Macrophage/Microglial Accumulation and Proliferating Cell Nuclear Antigen Expression in the Central Nervous System in Human Immunodeficiency Virus Encephalopathy

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This study was performed to quantitate and characterize the mononuclear phagocytes (MPs) in human immunodeficiency virus encephalopathy (HIVE) by immunohistochemistry in an effort to gain insights into potential mechanisms of central nervous system (CNS) accumulation. Single- and double-labeled studies using antibodies against CD14, CD16, CD68, proliferating cell nuclear antigen (PCNA), Ki-67, von Willebrand factor, and HIV-1 p24 were performed using brain tissue from patients with HIVE, HIV-1 infection without encephalitis, and seronegative controls. A substantial increase in MPs was observed in CNS tissue from patients with HIVE, relative to seronegative controls and patients with acquired immune deficiency syndrome but without encephalitis, as determined by CD68 and CD16 immunohistochemistry. A large proportion of CD16+ MPs in HIVE CNS tissue were PCNA+, but do not appear to be proliferating, based on limited Ki-67 positivity. Although virtually all cells positive for HIV-1 p24 were PCNA+, there were many PCNA+ cells where HIV-1 p24 expression was not detected. PCNA positivity was also observed in some endothelial cells and ependymal cells in HIVE CNS. Our results would support a role for HIV-1-induced alterations in MP trafficking and homeostasis in the pathogenesis of HIVE. (Am J Pathol 2004, 164:2089–2099)

Human immunodeficiency virus type 1 (HIV-1)-associated neurological disorders, including dementia and peripheral neuropathies, affect ~30% of adults and almost all children with HIV-1 infection and acquired immune deficiency syndrome (AIDS).1–3 HIV-1 dementia complex is characterized histologically as HIV encephalitis (HIVE) with accumulations of perivascular macrophages, multinucleated giant cells, nodular lesions with areas of focal necrosis, and white matter thinning.4–6 The transmission of virus to the central nervous system (CNS) by macrophages during acute HIV-1 infection has been proposed to account for HIV-1 dementia complex and HIVE, even though both occur much later in the course of the disease.7

Unchecked, an active reservoir of HIV-1 infection in the CNS would presumably promote the development of clinical dementia. Accordingly, viral load in CNS and cerebral spinal fluid correlates with the severity of dementia.8,9 Cells productively infected with HIV-1 in the CNS appear to be cleared by the immune system early in the course of infection, before immune deficiency. Studies in primate and murine models emphasize the role of T cells in the control and clearance of the active HIV-1 reservoir in the CNS and support the notion that changes in immune status and loss of control of viremia contribute to the development of HIVD.10,11 The active reservoir of HIV-1 infection in the CNS, therefore, likely reflects a balance between the accumulation and clearance of infected cells.

There is considerable debate as to whether peripheral blood-derived macrophages and/or resident microglia constitute the major CNS reservoir of productive HIV-1 infection. By standard immunohistochemical methods, CD14 and CD45(LCA) expression is detectable in perivascular macrophages, but not in resident microglia.12–15 Studies by our group and others have used these immunophenotypic markers to characterize mononuclear phagocytes (MPs) in the CNS in HIV.16,17 In the HIVE brain, the perivascular MPs are CD14- and CD45(LCA)-positive. These perivascular MPs also express the FcyII receptor (CD16) and HLA-DR, and are,

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for the most part, productively infected, as determined by HIV-1 p24 immunostaining.\textsuperscript{16} In addition, mononuclear cells within the nodular lesions and multinucleated giant cells are CD14\textsuperscript{+}/CD45(LCA)\textsuperscript{+} and CD16\textsuperscript{+}/HIV-1 p24\textsuperscript{+}.\textsuperscript{16} The prominent CD16 expression on these perivascular macrophages is evidence for immunophenotypic similarity with circulating MPs in HIVD, supporting the hypothesis put forth by Pulliam and colleagues,\textsuperscript{18} that an increased proportion of circulating monocytes in individuals with HIVD leads to CNS invasion and contributes to the pathogenesis of HIV-1 in the CNS. The immunophenotype of the perivascular MPs in HIVD, therefore, shows phenotypic similarity with that of MPs previously invaded the CNS from the periphery and taken on microglial characteristics, ie, loss of CD45(LCA) and CD14 expression, on differentiation. These alternatives are not necessarily mutually exclusive. To begin to address the issue of MP proliferation and/or invasion into the CNS, we performed quantitative immunohistochemical analysis of MP subsets in HIVE using markers for proliferation [proliferating cell nuclear antigen (PCNA) and Ki-67], activation (CD16), HIV-1 infection (HIV-1 p24), and monocyte/macrophage markers (CD14 and CD68).

### Materials and Methods

#### Human Tissue Samples

Paraffin-embedded brain tissue sections from patients with HIVE were obtained from the Manhattan Brain Bank National Neuro-AIDS Tissue Consortium.\textsuperscript{19} Specimens from seronegative and HIV-1-positive adults without CNS disease were obtained from the Drexel University College of Medicine autopsy service. A total of nine HIVE cases, three HIV-1-positive cases without CNS disease, and five seronegative controls were analyzed (Table 1).

#### Immunohistochemistry

Immunohistochemistry was performed as previously described\textsuperscript{16} on 4-\textmu m brain tissue sections. Mouse monoclonal anti-human antibodies were used as follows: CD68 (clone KP1; NovaCastra, Newcastle, UK) was used at a 1:50 dilution; CD14 (clone 7, NovoCastra) was used at 1:50 dilution; CD16 (clone 2H7, NovaCastra) was used at 1:40; PCNA (clone PC10; DAKO, Carpinteria, CA) was used at 1:50 dilution; CD14 (clone 7, NovoCastra) was used at 1:40; CD16 (clone 2H7, NovaCastra) was used at 1:40; and CD14 (clone 7, NovoCastra) was used at 1:40.

#### Table 1. Patient Data

<table>
<thead>
<tr>
<th>Accession no.</th>
<th>Age/gender</th>
<th>HIV-1 status</th>
<th>CNS pathology</th>
<th>Non-CNS pathology</th>
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<td>HIV-01</td>
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<td>+</td>
<td>HIVE</td>
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<td>HIVE</td>
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<td>HIVE</td>
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<td>Metastatic carcinoma, sepsis</td>
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<td>–</td>
<td>Hypoxic/ischemic changes</td>
<td>Hepatic failure</td>
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used at a 1:50 dilution; Ki-67 (clone MM1, NovoCastra) used at a 1:25 dilution; and HIV-1 p24 (clone Kal-1, DAKO) used at 1:5. A rabbit polyclonal antibody against human von Willebrand Factor (Chemicon International, Temecula, CA) was used at 1:300. Tonsil and autopsy intestine from HIV-1-seronegative individuals were used as positive controls. Negative controls included tonsil, intestine, and brain tissues incubated in blocking solution with IgG1 and IgG2 isotype control antibodies at concentrations equal to the highest antibody IgG concentration. Primary antibodies were detected with biotinylated anti-mouse (monoclonal primaries), or anti-rabbit (polyclonal primary) IgG, avidin-biotin complex, and alkaline phosphatase-Vector Red (Vector Laboratories, Burlingame, CA) or peroxidase-3,3′-diaminobenzidine (Sigma Chemical Co., St. Louis, MO) according to manufacturers’ instructions. A tyramide signal amplification method was used (Catalyzed Signal Amplification System, DAKO) for detection of Ki-67 as recommended by the manufacturer with the following exceptions: primary antibody was diluted in antibody diluent with background reducing components (DAKO) and allowed to incubate on tissues overnight at room temperature. Endogenous peroxidase was quenched by incubating sections in 3% H2O2/methanol.

Double-label immunohistochemistry for PCNA/CD16 and PCNA/von Willebrand factor was performed by sequential application of primary antibodies to the same tissue section, as previously described. Subsequent to overnight exposure with the first primary antibody, sections were incubated with biotinylated secondary antibody followed by avidin-biotin complex and 3,3′-diaminobenzidine. Tissues were then taken back to buffer and blocking reagent, incubated in the second primary antibody overnight, followed by secondary antibody, avidin-biotin complex, and alkaline phosphatase-Vector Red, according to the manufacturer’s instructions.

Double-label immunohistochemistry for PCNA/p24 was performed by sequential application of primary antibodies to the same tissue section, which were revealed by systems with fluorescein and Texas Red tags, as previously described. The double-stained sections were examined using an inverted fluorescence microscope (Nikon, Melville, NY) through both fluorescein isothiocyanate and rhodamine filters. A digital photographic system (Princeton Instruments; IP Lab) was used to superimpose images and evaluate the degree of co-localization of the fluorescent stain products.

Statistical Analysis

The number of CD68+, CD14+, CD16+, and Ki-67+ cells was determined for each case by averaging the number of positive cells observed over 10 random microscopic fields at ×400 magnification. This magnification allowed for the discrimination of cellular features, including overlying nuclei in perivascular inflammatory clusters, and yielded an adequate number of countable profiles/field for data collection. Data were obtained using two independent observers for a total of 20 microscopic fields. The mean of the two averages was accepted as the average number of positive cells per field for each case. In the case of CD68, the total number of positive cells and total parenchymal-positive cells were counted separately. The means for the three groups, HIV-, HIV+/HIVE−, and HIVE+, were compared by one-way analysis of variance followed by Tukey-Kramer multiple comparisons posttest using Graph Pad Prism version 3.00 software, San Diego CA.

Results

Increased brain macrophages in HIVE have been previously reported. In the studies presented here, we extend our previous studies identifying two immunophenotypically distinct subpopulations of activated MPs in HIVE CNS tissue. Here we performed quantitative immunophenotypic analysis with additional markers to evaluate mechanisms involved in the accumulation of MPs in HIVE, ie, increased trafficking into the CNS and/or MP proliferation. We previously reported an abundance of CD14+/CD16+ MPs localized perivascularly in HIVE. These cells appeared morphologically and immunophenotypically distinct from the parenchymal microglial cells, which expressed CD16 as a marker of MP activation, however, CD14 expression was not generally detectable by our immunohistochemical methods in these ramified MPs. To quantitate the total MPs in brain specimens from patients with HIVE, HIV-1 infection (AIDS) but without encephalitis and seronegative controls, immunohistochemistry was performed using a monoclonal antibody against CD68, a molecule of unknown function found intracellularly in monocytes and macrophages. Analysis of variance and Tukey-Kramer multiple comparisons analyses revealed a statistically significant increase in total CD68+ cells in HIVE brain tissue sections when compared to brain tissue from patients with HIV-1 infection without encephalitis (P = 0.016) and seronegative controls (P = 0.002) (Figure 1A). Clusters of CD68+ cells, generally observed in the perivascular spaces of HIVE samples, where the individual cell borders could not be distinguished, were counted as a single cell. As such, the actual number of total brain macrophages in HIVE we report here are most likely an underestimate of the total number of MPs in HIVE brain tissue. Because of the reduced density of parenchymal MPs, a more accurate quantitation of MPs in parenchyma could be obtained. Here, the total number of parenchymal CD68+ cells was also significantly increased in HIVE (Figure 1B). Similar increases in parenchymal MPs in HIVE were observed with tissue sections stained for CD16, relative to HIV+/HIVE− (P = 0.03) or normal (P = 0.005) (Figure 1C). Although we previously characterized the majority of parenchymal CD16+ MPs in HIVE as CD14−, we had noted scattered CD14+ cells with short processes. We observed restricted areas of parenchymal MP expression of CD14 in three of nine HIVE cases; overall there was only limited parenchymal CD14 positivity in HIVE brain. As illustrated graphically in Figure 1D, the difference in parenchymal CD14+ cells between seronegative, HIV-1/AIDS without encephalitis, and HIVE brain sections did
not reach statistical significance. We cannot rule out the possibility that an increase in CD14+/H11001 MPs in parenchyma in HIVE would reach significance with a larger number of samples. The P value was lower, P/0.114, yet did not reach statistical significance after combining the HIV/AIDS and seronegative groups and comparing the total non-encephalopathy group with HIVE by Student’s t-test (assuming unequal variances).

To begin to address potential mechanisms that could account for the increase in brain monocytes/macrophages in HIVE, we performed immunohistochemical analysis using antibodies recognizing markers associated with proliferation, ie, PCNA and Ki-67. PCNA+ nuclei were observed in the parenchyma of both cortex and white matter, as well as around blood vessels in patients with HIVE (Figure 2, C and G). Nodular lesions, including multinucleated giant cells, were also PCNA+ (Figure 2I). A smaller number of positive cells (an average of 0.1 cells per microscopic field) were seen perivascularly in the brains of patients with HIV-1 infection but without encephalitis (Figure 2F). Brain tissue from seronegative controls were negative for PCNA staining (Figure 2, A and E). The number of PCNA-positive cells was significantly increased in HIVE relative to seronegative controls using the Tukey-Kramer test for multiple comparisons (P/0.048) (see Figure 4A). The difference between HIV-1/AIDS and HIVE did not reach significance in multiple comparison tests, however the number of patients is small with reasonably large standard deviations because of local variations in staining within specimens. Furthermore, after combining the two HIV-1/AIDS and control groups into HIVE− and HIVE+, the encephalopathy group had increased PCNA positivity relative to the patients without encephalopathy (P/0.01) by Student’s t-test, assuming unequal variances as a conservative approach.

Because PCNA positivity is indicative of either DNA replication or DNA repair, additional studies were performed using an antibody against human Ki-67, a more definitive marker of cell proliferation. In contrast to the results with PCNA, Ki-67 positivity was detected in a relatively small number of parenchymal nuclei in HIVE specimens (Figure 3C). Rare positive cells were also observed in the perivascular cuffs in HIVE (Figure 3F),
but not in multinucleated giant cells (Figure 3H) as well as
in brain tissue from patients with HIV-1/AIDS but without
encephalitis and seronegative controls (Figure 3; A, B, D,
and E). Although there was a small increase in the num-
ber of Ki-67-positive cells in HIVE relative to HIV-1/AIDS
and seronegative controls, the differences were not sta-
tistically significant (Figure 4B). Additionally, these differ-
ences did not reach statistical significance after combin-
ing the HIV-1/AIDS and control groups ($P = 0.089$).

Human autopsy intestine, as a positive control, showed a
large proportion of positive nuclei confirming the sensi-
tivity of the tyramide signal amplification technique used
in this study in postmortem tissue (Figure 3G).

Previously, we reported CD16 expression in two pop-
ulations of cells representing the major reservoirs of
HIV-1 infection in the CNS, namely the CD14
perivas-
cular macrophage and CD14-negative parenchymal
ramified microglial cells (also shown in Figure 2D).$^{16}$ With
single-labeling immunohistochemistry, CD16+ cells were
once again observed perivascularly and within microglial
nodules (Figure 2, H and J). To determine the relationship
between these activated MPs and PCNA expression,
PCNA/CD16 double-label immunohistochemistry was
performed on the same CNS tissues. In HIVE, a consid-
erable number of PCNA+ cells were also CD16+ in the
perivascular space (Figure 5C), in parenchyma (areas
not in obvious association with blood vessels) (Figure 5,
A and B) and within nodular lesions (Figure 5, D and E).
Many multinucleated giant cells, not associated with nod-
ules, were also positive for both antigens (Figure 5F).

Although PCNA staining often co-localized with CD16,
not all CD16+ cells were PCNA+. Conversely, not all
PCNA+ cells were CD16+. Some PCNA+/CD16+ cells
observed along blood vessel walls were oblong, sug-
gesting endothelial cell morphology. Double-label immu-
nohistochemistry, using antibodies against PCNA and
d von Willebrand factor, confirmed the PCNA positivity of
some endothelial cells (Figure 6, A and B) in HIVE brain
tissue.

To determine whether PCNA expression occurred in
association with productive HIV-1 infection, double-label
immunofluorescence was performed on CNS tissues us-
ing antibodies against PCNA and the HIV-1 p24 gag
protein. PCNA/HIV-1 p24 double-labeling revealed that
HIV-1 p24 co-localized with PCNA in approximately half
of the PCNA+ cells observed in the parenchyma, around
blood vessels, and within microglial nodules (Figure 7; C,
F, and I, arrows) of patients with HIVE. In contrast, virtu-
ally all of the cells positive for HIV-1 p24 antigen were also positive for PCNA. Multinucleated giant cells were also generally positive for both antigens (Figure 7C, short-tailed arrows). Ependymal cells were positive for PCNA, but not HIV-1 p24 (Figure 7; J to L). Additional cells were PCNA-positive, but HIV-1 p24-negative in Figure 7, J and L, apparently within the subependymal region.

Discussion

Our results demonstrate an increase in the number of macrophages/microglia in the CNS in HIVE, in agreement with earlier reports suggesting an important role for MPs in the development of HIVE.5,6,20 Our previous studies demonstrated that there are two phenotypically distinct MP cell populations in HIVE CNS tissue. The perivascular macrophages are CD14+/CD16+, whereas the CD16+ cells in white matter, in areas not clustered around blood vessels, are CD14− with ramified morphology. These CD16+ MP populations seem to represent the predominant sources of CNS virus production in HIVE.16,21 In HIVE, perivascular macrophages share a similar immunophenotype with activated monocytes/macrophages in circulation,22 supporting a similar origin. The origin of the CD16+ ramified cells in parenchyma remains uncertain. The characterization of MPs accumulating around blood vessels and in microglial nodules as perivascular macrophages, rather than resident microglia, is based on differential expression of CD14 and CD45/LCA. Using standard immunohistochemical techniques (as in this study), these markers are detected on perivascular macrophages, but not resident microglia.14,23 Microglia are

Figure 3. Ki-67 immunohistochemistry. A and D: Normal brain. B and E: HIV-1 without encephalopathy. C, F, and H: HIVE. G: small intestine from a seronegative individual. The top row (A–C) illustrates white matter. D–F: Blood vessels. H: A multinucleated giant cell within a microglial nodule. PCNA+ brain tissue from patients with HIVE showed only occasional Ki-67-labeled nuclei, as demonstrated in C (white matter) and F (blood vessel). H: Multinucleated giant cells did not show Ki-67 positivity. Rare positivity was observed in white matter of patients with HIV-1 infection without dementia (B) and seronegative controls (A). E: Limited positive cells were also located perivascularly in HIVE+ brain tissue. G: Autopsy intestine from a seronegative individual shows abundant nuclear Ki-67 positivity with the CSA technique. Original magnifications: ×40 (A–G), ×100 (H).
Our results do not rule out the possibility that MP or MP precursor proliferation is altered outside the CNS, ie, in bone marrow. The number of CD14+/CD16+ cells has been demonstrated to be increased in circulation as well as in CNS tissue. If these activated MPs are indeed more invasive as has been suggested,18 the detection of high levels in both circulating and CNS compartments might suggest an increase in the generation kinetics of these cells, or an increase in survival time, possibly even recirculation of tissue MPs back into the circulation. Interestingly, bone marrow from patients with HIV-1 infection and AIDS has been reported to contain a statistically significant increase in PCNA+ macrophages, when compared to normal marrow, but these cells did not express detectable levels of topoisomerase IIα, a marker for DNA rep-
In the absence of corroborating evidence with topoisomerase IIα, it was proposed that the observed PCNA expression was because of virus-induced DNA damage, in accordance with the involvement of PCNA in unscheduled DNA repair. The PCNA+ cells, however, were not analyzed for viral gene expression in that study. In a recent study in SIV, co-localization of SIV p27 antigen with PCNA in bone marrow macrophages has been observed.33

In our study, HIV-1 infection was clearly associated with PCNA expression in CNS, in agreement with a similar study in SIV reported by Williams and colleagues.32 PCNA/p24 double-labeling revealed that all cells positive for p24 were PCNA+. In contrast, not all PCNA-expressing cells appeared to be productively infected, in which no more than half of the PCNA+ cells also had detectable levels of HIV-1 p24. Although many of the PCNA+/p24− cells were in the vicinity of productively infected cells, there were also large numbers of PCNA+/p24− cells that did not appear to be within the proximity of infected cells. We cannot exclude the possibility, however, that neighboring infected cells were outside the plane of the section. Additionally, PCNA positivity was observed in endothelial cells, without additional evidence for neovascularization in CNS. PCNA positivity was also observed in ependymal cells, which reportedly do not proliferate in adults.35 These results suggest that PCNA expression is induced by viral infection (ie, by the expression of viral gene products) and possibly by indirect mechanisms as well. The observation of PCNA-positive cells in the vicinity of MPs where HIV-1 p24

Figure 5. PCNA/CD16 immunohistochemistry. All panels show PCNA/CD16 double immunohistochemistry on brain tissue from a patient with HIVE (HIVE 08). In HIVE, PCNA (brown) and CD16 (pink) double-positive cells were observed in white matter (A and B), around blood vessels (C), and within microglial nodules (D and E). B: Inset of A. E: Inset of D. Arrows point to cells with co-localized PCNA and CD16 expression. Some PCNA+ cells in white matter and around blood vessels did not express detectable levels of CD16 (A–C, arrowheads). Multinucleated giant cells, not associated with microglial nodules, were also positive for both antigens (F). Original magnifications: ×40 (A–E), ×100 (F).

Figure 6. PCNA/von Willebrand factor immunohistochemistry. All panels show PCNA/von Willebrand factor double immunohistochemistry on brain tissue from a patient with HIVE (HIVE 08). In brain tissue from patients with HIVE, several endothelial cells, which are positive for von Willebrand Factor (pink), were also PCNA-positive (brown). Arrows indicate double-positive cells. Original magnifications: ×40 (A), ×100 (B).
was not detectable might suggest that PCNA expression may be also induced through cytokine dysregulation, secreted viral proteins, or other diffusible factors.36

Our results demonstrate a 10- to 20-fold increase in the total number of CD68+ macrophages/microglia in HIVE CNS tissue. Accordingly, no more than 5 to 10% of the microglia are probably long-term residents in this disease. Our results support the potential role of altered monocyte/macrophage trafficking in the pathogenesis of HIVE. In our study, many PCNA+ cells were also seen within blood vessel lumina. Therefore, cells that are already PCNA+ might enter the CNS compartment from the periphery. In support of this notion, sequence analysis of virus found in brain has been best correlated with virus in bone marrow.37 Although these results suggested trafficking of infected monocytes from bone marrow to the CNS, it is also possible that CNS and bone marrow are invaded by a common source of monocytes/macrophages.

The notion of altered trafficking kinetics of MPs into the CNS does not necessarily conflict with the notion of a compartmentalized infection in CNS. Although it has been demonstrated that HIV-1 envelope sequences in CNS are most closely related to sequences in peripheral blood and bone marrow,38 it is possible that some MPs are infected as they enter the CNS. Furthermore, there is

Figure 7. PCNA/p24 immunofluorescence. All panels show PCNA/p24 immunofluorescence on brain tissue from a patient with HIVE (HIVE 08). fluorescein isothiocyanate-labeled PCNA+ cells (green) were observed in white matter (A), around blood vessels (D), within microglial nodules (G), and in ependymal cells (J). Texas Red-labeled HIV-1 p24+ cells (red) were observed in white matter (B), around blood vessels (E), and within microglial nodules (H), but not in ependymal cells (K). Approximately half of the PCNA+ cells were also p24+ (yellow) (C, F, and I). Arrows point to cells that are positive for PCNA and p24. Some multinucleated giant cells, not associated with nodules, in white matter were also positive for both antigens (C, short-tailed arrows). Although virtually all p24+ cells were PCNA+, many PCNA+ cells were negative for HIV-1 p24, including cells located in white matter, perivascularly, and within microglial nodules (C, F, and I, arrowheads), as well as all ependymal cells (L, arrowheads). Original magnifications, ×40.
clearly sequence heterogeneity with respect to different areas of the brain. Virus and virus-infected cells entering the CNS from the periphery may present new opportunities for recombination, adaptation, and evolution within the brain microenvironment, as suggested by the mosaic nature of CNS isolates when compared to sequences within the periphery. The relationship between the blood compartment and the CNS is further demonstrated by biological comparison with respect to the frequency of co-receptor utilization specificities. It is possible that some of the differences that have been observed in comparison of brain sequence with other organs may reflect differences in cell populations infected by HIV-1 in particular tissues (ie, the abundance of T cells in lymphoid tissue, but not brain) or, alternatively, mechanisms of immune selection as has been inferred from differences in T-cell epitopes in viruses isolated from different organs. Further studies will be needed combining viral-genetic characterization with immunohistochemical analysis to characterize virus within individual cell populations in multiple organs.

In summary, our results demonstrate substantial increases in total numbers of perivascular macrophages and parenchymal microglia in HIVE. The magnitude of this increase, along with the demonstrated increase in circulating activated MPs in HIVD, suggests the role of altered monocyte/macroage trafficking and homeostasis in the pathogenesis of HIVD. This conclusion is further supported by the response of patients with HIVD to highly active anti-retroviral therapy, in which pharmacotherapies poorly penetrate the blood brain barrier,1,4,23 with the concomitant reduction in circulating CD16+ MPs. Additional studies will be necessary to elucidate the mechanisms involved in PCNA expression observed in cells of the CNS, particularly the monocyte/macrophage, and the potential role of cytokines and/or viral proteins in promoting up-regulation of this antigen. Furthermore, monocyte/macrophage trafficking studies will be needed to help identify alterations in homeostatic parameters contributing to the accumulation of MPs in the CNS in HIVE.

Acknowledgments

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