 256 × 180 mm, MA = 128 × 128 × 90.

T1-weighted 3D FLASH: TR/TE = 21/6 ms, FA = 30°, THK = 5 mm, 30% interslice gap, FOV = 230 mm, MA = 192 × 256.

PD-weighted spin echo (axial): TR/TE = 2000/14 ms, FA = 62°, THK = 5 mm, 3% interslice gap, FOV = 230 mm, MA = 192 × 256.

T1-weighted spin echo (axial, pre- and postcontrast): TR/TE = 610/14 ms, FA = 62°, THK = 5 mm, 3% interslice gap, FOV = 230 mm, MA = 192 × 256. Fixed receiver and reconstruction gains.

The axial T1-weighted spin-echo sequence was used to measure the time course of cerebral postcontrast enhancement. A minimum of three precontrast images were acquired and averaged to improve the estimate of the precontrast image intensities. The precontrast images were also used to obtain an estimate of the precontrast variance for each pixel in the image. The individual images had sufficient signal to noise to detect fractional enhancements of 1% or greater. Contrast agent (gadolinium-DTPA; Magnevist, 0.2 mmol/kg i.v.) was then administered, and collection of postcontrast T1-weighted images using identical acquisition conditions was begun. Collection of images continued without interruption (one set every ~2 min) for at least 30 min postcontrast, unless an earlier termination was requested by the patient.

Data analysis: For a given region of interest (ROI), the fractional enhancement (FE) at time t post contrast was defined as $\mathrm{FE}(t) = (S(t) - S(\mathrm{pre}))/S(\mathrm{pre})$, where $S(t)$ was the mean MRI signal in the ROI at time t post contrast, and S(pre) was the mean MRI signal in the same ROI prior to contrast administration. As noted earlier, a minimum of three precontrast images were acquired and averaged to improve the estimate of S(pre). Mean FE was determined for the basal ganglia and adjacent white matter as follows: ROI measurements for each time point pre and post contrast were collected using a mask traced to outline basal ganglia structures. These ROI measurements were combined to determine the mean FE in subcortical gray for each subject. Circular ROIs placed bilaterally in the anterior white matter tracts adjacent to the head of the caudate were used to assess white matter enhancement. As with the subcortical gray ROIs, the white matter ROI measurements were combined to determine a mean white matter FE for each subject. FE 30 min post contrast (FE30) were calculated and used as an index of BBB integrity (Berger *et al*, 2000).

MRS

^{1}H-MR spectra were obtained using a Siemens 1.5 T Vision MR scanner with the ^{1}H spectroscopy package running under Numaris vB33. Scout images were collected to confirm correct positioning, and then an automated global shim (MAPSHIM) was performed. A series of base images was then collected to guide placement of the volume of interest (VOI) in the basal ganglia and frontal white matter for localized spectroscopy. Each VOI was shimmed interactively to achieve a target water linewidth of less than

0.15 ppm, with good lineshape. A 20-ms TE STEAM sequence was used for measurement of relative levels of mI and Cr. Sequence parameters were TR = 1500, TE = 20, 1024 complex points, 1024 ms acquisition time (2 kHz SW), NS = 256.

Data analysis

Prior to postprocessing and analysis, ^{1}H-MRS data were inspected to ensure adequate shimming and water suppression. Peak areas were then measured using standard Siemens line fitting software (LUISE) for estimation of mI/Cr. When line-fitting yielded unusual mI/Cr ratios, the mI peak area was also determined as described by Alger *et al* (1993). If the discrepancy between methods was >10%, then the data were deemed a technical failure and discarded.

Statistics

The significance of relationships amongst continuous variables was determined using single and multiple linear regression models as appropriate. All analyses were implemented in JMP 5.0 (SAS Institute).

References

Alger JR, Symko SC, Bizzi A, Posse S, DesPres DJ, Armstrong MR (1993). Absolute quantitation of short TE brain 1H-MR spectra and spectroscopic imaging data. *J Comput Assist Tomogr* **17:** 191–199.

Andersson LM, Hagberg L, Fuchs D, Svennerholm B, Gisslen M (2001). Increased blood-brain barrier permeability in neuro-asymptomatic HIV-1-infected individuals—correlation with cerebrospinal fluid HIV-1 RNA and neopterin levels. *J NeuroVirol* **7:** 542–547.

Avison MJ, Nath A, Berger JR (2002). Understanding pathogenesis and treatment of HIV dementia: a role for magnetic resonance? *Trends Neurosci* **25:** 468–473.

Bagasra O, Lavi E, Bobroski L, Khalili K, Pestaner JP, Tawadros R, Pomerantz RJ (1996). Cellular reservoirs of HIV-1 in the central nervous system of infected individuals: identification by the combination of in situ polymerase chain reaction and immunohistochemistry. *AIDS* **10:** 573–585.

Barker PB, Lee RR, McArthur JC (1995). AIDS dementia complex: evaluation with proton MR spectroscopic imaging. *Radiology* **195:** 58–64.

Bell JE (1998). The neuropathology of adult HIV infection. *Rev Neurol (Paris)* **154:** 816–829.

Berger JR, Nath A, Greenberg RN, Andersen AH, Greene RA, Bognar A, Avison MJ (2000). Cerebrovascular changes in the basal ganglia with HIV dementia. *Neurology* **54:** 921–926.

Brew BJ, Rosenblum M, Cronin K, Price RW (1995). AIDS dementia complex and HIV-1 brain infection: clinical-virological correlations [see comments]. *Ann Neurol* **38:** 563–570.

Budka H, Costanzi G, Cristina S, Lechi A, Parravicini C, Trabattoni R, Vago L (1987). Brain pathology induced by infection with the human immunodeficiency virus (HIV). A histological, immunocytochemical, and electron microscopical study of 100 autopsy cases. *Acta Neuropathol* **75:** 185–198.

Burger DM, Boucher CA, Meenhorst PL, Kraayeveld CL, Portegies P, Mulder JW, Hoetelmans RM, Beijnen JH (1997). HIV-1 RNA levels in the cerebrospinal fluid may increase owing to damage to the blood-brain barrier. *Antivir Ther* **2:** 113–117.

Chang L, Ernst T, Leonido-Yee M, Walot I, Singer E (1999a). Cerebral metabolite abnormalities correlate with clinical severity of HIV-1 cognitive motor complex. *Neurology* **52:** 100–108.

Chang L, Ernst T, Leonido-Yee M, Witt M, Speck O, Walot I, Miller EN (1999b). Highly active antiretroviral therapy reverses brain metabolite abnormalities in mild HIV dementia. *Neurology* **53:** 782–789.

Cherner M, Masliah E, Ellis RJ, Marcotte TD, Moore DJ, Grant I, Heaton RK (2002). Neurocognitive dysfunction predicts postmortem findings of HIV encephalitis. *Neurology* **59:** 1563–1567.

Cinque P, Vago L, Ceresa D, Mainini F, Terreni MR, Vagani A, Torri W, Bossolasco S, Lazzarin A (1998). Cerebrospinal fluid HIV-1 RNA levels: correlation with HIV encephalitis. *AIDS* **12:** 389–394.

Clifford DB, McArthur JC, Schifitto G, Kieburtz K, McDermott MP, Letendre S, Cohen BA, Marder K, Ellis RJ, Marra CM (2002). A randomized clinical trial of CPI-1189 for HIV-associated cognitive-motor impairment. *Neurology* **59:** 1568–1573.

Conant K, McArthur JC, Griffin DE, Sjulson L, Wahl LM, Irani DN (1999). Cerebrospinal fluid levels of MMP-2, 7, and 9 are elevated in association with human immunodeficiency virus dementia. *Ann Neurol* **46:** 391–398.

Deutsch R, Ellis RJ, McCutchan JA, Marcotte TD, Letendre S, Grant I (2001). AIDS-associated mild neurocognitive impairment is delayed in the era of highly active antiretroviral therapy. *AIDS* **15:** 1898–1899.

Dhawan S, Weeks BS, Soderland C, Schnaper HW, Toro LA, Asthana SP, Hewlett IK, Stetler-Stevenson WG, Yamada SS, Yamada KM, *et al* (1995). HIV-1 infection alters monocyte interactions with human microvascular endothelial cells. *J Immunol* **154:** 422–432.

Dore GJ, Correll PK, Li Y, Kaldor JM, Cooper DA, Brew BJ (1999). Changes to AIDS dementia complex in the era of highly active antiretroviral therapy. *AIDS* **13:** 1249–1253.

Ernst T, Itti E, Itti L, Chang L (2000). Changes in cerebral metabolism are detected prior to perfusion changes in early HIV-CMC: A coregistered (1)H MRS and SPECT study. *J Magn Reson Imaging* **12:** 859–865.

Falangola MF, Hanly A, Galvao Castro B, Petito CK (1995). HIV infection of human choroid plexus: a possible mechanism of viral entry into the CNS. *J Neuropathol Exp Neurol* **54:** 497–503.

Fujimura RK, Goodkin K, Petito CK, Douyon R, Feaster DJ, Concha M, Shapshak P (1997). HIV-1 proviral DNA load across neuroanatomic regions of individuals with evidence for HIV-1-associated dementia. *J Acquir Immune Defic Syndr Hum Retrovirol* **16:** 146–152.

Gisolf EH, van Praag RM, Jurriaans S, Portegies P, Goudsmit J, Danner SA, Lange JM, Prins JM (2000). Increasing cerebrospinal fluid chemokine concentrations despite

undetectable cerebrospinal fluid HIV RNA in HIV-1-infected patients receiving antiretroviral therapy. *J Acquir Immune Defic Syndr* **25:** 426–433.

Gray F, Lescs MC, Keohane C, Paraire F, Marc B, Durigon M, Gherardi R (1992). Early brain changes in HIV infection: neuropathological study of 11 HIV seropositive, non-AIDS cases. *J Neuropathol Exp Neurol* **51:** 177–185.

Hickey WF (1999). Leukocyte traffic in the central nervous system: the participants and their roles. *Semin Immunol* **11:** 125–137.

Hofman FM, Dohadwala MM, Wright AD, Hinton DR, Walker SM (1994). Exogenous tat protein activates central nervous system-derived endothelial cells. *J Neuroimmunol* **54:** 19–28.

Hurwitz AA, Berman JW, Lyman WD (1994). The role of the blood-brain barrier in HIV infection of the central nervous system. *Adv Neuroimmunol* **4:** 249–256.

Jones MV, Bell JE, Nath A (2000). Immunolocalization of HIV envelope gp120 in HIV encephalitis with dementia. *AIDS* **14:** 2709–2713.

Kandanearatchi A, Williams B, Everall IP (2003). Assessing the efficacy of highly active antiretroviral therapy in the brain. *Brain Pathol* **13:** 104–110.

Kelder W, McArthur JC, Nance Sproson T, McClernon D, Griffin DE (1998). Beta-chemokines MCP-1 and RANTES are selectively increased in cerebrospinal fluid of patients with human immunodeficiency virus-associated dementia. *Ann Neurol* **44:** 831–835.

Kure K, Llena JF, Lyman WD, Soeiro R, Weidenheim KM, Hirano A, Dickson DW (1991). Human immunodeficiency virus-1 infection of the nervous system: an autopsy study of 268 adult, pediatric, and fetal brains. *Hum Pathol* **22:** 700–710.

Langford TD, Letendre SL, Larrea GJ, Masliah E (2003). Changing patterns in the neuropathogenesis of HIV during the HAART era. *Brain Pathol* **13:** 195–210.

Laubenberger J, Haussinger D, Bayer S, Thielemann S, Schneider B, Mundinger A, Hennig J, Langer M (1996). HIV-related metabolic abnormalities in the brain: depiction with proton MR spectroscopy with short echo times. *Radiology* **199:** 805–810.

Levy JA (1997). HIV neuropathogenesis. *J NeuroVirol* **3:** S14–S15.

Liuzzi GM, Mastroianni CM, Santacroce MP, Fanelli M, D'Agostino C, Vullo V, Riccio P (2000). Increased activity of matrix metalloproteinases in the cerebrospinal fluid of patients with HIV-associated neurological diseases. *J NeuroVirol* **6:** 156–163.

Masliah E, DeTeresa RM, Mallory ME, Hansen LA (2000). Changes in pathological findings at autopsy in AIDS cases for the last 15 years. *AIDS* **14:** 69–74.

McArthur JC, McClernon DR, Cronin MF, Nance Sproson TE, Saah AJ, St Clair M, Lanier ER (1997). Relationship between human immunodeficiency virus-associated dementia and viral load in cerebrospinal fluid and brain [see comments]. *Ann Neurol* **42:** 689–698.

Meyerhoff DJ, Weiner MW, Fein G (1996). Deep gray matter structures in HIV infection: a proton MR spectroscopic study. *AJNR Am J Neuroradiol* **17:** 973–978.

Moses AV, Bloom FE, Pauza CD, Nelson JA (1993). Human immunodeficiency virus infection of human brain capillary endothelial cells occurs via a CD4/galactosylceramide-independent mechanism. *Proc Natl Acad Sci USA* **90:** 10474–10478.

Neuen-Jacob E, Arendt G, Wendtland B, Jacob B, Schneeweis M, Wechsler W (1993). Frequency and topographical distribution of CD68-positive macrophages and HIV-1 core proteins in HIV-associated brain lesions. *Clin Neuropathol* **12:** 315–324.

Neuenburg JK, Brodt HR, Herndier BG, Bickel M, Bacchetti P, Price RW, Grant RM, Schlote W (2002). HIV-related neuropathology, 1985 to 1999: rising prevalence of HIV encephalopathy in the era of highly active antiretroviral therapy. *J Acquir Immune Defic Syndr* **31:** 171–177.

Nottet HS (1999). Interactions between macrophages and brain microvascular endothelial cells: role in pathogenesis of HIV-1 infection and blood-brain barrier function. *J NeuroVirol* **5:** 659–669.

Petito CK, Cash KS (1992). Blood-brain barrier abnormalities in the acquired immunodeficiency syndrome: immunohistochemical localization of serum proteins in postmortem brain. *Ann Neurol* **32:** 658–666.

Power C, Kong PA, Crawford TO, Wesselingh S, Glass JD, McArthur JC, Trapp BD (1993). Cerebral white matter changes in acquired immunodeficiency syndrome dementia: alterations of the blood-brain barrier. *Ann Neurol* **34:** 339–350.

Power C, Selnes OA, Grim JA, McArthur JC (1995). HIV Dementia Scale: a rapid screening test. *J Acquir Immune Defic Syndr Hum Retrovirol* **8:** 273–278.

Price RW, Brew BJ (1988). The AIDS dementia complex. *J Infect Dis* **158:** 1079–1083.

Pumarola Sune T, Navia BA, Cordon Cardo C, Cho ES, Price RW (1987). HIV antigen in the brains of patients with the AIDS dementia complex. *Ann Neurol* **21:** 490–496.

Robertson K, Fiscus S, Kapoor C, Robertson W, Schneider G, Shepard R, Howe L, Silva S, Hall C (1998). CSF, plasma viral load and HIV associated dementia. *J NeuroVirol* **4:** 90–94.

Sacktor N, Lyles RH, Skolasky R, Kleeberger C, Selnes OA, Miller EN, Becker JT, Cohen B, McArthur JC (2001). HIV-associated neurologic disease incidence changes: Multicenter AIDS Cohort Study, 1990–1998. *Neurology* **56:** 257–260.

Sacktor N, McDermott MP, Marder K, Schifitto G, Selnes OA, McArthur JC, Stern Y, Albert S, Palumbo D, Kieburtz K, De Marcaida JA, Cohen B, Epstein L (2002). HIV-associated cognitive impairment before and after the advent of combination therapy. *J NeuroVirol* **8:** 136–142.

Salvan AM, Vion-Dury J, Confort-Gouny S, Nicoli F, Lamoureux S, Cozzone PJ (1997a). Brain proton magnetic resonance spectroscopy in HIV-related encephalopathy: identification of evolving metabolic patterns in relation to dementia and therapy. *AIDS Res Hum Retroviruses* **13:** 1055–1066.

Salvan AM, Vion-Dury J, Confort-Gouny S, Nicoli F, Lamoureux S, Cozzone PJ (1997b). Cerebral metabolic alterations in human immunodeficiency virus-related encephalopathy detected by proton magnetic resonance spectroscopy. Comparison between sequences using short and long echo times. *Invest Radiol* **32:** 485–495.

Schmitt FA, Bigley JW, McKinnis R, Logue PE, Evans RW, Drucker JL (1988). Neuropsychological outcome of zidovudine (AZT) treatment of patients with AIDS and AIDS-related complex. *N Engl J Med* **319:** 1573–1578.

Schmitt FA, Wetherby M, Stern Y (1997). Neuropsychological changes in human immunodeficiency virus infection. In: *AIDS and the nervous system* (2nd ed). Berger JR, Levy RM (eds). Philadelphia: Lippincott-Raven. 401–417.

Seilhean D, Dzia-Lepfoundzou A, Sazdovitch V, Cannella B, Raine CS, Katlama C, Bricaire F, Duyckaerts C, Hauw JJ (1997). Astrocytic adhesion molecules are increased in HIV-1-associated cognitive/motor complex. *Neuropathol Appl Neurobiol* **23:** 83–92.

Silver NC, Good CD, Sormani MP, MacManus DG, Thompson AJ, Filippi M, Miller DH (2001a). A modified protocol to improve the detection of enhancing brain and spinal cord lesions in multiple sclerosis. *J Neurol* **248:** 215–224.

Silver NC, Tofts PS, Symms MR, Barker GJ, Thompson AJ, Miller DH (2001b). Quantitative contrast-enhanced magnetic resonance imaging to evaluate blood-brain barrier integrity in multiple sclerosis: a preliminary study. *Mult Scler* **7:** 75–82.

Smith TW, DeGirolami U, Henin D, Bolgert F, Hauw JJ (1990). Human immunodeficiency virus (HIV) leukoencephalopathy and the microcirculation. *J Neuropathol Exp Neurol* **49:** 357–370.

Soontornniyomkij V, Nieto Rodriguez JA, Martinez AJ, Kingsley LA, Achim CL, Wiley CA (1998). Brain HIV burden and length of survival after AIDS diagnosis. *Clin Neuropathol* **17:** 95–99.

Tracey I, Carr CA, Guimaraes AR, Worth JL, Navia BA, Gonzalez RG (1996). Brain choline-containing compounds are elevated in HIV-positive patients before the onset of AIDS dementia complex: a proton magnetic resonance spectroscopic study [published erratum appears in *Neurology* 1996 Jun;46(6):1787]. *Neurology* **46:** 783–788.

Tracey I, Hamberg LM, Guimaraes AR, Hunter G, Chang I, Navia BA, Gonzalez RG (1998). Increased cerebral blood volume in HIV-positive patients detected by functional MRI. *Neurology* **50:** 1821–1826.

Tyor WR, Glass JD, Griffin JW, Becker PS, McArthur JC, Bezman L, Griffin DE (1992). Cytokine expression in the brain during the acquired immunodeficiency syndrome. *Ann Neurol* **31:** 349–360.

van Gorp WG, Miller EN, Marcotte TD, Dixon W, Paz D, Selnes O, Wesch J, Becker JT, Hinkin CH, Mitrushina M, *et al* (1994). The relationship between age and cognitive impairment in HIV-1 infection: findings from the Multicenter AIDS Cohort Study and a clinical cohort. *Neurology* **44:** 929–935.

Weis S, Haug H, Budka H (1996). Vascular changes in the cerebral cortex in HIV-1 infection: I. A morphometric investigation by light and electron microscopy. *Clin Neuropathol* **15:** 361–366.

Wiley CA, Soontornniyomkij V, Radhakrishnan L, Masliah E, Mellors J, Hermann SA, Dailey P, Achim CL (1998). Distribution of brain HIV load in AIDS. *Brain Pathol* **8:** 277–284.

Woodman SE, Benveniste EN, Nath A, Berman JW (1999). Human immunodeficiency virus type 1 TAT protein induces adhesion molecule expression in astrocytes. *J NeuroVirol* **5:** 678–684.