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Macrophage-Induced Inflammation Affects Hippocampal Plasticity and Neuronal Development in a Murine Model of HIV-1 Encephalitis

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KEY WORDS
hippocampus; neurons; PSA-NCAM; cell proliferation; astrocytes; microglia; Ki-67

ABSTRACT
Cognitive, behavioral, and motor impairments, during progressive human immunodeficiency virus type 1 (HIV-1) infection, are linked to activation of brain mononuclear phagocytes (MP; perivascular macrophages and microglia). Activated MPs effect a giant cell encephalitis and neuroinflammatory responses that are mirrored in severe combined immunodeficient (SCID) mice injected with human monocyte-derived macrophages (MDM). Whether activated human MDMs positioned in the basal ganglia affect hippocampal neuronal plasticity, the brain subregion involved in learning and memory, is unknown. Thus, immunohistochemical techniques were used for detection of newborn neurons (polysialylated neuronal cell adhesion molecule [PSA-NCAM]) and cell proliferation (Ki-67) to assay MDM effects on neuronal development in mouse models of HIV-1 encephalitis. Immunodeficient (C.B.-17/SCID and nonobese diabetic/SCID, NOD/SCID) and immune competent (C.B.-17) mice were injected with uninfected or HIV-1-infected MDM. Sham-operated or unmanipulated mice served as controls. Neuronal plasticity was evaluated in the hippocampal dentate gyrus (DG) at days 7 and 28. By day 7, increased numbers of Ki-67$^+$ cells, PSA-NCAM$^+$ cells and dendrites in DG were observed in sham-operated animals. In contrast, significant reductions in neuronal precursors and altered neuronal morphology paralleled increased microglial activation in both HIV-1-infected and uninfected MDM-injected animals. DG cellular composition was restored at day 28. We posit that activated MDM induce inflammation and diminish DG neuronal plasticity. These data provide novel explanations for the cognitive impairments manifested during advanced HIV-1 infection.

INTRODUCTION

Progressive human immunodeficiency virus type 1 (HIV-1) infection commonly leads to cognitive, motor, and behavior deficits (reviewed in Lipton and Gendelman, 1995; Gendelman et al., 2004). Of all deficits, those affecting memory and learning are most debilitating (Navia et al., 1986; Pumarola-Sune et al., 1987; Rottenberg et al., 1987). HIV-1 infection induces an encephalopathy mediated by brain mononuclear phagocytes (MP; perivascular macrophages and microglia), MP neurotoxic secretions initiate from subcortical brain regions (Neuen-Jacob et al., 1993; Kieburtz et al., 1996; Wiley et al., 1998; Patel et al., 2002). Over time, this can lead to HIV-1-associated dementia (HAD) (McArthur et al., 2003). Neuropathologic abnormalities associated with HAD include multinucleated giant cell formation, myelin pallor, astrogliosis, and neuronal dropout and are termed HIV-1 encephalitis (HIVE) (reviewed in De Girolami et al., 2004).

A recurring question in the neuropathogenesis of HIV-1 infection is how subcortical disease affects global memory deficits. One explanation is that an autocrine and paracrine amplification of cellular and viral products released from activated and HIV-1-infected macrophages affects hippocampal learning and memory (Zink et al., 2002; Anderson et al., 2003). Alternatively, but not mutually exclusive, is the fact that MP inflammatory responses directly affect hippocampal development. Neurogenesis (formation of newborn neurons) occurs within the dentate gyrus (DG) of the hippocampus (Altman and Das, 1966; Ekdahl et al., 2003; Krathwohl and Kaiser, 2004) and in adulthood is identified as an integral part of mammalian neuronal plasticity. Adult neurogenesis and neuronal plasticity (survival of newborn neurons, growth, branching, elongation, and incorporation in neuronal circuit) are stimulated by traumatic injury, neurotrophins, and environmental cues. In contrast, the identical processes are suppressed by age, stress, toxins, and inflammation (Ekdahl et al., 2003; Monje et al., 2003; Parent, 2003).

In the current study, HIV-1-infected monocyte-derived macrophages (MDM) were injected into the brains of severe combined immunodeficient (SCID) mice. Adaptive immunity was assessed using nonobese diabetic/SCID (NOD/SCID) mice reconstituted with human peripheral blood lymphocytes (hu-PBL-NOD/SCID) (Poluektova

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et al., 2002, 2004) before MDM brain injections. Parental C.B.-17 mice served as controls for mutations in DNA-dependent protein kinase (DNA-PK) present in SCID mice (Culmsee et al., 2001). Traumatic injection into the basal ganglia stimulated neuronal development and glial reactions in DG of SCID and NOD/SCID mice with or without hu-PBL reconstitution. Significant inhibition of neuronal plasticity and neurogenesis was observed in SCID mice injected with uninfected or HIV-1-infected human MDM. The number of newborn PSA-NCAM$^+$ neurons in the DG subgranular layer was reduced at 1 week after injection, but was restored at 28 days. Severe inflammation triggered by HIV-1-infected MDM diminished neuronal development in wild-type C.B.-17 mice. We conclude that inflammatory responses induced by activated MDM, rather than HIV-1 infection, per se, reduce neuronal development in the DG. These observations provide yet another explanation for how cognitive and memory deficits can occur in advanced HIV-1 disease.

MATERIAL AND METHODS

Cells and Viruses

Monocytes and PBL obtained from HIV-1, HIV-2, and hepatitis B seronegative donor leukopaks, were separated by countercurrent centrifugal elutriation as previously described (Gendelman et al., 1988). Monocytes were cultivated in DMEM (Sigma-Aldrich, St. Louis, MO) supplemented with 10% heat-inactivated pooled human serum, 1% glutamine, 50 μg/ml gentamicin, 10 μg/ml ciprofloxacin (Sigma), and 1,000 U/ml highly purified recombinant human macrophage colony-stimulating factor (CSF; a generous gift from Wyeth-Pharmaceuticals, Cambridge, MA). After 7 days in culture, MDM were infected with HIV-1_{ADA} (Gendelman et al., 1988) at a multiplicity of infection (MOI) of 0.01.

Mice and Cell Injections

Four-week-old male C.B.-17 and C.B.-17/SCID mice were purchased from Charles River Laboratories (Wilmington, MA). NOD/SCID mice were purchased from the Jackson Laboratory (Bar Harbor, ME). Animals were maintained in sterile microisolator cages under pathogen-free conditions in accordance with the ethical guidelines for care of laboratory animals at the University of Nebraska Medical Center and the National Institutes of Health (NIH); 10 μl of saline (sham-operated), 3×10^5 cells/10 μl of uninfected MDM (MDM group) or HIV-1_{ADA}-infected MDM (HIVE group) were injected intracerebrally (i.c.) into the basal ganglia with previously described coordinates: 3.5 mm behind the bregma, 3.5 mm lateral from the sagittal midline, a depth and angle of 4.0 mm and 35° from the vertical line (Persidsky et al., 1996). NOD/SCID mice were reconstituted with human PBL (50×10^6 cells/mouse) and after 7 days injected i.c. with syngeneic HIV-1-infected (HIVE) or uninfected autologous MDM. Replicate PBL reconstituted animals did not receive MDM injections. Animals were included in the analysis if ≥30% of the total spleen cells were human CD45$^+$/CD3$^+$ leukocytes. C.B.-17/SCID mice were sacrificed at days 7 and 28. NOD/SCID and C.B.-17 control mice were sacrificed at day 7. Unmanipulated animals served as age-matched controls (per each strain and time point).

Immunocytochemical Analysis

Brain tissue was collected at necropsy, fixed in 4% phosphate-buffered paraformaldehyde, and embedded in paraffin or frozen for later use. Blocks were cut to identify the injection site. For each mouse, ~100 serial (5-μm-thick) sections were cut from the injection site and at the level of the hippocampus. Sections were deparaffinized with xylene and hydrated in gradient alcohols. Immunohistochemical staining utilized followed a basic indirect protocol, using antigen retrieval by heating to 95°C in 0.01 M citrate buffer for 30 min for all antigen markers except polysialylated neuronal cell adhesion molecules (PSA-NCAM), which is acid-sensitive. On serial sections, immature neurons were localized with Ab to PSA-NCAM (mouse IgM, 1:1,000, generously provided by Dr. T. Seki, Jutendo University School of Medicine, Tokyo, Japan). Murine microglia and astrocytes were detected with rabbit polyclonal Abs to ionized calcium-binding adapter molecule 1 (Iba-1; 1:500; Wako, Richmond, VA) and glial fibrillary acidic protein (GFAP, 1:1,000; Dako, Carpenteria, CA), respectively. Dividing cells were detected with monoclonal antibody (mAb) to Ki-67 (1:50, mouse IgG clone B56, BD PharMingen, San Jose, CA). The polymer-based horseradish peroxidase (HRP)-conjugated anti-mouse and anti-rabbit Dako EnVision systems and polymer-based alkaline phosphatase-conjugated PowerVision™ (ImmunoVision Technologies, Daly City, CA) systems were used as secondary detection reagents, and 3,3′-diaminobenzidine (DAB) and Permanent Red (Dako) were used as respective chromogens. For double staining, primary Ab was applied simultaneously, and development steps were performed separately. All paraffin-embedded sections were counterstained with Mayer's hematoxylin. Deletion of primary Ab served as a control. Tissue examination was performed with a Nikon Eclipse E800 (Nikon Instruments, Melville, NY). Images were obtained by Optronics digital camera (Buffalo Grove, IL) with MagnaFire 2.0 software (Goleta, CA) and processed by Adobe® Photoshop® 7.0 software.

In Vivo Cell Labeling

Ten C.B.-17/SCID 4-week-old mice were used in studies of *in vivo* analysis cell proliferation. The thymidine analogue BrdU (Sigma) was administered by continuous infusion into the left lateral ventricle using an Alzet 2004 osmotic pump. The flow rate/concentration of BrdU administered in 0.9% saline was 0.25 μl/150 mg/ml/h (Zhao et al., 2003) for 7 or 28 days, at the time of animal

TABLE 1. Mouse Strains, Experimental Conditions, and Numbers of Brain Slices Analyzed

Mouse strains	Experiments performed	Experimental conditions	Brain sections analyzed[a]
C.B.-17/SCID	3	Control,[b] $n = 6$	26
		Sham-operated, $n = 6$	24
		MDM, $n = 12$	47
		HIV-1 MDM, $n = 13$	52
NOD-SCID	2	Control,[b] $n = 9$	40
		hu-PBL, $n = 3$	12
		hu-PBL, sham-operated, $n = 3$	14
		hu-PBL, MDM, $n = 6$	28
		hu-PBL, HIV-1 MDM, $n = 12$	45
		HIV-1 MDM, $n = 9$	36
C.B.17	1	Control,[b] $n = 3$	12
		Sham-operated, $n = 3$	12
		MDM, $n = 3$	12
		HIV-1 MDM, $n = 3$	12

[a]$\geq$4 sets of 5-μm serial brain tissue sections were analyzed after immunostainings for PSA-NCAM, GFAP, Iba-I, and Ki-67 antigens.
[b]Control animals were unmanipulated. Hu-PBL were those mice reconstituted with human lymphocytes. Sham-operated animals were injected i.c. with saline only. HIV-1_{ADA}-infected MDM (HIV-1 MDM) induced a focal encephalitis consisting of multinucleated giant cells, astrogliosis, and neuronal drop-out analogous to what is observed in human HIV-1 encephalitis.

sacrifice. Before staining for BrdU (mouse mAb clone BU33, 1:100, Sigma), DNA was denatured and unmasked using 2N HCl and 0.4% pepsin.

DG Neural Cell Composition

Sets of serial sections were selected at defined distances from bregma (−1.4 to −2.8 mm) and were ~50 μM apart (Table 1). Images were obtained and analyzed under magnification ×100. Cells within the DG were counted by computer-assisted image analysis (Image-Pro®Plus, Media Cybernetics, Silver Spring, MD). A manual trace of the outline of each neuron (cell bodies and processes), astrocytes, and microglia, together with digital measurements of the length and precise path of each neurite, was made. Cell analysis of brain tissue sections were done in a blinded fashion by two investigators as previously described (Zheng et al., 2004). PSA-NCAM$^+$ cells were distributed in linear fashion along the inner border of the granular layer. To normalize data collected from diverse brain tissue sections, the density of PSA-NCAM$^+$ cells was adjusted to 1 mm of the line length/section. Dividing Ki-67$^+$ cells, astrocytes, and microglia were distributed throughout the DG, including the hilus. For normalization, these cell numbers were adjusted to 1 mm^2 of DG surface area/section (including the granular layer and hilus) (Fig. 1). No significant differences between cell numbers in the ipsilateral and contralateral hemispheres were found within any group.

Statistical Analysis

Mean value and standard error of the mean (SEM) were calculated for each parameter tested. Student's *t*-test and one-way analysis of variance (ANOVA) were performed using Microsoft Excel. Differences were considered statistically significant at $P < 0.05$.

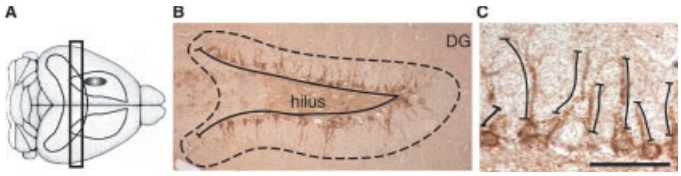

Fig. 1. Cytological analyses. **A:** Brain subregion selected for analysis in the hippocampus (black rectangle), and excludes the injection area (oval). **B:** Outlined area of analysis that includes the DG granule cell layer and hilus (dashed line, measured in mm^2/section). Solid line represents the length of SGL (measured in mm/section). The number of PSA-NCAM$^+$ cells was adjusted to the length of the DG (solid line). **C:** Measurements of the length of PSA-NCAM$^+$ dendrites. Section stained with PSA-NCAM. B, ×100; C, ×1,000. Scale bar = 25 μm in C.

RESULTS

Effects of Trauma and MDM-Induced Inflammation on Hippocampal Neurogenesis

Hippocampal neural stem cells are present within the inner border of the granule cell layer (GCL) and during asymmetric division produce PSA-NCAM$^+$ transit-amplifying cells (neuroblasts), which in turn produce PSA-NCAM$^+$ immature neurons (Doetsch et al., 1997; Kempermann et al., 2004a,b). Inflammatory responses generated from HIV-1-infected and uninfected MDM injected in the basal ganglia affected neuronal plasticity and neurogenesis in the DG. Sham-operated mice served as positive neurogenesis controls, since trauma alone stimulates generation of new neurons (Dash et al., 2001; Kernie et al., 2001; Yoshimura et al., 2003). Unmanipulated mice served as negative controls. Evaluation of histopathology, glial inflammation, and neurogenesis was performed on days 7 and 28 after injection. Table 1 shows the number of experiments, mouse strains, experimental conditions, and numbers of brain tissue sections analyzed for all experiments performed.

Neurogenesis and plasticity were assessed by PSA-NCAM$^+$ and Ki-67$^+$ staining within the DG. These represent newborn neurons, as small round PSA-NCAM$^+$ cells with dark nuclei bordering the hilus and the DG GCL, as well as ongoing neuronal plasticity, as dendrites outgrowth (cells with apical dendrites or with a marked dendritic processes extending from the GCL up to molecular layer) (Seki, 2002a,b) (Fig. 2A). Fewer large PSA-NCAM$^+$ cells and their dendrite extensions were located inside the GCL and were co-localized with GFAP$^+$ astrocytes (Fig. 3A).

At day 7 after traumatic injury, the density of small PSA-NCAM$^+$ cells, numbers of dendrites, and dendrite length increased significantly compared with unmanipulated animals (Fig. 2B). MDM injection (uninfected or HIV-1-infected) into the basal ganglia reduced the numbers of PSA-NCAM$^+$ cells in the DG when compared with sham-operated controls ($P < 0.001$). Neural cell morphology was also changed. Dendrite length was significantly reduced (Fig. 2C, $P < 0.02$). At 28 days after

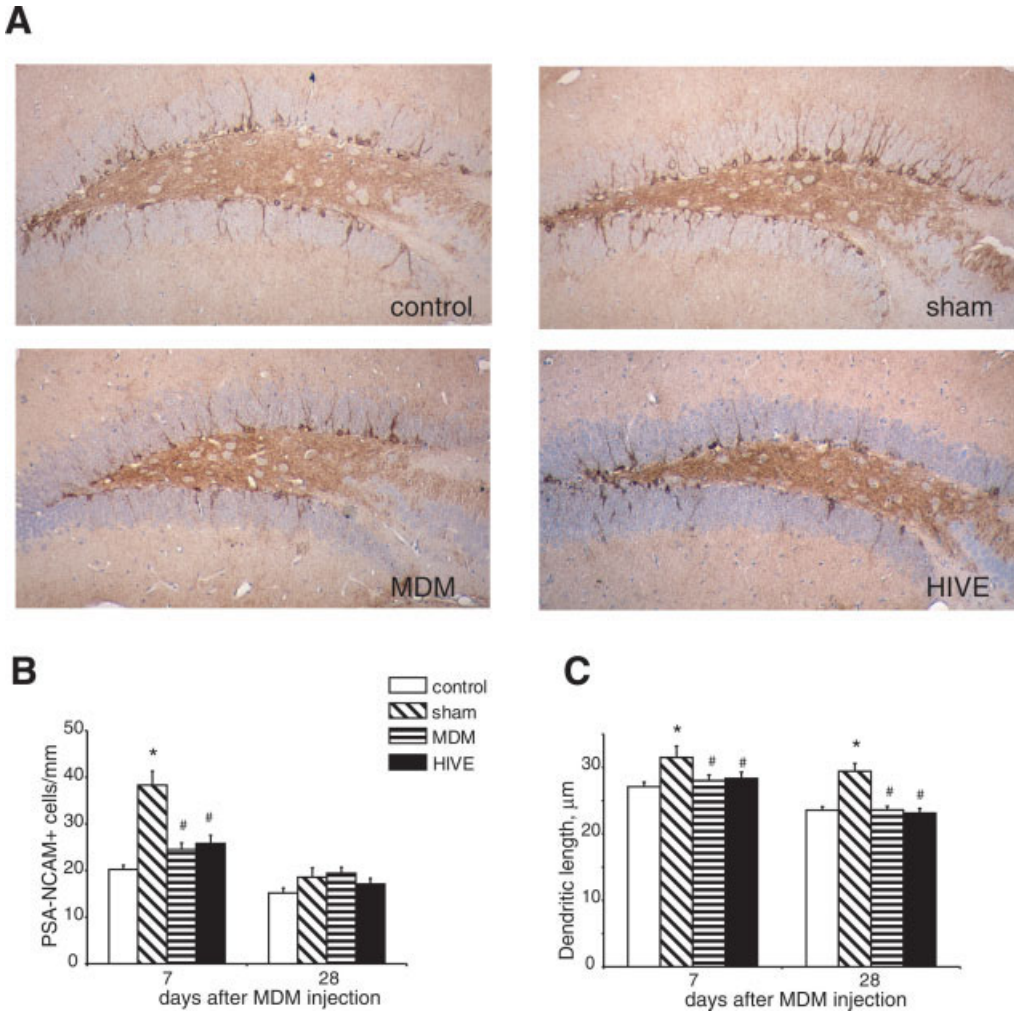

Fig. 2. Quantitative morphologic determinations of PSA-NCAM$^+$ cells. Expression of PSA-NCAM in DG was upregulated in sham-operated animals, but reduced in mice injected with uninfected or HIV-1-infected MDM. **A:** Morphological changes in sham-operated, MDM and HIVE mice compared with uninjected animals. Stained with PSA-NCAM, counterstained with hematoxylin. **B:** Statistical analysis (mean ± SEM) of the number of cells adjusted to the length of DG ($P < 0.001$; ANOVA). **C:** Average length of dendrites ($P < 0.02$; ANOVA). *Differences with uninjected mice; #differences with sham-operated animals. A, ×100.

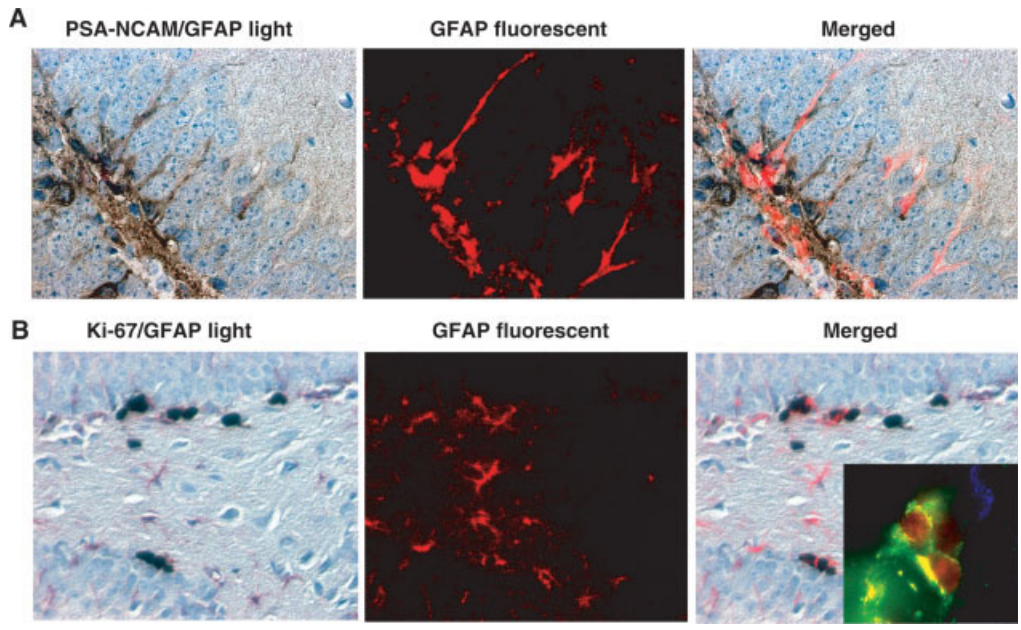

Fig. 3. Co-localization of GFAP$^+$ astrocytes (Permanent Red) with PSA-NCAM (A) and Ki-67 (B) (brown, DAB). Counterstained with hematoxylin. Insert (B, right) represents triple fluorescent staining for Ki-67 (red), PSA-NCAM (green), and GFAP (blue). A, ×600; B, ×1,000.

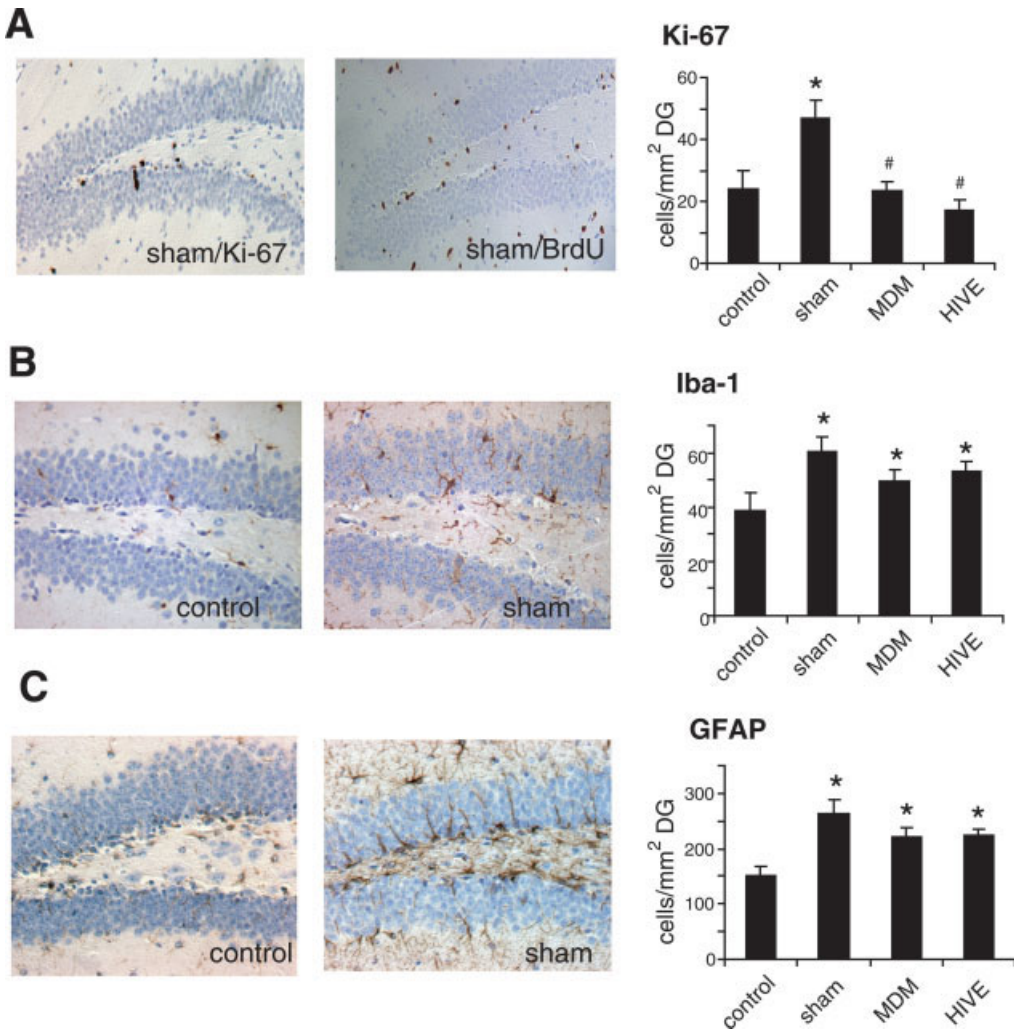

Fig. 4. Quantitative and morphologic analysis of the DG cellular composition on day 7 post-injection in SCID mice. MDM-induced inflammatory responses reduced the number of proliferating cells, but did not affect injury-associated increase of the number of glial cells. **A:** Sections stained with Ab to glial Ki-67 and BrdU (DAB, brown) in sham-operated animals. Graph represents quantification of the number of dividing Ki-67$^+$ cells per mm^2 of the DG. *Differences with uninjected mice; $^{\#}$differences sham-operated animals ($P < 0.001$; ANOVA). **B:** Sections stained with Ab to microglial marker Iba-1 in control (unmanipulated) and sham-operated mice. Graph represents quantification of the number of Iba-1$^+$ cells per mm^2 of the DG. *Differences with uninjected mice ($P < 0.02$; ANOVA). **C:** Sections stained with Ab to GFAP in control and sham-operated mice. Graph represents quantification of the number of GFAP$^+$ cells per mm^2 of the DG. *Differences with uninjected mice ($P < 0.03$; ANOVA). C, × 400.

injection, no differences were seen in PSA-NCAM$^+$ neuron numbers between groups with the exception of a modest, but significant, reduction in dendrite length in MDM and HIVE animals compared with sham-operated controls ($P < 0.02$) (Fig. 2C).

Glial Inflammatory Responses and MDM-Induced Reduction of Cell Division

We examined the affects of MDM on neural proliferation and glial inflammatory responses within the DG. Common dividing cells in the DG are neuronal lineage-restricted doublecortin-positive and PSA-NCAM$^+$ transit-amplifying type cells (neuroblasts) (Fig. 3B, insert). DG cell proliferation was determined by immunoreactivity to Ki-67, a biomarker for cell division, which is expressed at all phases of the cell cycle except for G_o (Kee et al., 2002). *In vivo* labeling of DG cell proliferation after intraventricular infusion of BrdU in SCID mice showed similar distribution of dividing cells in DG, with some accumulation in subgranular cell layer (SGL) and diffuse distribution throughout the brain parenchyma as reported by others (Zhao et al., 2003). In sham-operated animals, increased numbers of proliferating cells and cell clusters were observed compared with unmanipulated mice ($P < 0.001$). The number of BrdU$^+$ cells exceeded the numbers of Ki-67$^+$ cells in the subgranular layer of DG (Fig. 4B). We co-localized few Ki-67$^+$ cells with GFAP$^+$ astrocytes and Iba-1$^+$ microglia, whereas 30-50% of Ki-67$^+$ expressed PSA-NCAM (Fig. 3B, insert). The presence of human MDM in the basal ganglia, either infected or uninfected, significantly reduced the numbers of Ki-67$^+$ cells at day 7 compared with sham-operated controls ($P < 0.001$) (Fig. 4A).

Hippocampi from unmanipulated control mice contained small numbers of ramified Iba-1$^+$ microglia located in the hilus, within and around the GCL. Traumatic injury increased numbers of Iba-1$^+$ microglia in the DG, which displayed thicker processes. Moreover, those cells were in closer proximity to granule neurons (Fig. 4B). Microglia from sham-operated, MDM, and HIVE animals had similar morphological phenotype and distribution patterns.

GFAP$^+$ astrocytes in the innermost region of GCL showed morphology analogous to that of residual radial glia cells (Kosaka and Hama, 1986). Astroglial processes extended to the molecular layer in direct contact with

PSA-NCAM$^+$ dendrites (Fig. 3A). Few astrocytes in the hilus and the outer line of the DG showed "star" morphology. The DG of sham-operated animals showed statistically significant increases in GFAP$^+$ astrocytes numbers (Fig. 4A, $P < 0.03$). Equivalent numbers of astrocytes were detected in MDM, HIVE, and sham-operated mice (Fig. 4A).

Neurogenesis in Lymphocyte-Reconstituted NOD/SCID Mice

To assess whether the addition of the effectors of adaptive immunity (lymphocytes) affects neuronal plasticity and the diminution of neurogenesis by innate immunity (macrophages), we reconstituted NOD/SCID with human PBL and then injected autologous HIV-1-infected MDM (hu-PBL-NOD/SCID HIVE) or uninfected MDM (hu-PBL-NOD/SCID MDM) in the basal ganglia. Unmanipulated mice, with or without reconstitution, and mice injected with HIV-1-infected macrophages, but without reconstitution, served as controls. Significant lymphocyte infiltration into the meninges and area of injection in the basal ganglia were observed in hu-PBL-NOD/SCID HIVE and hu-PBL-NOD/SCID MDM mice compared with sham-operated controls (Poluektova et al., 2004), indicating that trauma alone was incapable of inducing lymphocytic infiltrate. PBL reconstitution did not affect hippocampal DG cell composition (data not shown). Significant differences in DG cell composition were not observed in SCID and NOD/SCID mice, with one exception. Microglial cell numbers were nearly 2-fold higher in NOD/SCID than in SCID mice. These results could reflect the differing genetic backgrounds and phenotypic features of the mouse strains (Kempermann and Gage, 2002). Traumatic injury increased the numbers of newborn PSA-NCAM$^+$ neurons, dividing cells, and astrocytes in the DG of sham-operated mice (Fig. 5). Microglial cells were similar in number to unmanipulated animals, but showed an activated morphological phenotype (data not shown). After injection of HIV-1-infected or -uninfected MDM, identical reductions in cell division, new neurons, dendrites, and dendrite length were observed in lymphocyte-reconstituted and nonreconstituted mice. This reduction was accompanied by increased numbers of microglia (Fig. 5). Three weeks later, the number of PSA-NCAM$^+$ cells were similar in all injected groups (20–22 cells/mm) and higher than in unmanipulated mice of the same age (13 ± 3 cells/mm, $P < 0.01$).

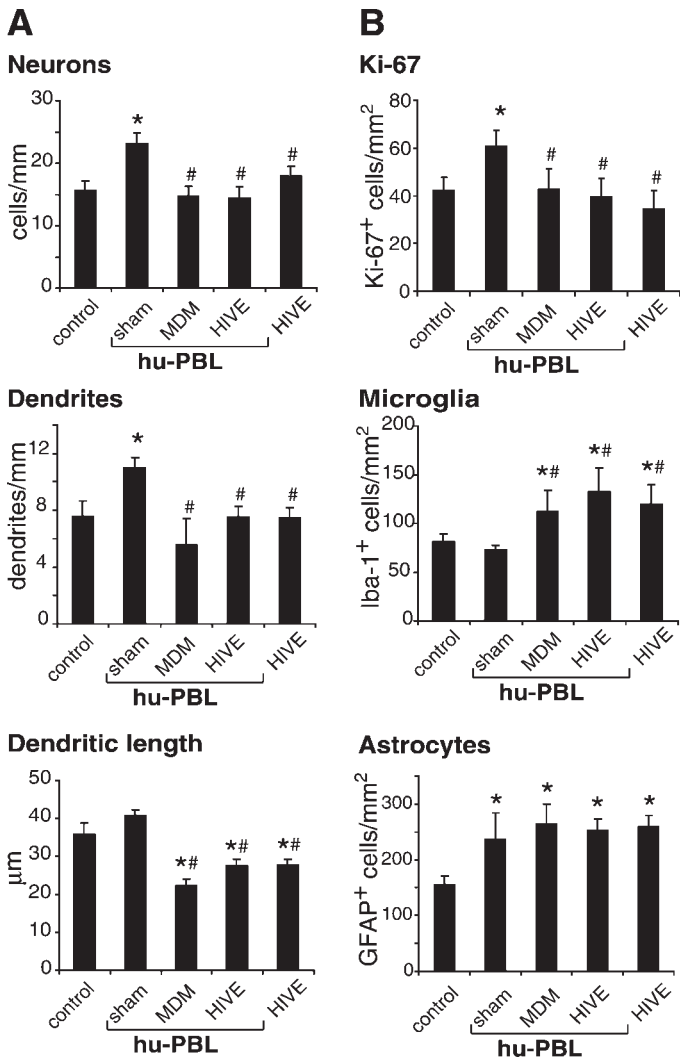

Fig. 5. Quantitative analysis of the DG cellular composition on day 7 post-injection in hu-PBL-NOD/SCID mice. The number of Ki-67$^+$ and PSA-NCAM$^+$ cells in DG was increased in sham-operated animals, but reduced in mice injected with uninfected or HIV-1-infected MDM. The reduction of the numbers of proliferating cells did not affect total number of glial cells. **A:** Statistical analysis (mean ± SEM) of the number of PSA-NCAM$^+$ cells adjusted to the length of the DG, the average number of dendrites, and dendrite length. *Differences between MDM and unmanipulated mice; #differences between unmanipulated and sham-operated animals ($P < 0.002$ for neurons, $P < 0.02$ for dendrites and $P < 0.001$ for dendritic length; ANOVA). **B:** Quantification of the number of dividing Ki-67$^+$ cells, microglia and astrocytes per mm^2 of the DG. *Differences between MDM and unmanipulated mice; #differences between unmanipulated and sham-operated animals ($P < 0.05$ for Ki-67$^+$ cells, $P < 0.02$ for microglia and $P < 0.005$ for GFAP$^+$ cells; ANOVA).

MDM-Induced Inflammation Affects Newborn Neuronal Morphology

Hippocampal neurons in SCID mice that contain deficiencies in DNA-PK show enhanced vulnerability to DNA damage, oxidative stress, excitotoxicity (Culmsee et al., 2001), and diminished cell survival (Chechlacz et al., 2001). To exclude this as a putative experimental variable, we assessed all experimental parameters of neurogenesis, plasticity, and gliosis in C.B.-17 mice (the wild-type parental strain) injected with HIV-1 or uninfected MDM in the basal ganglia and compared those with similarly treated SCID mice. The cellular composition of DG was distinct in C.B.-17 than in SCID mice (Fig. 6). C.B.-17 mice displayed 1.8 times more PSA-NCAM$^+$ cells/section than DNA-PK deficient SCID mice (36.4 ± 2.5 vs. 20.2 ± 0.9, $P < 0.001$). However, similar dendrite numbers (9.7 ± 0.9 vs. 8.0 ± 0.9) and dendrite

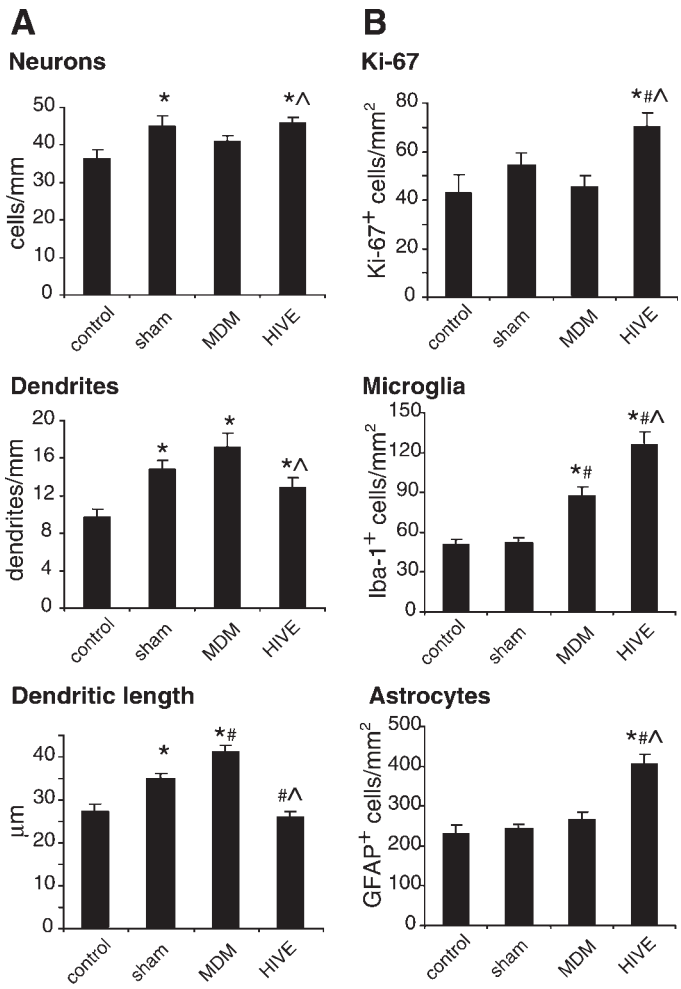

Fig. 6. Quantitative analysis of the DG cellular composition on day 7 post-injection in parental C.B.-17 mice. The numbers of PSA-NCAM$^+$ cells in DG was increased in sham-operated animals and mice injected with HIV-1-infected MDM. Injection of macrophages did not reduce the number of proliferating cells. **A:** Statistical analysis (mean ± SEM) of the number of PSA-NCAM$^+$ cells adjusted to the length of DG, the average number of dendrites, and dendrite length. **B:** Quantification of the number of dividing Ki-67$^+$ cells, microglia and astrocytes per mm^2 of DG. *Differences between MDM and unmanipulated mice; #differences between sham-operated and unmanipulated animals; ^Differences between MDM and HIVE animals ($P < 0.02$ for neurons, $P < 0.01$ for Ki-67$^+$ cells, $P < 0.0001$ for number of dendrites and dendritic length, microglia, and astrocytes).

length (28.8 ± 1.1 µm vs. 27.1 ± 0.8 µm) were seen in both parental and SCID animals. The dynamics of cell proliferation and variability of neurite numbers could reflect the SCID mutations and mouse genetic backgrounds (van Pelt et al., 1997; Hayes and Nowakowski, 2002; Amrein et al., 2004). No differences were observed in microglia and astrocyte morphology and numbers from unmanipulated C.B.17 compared with SCID mice.

As expected, the presence of xenogeneic human cells induced quick graft rejection for transplanted human MDM with significant mononuclear/lymphocyte infiltration in the meninges and in the area of injection within the basal ganglia (data not shown).

Traumatic injury increased small PSA-NCAM$^+$ cell numbers by 15–25% in C.B.-17 mice. At day 7, sham-operated animals showed no changes in numbers of microglia or astrocytes; and this was distinct from what was previously demonstrated in SCID animals (Fig. 4). The presence of MDM-induced inflammation and xenoreaction neither reduced the number of newborn PSA-NCAM$^+$ neurons nor affected their morphology (Fig. 6). However, in HIVE animals, a statistically diminished number of dendrites and dendrite length was demonstrated ($P < 0.0001$) (Fig. 6A). This observation occurred in parallel with a dramatic expansion in astrocyte and microglial responses ($P < 0.0001$) in HIVE mice. The number of activated microglial cells was also increased in animals injected with uninfected MDM ($P < 0.0001$). Increased numbers of dendrites and dendrite length was observed in the brains of mice injected with uninfected MDM than in HIVE animals. Ki-67$^+$ cell numbers were also increased significantly in HIVE, likely representing ongoing division of neuronal and glial cells.

DISCUSSION

Generation of new neurons in the hippocampus continues throughout life and involves multiple steps. These include proliferations of neural stem and precursor cells, as well as prolonged cell survival, migration, differentiation, and incorporation into an existing neural network. Studies on adult rat DG demonstrated that newborn neurons during the first 3 days of development extend their apical dendrite into the GCL. After 7 days, the developing neurons will reach the molecular layer (Seki and Arai, 1993; Seki, 2002a,b). This process is known to be stimulated by mechanical injury (Dash et al., 2001; Kernie et al., 2001; Yoshimura et al., 2003). Using brain injury as an experimental paradigm, we investigated relationships between neurogenesis and plasticity and brain inflammation in a murine model of HIVE (Gendelman et al., 1993; Persidsky et al., 1996, 1997; Persidsky and Gendelman, 2002; Poluektova et al., 2004). We used the trauma-induced neurogenesis to evaluate the effects of secondary inflammatory responses in neural tissue. SCID mice, allowed prolonged survival of transplanted cells and showed increased sensitivity of hippocampal neurons to MP neurotoxins (Chechlacz et al., 2001; Culmsee et al., 2001). Reconstituted NOD/SCID mice evaluated xenogeneic human adaptive immunity while the use of parental mouse strains assessed SCID mutation effects on neurogenesis. Lastly, the use of both uninfected and HIV-1-infected MDM-injected animals evaluated the contribution of viral proteins to neuronal development.

We showed that neurogenesis was attenuated by MP inflammatory responses in SCID mice. The reduction in the numbers of new PSA-NCAM$^+$ neurons was associated with decreased cell division. However, such changes did not affect astrocyte and microglial responses. This led to the idea that attenuation of neurogenesis in SCID mice was associated with high sensitivity of proliferating PSA-NCAM$^+$ neuronal precursors to human MP inflammatory products. We previously

demonstrated that activated macrophages affect neuroinflammatory responses, neuronal plasticity, long-term potentiation, and behavior. Although the levels seen were lower than observed for infected macrophages, they were statistically different from what was seen in sham-operated animals (Zink et al., 2002; Anderson et al., 2002, 2003). Indeed, the numbers of activated macrophages per se, rather than the absolute levels of virus, are the best indicators for cognitive dysfunction in patients with progressive HIV infection in its human host (Glass et al., 1995; Johnson et al., 1996).

HIV-1 infected and activated MP secrete viral and cellular products that negatively affect neuronal function and inevitably lead to neural dropout (reviewed in Gendelman, 2004). However, it is unknown whether the neuronal dysfunction represents MP inflammatory-induced damage or deficits in repair, protection, or regeneration. A leading hypothesis for HIV neuropathogenesis is that widespread MP-induced inflammation affects hippocampal learning and memory. We previously demonstrated that this could occur through paracrine amplification of pro-inflammatory factors from the basal ganglia to the hippocampus (Zink et al., 2002). Indeed, previous studies showed that lipopolysaccharide, which elicits broad microglia activation, reduces hippocampal neurogenesis when administered to areas where neurons are born. Increased neurogenesis triggered by brain insults are also reduced by microglia activation (Ekdahl et al., 2003).

Lack of observed differences in neurogenesis and neuronal plasticity between virus-infected and -uninfected MDM in SCID mice supports a reduced role for viral proteins in the attenuation of neurogenesis by MP. SCID mutations increase the sensitivity of rapidly dividing cells to DNA damage and can mask possible differences associated with direct viral toxicity (Chechlacz et al., 2001; Culmsee et al., 2001). Indeed, DNA-PK catalytic subunit is an important component of DNA repair and cell signaling (Yang et al., 2003). Nonetheless, viral proteins could directly affect neuronal stem cells (Peng et al., 2004) as well as immature and mature neurons. This includes HIV-1gp120, whose effects require expression of G-protein-coupled CXCR4 and CCR5 receptors (Lipton, 1994; Medina et al., 1999; Krathwohl and Kaiser, 2004), along with HIV-1gp41 (Adamson et al., 1996), Tat (Nath et al., 1996), and Vpr (Piller et al., 1998; Patel et al., 2000).

The observation that viral proteins and lymphocyte reconstitution did not play roles in neurogenesis came as a surprise. SCID and NOD/SCID mice are of different backgrounds and show divergent microglia numbers (Saravia et al., 2002). Reconstituted and nonreconstituted NOD/SCID mice show dramatic differences in brain tissue damage after MDM injections. In this regard, expression of pro-inflammatory cytokines, such as interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and inducible nitric oxide synthase (iNOS) (Poluektova et al., 2004), are upregulated in brains of hu-PBL-NOD/SCID HIVE mice. One of these factors, IL-6, directly reduces the proliferation rates of neuronal progenitor cells (Vallieres et al., 2002; Monje et al., 2003). More complex effects could be associated with released NO (Zhang et al., 2001). In this NOD/SCID model, the absence of detrimental effects of lymphocyte infiltration on neurogenesis could be associated with its dual role in inflammation and growth factor production (Moalem et al., 2000; Barouch and Schwartz, 2002).

The negative effects of inflammation on neuronal plasticity were confirmed in wild type C.B.-17 mice. Normal mice of the same age as SCID animals had nearly two times more PSA-NCAM$^+$ cells. This results in more rapid elimination of human cells than in SCID animals. Proliferation of progenitor cells (PSA-NCAM$^+$ and Ki-67$^+$) was not affected by the induced inflammation. However, PSA-NCAM$^+$ dendrites and dendrite length was reduced significantly. This suggests that inflammation, triggered by HIV-1-infected cells, is a major factor in inhibition of hippocampal neuronal development.

Elimination of human MDM and reduction of inflammatory responses was accompanied by restoration of PSN-NCAM$^+$ cells in the DG. The functional significance of adult neurogenesis and the potential contribution to cognition are most likely to be found in long-term adaptations (Kempermann et al., 2004b). Links were established between immune activation, inflammation, and neuronal development. It is especially relevant as DG neurogenesis is a critical event for spatial and episodic memory (Lisman, 1999; Burgess et al., 2002). Recent data support the idea that newly generated neurons facilitate synaptic plasticity and are needed for the formation of new memories (Kempermann et al., 2004b; Schmidt-Hieber et al., 2004). Such observations have direct application to the murine HIVE model described in this work. Research in our laboratory demonstrated that impaired synaptic transmission and long-term potentiation in hippocampal CA1–3 regions were directly associated with behavioral deficits in HIVE mice (Zink et al., 2002; Anderson et al., 2002, 2003). It is reasonable to conclude that deficits in neurogenesis and newborn neurons connections may also contribute to the functional deficits seen in these animals.

Our findings may have widespread relevance to other neurodegenerative disorders, such as Parkinson's and Alzheimer's disease, where inflammation, induced as a consequence of microglial activation, is a principal part of the disease processes (McGeer and McGeer, 1998). In each case, microglial and brain macrophage inflammatory responses may have direct effects both on neuronal function and on perturbing repair or regenerative processes operative in neurogenic regions. Studies of neurogenesis have sparked new research efforts into studies of brain repair during disease (Kuhn et al., 2001). Neurogenesis was previously studied in rodent models of human neurological diseases, including stroke (Iwai et al., 2002; Tanaka et al., 2004), radiation injury (Monje et al., 2003; Raber et al., 2004), subarachnoid hemorrhage (Mino et al., 2003), and seizures (Sato et al., 2002; Huttmann et al., 2003). The means to reverse or attenu-

ate deficits in the production of new neurons may inevitably have therapeutic implications.

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REFERENCES

Adamson DC, Wildemann B, Sasaki M, Glass JD, McArthur JC, Christov VI, Dawson TM, Dawson VL. 1996. Immunologic NO synthase: elevation in severe AIDS dementia and induction by HIV-1 gp41. Science 274:1917–1921.

Altman J, Das DG. 1966. Autoradiographic and histological studies of postnatal neurogenesis. I. A longitudinal investigation of the kinetics, migration and transformation of cells incorporating tritiated thymidine in neonate rats, with special reference to postnatal neurogenesis in some brain regions. J Comp Neurol 126:337–389.

Amrein I, Slomianka L, Poletaeva II, Bologova NV, Lipp HP. 2004. Marked species and age-dependent differences in cell proliferation and neurogenesis in the hippocampus of wild-living rodents. Hippocampus 14:1000–1010.

Anderson E, Zink W, Xiong H, Gendelman HE. 2002. HIV-1-associated dementia: a metabolic encephalopathy perpetrated by virus-infected and immune-competent mononuclear phagocytes. J Acquir Immune Defic Syndr 31(suppl 2):S43–54.

Anderson ER, Boyle J, Zink WE, Persidsky Y, Gendelman HE, Xiong H. 2003. Hippocampal synaptic dysfunction in a murine model of human immunodeficiency virus type 1 encephalitis. Neuroscience 118: 359–369.

Barouch R, Schwartz M. 2002. Autoreactive T cells induce neurotrophin production by immune and neural cells in injured rat optic nerve: implications for protective autoimmunity. FASEB J 16:1304–1306.

Burgess N, Maguire EA, O'Keefe J. 2002. The human hippocampus and spatial and episodic memory. Neuron 35:625–641.

Chechlacz M, Vemuri MC, Naegele JR. 2001. Role of DNA-dependent protein kinase in neuronal survival. J Neurochem 78:141–154.

Culmsee C, Bondada S, Mattson MP. 2001. Hippocampal neurons of mice deficient in DNA-dependent protein kinase exhibit increased vulnerability to DNA damage, oxidative stress and excitotoxicity. Brain Res Mol Brain Res 87:257–262.

Dash PK, Mach SA, Moore AN. 2001. Enhanced neurogenesis in the rodent hippocampus following traumatic brain injury. J Neurosci Res 63:313–319.

De Girolami U, Sharer LR, Gabuzda D, Sotrel A. 2004. Neuropathology of AIDS. London: Elsevier. 803 p.

Doetsch F, Garcia-Verdugo JM, Alvarez-Buylla A. 1997. Cellular composition and three-dimensional organization of the subventricular germinal zone in the adult mammalian brain. J Neurosci 17:5046–5061.

Ekdahl C, Claasen JH, Bonde S, Kokaia Z, Lindvall O. 2003. Inflammation is detrimental for neurogenesis in adult brain. Proc Natl Acad Sci USA 100:13632–13637.

Gendelman HE, Orenstein JM, Martin MA, Ferrua C, Mitra R, Phipps T, Wahl LA, Lane HC, Fauci AS, Burke DS. 1988. Efficient isolation and propagation of human immunodeficiency virus on recombinant colony-stimulating factor 1-treated monocytes. J Exp Med 167:1428–1441.

Gendelman HE, Genis P, Jett M, Bernton EW, Gelbard HA, Cvetkovich TA, Lazar ES, Cerro MD, Epstein LG. 1993.Experimental model systems for studies of HIV-associated CNS disease. In: Montagnier L, Gougeon M-L, editors. New concepts in AIDS pathogenesis. New York: Marcel Dekker. p 89–113.

Gendelman HE, Diesing S, Gelbard H, Swindells S. 2004.The neuropathogenesis of HIV-1 infection. In: Wormser GP, editor. AIDS and other manifestations of HIV infection;4th Ed. Amsterdam: Elsevier. p 95–116.

Glass JD, Fedor H, Wesselingh SL, McArthur JC. 1995. Immunocytochemical quantitation of human immunodeficiency virus in the brain: correlation with dementia. Ann Neurol 38:755–762.

Hayes NL, Nowakowski RS. 2002. Dynamics of cell proliferation in the adult dentate gyrus of two inbred strains of mice. Brain Res Dev Brain Res 134:77–85.

Huttmann K, Sadgrove M, Wallraff A, Hinterkeuser S, Kirchhoff F, Steinhauser C, Gray WP. 2003. Seizures preferentially stimulate proliferation of radial glia-like astrocytes in the adult dentate gyrus: functional and immunocytochemical analysis. Eur J Neurosci 18: 2769–2778.

Iwai M, Sato K, Omori N, Nagano I, Manabe Y, Shoji M, Abe K. 2002. Three steps of neural stem cells development in gerbil dentate gyrus after transient ischemia. J Cereb Blood Flow Metab 22:411–419.

Johnson RT, Glass JD, McArthur JC, Chesebro BW. 1996. Quantitation of human immunodeficiency virus in brains of demented and nondemented patients with acquired immunodeficiency syndrome. Ann Neurol 39:392–395.

Kee N, Sivalingam S, Boonstra R, Wojtowicz JM. 2002. The utility of Ki-67 and BrdU as proliferative markers of adult neurogenesis. J Neurosci Methods 115:97–105.

Kempermann G, Gage FH. 2002. Genetic influence on phenotypic differentiation in adult hippocampal neurogenesis. Brain Res Dev Brain Res 134:1–12.

Kempermann G, Jessberger S, Steiner B, Kronenberg G. 2004a. Milestones of neuronal development in the adult hippocampus. Trends Neurosci 27:447–452.

Kempermann G, Wiskott L, Gage FH. 2004b. Functional significance of adult neurogenesis. Curr Opin Neurobiol 14:186–191.

Kernie SG, Erwin TM, Parada LF. 2001. Brain remodeling due to neuronal and astrocytic proliferation after controlled cortical injury in mice. J Neurosci Res 66:317–326.

Kieburtz K, Ketonen L, Cox C, Grossman H, Holloway R, Booth H, Hickey C, Feigin A, Caine ED. 1996. Cognitive performance and regional brain volume in HIV-1 infection. Arch Neurol 53:155–158.

Kosaka T, Hama K. 1986. Three-dimensional structure of astrocytes in the rat dentate gyrus. J Comp Neurol 249:242–260.

Krathwohl MD, Kaiser JL. 2004. HIV-1 promotes quiescence in human neural progenitor cells. J Infect Dis 190:216–226.

Kuhn HG, Palmer TD, Fuchs E. 2001. Adult neurogenesis: a compensatory mechanism for neuronal damage. Eur Arch Psychiatry Clin Neurosci 251:152–158.

Lipton SA. 1994. HIV-related neuronal injury. Potential therapeutic intervention with calcium channel antagonists and NMDA antagonists. Mol Neurobiol 8:181–196.

Lisman JE. 1999. Relating hippocampal circuitry to function: recall of memory sequences by reciprocal dentate-CA3 interactions. Neuron 22:233–242.

Lipton SA, Gendelman HE. 1995. Dementia associated with the acquired immunodeficiency syndrome. N Engl J Med 16:934–940.

McArthur JC, Haughey N, Gartner S, Conant K, Pardo C, Nath A, Sacktor N. 2003. Human immunodeficiency virus-associated dementia: an evolving disease. J Neurovirol 9:205–221.

McGeer PL, McGeer EG. 1998. Glial cell reactions in neurodegenerative diseases: pathophysiology and therapeutic interventions. Alzheimer Dis Assoc Disord 12(suppl 2):S1–6.

Medina I, Ghose S, Ben-Ari Y. 1999. Mobilization of intracellular calcium stores participates in the rise of $[Ca^{2+}]_i$ and the toxic actions of the HIV coat protein GP120. Eur J Neurosci 11:1167–1178.

Mino M, Kamii H, Fujimura M, Kondo T, Takasawa S, Okamoto H, Yoshimoto T. 2003. Temporal changes of neurogenesis in the mouse hippocampus after experimental subarachnoid hemorrhage. Neurol Res 25:839–845.

Moalem G, Gdalyahu A, Shani Y, Otten U, Lazarovici P, Cohen IR, Schwartz M. 2000. Production of neurotrophins by activated T cells: implications for neuroprotective autoimmunity. J Autoimmun 15:331–345.

Monje ML, Toda H, Palmer TD. 2003. Inflammatory blockade restores adult hippocampal neurogenesis. Science 302:1760–1765.

Nath A, Psooy K, Martin C, Knudsen B, Magnuson DS, Haughey N, Geiger JD. 1996. Identification of a human immunodeficiency virus type 1 Tat epitope that is neuroexcitatory and neurotoxic. J Virol 70:1475–1480.

Navia BA, Cho ES, Petito CK, Price RW. 1986. The AIDS dementia complex. II. Neuropathology. Ann Neurol 19:525–535.

Neuen-Jacob E, Figge C, Arendt G, Wendtland B, Jacob B, Wechsler W. 1993. Neuropathological studies in the brains of AIDS patients with opportunistic diseases. Int J Legal Med 105:339–350.

Parent JM. 2003. Injury-induced neurogenesis in the adult mammalian brain. Neuroscientist 9:261–272.

Patel CA, Mukhtar M, Pomerantz RJ. 2000. Human immunodeficiency virus type 1 Vpr induces apoptosis in human neuronal cells. J Virol 74:9717–9726.

Patel SH, Kolson DL, Glosser G, Matozzo I, Ge Y, Babb JS, Mannon LJ, Grossman RI. 2002. Correlation between percentage of brain parenchymal volume and neurocognitive performance in HIV-infected patients. AJNR Am J Neuroradiol 23:543–549.

Peng H, Huang Y, Rose J, Erichsen D, Herek S, Fujii N, Tamamura H, Zheng J. 2004. Stromal cell-derived factor 1-mediated CXCR4 signaling in rat and human cortical neural progenitor cells. J Neurosci Res 76:35–50.

Persidsky Y, Gendelman HE. 2002. Murine models for human immunodeficiency virus type 1-associated dementia: the development of new treatment testing paradigms. J Neurovirol 8(suppl 2):49–52.

Persidsky Y, Limoges J, McComb R, Bock P, Baldwin T, Tyor W, Patil A, Nottet HSLM, Epstein L, Gelbard H, Flanagan E, Reinhard J, Pirruccello S, Gendelman HE. 1996. Human immunodeficiency virus encephalitis in SCID mice. Am J Pathol 149:1027–1053.

Persidsky Y, Buttini M, Limoges J, Bock P, Gendelman HE. 1997. An analysis of HIV-1-associated inflammatory products in brain tissue of humans and SCID mice with HIV-1 encephalitis. J Neurovirol 3:401–416.

Piller SC, Jans P, Gage PW, Jans DA. 1998. Extracellular HIV-1 virus protein R causes a large inward current and cell death in cultured hippocampal neurons: implications for AIDS pathology. Proc Natl Acad Sci USA 95:4595–4600.

Poluektova L, Gorantla S, Faraci J, Birusingh K, Dou H, Gendelman HE. 2004. Neuroregulatory events follow adaptive immune-mediated elimination of HIV-1-infected macrophages: studies in a murine model of viral encephalitis. J Immunol 172:7610–7617.

Poluektova LY, Munn DH, Persidsky Y, Gendelman HE. 2002. Generation of cytotoxic T cells against virus-infected human brain macrophages in a murine model of HIV-1 encephalitis. J Immunol 168: 3941–3949.

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.

Raber J, Fan Y, Matsumori Y, Liu Z, Weinstein PR, Fike JR, Liu J. 2004. Irradiation attenuates neurogenesis and exacerbates ischemia-induced deficits. Ann Neurol 55:381–389.

Rottenberg DA, Moeller JR, Strother SC, Sidits JJ, Navia BA, Dhawan V, Ginos JZ, Price RW. 1987. The metabolic pathology of the AIDS dementia complex. Ann Neurol 22:700–706.

Saravia FE, Revsin Y, Gonzalez Deniselle MC, Gonzalez SL, Roig P, Lima A, Homo-Delarche F, De Nicola AF. 2002. Increased astrocyte reactivity in the hippocampus of murine models of type 1 diabetes: the nonobese diabetic (NOD) and streptozotocin-treated mice. Brain Res 957:345–353.

Sato K, Iwai M, Nagano I, Shoji M, Abe K. 2002. Changes of localization of highly polysialylated neural cell adhesion molecule (PSA-NCAM) in rat hippocampus with exposure to repeated kindled seizures. Brain Res 946:323–327.

Schmidt-Hieber C, Jonas P, Bischofberger J. 2004. Enhanced synaptic plasticity in newly generated granule cells of the adult hippocampus. Nature 429:184–187.

Seki T, Arai Y. 1993. Highly polysialylated neural cell adhesion molecule (NCAM-H) is expressed by newly generated granule cells in the dentate gyrus of the adult rat. J Neurosci 13:2351–2358.

Seki T. 2002a. Expression patterns of immature neuronal markers PSA-NCAM, CRMP-4 and NeuroD in the hippocampus of young adult and aged rodents. J Neurosci Res 70:327–334.

Seki T. 2002b. Hippocampal adult neurogenesis occurs in a microenvironment provided by PSA-NCAM-expressing immature neurons. J Neurosci Res 69:772–783.

Tanaka R, Yamashiro K, Mochizuki H, Cho N, Onodera M, Mizuno Y, Urabe T. 2004. Neurogenesis after transient global ischemia in the adult hippocampus visualized by improved retroviral vector. Stroke 35:1454–1459.

Vallieres L, Campbell IL, Gage FH, Sawchenko PE. 2002. Reduced hippocampal neurogenesis in adult transgenic mice with chronic astrocytic production of interleukin-6. J Neurosci 22:486–492.

van Pelt J, Dityatev AE, Uylings HB. 1997. Natural variability in the number of dendritic segments: model-based inferences about branching during neurite outgrowth. J Comp Neurol 387:325–340.

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.

Xiong H, Zeng YC, Zheng J, Thylin M, Gendelman HE. 1999. Soluble HIV-1 infected macrophage secretory products mediate blockade of long-term potentiation: a mechanism for cognitive dysfunction in HIV-1-associated dementia. J NeuroVirol 5:519–528.

Yang J, Yu Y, Hamrick HE, Duerksen-Hughes PJ. 2003. ATM, ATR and DNA-PK: initiators of the cellular genotoxic stress responses. Carcinogenesis 24:1571–1580.

Yoshimura S, Teramoto T, Whalen MJ, Irizarry MC, Takagi Y, Qiu J, Harada J, Waeber C, Breakefield XO, Moskowitz MA. 2003. FGF-2 regulates neurogenesis and degeneration in the dentate gyrus after traumatic brain injury in mice. J Clin Invest 112:1202–1210.

Zhang R, Zhang L, Zhang Z, Wang Y, Lu M, Lapointe M, Chopp M. 2001. A nitric oxide donor induces neurogenesis and reduces functional deficits after stroke in rats. Ann Neurol 50:602–611.

Zhao M, Momma S, Delfani K, Carlen M, Cassidy RM, Johansson CB, Brismar H, Shupliakov O, Frisen J, Janson AM. 2003. Evidence for neurogenesis in the adult mammalian substantia nigra. Proc Natl Acad Sci USA 100:7925–7930.

Zheng J, Zhuang W, Yan N, Kou G, Peng H, McNally C, Erichsen D, Cheloha A, Herek S, Shi C. 2004. Classification of HIV-1-mediated neuronal dendritic and synaptic damage using multiple criteria linear programming. Neuroinformatics 2:303–326.

Zink WE, Anderson E, Boyle J, Hock L, Rodriguez-Sierra J, Xiong H, Gendelman HE, Persidsky Y. 2002. Impaired spatial cognition and synaptic potentiation in a murine model of human immunodeficiency virus type 1 encephalitis. J Neurosci 22:2096–2105.