Quantitation of Human Immunodeficiency Virus, Immune Activation Factors, and Quinolinic Acid in AIDS Brains

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Abstract

HIV encephalitis is unusual in that neurologic damage occurs in the absence of significant infection of neuronal or glial cells. Because the predominant infected cell in the brain is the macrophage, it has been proposed that release of viral or immune activation factors from macrophages may mediate neurologic damage. Numerous studies have examined the concentration of immune activation factors in the cerebrospinal fluid (CSF), however, there has been no correlation between these CSF measurements and severity of HIV encephalitis (Wiley, C. A., C. L. Achim, R. D. Schrier, M. P. Heyes, J. A. McCutchen, and I. Grant. 1992. *AIDS (Phila.)*. 6:1299–1307. Because CSF measurements may not represent tissue concentrations of these factors, we examined the concentrations of HIV p24, quinolinic acid (QUIN), IL-1, IL-3, IL-6, TNF-α, and GMCSF within the brains of 10 AIDS autopsies. Homogenization and extraction of cortical gray, cortical white and deep gray matter showed a good correlation between the amount of HIV gp41 immunostaining and extracted HIV gag protein p24. The concentrations of cytokines were low in the tissue extracts and showed no correlation with severity of HIV encephalitis. Brain extracts from mild cases of HIV encephalitis showed elevated levels of TNF-α in deep gray matter, while in more severe cases, elevated TNF-α levels were also found within cortical white and cortical gray matter. Brain tissue and CSF QUIN concentrations were substantially increased compared to control values. QUIN concentrations were not correlated with the severity of HIV encephalitis. We conclude that increased tissue levels of TNF-α and QUIN may have a role in the etiology of HIV-related neurologic dysfunction. (*J. Clin. Invest.* 1993. 91:2769–2775.) Key words: cytokine • central nervous system • enzyme-linked immunosorbent assay • encephalitis • macrophage

Introduction

Neurologic deficits during life are increasingly being recognized as an important feature of infection with HIV. Further, ~ 30% of terminally ill AIDS patients develop neurologic disease presumably caused by HIV infection of the brain (1–4). Neuropathologic study of these patients demonstrated microglial nodules and occasionally multinucleated giant cells. Subsequent immunohistochemical and in situ hybridization studies demonstrated HIV within these lesions, and this pathology has been named HIV encephalitis (HIV-E) (5). While "viral burden" appears to be tightly associated with neuropathology (6), such an association has not yet been made with clinical neurologic disease (dementia). After systemic HIV infection, viral invasion of the central nervous system (CNS)[1] occurs rapidly and frequently (7), however, the timing of significant CNS parenchymal infection is unknown. In unselected AIDS autopsies, there is a wide range in the severity of HIV encephalitis (8). Between 20 and 30% of AIDS patients develop severe HIV encephalitis in terminal stages of the disease (9–11).

A peculiar feature of this chronic encephalitis is that there is no evidence of significant neuronal or glial infection (12–14). In the absence of direct lytic infection, it has been hypothesized that indirect effects of HIV infected macrophages mediates neuronal damage (15). In addition to being infected, CNS macrophages are clearly affected by HIV, showing morphologic transformation with abundant cytoplasm and processes, in addition to elevated expression of major histocompatibility complex class I and II molecules (16). It is reasonable to hypothesize that such activated macrophages, and perhaps other cells, could release neurotoxic factors. Some of these factors (e.g., lipases and proteases) could mediate neurotoxicity nonspecifically while other factors (e.g., cytokines) mediate potent toxic effects by binding to cell surface receptors. Such factors proposed to mediate neurotoxicity in AIDS include IL-1β, TNF-α, IL-6 (18), gp120 (19), and quinolinic acid (QUIN) (19), as well as unidentified factors (20, 21) whose presence has been demonstrated only in vitro. Quantitation of these factors in the brain of HIV-infected patients has not been described. With respect to cytokines little is known about specific neuronal or glial surface receptors, except for IL-1 where hypothalamic receptors are well documented (22).

Several investigators have examined the concentration of candidate neurotoxic immune activation factors found in the cerebrospinal fluid (CSF) of AIDS patients (reviewed by Wiley et al. in reference 23). None of these previous studies have shown a consistent correlation between presence of these factors [e.g., IFN-γ, IL-1β, TNF-α, and IL-6] and the severity of neurologic damage. However, increased CSF concentrations of the excitotoxin and *N*-methyl-D-aspartate receptor agonist quinolinic acid (QUIN) are correlated with both the severity of neurologic impairments and markers of immune activation during life in both HIV-infected patients (not assessed for presence of HIV encephalitis) and simian immunodeficiency virus–infected macaques; the highest CSF and brain QUIN levels occur in conditions of macrophage infiltration and gliosis within the central nervous system (23, 24–29).

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1. *Abbreviations used in this paper:* CNS, central nervous system; CSF, cerebrospinal fluid; HIV-E, encephalitis; QUIN, quinolinic acid.

Recently, we have examined the relationship between a variety of immune activation factors in the CSF and severity of HIV encephalitis. While significant intercorrelation between immune activation markers were found, no correlations were observed between these factors in the CSF and the severity of HIV encephalitis (23). Because the CSF may not reflect local CNS tissue concentrations of these factors, we extracted specific CNS regions (frontal cortex, cerebral white matter, and basal ganglia) and directly evaluated the concentrations of immune activation factors and HIV in relationship to the severity of HIV encephalitis.

Methods

CNS material from 10 AIDS autopsies (Table I) that had a postmortem time < 24 h were obtained. Based on previous screening for the severity of HIV encephalitis and availability of suitable frozen material, we selected three patients with low levels of HIV and seven with high levels of HIV (see below for quantification and references). We excluded cases with opportunistic CNS infections or intermediate levels of HIV in the brain.

CSF was obtained through cervical spinal tap at the time of autopsy and stored at −70°C until used for analysis. Routine tissue blocks from the following CNS regions were paraffin embedded for histopathologic diagnosis: frontal cortex, caudate nucleus, insular cortex, basal ganglia, thalamus, hypothalamus, hippocampus, superior cerebellum, midbrain, pons, medulla, and, when available, three levels of the spinal cord. To assess histopathology, routine hematoxylin and eosin staining was done on all of these blocks.

Immunocytochemistry for HIV envelope protein gp41 and scoring of HIV-E was performed as reported previously (9). Sections of basal ganglia and cerebral cortex were mounted (Vectabond; Vector Laboratories, Burlingame, CA) treated slides. To identify HIV antigens, we used a monoclonal antibody obtained from Genetic Systems (Seattle, WA) that specifically recognizes the transmembrane portion of the HIV envelope protein gp41. Sections were deparaffinized (Histo-Clear; National Diagnostics, Manville, NJ), treated with 1% H_2O_2 in methanol (to block endogenous peroxidase), rehydrated through graded alcohols, and rinsed in PBS; tissues were incubated with primary antibody for 2 h at 37°C or overnight at 4°C followed by PBS rinses and another incubation with biotinylated secondary antiserum (goat anti-mouse) (Tago, Inc., Burlingame, CA) for 30 min at room temperature. After rinsing and a 30-min reaction with an avidin-biotin-peroxidase complex (ABC kit; Vector Laboratories) at room temperature, the enzyme was developed with 3-amino-9-ethylcarbazole and the sections were counterstained with Meyer's hematoxylin.

Tissue sections from all 10 cases were studied for presence of HIV gp41 in three major cerebral regions: frontal cortical gray matter, cortical white matter, and deep gray matter. Levels of HIV antigen expression were assessed separately for each region and were scored on a scale from 0 to 2: 0, no cells stained for gp41; 1 = less than two cells stained for gp41 in an average of five fields with a magnification of 20; and 2,

Table I. Summary of the AIDS Cases Studied

ID number	Age	PMT	Neuropathologic diagnosis	Systemic disease
		h		
HIVgp41 sum = 0–1				
A90-67	49	9	Mild chronic meningitis	Thrombocytopenia with GI hemorrhage, splenectomy, hepatitis, KS of lymph nodes
A90-87	37	19	Chronic meningitis, hippocampal ischemic changes in CA1	PC pneumonia
A90-198	31	24	Microglial nodular encephalitis, scattered calcifications, vermal atrophy	CMV adrenalitis and pneumonia, Candida septicemia, acute gastroenteritis, mediastinal adenopathy
HIVgp41 sum = 4–6				
A89-154	41	10	HIV encephalitis, CMV neuritis, vacuolar myelopathy	MAI: spleen, lymph nodes and intestines, PC pneumonia; CMV: lungs, nerve root, adrenal; nocardia: lungs, thyroid, liver, intestine
A90-30	37	18	Microglial nodular encephalitis, mild chronic meningitis, vacuolar myelopathy	Disseminated MAI, Gram-positive bacteria pneumonia, lymphadenopathy
A90-99	53	21	Microglial nodular encephalitis	Disseminated PC, CMV
A90-211	44	10	Meningitis, microglial nodular encephalitis, hydrocephalus	Clostridial septicemia, bacterial and CMV colitis, PC pneumonia, herpes proctitis
A91-52	36	12	HIV encephalitis, chronic meningitis	Pneumococcal and MAI pneumonia, CMV retinitis
A91-193	37	15	HIV encephalitis	Disseminated KS, CMV adrenalitis
A91-207	36	12	CNS lymphoma, mild microgial nodular encephalitis	Bronchopneumonia, disseminated KS, MAI splenitis, chronic pancreatitis, HSV esophagitis

GI, gastrointestinal; KS, Kaposi's sarcoma; MAI, mycobacterium avium-intracellulare; PC, pneumocystis carinii; PMT, postmortem time. HIV-E was defined as microglial nodular encephalitis accompanied by multinucleated giant cells.

more than two cells stained for gp41 in an average of five fields with a magnification of 20. All assessments were done blindly and independently by two authors (C. A. Wiley and C. L. Achim). A composite gp41 score for each autopsy was derived by summing the individual scores of the three regions (ranges were 0–6).

Frozen brain material was minced by using the commercially available "Minibead beater" (Biospec Products, Inc., Bartlesville, OK). Aliquots of 10% (wt/vol) homogenates from all regions of interest (cortical gray, cortical white, globus pallidus, and putamen) were obtained by homogenizing ~ 100 mg of tissue in Tris buffer with 1-mm glass beads.

Equal aliquots of tissue homogenates from each region were used for ELISA antigen capture assays. For detection of HIV p24 we used the commercially available kit from Coulter Corp. (Hialeah, FL). The cytokines IL-1β, TNF-α, IL-3, IL-6, and GM-CSF were detected with antigen capture kits from R&D Systems, Inc., (Minneapolis, MN). All assays were performed following manufacturer's protocols.

Quinolinic acid was assayed in 100-μl aliquots of 5% tissue homogenate by a modified gas chromatography/mass spectrometric method (27, 29, 30).

Statistical analysis was performed using software (StatView II; Abacus Concepts, Inc., Berkeley, CA); correlation matrix for Pearson coefficient and simple linear regression were determined using 95% confidence intervals.

Results

The concentrations of viral and immune activation factors in the CNS tissue extracts and previously reported CSF (23) are shown in Table II. Only cases with severe HIV encephalitis (as assessed by high numbers of macrophages immunostained for HIV gp41 (8) had high concentrations of HIV p24 in tissue extracts (Table II). However, half of the cases with severe HIV

Table II. Summary of Assay Results

	CNS tissue concentrations								CSF concentrations (23)					
Patient ID	gp41	HIVp24	QUIN	TNFa	GMCSF	IL-1	IL-3	IL-6	HIVp24	QUIN	TNFa	IL-1b	IL-3	IL-6
HIVgp41 sum = 0–1														
A90-67/A	0	< 10	2,400	< 10	< 10	< 10	< 10	< 10						
- B	0	< 10	1,960	11	< 10	< 10	< 10	12	< 10	8.9	13	17	< 10	48
- C	1	13	4,720	26	< 10	< 10	11	< 10						
A90-87/A	0	< 10	120	< 10	< 10	< 10	< 10	< 10						
- B	0	< 10	80	37	< 10	< 10	< 10	< 10	< 10	8.8	10	20	< 10	< 10
- C	0	14	160	34	< 10	< 10	< 10	< 10						
A90-198/A	0	< 10	1,400	26	< 10	< 10	< 10	19						
- B	0	< 10	1,440	13	< 10	< 10	< 10	< 10	< 10	4.4	13	39	27	2,217
- C	1	< 10	1,660	47	< 10	< 10	< 10	< 10						
HIVgp41 sum = 4–6														
A89-154/A	2	894	220	19	< 10	< 10	< 10	< 10						
- B	2	1,135	160	27	< 10	< 10	< 10	10	30	1.9	11	31	< 10	1,905
- C	2	986	180	16	< 10	< 10	< 10	< 10						
A90-30/A	2	13	4,640	71	< 10	< 10	20	10						
- B	1	11	3,500	22	< 10	< 10	< 10	12	< 10	5.9	12	20	< 10	< 10
- C	2	13	5,160	13	< 10	< 10	< 10	< 10						
A90-99/A	2	11	400	38	< 10	< 10	< 10	< 10						
- B	2	11	320	40	< 10	< 10	< 10	< 10	< 10	2.8	10	17	< 10	1,046
- C	2	11	480	92	< 10	< 10	< 10	< 10						
A90-211/A	2	< 10	960	24	< 10	< 10	< 10	< 10						
- B	2	< 10	880	11	< 10	< 10	< 10	< 10	< 10	2	10	50	10	1,070
- C	2	12	1,900	21	< 10	< 10	< 10	< 10						
A91-52/A	2	11	2,460	31	< 10	< 10	< 10	15						
- B	2	189	2,380	49	< 10	< 10	11	< 10	24	6.1	43	< 10	14	17
- C	2	580	4,220	91	< 10	< 10	13	< 10						
A91-193/A	2	< 10	300	36	< 10	< 10	11	< 10						
- B	1	< 10	1,440	44	< 10	< 10	< 10	10	13	N/A	23	< 10	14	992
- C	2	557	1,200	42	< 10	< 10	17	22						
A91-207/A	2	17	1,520	56	< 10	< 10	11	11						
- B	1	14	220	43	< 10	< 10	18	< 10	31	N/A	16	< 10	< 10	228
- C	2	913	13,580	34	< 10	< 10	17	21						

The values for HIVp24 and cytokines represent pg in 5 mg of tissue extracted; QUIN is expressed as nanomoles per kilograms; the values for gp41 represent scores (on a 0–2 scale) determined by immunocytochemistry for HIVgp41. The CSF levels of HIVp24 and cytokines are expressed as pg/ml; QUIN values represent μM. For all cases *A* represents cortical grey matter, *B* represents cortical white matter, and *C* is the average of putamen and globus pallidus. N/A, not available. We chose 10 pg as the cutoff value for the cytokine immunoassays (the sensitivity suggested by manufacturer was 1–7 pg/ml).

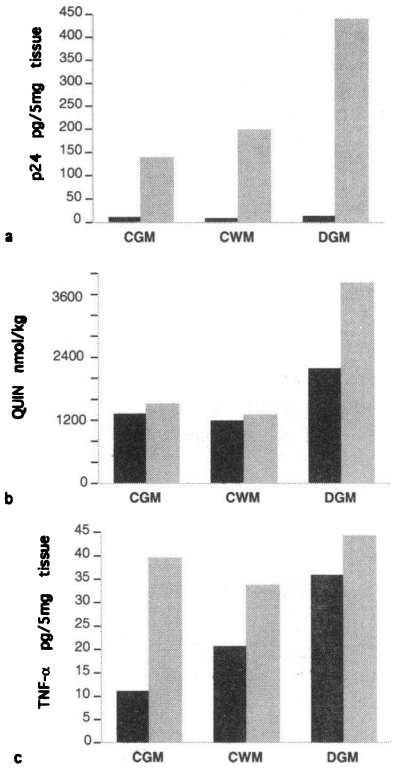

Figure 1. Regional analysis of HIV p24 (*a*), QUIN (*b*), and TNFα (*c*) in tissue homogenates from AIDS brains with low ($n = 3$) and high ($n = 7$) HIV gp41 immunocytochemistry scores.

encephalitis had little p24 in brain tissue extracts. Regional distribution of extracted p24 also correlated with gp41 measurements showing high concentrations in deep gray structures (Fig. 1 *a*).

Comparison of extract concentrations with CSF concentrations of p24, showed that only those cases with high tissue concentrations also had high CSF concentrations (Table II). These correlations were statistically significant (Table III and Fig. 2). While difficult to compare, the concentrations of p24 in the tissue extracts were substantially higher than those found in CSF (Table II).

The highest concentrations of QUIN were also detected in severe HIV encephalitis (Table II). In general, QUIN in tissue extracts correlated with its concentration in CSF (Fig. 2). Commensurate with the neuropathology (gliosis, vacuolation, and microglial infiltration), regional analysis of QUIN concentrations showed higher levels in basal ganglia than in other brain regions (Fig. 1 *b*), however, these differences were not statistically significant.

*Table III. Correlation Matrix for Brain and CSF Levels of HIVp24, TNFa, QUIN and Scores for HIVgp41 ICC**

	HIVgp41 (ICC)	Brain HIVp24	Brain QUIN	Brain TNFa
HIVp24 (brain)	0.384			
QUIN (brain)	0.175	−0.155		
TNFa (brain)	0.524	0.073	0.206	
HIVp24 (CSF)	0.446	0.803	0.283	0.245
QUIN (CSF)	−0.669	−0.420	0.388	−0.142

* The values for brain levels of HIVp24, TNFa and cytokines used in this correlation matrix were averages of cortical grey, cortical white, and deep grey matter.

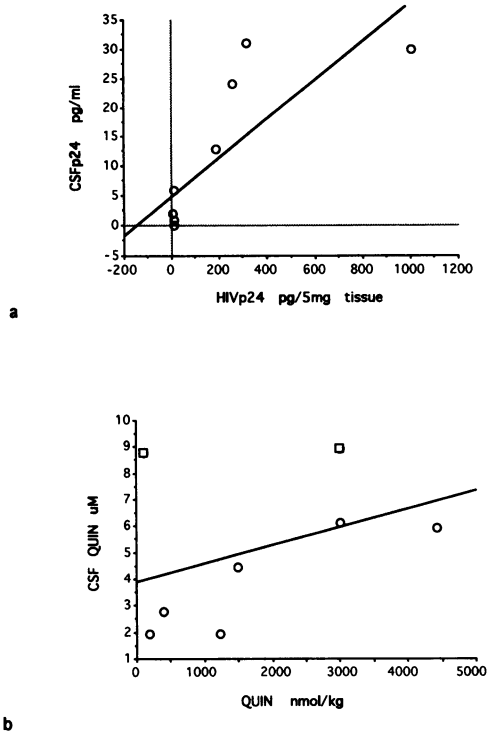

Figure 2. Regression analysis of HIV gp41 scores, HIV p24 and QUIN levels in the CNS. (*a*) Correlation between brain (average of cortical grey, white, and deep grey matter) and CSF HIV p24 ($n = 10$, $r^2 = 0.645$). (*b*) correlation between brain and CSF QUIN ($n = 8$, $r^2 = 0.151$). ■, gp41 = 0–1; □, gp41 = 4–6.

Cytokine concentrations in the extracted tissues were uniformly low except for an occasional modest level of TNF-α (Table II). Comparison with CSF concentrations of these same monokines showed no significant correlation. Despite strikingly high concentrations of IL-6 in the CSF of many autopsies, these levels were not reflected in the parenchyma.

Regional analysis of cytokines showed higher concentrations of TNF-α in the basal ganglia (Fig. 1 *c*). Regional analysis of cytokines in cases with minimal HIV encephalitis showed a gradient of TNF-α with highest concentration in basal ganglia (Fig. 1 *c*). A similar analysis in cases with severe HIV encephalitis showed a uniform elevation in all three regions.

Discussion

Analysis of neurologic disease in AIDS is complicated by frequent opportunistic CNS infections. In the present study, we evaluated brains from autopsy cases where complete neuropathologic exam was possible. When initially described by Navia et al. in 1986 (2) those patients with AIDS dementia had neuropathology of microglial nodules some with multinucleated giant cells. Strangely, since that initial study there have been few reports carefully correlating the clinical symptomatology with neuropathologic finings. In part this may be because of the complexity of the task given the plethora of opportunistic infections (in particular cytomegalovirus [CMV]) that can affect AIDS patients and make a one to one disease association difficult. Additionally, the definition of HIV encephalitis has

evolved and the insensitive histopathological criteria of microglial nodules with multinucleated giant cells have been replaced with immunocytochemical and in situ hybridization detection of HIV (5). The association of dementia with new sensitive and quantitative criteria needs to be studied methodically. Since opportunistic pathology is a potential source of immune activation in the brain of AIDS patients, we focused our attention on the neuropathologic changes associated with a significant HIV burden (i.e., HIV encephalitis), and define a pattern of immune activation specific to this condition.

Studies of a chronic viral encephalitis like that caused by HIV, require some quantitative appreciation for the severity of the encephalitis. As we have seen in previous studies (8), HIV encephalitis is not an all or nothing event, but rather consists of a spectrum of viral burden. In our original definition of a scale for this spectrum, we used semiquantitative immunohistochemistry for HIV gp41. The present assessment of viral burden with HIV p24 antigen capture assay is in substantial agreement with the previously published gp41 assay. All subjects with high levels of HIV p24 in tissue extracts also had high CSF levels of p24 and high numbers of tissue macrophages that immunostained for HIV gp41. However, there were several cases with high numbers of gp41 immunostained macrophages without elevated CSF or tissue p24 levels (Table II, cases A90-30, A90-99, and A90-211). There could be multiple explanations for this disparity.

One explanation would be that HIV gp41 immunohistochemistry excessively labeled activated but not infected macrophages (false positive cells). We consider this possibility unlikely, since immunostains of other neurologic diseases (e.g., stroke, progressive multifocal leukoencephalopathy) (9) with abundant activated macrophages, do not show false positive staining. A second explanation is that HIV p24 antigen is less stable during the time between death and autopsy and therefore absence of detection is a false negative. This explanation also does not appear likely because there was no association between the postmortem interval and the antigen capture results. However, it has been our and others experience (31), that HIV p24 antigen is more difficult to preserve than gp41; e.g., immunohistochemistry for p24 requires frozen rather than paraffin sections. A third explanation is that those observations of a disjunction between gp41 and p24 concentrations are not artifact, but rather are evidence of differential transcription or translation of HIV in the CNS. Envelope proteins (e.g., gp41) are translated mostly from doubly-spliced mRNA, while gag proteins (e.g., p24) are translated from unspliced mRNA. Splicing is under exquisitely fine control of transcriptionally active regulator proteins like Tat and Rev. It would be reasonable to hypothesize that within CNS macrophages, there is preferential production of HIV envelope proteins leading to greater sensitivity of detecting infected cells with envelope markers. We favor this interpretation despite the fact that it is counter to previously published data (32), suggesting that in vitro macrophages produce less envelope protein.

HIV replication in CNS macrophages of AIDS patients would be expected to perturb the functions of these cells, including production of monokines (which could also affect the function of noninfected cells). As others have done, we chose antigen capture assays to study cytokines within the CNS in part because of the ease of quantitation. An additional advantage of this technology is that after various post mortem intervals, antigenicity would be expected to be more reliably preserved than biologic activity. Even with reliable quantitation, without knowledge of biologic inhibitors and cellular receptors, it may not be possible to predict biologic activity; e.g. high levels of cytokines may be inactive in the presence of high levels of biological inhibitors or absence of receptors).

Except for elevated TNF-α levels, the tissue concentrations of all of the cytokines were low. This may reflect the inadequacies of single end point assessments in a chronic disease or low sensitivity of the assay system. Temporally and focally elevated cytokines could cause microscopic damage that after several years would sum to significant CNS damage, and yet sampled at any one terminal time point, global tissue concentrations of individual cytokines may seem unremarkable. At the end stage when we assessed cytokine levels, target and producer cells may have been depleted (e.g., macrophages died because of HIV infection). Assessment of nonterminal timepoints will require appropriate animal models. Post mortem degradation of cytokines might also diminish signal; however, we chose cases with < 24 h postmortem time and we saw no correlation between postmortem time and cytokine levels. Several other disadvantages of the antigen capture technology also could be hypothesized to explain our inability to detect significant tissue concentrations of cytokines. The most serious disadvantage results from the extraction procedure. Locally concentrated cytokines may be diluted to low levels by mixing abundant extra- and intracellular compartments. Another disadvantage is that the tissue extracts are contaminated by vascular contents. However, given our predominantly negative findings of serum cytokine responses, concern about serum cytokine contamination would be limited to dilutional effects. To avoid these problems, some investigators have used immunohistochemistry to detect cytokines in CNS tissues (33); however, this nonquantitative technique is subject to its own vagaries, caused by diffusion of small soluble proteins. Although antigen capture ELISA is a sensitive technique, for a variety of technical reasons, it may underestimate the presence of biologically significant cytokines. We are in the process of assessing immune activation in the brain using chemiluminescent Western blots and mRNA-PCR.

Previous autopsies studies have suggested that HIV infection begins in the deep gray matter and later spreads to the neocortex. Elevated TNF-α levels appear to mimic this progression, being high in deep gray matter in "early" HIV encephalitis (low gp41 expression) and more equally elevated in cortical gray and white matter in severe HIV encephalitis (Fig. 1 *c*). The strong correlation between gp41 scores and TNF-α in the cortical gray matter may reflect the late arrival of HIV to the neocortex with late stimulation of TNF-α production.

Striking differences were found between CSF and tissue concentrations of the various compounds. There was no correlation between CSF and tissue measurements of IL-6; however, it is interesting to note that IL-6 was very high in the CSF and low in the parenchyma, while the opposite was the case with p24 measurements. This would suggest that while CSF can reflect tissue findings, the CSF and CNS parenchyma are clearly very different compartments of the body. Changes in one compartment may be completely independent of the other. There was a good correlation between CSF and tissue measurements of p24. This could imply good diffusion or mix-

ing of between these two compartments or alternatively systemic conditions permitting changes in the tissue compartment equally affect the CSF.

Brain parenchyma QUIN levels were markedly increased in the HIV-infected patients, particularly in the basal ganglia. Previously we have observed striking regional differences in CNS QUIN levels in conditions of focal inflammatory disease with significantly more QUIN in inflamed regions than in noninflamed ones (27, 29). These studies suggest that the transfer of QUIN from tissue into CSF occurs readily, the transfer of QUIN from the CSF into the brain parenchyma is slight. In the present study, the two cases where CSF QUIN levels were markedly increased without substantial elevations in tissue QUIN levels (Fig. 2 *b, squares*) were the patients with meningitis without signs of HIV encephalitis. It is likely that QUIN has been synthesized within the meninges rather than the brain parenchyma; when these two cases were excluded from the regression analysis, the correlation between CNS and CSF QUIN concentration was 0.88. The elevated CSF QUIN levels probably reflect locally synthesized QUIN in inflamed meninges rather than the brain parenchyma. HIV-infected patients with aseptic meningitis have substantial elevations in CSF QUIN but may have no or minimal symptoms of the AIDS dementia complex (26). Such lack of CNS dysfunction, in the face of elevated CSF levels of the neurotoxin, may therefore be explained by a lack of QUIN increases in the brain parenchyma. From a functional perspective, the source of QUIN is immaterial to the fact that QUIN levels are increased within the brain parenchyma of all patients compared to normal values.

Recently, we have reported that human macrophages in vitro stimulated with IFN-γ produce large amounts of QUIN, that is attributable to induction of the synthetic enzymes for QUIN, including indoleamine-2,3-dioxygenase (19, 27, 34). Indeed, increases in CNS QUIN levels and indoleamine-2,3-dioxygenase induction occur across a broad spectrum of inflammatory neurologic disease and brain injury, independent of HIV infection, and the increases in CNS QUIN are correlated with markers of macrophage and immune activation (neopterin, β_2-microglobulin, IgG and pleocytosis, if present) (23, 28, 35). While human post mortem studies of IDO are difficult because of enzyme lability, the present results tend to show that QUIN levels are related to the severity of inflammatory responses within the CNS. It is of note that the actual molar QUIN concentrations in the brain parenchyma that were achieved (up to 13,580 vs 80 nmol/kg in noninfected controls) exceeded the nanomolar/low micromolar levels already demonstrated to be neurotoxic to certain neuronal populations including organotypic corticostriatal neurons (20, 36). Interestingly, parenchymal levels of QUIN were highest in the basal ganglia, a region rich in the NMDA receptors that mediate the neurologic effects of QUIN (37). Because motor deficits and inflammatory neuropathologic lesions in these areas are characteristics of HIV encephalitis, it would be reasonable to hypothesize that QUIN may act as a pathological agonist of this receptor to mediate neurologic damage in these regions.

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