Dexamethasone Therapy Worsens the Neuropathology of Human Immunodeficiency Virus Type 1 Encephalitis in SCID Mice

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Human immunodeficiency virus (HIV) dementia is a late complication of viral infection. Cognitive dysfunction revolves around the secretion of neurotoxins from immunologically competent virus-infected brain macrophages and microglia. Such macrophage neurotoxins are inflammatory factors that produce selective neuronal dysfunction and ultimately cell death. To evaluate the potential efficacy of antiinflammatory therapy for HIV dementia, dexamethasone was administered to severe combined immunodeficient mice with HIV-1 encephalitis. Mice were given therapeutic doses of dexamethasone before intracerebral inoculation with HIV-1-infected human monocytes. Histochemical evaluation showed a worsening of neuropathology after treatment, with astrogliosis and increased apoptosis of neurons. Laboratory investigation of the mechanisms for the dexamethasone effect revealed increased viability of HIV-infected macrophages and incomplete suppression of neurotoxic inflammatory secretions. The results suggest the need for caution in administering glucocorticoids for treatment of HIV encephalitis in humans.

Human immunodeficiency virus (HIV) dementia, also termed the HIV-1-associated cognitive-motor complex, is a devastating and usually late complication of viral infection. The clinical syndrome affects 20% of adults and 50% of children with AIDS [1]. Signs of disease begin with subtle cognitive changes and physical slowing, progressing to florid memory loss, behavioral abnormalities, incontinence, hallucinations, seizures, and ultimately death [2–5]. Patients affected with severe forms of HIV dementia often have multinucleated giant cell encephalitis [5]. The neuropathologic hallmarks of HIV-1 encephalitis include accumulation or activation of astrocytes (astrocytosis) and macrophages (microglial nodules, multinucleated giant cells) in subcortical white and gray matter. Neuronal injury and death often accompany the high levels of HIV replication seen in the brain during advanced immunosuppression [6–8]. While most persons with HIV dementia and advanced immunosuppression have high levels of virus in the brain [4], the converse is not always true [4, 9–11]. This suggests that both viral and cellular factors are involved in disease pathogenesis.

Macrophages are the major reservoir for HIV-1 in brain tissue [6, 12]. This differentiates HIV-1 encephalitis from other encephalitides (e.g., herpes simplex virus 1 and 2, JC virus, and cytomegalovirus infections). The latter neuroinvasive viruses are tropic for neurons, glia, or both. Importantly, in HIV dementia, both astrocytic and macrophage reactions are associated with brain immune activation initiated by macrophages and microglia [11]. Activated HIV-1-infected brain macrophages secrete a number of factors, many of which are associated with neuronal injury [13]. These include but are not limited to tumor necrosis factor (TNF)-α, platelet activating factor, eicosanoids, and nitric and quinolinic acids [14–22]. Such macrophage secretory products can also cause leakiness of the blood-brain barrier, permitting the transendothelial migration of monocytes into the central nervous system (CNS) and perpetuation of disease.

If the neuropathologic changes of HIV encephalitis are caused by inflammatory factors released by immune-activated macrophages, it would follow that treatment with antiinflammatory or immunosuppressive agents might positively affect virus-associated CNS pathology. To test this hypothesis, we used a murine model of HIV encephalitis in which virus-infected human monocytes were inoculated into the brains of severe combined immunodeficient (SCID) mice [23]. Such monocyte inoculation into the brain is accompanied by microglial nodule formation, astrocytosis, and neuronal dropout. These changes persist up to 5 weeks and parallel what is seen in human HIV encephalitis. Dexamethasone was chosen as the first candidate therapeutic agent because experiments in our laboratory and elsewhere showed it to be effective in decreasing macrophage secretions, including TNF-α and arachidonic acid metabolites, both of which play a role in the pathogenesis of HIV encephalitis. In addition, dexamethasone is used widely as a CNS antiinflammatory agent for trauma-induced edema, cancer, and chemotherapy-related brain swelling. In the present work, we treated SCID mice with HIV-1 encephalitis with...
therapeutic doses of dexamethasone 24 h before and continuously following intracerebral macrophage inoculation.

**Material and Methods**

*Isolation and culture of primary human monocytes.* Monocytes obtained by leukapheresis of HIV-1- and hepatitis B–seronegative donors were purified by counter current centrifugal elutriation [24]. Cell suspensions were documented as >98% monocytes by cell morphology criteria in Wright-stained cytospins, granular peroxidase, nonspecific esterase, and CD68 immunolabeling. Monocytes were cultured in Teflon flasks (2 × 10^6 cells/mL, 150 × 10^6 cells/flask) in Dulbecco’s MEM (DMEM; Sigma, St. Louis) with 10% heat-inactivated pooled human serum, 1% glutamine, 50 μg/mL gentamicin (Sigma), 10 μg/mL ciprofloxacin (Sigma), and 1000 U/mL highly purified recombinant human macrophage colony-stimulating factor (M-CSF; gift of Genetics Institute, Cambridge, MA). M-CSF was placed in culture for the initial 5 days of monocytocyte cultivation, then removed. All tissue-culture reagents were screened before use and were negative for endotoxin (<10 pg/mL; Associates of Cape Cod, Woods Hole, MA) and mycoplasma contamination (Gen-Probe II; Gen-Probe, San Diego).

*HIV-1 infection of monocytes.* After 7 days in Teflon flask suspension culture, monocytes were infected with HIV-1sd, at an MOI of 0.1 infectious virus per target cell [24]. Culture medium was changed every 3 days. Reverse transcriptase (RT) activity was determined in triplicate samples of culture fluids added to a reaction mixture of 0.05% Nonidet P-40 (Sigma), 10 μg/mL poly(A), 0.25 μg/mL oligo(dt) (Pharmacia Fine Chemicals, Piscataway, NJ), 5 mM dithiothreitol (Pharmacia), 150 mM KCl, 15 mM MgCl₂, and [3H]dTTP (2 Ci/mmol; Amersham, Arlington Heights, IL) in TRIS-HCl buffer (pH 7.9) for 24 h at 37°C. Radiolabeled nucleotides were precipitated with cold 10% trichloroacetic acid and 95% ethanol in an automatic cell harvester (Skatron, Sterling, VA) on paper filters. Radioactivity was estimated by liquid scintillation spectroscopy [25]. The percentage of HIV-infected monocytes was determined by immunostaining with anti--HIV-1 p24 monoclonal antibody (MAb; Dako, Carpinteria, CA). Cytosin preparations of HIV-1–infected or noninfected cells were fixed in cold absolute acetone/methanol (1:1) for 10 min at −20°C; anti-p24 MAb (1:10; Dako) was applied for 60 min at room temperature, followed by treatment with a fluorescein isothiocyanate (FITC)–conjugated anti-mouse IgG F(ab')₂ fragment (1:100; Boehringer Mannheim, Indianapolis) for 1 h at room temperature. Viral antigen–positive cells were counted microscopically (Microphot-FXA; Nikon, Tokyo) using a ×20 objective in 10 random fields and an FITC filter. All experiments were done in triplicate.

*Animals and intracerebral injections in the SCID mouse model.* SCID mice (male C.B-17/Icr-Crl-SCID-bgBR, 3–4 weeks old) were purchased from Charles River Laboratories (Wilmington, MA). Animals were maintained in sterile microisolator cages under pathogen-free conditions in the Laboratory of Animal Medicine, University of Nebraska Medical Center. All animal manipulations (including intracranial inoculations) were done in a laminar flow hood. Intracerebral injections of HIV-1sd–infected or control uninfected monocytes were done under anesthesia (100 mg/kg ketamine, 16 mg/kg xylazine). Cells were pelleted on the day of brain inoculation (postinfection day 7). A monocyte suspension (1 × 10^7 cells/mL in serum-free DMEM) was prepared for injections. Anesthetized mice were placed in a stereotactic apparatus (Stoetling, Wood Dale, IL) designed specifically for mice. The animal’s head was secured by ear bars and mouthpiece as previously described [26]. The coordinates for inoculation (putamen), confirmed by control indium injections, were 3.5 mm behind the bregma, 3.5 mm lateral from the sagittal midline at a depth of 4.0 mm and 35° from the vertical line. For cell injections, we used a 100-μL Hamilton syringe and a 26-gauge needle. Each animal brain received 15 μL of suspension containing 3.0 × 10^5 HIV-infected or uninfected cells. Animals were sacrificed from 4 days to 4 weeks after inoculation. Similar numbers of human monocytes were identified in HIV-1–infected and control uninfected monocytes inoculated in each of the three independent experiments by morphologic and immunohistochemical analyses.

*Dexamethasone administration.* Animals were inoculated with HIV-1–infected and control uninfected monocytes as described above. At 24 h before intracerebral injection of the monocytes, half of the mice (treated group) were injected subcutaneously with dexamethasone acetate (Schein Pharmaceutical, Florham Park, NJ). A repository form of the drug was used, with an average duration of action of 7–9 days. Mice were given dexamethasone (14 mg/kg/week) until sacrifice. Mice from each treatment arm were sacrificed at 4, 7, 14, 21, and 28 days after injection. Control mice were treated with dexamethasone or left untreated, injected with media alone (sham), and sacrificed 1 and 4 weeks after injection. Three additional control mice received no intracerebral injections but were treated with dexamethasone at equivalent dosages and sacrificed at weeks 1, 3, and 4. Table 1 shows total mice analyzed.

**Histopathology and immunohistochemistry.* Brain tissue was collected at necropsy. Tissue was either fixed in 4% phosphate-buffered paraformaldehyde and paraffin-embedded or frozen in

| Table 1. Analysis of dexamethasone treatment for SCID mice with HIV-1 encephalitis. |
|---------------------------------|--------|--------|--------|--------|--------|
| Treatment                        | Days    |
|                                  | 4      | 7      | 14     | 21     | 28     |
| Uninfected monocytes             | 2 (2)  | 7 (6)  | 2 (2)  | 2 (2)  | 5 (4)  |
| Uninfected monocytes + dexamethasone | 2 (2)  | 7 (6)  | 2 (2)  | 2 (2)  | 5 (4)  |
| HIV-1–infected monocytes         | 2 (2)  | 7 (6)  | 2 (2)  | 2 (2)  | 5 (3)  |
| HIV-1–infected monocytes + dexamethasone | 2 (2)  | 7 (6)  | 2 (2)  | 2 (1)  | 5 (4)  |
| Sham-inoculated                  | 0      | 2      | 0      | 0      | 2      |
| Sham-inoculated mice + dexamethasone | 0      | 2      | 0      | 0      | 2      |

NOTE. Data are no. of animals analyzed at each time point after monocyte inoculations; parentheses show no. of animals (from total at each time point) where human monocytes were identified immunocytochemically by CD68 immunostaining.
OCT mounting medium (Miles, Elkhart, IN) in cassettes for cryostat sectioning. After freezing or fixation, six coronal blocks (~1 mm thick) were prepared from each brain. Ten sections (5 μm each) were cut from each block and stained by hematoxylin-eosin or immunocytochemically labeled with antibodies to glial fibrillary acidic protein (GFAP) and CD68 to identify the inoculation site. At the inoculation site, 45 extra serial sections were prepared and stained with HIV-1 p24 antigen, HLA-DR, neurofilament, or vimentin. Two adjacent blocks were similarly sectioned and stained. This approach permitted the analysis of morphologic changes at a known distance from the site of human monocyte inoculation.

Immunohistochemistry was done on 5-μm paraformaldehyde-fixed paraffin-embedded or cryostat sections fixed in absolute acetone/methanol (1:1) for 10 min at ~20°C. Human monocytes were identified immunocytochemically with anti-CD68 KP-1 (1:100; Dako) or anti-vimentin (1:25; Boehringer Mannheim) MAb.s. Human TNF-α, interleukin (IL)-1β, and IL-6 were detected with polyclonal rabbit anti-human TNF-α, IL-1β, and IL-6 antibodies (Genzyme, Cambridge, MA) at a dilution of 1:25. Mouse endothelial cells or astrocytes were recognized with polyclonal antibodies against von Willebrand factor and GFAP (both from Dako) at 1:1000 dilution. Mouse neurons were labeled with MAb.s for the phosphorylated form of neurofilament and neuronal-specific enolase (both from Dako) at 1:100 dilution against microtubule-associated protein (MAP)-2 (Boehringer Mannheim) and PGP 9.5 (Accurate Chemical Scientific, Westbury, NY) at dilutions of 1:100 and 1:10, respectively.

Mouse microglia and macrophages were identified with rat MAb F4/80 (Serotech, Kidlington, UK). Rat MAb.s to vascular cell adhesion molecule-1 and very late antigen (VLA)-4 (PharMingen; San Diego) were used at a 1:25 dilution and recognized mouse antigens. Mouse intercellular adhesion molecule (ICAM)-1 was detected with hamster MAb (PharMingen) and used at a 1:50 dilution. Mouse cytokines were detected by polyclonal anti–TNF-α and anti–IL-1β MAb.s (Biosource, Camarillo, CA), and IL-6 was seen with rat MAb (Genzyme Diagnostics). Human HLA-DR (Boehringer Mannheim) at a 1:25 dilution and HIV-1 p24 antigen (Dako) at a 1:10 dilution (MAb.s) were used to detect cellular and viral gene products, respectively.

Indirect immunofluorescence detection (secondary antibodies) was done with FITC-coupled anti-mouse IgG F(ab′)2, and anti-rabbit IgG antibody F(ab′)2 (Boehringer Mannheim; 1:100 dilution) labeled with rhodamine or goat anti-rat antibody F(ab′)2 (fragment Biosource), coupled with FITC. Immunostained tissues were analyzed microscopically (Microphot-FXA; Nikon). Double labeling (with anti-CD68 and anti-GFAP) was done on replicate tissue sections to characterize astrocyte and human monocyte reactions. Deletion of the primary antibody or use of mouse IgG or rabbit IgG (Dako) served as controls. To detect antigens (CD68, HLA-DR, HIV-1 p24, GFAP, vimentin, neuronal-specific enolase, MAP-2, neurofilaments, and PGP 9.5) on paraffin sections, avidin-biotin immunoperoxidase staining (Vectastain Elite ABC kit; Vector Laboratories, Burlingame, CA) was used with 3,3′-diaminobenzidine as the chromogen. All sections were counterstained with Mayer’s hematoxylin.

In situ detection of apoptotic cells. Labeling of cells for apoptosis (programmed cell death) was done on paraffin sections using an in situ cell death detection kit (Boehringer Mannheim). The system utilizes the incorporation of FITC-labeled nucleotides into DNA strand breaks induced during apoptosis and is known as terminal deoxynucleotidyl transferase–mediated dUTP nick end-labeling (TUNEL). Briefly, dewaxed sections were digested with pepsin (0.5% in HCL, pH 2, for 10 min at 37°C) and incubated with the TUNEL reaction mixture (for 60 min at 37°C). Slides were analyzed under a fluorescent microscope (Microphot-FXA), or incorporated FITC was detected with anti-fluorescein antibodies (F’ab fragment from sheep) conjugated with horseradish peroxidase. After the substrate reaction (with 3,3′-diaminobenzidine used as the chromogen), stained sections were examined under light microscopy. The exclusion of terminal transferase from the TUNEL reaction mixture was used as a negative control. Pretreatment of slides with DNase (10 min at room temperature) to induce DNA strand breaks served as a positive control. Normal human tonsil and small intestine tissues were used as additional controls for the TUNEL assay; both tissues have a high cell turnover and concomitant apoptosis under normal physiologic conditions.

Reversed-phase high-performance liquid chromatography (RP-HPLC). Arachidonic acid metabolites (AAMs) and unlaabeled arachidonic acid (AA) were purchased (Sigma) as were [5,6,8,9,11,12,14,15-3H(N)]AA (200 μCi/mmol) and 8,11,14-[1-14C]icosatrienoic acid (55 μCi/mmol; American Radiolabeled Chemicals, St. Louis). Prostaglandin standards were obtained from Biomole (Plymouth Meeting, PA). Standards were injected separately during each RP-HPLC run to determine the elution times. Simultaneous analysis of AAMs was then done by pooling 5 mL of each standard for injection to determine if AAMs could be detected and quantitated in such a reaction mixture. The cocktail was transferred to a 150-μL HPLC vial (Hewlett Packard, Wilmington, DE), 5 mL was injected, and analysis was done by RP-HPLC using an on-line solid-phase extraction column (Nova-Pak C18, 2 × 150 mm; Waters, Milford, MA). Simultaneous UV detection was done at 235 and 280 nm with a photodiode array detector (1050 variable wavelength detector; Hewlett Packard). The conditions for the RP-HPLC included a gradient from 40% mobile phase A (1% acetic acid in water) to 100% mobile phase B (1% acetic acid in acetonitrile) and 60% (vol/vol) mobile phase A (1% acetic acid in water) to 100% mobile phase B.

Immune activation of monocytes and determination of AAMs. Lipopolysaccharide (LPS) obtained from Pseudomonas aeruginosa serotype 10 and zymosan A obtained from Saccharomyces cerevisiae were purchased (Sigma). Opsonized zymosan (OpZ) was prepared by boiling 0.05 g of zymosan in 36 mL of PBS for 1 h, centrifuging at 1200 × g for 8 min, and washing with PBS. The zymosan was then incubated for 30 min with 20% freshly prepared human serum, washed twice with PBS, and stored at −70°C.

For detection of AAMs in immune-stimulated monocytes, cells (3.6 × 10⁶/well) were cultured in Primaria 9.6-cm² wells (Fisher, Pittsburgh) in DMEM containing M-CSF. HIV infection of cells was done as described above. AAMs were analyzed on days 5, 10, and 14 after viral infection. Briefly, 10 μCi of [3H]AA was added to each well for 18 h. Dexamethasone (EtOH soluble; Sigma) was then added to cultures of HIV-1–infected and control uninfected cells at 10⁻⁴ M 45 min before stimulation with LPS (10 μg/mL) or OpZ (300 μg/mL). The reaction was stopped 2 h after stimulation by addition of 10 μL of formic acid, 25 μL of butylated hydroxytoluene (10 mg/mL), and 0.6 mL of methanol. The plates were quickly frozen in a dry ice–ethanol mixture and stored at −70°C. Cells were thawed, the lysate–culture supernatant
mixtures from each well were placed in 1.8-mL Ependorf tubes, and 200 \( \mu \)L of methanol was added to each well. Cells were scraped using a Foster disposable cell scraper (Fisher), followed by a second 200-\( \mu \)L methanol wash and centrifugation (5000 g for 5 min). The resulting fractured cell supernatant was pooled with the previous lysate–culture supernatant mixtures in 14-mL polypropylene tubes. An internal standard, \([^{12}C]icosatetraenoic \) acid (1.3 \( \mu \)Ci/mL) in known concentrations, was added to the clarified supernatant, and the samples were placed under argon at \(-20^\circ\)C. The pH of each 3-mL sample was adjusted to 4.0 with 9.0 mL of acified water (1 \( \mu \)L of 22 \( \mu \)Ci/mL) in known concentrations, was added to the clarified supernatant, and the samples were placed under argon at \(-20^\circ\)C. The pH of each 3-mL sample was adjusted to 4.0 with 9.0 mL of acified water (1 \( \mu \)L of 22 \( \mu \)Ci/mL).  

A cartridge (C18 Sep-Pak; Waters) was activated by adding 6 mL of HPLC-grade methanol to the cartridge and washing with 7 mL of Milli-Q water. The sample was loaded onto the Sep-Pak cartridge and washed with 0.2% ethanol, and AAMs were eluted with a mixture of 85% acetonitrile and 15% methanol. The eluent was lyophilized with a concentrator (Labconco, Kansas City, MO). The AAMs were dissolved in 1 mL of methanol, vortexed, freeze-dried, resuspended in 100 \( \mu \)L of methanol, transferred to 0.8-mL Ependorf tubes, and centrifuged at 5000 g for 10 min. After centrifugation, the AAMs were transferred to 150-mL HPLC (Hewlett Packard) vials for injection into the RP-HPLC by use of a flow-through scintillation analyzer (Radiomatic 525 TR; Hewlett Packard). For quantitation of the AAMs, the area under the curve for each metabolite was compared with the internal standard, and the quantity of the metabolite was calculated from the chromatographic tracings.  

**Dexamethasone treatment of monocytes.** Human monocytes were obtained and purified as previously described [24]. Monocytes were plated into sterile 96-well microtiter plates (Costar, Cambridge, MA) and cultured for 7 days. One day before infection with HIV-1 ADA, dexamethasone (water soluble from Sigma or dexamethasone acetate from Schein; 2 separate experiments) was added to the treatment wells at 10\(^{-4}\) or 10\(^{-6}\) M. Replicate cells were left untreated. All treatments, including controls, were done in replicates of 16. Aliquots (50 \( \mu \)L) of supernatant, in duplicate, were taken from each well every other day (days 2–14) following infection and placed in sterile 96-well microtiter plates for subsequent RT analysis (described previously). TNF bioassays followed standard laboratory procedures [15, 27]. Viability of macrophages was assessed on days 7 and 14 after infection by conversion of MTT bromide. Color intensity was measured at 490 nm [28, 29].  

**Statistical analyses.** Data were analyzed using InStat 1.12 (1992) software for Macintosh, with two-tailed unpaired \( t \) test for between-group comparisons.  

**Results**  
The neuropathogenesis of HIV-1 encephalitis remains poorly understood, and there are few therapeutic options. Much current research in HIV dementia is directed toward understanding how secretions from immune-activated HIV-1–infected macrophages produce the pathologic changes, including the hallmark neuronal loss. Because corticosteroids are broad-acting potent antiinflammatory drugs, they were our initial choice for therapeutic development for HIV dementia. A long-acting (repository) form of dexamethasone acetate was chosen to decrease handling stress of the mice.  

**Biologic consequences of dexamethasone on SCID mice.** SCID mice were treated with dexamethasone acetate in a dose equivalent to 2 mg/kg/day during the course of the experiment. This dose is in a supraphysiologic therapeutic range that optimizes down-modulation of all inflammatory events [30–33]. Treated mice had adrenal suppression and failure to thrive (figure 1). At autopsy, dexamethasone-treated mice had atrophy of the adrenal cortex (figure 1A, B). The weight differences between treated and untreated groups of mice were statistically significant (\( P < .002; \) figure 1C). There were no weight differences between groups injected with uninfected or HIV-1–infected monocytes (data not shown).  

**Effect of dexamethasone on the neuropathology-neuroimmunology of HIV encephalitis in SCID mice.** Brains from all groups of dexamethasone-treated and untreated mice were analyzed. As observed in our previous studies, the inflammatory component of HIV encephalitis in the mouse brains was greatest 7 days after injection. There was negligible needle tract–induced inflammation. For the purposes of illustration, data are shown only for the 7 day (postinjection) time point; however, differences between the dexamethasone-treated and untreated groups were seen at all time points throughout the study (data not shown).  
The effect of dexamethasone on astrogliosis was investigated first. Figure 2 shows neuropathology in brains of mice injected with uninfected macrophages. Controls (untreated) are on the left; dexamethasone-treated mice are on the right. Uninfected monocytes (15–110 cells/section, mean 60; figure 2a) were found in 6 of 7 control animals and were distributed in the putamen and globus pallidus and to a lesser extent throughout the cortex. Eighty percent of the inoculated human monocytes expressed HLA-DR (figure 2c). The presence of human cells induced an astrogliosis (figure 2e, g) \( \sim 500 \) \( \mu \)m from the injection site. This astrogliosis (hypertrophy of cell processes and increased GFAP immunostaining) was in proportion to the number of human monocytes detected. Dexamethasone treatment had no effect on the number or distribution of the monocytes identified in the sections (figure 2b). Human monocytes were found in 6 of 7 dexamethasone-treated animals. HLA-DR expression on the cell surface of the monocytes (figure 2d) and the intensity and distribution of surrounding astrogliosis (figure 2f, h) were not affected by dexamethasone treatment. In addition, GFAP content was not affected by glucocorticoid treatment in the contralateral (noninjected) hemispheres in places of usual astrocyte accumulation (corpus callosum, internal and external capsules and walls of ventricles, brainstem, and major fiber tracks; data not shown).  
Morphologic analysis of sham-inoculated mice demonstrated a very focal and minimal astrocytic response along the needle track in basal ganglia and cortex 1 week after animal manipulation. Sham-inoculated dexamethasone-treated mice showed enhanced GFAP immunostaining of astrocytes that was not restricted to the needle track. Moderately hypertrophied astrocytes were found in basal ganglia and around ventricles.
treated) are on the left and dexamethasone-treated mice are on the right. HIV-1–infected cells were seen in 6 of 7 control (untreated) animals (12–160/section, mean 62; figure 3a) and were distributed similarly to the uninfected cells. In total, 37% of monocytes immunostained with HIV-1 p24, 80% expressed HLA-DR (figure 3e), and many multinucleated giant cells were demonstrated (figure 3c). A notable astrogliosis again was seen around the injection site but was more widespread (800 μm) and intense than observed with uninfected cells. As previously shown, dexamethasone treatment did not affect the mean number or distribution of monocytes (figure 3b; monocytes were found in 6/7 dexamethasone-treated animals), p24 antigen expression, giant cell formation (figure 3d), or HLA-DR expression (figure 3f). The intensity or distribution of astrogliosis in the injected hemisphere was either not affected or was slightly increased following treatment (figure 3h, j). Dexamethasone had no effect on astrocyte responses in the contralateral (noninjected) hemisphere (data not shown).

We next evaluated the effect of dexamethasone treatment on neuronal injury. Figure 4 illustrates brain pathology of mice injected with uninfected monocytes with controls (untreated) on the left and dexamethasone-treated mice on the right. Figure 4(a, b) shows the injected human monocytes in the area of analysis. Shrunken neurons with hyperchromic nuclei were in and around the injection site. Cells containing nuclei with condensed chromatin were occasionally found around uninfected monocytes in the cortex and putamen. The TUNEL technique showed neural cells undergoing programmed cell death (detected by DNA fragmentation). Apoptosis was minimal in mice injected with uninfected monocytes (detected in 1/7) and was not observed in the contralateral hemisphere (figure 4c, e, respectively). Few cells smaller than neurons (of unidentified origin) had clear evidence of apoptosis. Cortical neurons showed increased aggregation of neurofilaments in cell bodies, while the neurofilament content in axons was decreased (figure 4g). The contralateral (noninjected) hemispheres showed normal distribution of neurofilament (figure 4i). These changes were accompanied by a modest local vacuolation of neuronal processes around human cells. Dexamethasone treatment caused modest increases in the level of apoptosis (detected in 2/7 mice) and no detectable apoptosis in the contralateral hemisphere (figure 4f). Dexamethasone did not affect neurofilament aggregation in the injected hemisphere (figure 4h). Normal distribution of neurofilament was observed in the contralateral (noninjected) hemisphere (figure 4i).

Neuronal death was evaluated in dexamethasone-treated HIV-1–infected monocyte-inoculated mice. HIV-1–infected cells were injected (figure 5; controls [untreated] are on the left and dexamethasone-treated animals on the right). Figure 5(a, b) shows a representative distribution of numbers of monocytes in affected brain areas. Many neurons undergoing programmed cell death were detected by apoptotic staining in 5 of 7 control mice (figure 5c), but no apoptotic nuclei were detected in contralateral hemispheres (figure 5e). Changes of...
Figure 2. Astrocytic and immune responses in mouse brains 7 days after uninfected control monocyte inoculation and dexamethasone treatment. Equal nos. of monocytes are in putamen along needle track in untreated (a) and dexamethasone-treated (b) mice. Monocytes express HLA-DR in both untreated (c) and dexamethasone-treated (d) mice. Astrocytic responses are in and around monocytes (arrows) in putamen of untreated (e) and treated (f) mice. Astrocytes are hypertrophied and show increased glial fibrillary acidic protein immunostaining intensity in both control (g) and treated (h) mice. Serial coronal sections from control (a, c, e, g) and treated (b, d, f, h) mice were immunostained with antibodies to CD68 (a, b), HLA-DR (c, d), and glial fibrillary acidic protein (e–h). Tissue sections were counterstained with Mayer’s hematoxylin. Original magnifications, ×200 (a, b, g, h); ×400 (c, d); ×40 (e, f).

Neurofilament aggregation and axonal damage were more pronounced than with uninfected cells (figure 5g), and no changes were found in the contralateral hemisphere (figure 5i). With dexamethasone treatment, programmed cell death of neurons was detected in 6 of 7 animals, and changes were more widespread than in untreated HIV-1–infected monocyte-inoculated animals (figure 5d). Such neuronal changes were absent in the contralateral hemispheres (figure 5f). Dexamethasone treatment
Figure 3. Astrocytic, viral antigen, and immune responses in mouse brains 7 days after HIV-1–infected monocyte inoculation and dexamethasone treatment. Numerous single and multinucleated cells expressing CD68 antigen are in putamen in both untreated (a) and dexamethasone-treated (b) mice. Human monocytes expressed HIV-1 p24 antigen (c, d). No differences are seen between untreated (c) and treated (d) mice. HLA-DR is expressed on monocytes in both untreated (e) and treated (f) mice. More pronounced and widely spread astrocytic reaction surrounds HIV-1–infected cells (arrows) in treated (h) than in untreated (g) mice. Cellular processes of astrocytes are hypertrophied and show significantly more glial fibrillary acidic protein (GFAP) immunoreactivity in treated (j) than in untreated (i) mice. Serial coronal sections from untreated (a, c, e, g, i) and treated (b, d, f, h, j) mice immunostained with antibodies to CD68 (a, b), HIV-1 p24 antigen (c, d), HLA-DR (e, f), and GFAP (g–j). Tissue sections were counterstained with Mayer’s hematoxylin. Original magnifications, ×200 (a–d, i,j); ×400 (e, f); ×40 (g, h).
Figure 4. Neuronal alterations in serial coronal sections of mouse brains 7 days after uninfected control (a, c, e, g, i) monocyte inoculation and dexamethasone treatment (b, d, f, h, j). Equal nos. of CD68-positive monocytes were found in cortex of untreated (a) and dexamethasone-treated (b) mice. Rare neurons and other unidentified cells underwent apoptosis (DNA fragmentation) in untreated mice (c); treated (d) mice had modestly increased apoptosis. Contralateral hemispheres of untreated (e) and treated (f) mice failed to show neuronal apoptosis. Redistribution of neurofilaments is seen in same areas on serial sections of untreated (g) and treated (h) mice. Neurofilament distribution was normal in contralateral hemispheres (i, j). Tissue was immunostained with antibodies to CD68 (a, b) or neurofilaments (g – j) or labeled for apoptosis by transferase-mediated dUTP nick end-labeling method (c – f). Tissue sections (a, b, g – j) were counterstained with Mayer’s hematoxylin. Original magnifications, ×200 (a, b); ×100 (c – j).
Figure 5. Neuronal alterations in mouse brains 7 days after HIV-1–infected human monocyte inoculation and dexamethasone treatment. CD68-positive monocytes were equally distributed in cortex of untreated (a) and dexamethasone-treated (b) mice. Nuclei of neurons and other unidentified cells stained for DNA fragmentation in areas adjacent to HIV-1–infected monocytes in untreated (c) and to larger degree in treated (d) mice. No staining was seen in respective contralateral hemispheres (e, f). Affected neurons accumulated neurofilaments in cell bodies in untreated (g) mice. Treated mice (h) had increased cell bodies containing neurofilaments. Contralateral hemispheres of untreated (i) and treated (j) mice had normal neurofilament distribution. Serial coronal sections from untreated (a, c, e, g, i) and treated (b, d, f, h, j) mice immunostained with antibodies to CD68 (a, b), neurofilament (g–j), or labeled for apoptosis by transferase–mediated dUTP nick end-labeling method (e–f). Tissue sections (a, b, g–j) were counterstained with Mayer’s hematoxylin. Original magnifications, ×200 (a, b); ×100 (c–j).
had no effect on neurofilament redistribution (figure 5H) or on neurofilament immunostaining in the contralateral hemisphere (figure 5J).

The effect of dexamethasone on the microglial nodule formation was next investigated. As found in our previous studies, F4/80-positive microglia formed aggregates around human monocytes 4 days after injection and peaked 7–12 days after monocyte inoculation. The degree of microglial accumulation and signs of cellular activation were dependent upon the number of HIV-1–infected monocytes. Mice injected with uninfected monocytes showed minimal microglial accumulation, which was unaffected by dexamethasone treatment (data not shown). Figure 6 shows mice injected with HIV-infected monocytes (untreated controls, left; dexamethasone-treated on right). Control mice showed focal aggregation of microglia (figure 6a) around HIV-infected monocytes (figure 6c); contralateral hemispheres showed no signs of cellular (microglial) activation (figure 6e). Dexamethasone-treatment resulted in increased microglial accumulation and activation. These microglial reactions were more diffuse with less distinct nodule formation (figure 6b); no effect on microglial formation was observed in the noninjected hemisphere (figure 6f).

Vascular cell adhesion molecule-1 (more pronounced on microvessels in hemispheres receiving HIV-1–infected monocytes) and ICAM-1 expression (more obvious in mouse brains inoculated with control cells) were down-regulated in mice treated with dexamethasone. VLA-4 expression on microglia and mouse macrophages was unaffected by glucocorticoids (data not shown).

Taken together, the results obtained in this study confirm that injection of HIV-1–infected monocytes into the brains of SCID mice result in increased levels of astrocytosis, microglial nodule formation, and neuronal alterations than seen with uninfected monocytes. Dexamethasone treatment of the animals overall caused increased neuronal changes in both groups of mice (uninfected and HIV-1–infected). The neuropathologic effects in the HIV-1–infected, dexamethasone-treated group were the most severe. Of interest, dexamethasone worsened astrocytosis in SCID mouse brains with needle trauma (sham inoculated), signaling that glucocorticoids may actually enhance vulnerability of brain tissue to other (nonviral) types of harmful insults.

**Effect of dexamethasone on TNF and prostaglandin E2 (PGE2) secretion from human macrophages.** Because of the effect of dexamethasone on the neuropathology of our SCID mouse model of HIV encephalitis, we evaluated possible mechanisms for these observations. TNF is one of many macrophage secretory products thought to contribute to the neuropathology of HIV encephalitis. To evaluate the effect of dexamethasone on TNF secretion, both uninfected and HIV-1–infected monocytes were treated with dexamethasone in increasing concentrations prior to stimulation with LPS. Samples were collected 4–24 h after stimulation and analyzed for TNF bioactivity. Figure 7 shows TNF production for HIV-1–infected cells 12 h after LPS stimulation. Dexamethasone decreased TNF secretion to <80% of that in control (untreated) cells. Maximal effects were seen only at the highest concentration, 10^{-4} M, whereas at 10^{-6} M, only a small (~20%) decrease in the TNF level was observed. Given the volume of distribution of dexamethasone in the mice, the highest molar concentrations achievable in animals is projected to be 10^{-6}–10^{-4} M.

A number of AAMs are thought to play a role in the neuronal damage of HIV-1 encephalitis. To evaluate the effect of dexamethasone on AA metabolism, HIV-1–infected and control uninfected macrophages were treated in vitro with dexamethasone (10^{-4} M) before stimulation by OpZ. After cells and super-
natants were collected and extracted, PGE₂ production was measured by RP-HPLC. The results (figure 8) demonstrate a trend toward decreased PGE₂ production by HIV-1-infected macrophages in the presence of the highest doses of dexamethasone, but again, suppression was incomplete.

**Effect of dexamethasone on HIV replication.** If dexamethasone caused an increase in HIV replication, this might account for the therapeutic failure of dexamethasone in our mouse model. Indeed, the concern for increased viral replication in the presence of corticosteroids has tempered the use of such drugs during progressive HIV disease for problems such as idiopathic thrombocytopenic purpura. A recent study, however, suggests this may not be biologically relevant. In that study, AIDS patients treated with systemic steroids had improved CD4⁺ T cell counts [34]. To evaluate the possibility that dexamethasone could alter HIV-1 replication in monocytes and thereby modulate virus-induced neurotoxicity, we treated cell cultures (both HIV-1-infected and uninfected) with dexamethasone at 10⁻⁴ and 10⁻⁶ M. Samples were collected every other day and analyzed for RT activity. Dexamethasone treatment decreased RT activity compared with that of untreated HIV-infected controls, but only at the highest concentration used (10⁻⁴ M; figure 9). The effect of dexamethasone on progeny virion production was only modest.

**Effect of dexamethasone on macrophage viability.** Another possible mechanism for the worsened pathology in the dexamethasone-treated mice, despite decreased monocyte secretions, might occur if dexamethasone increased cell viability, leading to absolute increases in monocytes in the brain. To
evaluate this issue, cultured monocytes (HIV-infected and uninfected) were treated with dexamethasone (two different preparations) at $10^{-4}$ and $10^{-6}$ M. Viability was measured by MTT conversion assays 7 and 14 days after infection. Dexamethasone increased viability in HIV-1–infected cells compared with controls (untreated cells; figure 10). These differences were statistically significant ($P < .001$) but were seen only in infected cells. Paradoxically, dexamethasone decreased cell viability in uninfected monocytes.

**Discussion**

We used a SCID mouse model for HIV-1 encephalitis to investigate the therapeutic efficacy of glucocorticoids. Our hypothesis was based on the idea that HIV-1–infected macrophages or microglia are the main reservoir for virus in brain and are the perpetrators for neurotoxic secretions, and that their numbers correlate with the clinical severity of HIV-1 dementia. We anticipated that brain pathology (including astrogliosis, microglial nodule formation, and neuronal injury) would diminish after dexamethasone treatment. Even though high levels of drug were achieved, signs of monocyte-induced damage in the CNS were in many instances surprisingly exacerbated.

Multiple mechanisms could have been involved in this apparent therapeutic failure. Viral gene products (gp120, tat, nef, and others) are involved in neurotoxic activation of HIV-1–infected macrophages [13]. Although dexamethasone decreased HIV-1 replication in cultured monocytes (as measured by RT activity), its effect was modest. The failure of dexamethasone to affect pathology in our model of HIV-1 encephalitis supports the notion that specific viral factors play a role in disease pathogenesis.

Our in vitro studies showed that although dexamethasone could induce a modest decrease in TNF and PGE$_2$ production from HIV-1–infected monocytes, an increase in cell viability of the infected monocyte likely negated any concomitant decrease in neurotoxic secretions. These results are in agreement with previously published works. In Borna disease–induced encephalitis, dexamethasone treatment did not down-regulate TNF-$\alpha$, IL-6, macrophage inflammatory protein-1$\beta$, and mob-1 in the CNS of rats [35]. These findings, in total, suggest a refractoriness of resident brain macrophages and microglia to corticosteroid treatments. The down-regulation of TNF and PGE$_2$ by dexamethasone in our model may not indicate that these molecules do not play a role in disease. Low-level production of TNF and PGE$_2$ may be necessary, but not sufficient, to induce neuropathology. Moreover, both factors are part of a complicated loop of autocrine and paracrine inflammatory factors known to affect various aspects of neuronal function and viability. In the context of this single study utilizing one broadly acting antiinflammatory drug, it is difficult to specify the exact roles played by TNF and PGE$_2$ in disease pathogenesis. Nevertheless, the reduction of both of these factors failed to affect the neuropathologic outcomes for disease. This suggests, at the least, that multiple factors are involved in the neurotoxicity observed during CNS HIV-1 infection.

An additional possibility for the therapeutic failure of dexamethasone in this model revolves around the complex and diverse actions of glucocorticoids in the brain. As reviewed by Sapolsky [36], glucocorticoids can have deleterious effects on CNS function. Sustained exposure to high levels of glucocorticoids in brain tissue can directly damage or atrophy the hippocampus (assessed as neuronal loss) in various animal species (guinea pigs, rats, mice, and Old World primates) and accelerate hippocampal aging. Hippocampal atrophy, as measured by magnetic resonance imaging, is often seen in human Cushing’s syndrome. In major depression disorders (in which about half of affected persons are hypercortisolemic), hippocampal atrophy is also present. Glucocorticoids also impair glucose uptake. This renders neurons more susceptible to injury from excitotoxic and metabolic insults through augmentation of extracellular excitotoxic amino acid accumulation, free cytosolic calcium, and an exacerbation of calcium-dependent neurodegeneration. Such mechanisms help to explain the findings seen here of worsened HIV-1–associated neuropathology, including increased neuronal apoptosis after dexamethasone treatment.

The effects of dexamethasone in worsening needle trauma–induced neuropathology indicates that such treatments may impair the ability of the brain to recover from CNS trauma and perhaps other noxious insults. Others have reported increased neuronal apoptosis in the hippocampus in a rabbit model of pneumococcal meningitis following treatment with dexamethasone.
Acknowledgments

seen commonly in children given high doses of glucocorticoids, treated mice showed signs of failure to thrive. Such findings are seen commonly in children given high doses of glucocorticoids, along with adrenal suppression and neurologic deficits. The inanition and adrenal suppression observed in the study animals may thus have played a role(s) in the increased neuropathologic findings seen in the dexamethasone-treated mice.

The effects seen in brain tissue after systemic administration of dexamethasone may be related to the stage of HIV brain disease. The pathogenesis of HIV encephalitis is complex, involving the presence of infected macrophages in the CNS with subsequent immune activation. Neurotoxic secretory responses from immunologically competent macrophages ultimately induce alterations in blood-brain barrier permeability and macrophage chemokine production, with further transendothelial migration of infected monocytes into the CNS. This results in a vicious cycle of inflammation and sustained neurotoxicity.

Importantly, our animal model of HIV encephalitis involved the direct placement of HIV-infected monocytes into the brain, thus bypassing the blood-brain barrier and inflammatory macrophage recruitment steps of disease. This process mimics only the later stages of HIV-1 CNS disease. In a study by Morimoto et al. [35], dexamethasone treatment early in the course of Borna disease prevented the development of neurologic signs and neuropathologic lesions associated with viral infection. This was accomplished through the prevention of inflammatory cell transendothelial migration into the brain and was likely mediated by down-regulation of inflammatory molecule production, preventing cell access across the blood-brain barrier (e.g., bradykinin, PGE₂, and nitric oxide). Thus, while dexamethasone may fail to block the neurotoxic responses late in HIV encephalitis, it may still play an important role early in the disease by preventing the influx of inflammatory cells (macrophages) into the brain.

The failure of dexamethasone to ameliorate the neuropathology in our SCID mouse model of HIV-1 encephalitis may have important implications in therapeutic trials in AIDS patients. Unfortunately, no effective medicines are available for HIV-1 dementia, and few clinical trials are currently planned with drugs other than those directed against a component of the virus life cycle. Our results suggest the need for caution when administering high-dose dexamethasone to treat late stages of HIV-1 dementia. Caution should also be exercised in extrapolating these experimental findings to the general use of steroids in virus-infected humans. Certainly, these observations should not be generalized so that glucocorticoids are avoided in other HIV-associated complications where they are clearly warranted (e.g., idiopathic thrombocytopenic purpura and chronic aphthous ulcers). Studies are now underway in our laboratories to investigate antiinflammatory drugs with specific modes of action. These will be tested in conjunction with antiretroviral therapies that cross the blood-brain barrier. Such combined therapies may ultimately prove invaluable to prevent or treat this important complication of HIV-1 infection in humans.

References