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Regulation of Lentivirus Neurovirulence by Lipopolysaccharide Conditioning: Suppression of CXCL10 in the Brain by IL-10

Ferdinand Maingat,*¹ Serena Viappiani,*¹ Yu Zhu,* Pornpun Vivithanaporn,*⁺ Kristofer K. Elsestad,* Janet Holden,‡ Claudia Silva,‡ and Christopher Power*⁺⁺

Lentivirus infections including HIV and feline immunodeficiency virus (FIV) cause neurovirulence, which is largely mediated by innate immunity. To investigate the interactions between neurovirulence and repeated conditioning by innate immune activation, models of lentivirus infection were exposed to LPS. Gene expression in HIV-infected (HIV+) and control (HIV−) patient brains was compared by real time RT-PCR and immunocytochemistry. Supernatants from mock and HIV-infected monocyte-derived macrophages exposed to LPS were applied to human neurons. FIV-infected (FIV+) and control (FIV−) animals were exposed repeatedly to LPS postinfection together with concurrent neurobehavioral testing, viral load, and host gene analyses. Brains from HIV+ individuals exhibited induction of CD3ε, CXCL10, and granzyme A expression (p < 0.05). Supernatants from HIV+ monocyte-derived macrophages induced CXCL10 expression in neurons, which was diminished by IL-10 treatment (p < 0.05). LPS-exposed FIV+ animals demonstrated lower plasma and brain viral loads (p < 0.05). Neuronal CXCL10 expression was increased in FIV+ animals but was suppressed by LPS exposure, together with reduced brain CD3ε and granzyme A expression (p < 0.05). In conjunction with preserved NeuN-positive neuronal counts in parietal cortex (p < 0.05), FIV+ animals exposed to LPS also showed less severe neurobehavioral deficits (p < 0.05). Repeated LPS exposures suppressed CXCL10 in the brain and ensuing T cell infiltration with a concomitant reduction in neurovirulence. Thus, innate immune chronic conditioning exerted beneficial effects on neurovirulence through suppression of a specific chemotactic factor, CXCL10, mediated by IL-10, leading to reduced leukocyte infiltration and release of neurotoxic factors. The Journal of Immunology, 2010, 184: 1566–1574.

Lentiviral infections exhibit several common features, including macrophage tropism and the occurrence of neurologic disease, also termed neurovirulence (1). The immunosuppressive lentiviruses including HIV, SIV, feline immunodeficiency virus (FIV), and bovine immunodeficiency virus (BIV) are defined by infection and depletion of T cells with chronic dysregulation of innate immunity (2). Neurovirulence in lentivirus infections stems from the viruses’ capacity to infect all levels of the neural axis early in the course of infection, leading to a constellation of neurologic phenotypes (3). However, infection of the brain represents a particular deleterious outcome of lentivirus infection with ensuing neurovirulence as a consequence of two predominant processes, including aberrant activation of host innate immune mechanisms in the brain together with local cytotoxic effects of viral proteins (4). Infiltrating leukocytes, including activated/infected macrophages and lymphocytes, represent the principal cell types involved in increased trafficking into the brain, allowing lentivirus infection of permissive resident brain cells including microglia and astrocytes within the neural parenchyma. The underlying molecular mechanisms have received increasing attention with a focus on chemokine expression (5). The chemokines CXCL10, CXCL12, and CCL2 have drawn substantial attention because of their pluripotent effects, including chemotaxis and neurotoxicity together with their cognate receptors’ important downstream effects (6). For example, CXCL10, also termed interferon-γ inducible protein-10, is upregulated in HIV- and SIV-infected brains (7), whereas its receptor CXCR3 appears to mediate neurotoxicity that depends on the individual isoform (8, 9). Hence, this group of soluble molecules represents important pathogenic factors in neurovirulence as proposed for other nervous system infections, perhaps by multiple mechanisms including chemotaxis of circulating leukocytes and direct neurotoxic actions.

Both the nervous and gastrointestinal systems are major targets for lentivirus infection, resulting in neural tissue damage (4) or depletion of GALT with ensuing injury to the small bowel (10), respectively. Damage to the small bowel results in increased gut permeability and microbial translocation from the gut lumen into the circulation (11). These events are assumed to contribute to systemic innate immune activation and perhaps drive immunodepletion and end-organ damage (12, 13). The development of neurologic disease in HIV infection has been associated with microbial translocation (14). However, innate immune activation induced by bacterial products exerts diverse effects on neurologic disease. In some human diseases (e.g., graft versus host disease, inflammatory bowel disease) microbial translocation has no apparent effect on the nervous system, whereas in others (e.g., Alzheimer’s disease, septic encephalopathy) concurrent systemic...
infection exacerbates the disease process. Indeed, early life infections appear to reduce the occurrence and severity of some neurologic diseases such as multiple sclerosis (15, 16). In models of neurodegenerative diseases, repeated exposure to the bacterial cell wall constituent LPS accelerates the disease course (17, 18). Conversely, in other models of neurologic disease, in which LPS was applied chronically, the disease outcome was improved (19). Thus, LPS and other microbe-derived factors have varied effects on neurologic diseases, depending on the type of immune activator and several host factors including age, sex, and host response to the underlying disease.

Immunosuppressive lentivirus infections are frequently accompanied by systemic (opportunistic) infections (20), but the impact of these concurrent infections on neurologic disease remain uncertain. In this study, we investigated in vitro and in vivo models of lentivirus neuropathogenesis in which HIV- or FIV-infected animals or, respectively, were repeatedly exposed to LPS, and systemic variables together with neurologic outcomes were assessed. These studies showed that LPS exposure suppressed viral load in vivo and improved neurologic outcomes through a mechanism involving suppressed chemokine expression accompanied by reduced lymphocyte infiltration into the brain.

Materials and Methods

Viruses
Culture supernatants from feline PBMCs infected with an infectious neurovirulent molecular clone (FIV-Ch) served as sources of infectious FIV (21) for the in vivo experiments. Supernatants from human PBMCs infected with a neurotropic HIV-1 strain (HIV-1 SF162) was prepared similarly and used for in vitro infection experiments. Viruses were titrated by limiting dilution as previously reported (22).

Human brain samples
Human CNS tissue (frontal lobe) was collected at autopsy with consent and stored at −80°C from HIV-infected (HIV encephalitis, n = 2; toxoplasma encephalitis [remote from lesion], n = 3) and noninfected (stroke, n = 2; sepsis, n = 3; leukemia, n = 2) control patients with consent. All HIV-infected individuals were AIDS-defined and died of AIDS-related causes. Controls comprised other neurologic diseases including Alzheimer’s disease, multiple sclerosis, and stroke (23, 24).

Animals and virus infection
Adult, specific-pathogen-free pregnant cats (queens) were housed according to Universities of Alberta and Calgary animal care facilities’ guidelines in agreement with Canadian Committee on Animal Care guidelines. All queens were negative for feline retroviruses (FIV, FeLV) by PCR analysis and serologic testing. At day 1 postnatal, animals were inoculated (right frontal lobe) with 200 μl FIV-Ch29 at 10^7 TCID50/ml using a 30-gauge needle and syringe via intracranial injection in accordance with Canadian Committee on Animal Care guidelines, as described previously (22, 25). Control animals (FIV−) received heat-inactivated virus. Adolescent animals were weaned at 6 wk, and at weeks 7, 9, and 11 postinfection, LPS (Sigma-Aldrich, St. Louis, MO) for 3 d at 37°C, 10% CO2. Primary human fetal neurons (hFNs) were obtained in accordance with the University of Alberta Ethics committee, prepared as previously reported (31), and cultured in polyornithine-coated (Sigma-Aldrich) plates in serum-free AIM-V (PBS; Life Technologies) (23). Human monocyte-derived macrophages (MDMs) were prepared from healthy HIV-seronegative controls by initially isolating PBMCs on a Ficoll gradient and plastic adherence, and thereafter maintained in RPMI (Life Technologies) supplemented with 10% L929 medium and 20% FBS (23). MDMs were cultured at 37°C, 10% CO2 for 1 wk before use. Mock-infected (HIV−) cells served as controls.

LPS exposure to hMDMs
Human MDMs—mock or HIV-infected—were exposed to LPS (100 ng/ml; Sigma-Aldrich) or PBS in growth media 7 d postinfection. At 3, 6, and 9 d after initial LPS exposure, half of the culture supernatant was collected and replaced with fresh media supplemented with LPS (100 ng/ml). A reverse transcriptase assay was performed on the harvested supernatants to monitor levels of viral release, as previously reported (23). The supernatants harvested at days 3, 6, and 9 were applied to hNBs and hFNs for 48 h at 37°C, 5% CO2. Cultures were then fixed and processed for in-cell Western of β-tubulin immunoactivity and DAPI staining.

MDM-derived supernatant application to hNB and hFN cells
Human MDMs were infected with HIV-1 (SF162) or mock-infected. Initial infection of MDM was achieved by a 6-h virus exposure followed by a media change every 3 d for a period of 7 d at 37°C, 5% CO2.
Supernatants were then harvested and applied to cultured hNB and hFN cells for 6 or 48 h at 37 °C, 5% CO₂. Cultures were then fixed and processed for in-cell Western of β-tubulin and CXCL10 immunoreactivity, followed by DAPI staining.

Reverse transcriptase assay
Reverse transcriptase (RT) activity in culture supernatants was measured using a protocol described previously (32). Ten microliters of culture supernatant was cleared of cellular debris by high-speed centrifugation and incubated with 40 ml reaction mixture containing [α-32P]TTP for 2 h at 37°C. Samples were plated on DE81 Ion Exchange Chromatography Paper (Whatman International, Maidstone, U.K.) and washed three times for 5 min in 2 x SSC and twice for 5 min in 95% ethanol (1 x SSC is 0.15 M NaCl plus 0.015 M sodium citrate). RT levels were measured by liquid scintillation counting. All assays were performed in duplicate and repeated a minimum of two times.

IL-10 treatment of neurons
Cultures of hNB and hFN cells were pretreated in growth media supplemented with recombinant human IL-10 (Peprotech, Rocky Hill, NJ) or PBS for 3 and 16 h. Mock (HIV−) or HIV-infected (HIV+) supernatants were applied to cultured hNB and hFN cells for 48 h at 37 °C, 5% CO₂, followed by processing of cultures for in-cell Western analyses of CXCL10 immunoreactivity.

In-Cell Western/DAPI staining
hNB cells and primary hFNs were cultured for 24 h with supernatants from HIV-mock or HIV-infected MDMs at 37 °C, 10% CO₂. An in-cell Western ELISA (LI-COR, Lincoln, NE) using Abs against β-tubulin or CXCL10 was used to assess neuronal viability and CXCL10 expression, respectively. After treating hFNs and hNBs with MDM-derived supernatants, cells were fixed in 2% formalin, washed in PBS, permeabilized with 0.5% Triton X-100 in PBS, and blocked in LI-COR blocking buffer. Fixed cells were incubated overnight with anti β-tubulin (1/800; Sigma-Aldrich) and CXCL10 (1/500; R&D Systems, Minneapolis, MN) Abs, washed, and incubated with secondary Alexa fluor 680 (Invitrogen, Burlington, Ontario, Canada) and IRDye 800 (Rockland, Gilbertsville, PA) conjugated Abs, respectively. After the final washing, neuronal viability and CXCL10 induction were quantified using an Odyssey Infrared Imaging System (LI-COR). Fixed cells were exposed to 0.01 mg/ml DAPI stain (Molecular Probes, Eugene, OR) for 5 min in 2 x SSC and twice for 5 min in 95% ethanol (1 x SSC is 0.15 M NaCl plus 0.015 M sodium citrate). RT levels were measured by liquid scintillation counting. All assays were performed in duplicate and repeated a minimum of two times.

Immunodetection in tissue sections
Immunohistochemical and immunofluorescent labeling was performed using 6-μm, paraffin-embedded serial human and feline brain sections, respectively, prepared as previously described (33). Coronal brain sections were deparaffinized and hydrated using decreasing concentrations of ethanol. Ag retrieval was performed by boiling the slides in 0.01 M trisodium citrate buffer, pH 6.0, for 10 min. Sections were blocked in PBS containing 10% normal goat serum, 2% BSA, and 0.1% Triton X-100 overnight at 4°C. Afterward, feline brain sections were incubated overnight at 4°C with Abs against ionized calcium binding adaptor molecule 1 (Iba-1; 1:200; Wako, Tokyo, Japan), calcium binding protein 1 (Cahbp1; 1:100; Abcam, Cambridge, MA), CXCL10 (1:100; R&D Systems, Minneapolis, MN), and IL-10 (1:100; Biologend, San Diego, CA); they were then washed in PBS and incubated with biotin-conjugated goat Abs followed by avidin-biotin-peroxidase amplification (1:500 dilution; Vector Laboratories, Burlingame, CA) for 2 h at room temperature, and washed again in PBS. Subsequent immunoreactivity was detected by 3,3'-diaminobenzidine tetrachloride staining (Vector Laboratories). All sections were examined with a Zeiss Axioskop 2 upright microscope (Oberlenchen, Germany) and an LSM510 META (Carl Zeiss MicroImaging) confocal laser-scanning microscope and analyzed using LSM 5 Image Browser (Carl Zeiss, Jena, Germany). The specificity of staining was confirmed by omitting the primary Ab.

Neuronal counts
To assess neuronal viability in the cortex of FIV- and mock-infected animals, NeuN-immunopositive neurons in the left parietal cortex and hippocampus were counted at +10 mm from the bregma. For the parietal cortex, immunopositive cells were counted at 400× magnification in all layers of the cortex in five separate nonoverlapping fields for each animal. Similarly, the number of neurons was counted in five different fields within the dentate gyrus at 400× magnification for each animal. The total number of cells was summed for each animal and averaged across groups.

Statistical analysis
Statistical analyses were performed by Student t test when comparing two different groups or by ANOVA test with Tukey-Kramer or Bonferroni as post hoc tests, using GraphPad Instat version 3.0 (GraphPad, San Diego, CA); p < 0.05 was considered significant.

Results
Neuroinflammatory gene expression during HIV infection
Prior studies have reported that brains from HIV-infected persons exhibit inflammatory changes, defined by glial activation and in some instances lymphocyte infiltration (34, 35), which contribute to neurodegenerative changes manifesting as HIV-associated dementia (6). To extend these studies, we investigated the expression of several inflammatory genes in brains from HIV+ and HIV− persons, which revealed that the T cell marker CD3ε (Fig. 1A), the astrocyte marker CD68 (Fig. 1B), the macrophage marker Iba-1 (Fig. 1C), and granzyme A (Fig. 1D) were also elevated in HIV+ brains, whereas IL-10 (Fig. 1E) transcript levels did not differ significantly. Immunocytochemical analysis revealed that CD3 immunoreactivity was negligible in HIV− brains (H), but evident in HIV+ brains (G). In contrast, IL-10 was present on macrophage-resembling cells in HIV− brains (I), but not in HIV+ brains (L; mean ± SD; Student t test; p < 0.05). G, I, J, L, original magnification ×400; H K, original magnification ×200.
GFAP (Fig. 1B), and the myeloid cell marker HLA-DRA (Fig. 1C) transcripts (Table I) were increased in white matter from HIV-infected brains. Similarly, the chemokine CXCL10 (Fig. 1D) and the proteolytic enzyme granzyme A were induced in HIV-infected brains, whereas IL-10 (Fig. 1F) did not differ significantly between groups. Of note, bacterial ribosomal 16S RNA levels in the brain did not differ between groups (Supplemental Fig. 1A). Immunocytochemical studies revealed that CD3 immunoreactivity was minimal in HIV− brains (Fig. 1G), but was evident in small round cells, resembling lymphocytes in HIV+ brains (Fig. 1J). Likewise, CXCL10 immunoreactivity was negligible in HIV− brains (Fig. 1H), but was apparent in HIV+ brains, particularly in cells with a neuronal appearance (Fig. 1K). Conversely, IL-10 immunoreactivity was detected in cells resembling macrophages in HIV− brains (Fig. 1I), but was minimally present in HIV+ brains (Fig. 1L). These data underscored the capacity for HIV infection to induce CXCL10 in the brain in conjunction with T cell infiltration of the brain. 

CXCL10 and IL-10 interactions during HIV infection

Earlier studies suggested that CXCL10 was induced in neurons during HIV Infection, although the mechanism is uncertain (36). To explore this observation further, we exposed human neurons to supernatants from HIV-infected MDMs (Fig. 2B), which is indicative of process retraction but not in primary hFNs following exposure to supernatants from HIV+ MDMs (Fig. 2B). Cellular viability as measured by DAPI staining of both human neuronal cell lines was unaffected by the same supernatants (Fig. 2C, 2D), but CXCL10 expression was significantly enhanced in both human neuroblastoma cells (Fig. 2E) and human fetal neurons (Fig. 2F) after exposure to supernatants derived from HIV+ MDMs. However, prior treatment of neurons with IL-10 (50 ng/ml) for 3 or 16 h suppressed CXCL10 expression in human fetal neurons when supernatants from HIV-infected MDMs were applied (Fig. 2G). Thus, HIV infection induced CXCL10 expression in neurons, which could be regulated by prior IL-10 exposure. These observations recapitulated the findings in human brains in that CXCL10 was induced in HIV+ brains in conjunction with lower IL-10 expression.

LPS effects on HIV infection and neuronal viability

LPS exposure is a consequence of Gram-negative bacterial infections, which lead to engagement of TLR4 and ensuing activation of monocytes (37). To investigate the effects of LPS on HIV infection, HIV− and HIV+ MDMs were exposed to PBS or LPS at days 3, 6, and 9 after HIV infection, disclosing that HIV replication, measured by RT activity, was increased significantly at days 3, 6, and 9 after LPS exposure (Fig. 3A). Application of supernatants from HIV-infected MDMs exposed to LPS for 48 h exerted differential effects on neuronal viability in terms of β-tubulin expression (Fig. 3B) and DAPI staining (Fig. 3C). At days 3 and 6 (Fig. 3B), supernatants from HIV+ MDMs exposed to LPS applied to neurons showed that neuronal β-tubulin immunoreactivity was reduced compared with supernatants from PBS-exposed MDMs, whereas at day 9, supernatants from LPS-exposed HIV+ MDMs showed a significant protective effect. Similar findings were observed in the DAPI-stained cultures (Fig. 3C), whereas supernatants from HIV− MDMs were neurotrophic at each time point (data not shown). Analyses of changes in neuronal viability over time exhibited a significant increase for cells exposed to the supernatants of chronically stimulated MDMs, as evaluated by β-tubulin immunoreactivity (Fig. 3B; p = 0.0005) and DAPI reactivity (Fig. 3C; p = 0.0025). These observations emphasized the differential effects of acute versus chronic LPS exposure on neuronal survival, presumably owing to an LPS-related tolerogenic effect. These findings indicate that repeated stimulation of HIV-infected MDMs with LPS diminishes the production of neurotoxic factors over time in contrast to cells acutely exposed to LPS.

Systemic LPS exposure reduced viral burden

Like HIV infection, IVF-induced immunosuppression (and ensuing neurologic disease) occurs largely through infection of CD4+ T cells and their subsequent depletion. Viral load is a key marker of in vivo viral burden, and its measurement in blood is a useful indicator of systemic disease status (38, 39). Animals that were repeatedly exposed to LPS showed signs of sickness behavior for ~1–2 h after injection, as evidenced by reduced motor activity, sleep, and ruffled coat, but promptly recovered in all cases. IVF

Table I.  Primers used for real-time RT-PCR analyses

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<th>Primer</th>
<th>Sequence (5′-3′)</th>
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<td>56–60</td>
<td>Human/feline</td>
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<tr>
<td>GAPDH rev</td>
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<td>56–60</td>
<td>Human/feline</td>
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<td>56</td>
<td>Human/feline</td>
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<td>GFAP rev</td>
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<td>56</td>
<td>Human/feline</td>
</tr>
<tr>
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<td>56</td>
<td>Human/feline</td>
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<tr>
<td>Granzyne A rev</td>
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<td>56</td>
<td>Human/feline</td>
</tr>
<tr>
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infection resulted in reduced CD4+ T cell levels in the blood of
animals with repeated LPS or PBS exposures (Fig. 4A), compared
with FIV- animals. Conversely, CD8+ T cell levels in blood were
increased in PBS-exposed FIV+ animals, and this effect was
suppressed in FIV+ animals that were repeatedly exposed to LPS
(Fig. 4B). Analyses of weight gain in the four experimental groups
(Fig. 4C) revealed that FIV+ animals treated with PBS exhibited
significantly lower levels of weight gain over time. Conversely,
relative weight gain in LPS-exposed FIV+ and FIV- animals did
not differ significantly from that in PBS-treated FIV- animals
over time. Viral load analyzed in blood or brain samples from FIV+
animals with repeated PBS or LPS exposures indicated that
repeated LPS exposure was associated with a ∼2 log10 copies/mL viral
reduction in plasma viral load (Fig. 4D). Likewise, LPS exposure
resulted in a ∼1 log10 reduction in viral load in the parietal cortex
(Fig. 4E) and basal ganglion (Fig. 4F), although viral load in the
brain was ∼0.5 log10 less than in blood. These findings indicated
that repeated LPS exposures suppressed blood CD8+ T cell levels
and viral burden in different tissue compartments of FIV+ animals
together with eliminating the delay in weight gain in the same
animals.

**Neuroinflammatory gene expression in FIV infection**

Like HIV infection, increased neuroinflammatory gene expression
is a feature of FIV infection, although the impact of repeated LPS
(versus PBS) exposures remained unclear. Examination of host gene
expression in both the parietal cortex and the basal ganglia revealed
that the macrophage activation marker F4/80 (Fig. 5B), CD3ε (Fig.
5A), and GFAP (Fig. 5C) transcript levels were significantly in-
creased in FIV+ animals with PBS exposures compared with FIV-
animals. However, day 9-derived supernatants from HIV-infected MDMs exposed to LPS exerted a comparative neuroprotective effect. Moreover, both methods of measuring neuronal viability revealed an increasing neuroprotective effect with repeated LPS exposures to HIV+ MDMs (B, C; mean ± SD; Student t test; *p < 0.05).

ANOVA of a linear trend over time exhibited a significant increase in
neuronal survival in cells treated with supernatants from macrophages chronically stimulated with LPS, as assessed by β-tubulin immunoreactivity (B, p = 0.0005) and DAPI reactivity (C, p = 0.0025).
the FIV+ brains, but these changes were abrogated by concurrent LPS exposures. IL-10 transcript levels were significantly reduced in the cortex of the FIV+ animals compared with FIV- animals, but IL-10 transcript abundance in LPS-exposed FIV+ brains did not differ from both FIV- groups (Fig. 5F); a similar (nonsignificant) trend was observed for the basal ganglia. Of note, bacterial ribosomal 16S RNA levels in the brain (and plasma) did not differ between groups (Supplemental Fig. 1B). Hence, these findings recapitulated earlier neuroinflammation results in human brains (Fig. 1), but also demonstrated that repeated LPS exposures reduced expression of the proinflammatory genes and restored IL-10 expression.

Neuropathologic and neurobehavioral effects of LPS exposure

Previous studies have shown that glial activation and neuronal injury are cardinal features of lentivirus infections (40–42). To define the effects of repeated LPS exposures in terms of the neuropathologic changes, brain sections from each experimental group were assessed, revealing limited Iba-1 immunoreactivity in microglia-like cells on PBS-exposed (Fig. 6A) and LPS-exposed (Fig. 6B) FIV- animals, whereas Iba-1 immunoreactivity was increased in terms of the number of cells and cellular hypertrophy in FIV+ brains (Fig. 6C); these latter changes were reduced in LPS-exposed FIV+ animals (Fig. 6D). CD3-immunopositive cells were rarely detected in the FIV- group (Fig. 6A, inset), whereas more CD3 immunoreactivity was evident in the FIV+ groups (Fig. 6C, inset). Similarly, GFAP immunoreactivity in astrocytes was detected in PBS-exposed (Fig. 6E) and LPS-exposed (Fig. 6F) FIV- animals; however, GFAP immunoreactivity was increased in the brains of PBS-exposed FIV+ animals (Fig. 6G), which was reduced by repeated LPS exposure (Fig. 6H). NeuN immunoreactivity was evident in PBS-exposed (Fig. 6I) and LPS-exposed (Fig. 6J) FIV- animals. Fewer NeuN-immunopositive cells were apparent in the brains of PBS-exposed FIV+ animals (Fig. 6K) and were restored by repeated LPS exposure (Fig. 6L). These latter observations were supported by quantitative analyses of NeuN-immunopositive neurons in the parietal cortex, which showed that mean neuronal counts/high power field were significantly reduced in the FIV+ animals exposed to PBS. However, there were no differences among the other three experimental groups (Fig. 6M). These observations were corroborated by neurobehavioral testing, which revealed that the FIV+ animals with repeated PBS exposures exhibited significant deficits, as indicated by increased cumulative mean deficit scores over the latter 6-wk period of infection, compared with the other three groups (Fig. 6N). Thus, these neurobehavioral data mirrored the in vivo neuropathologic and gene expression studies.

Discussion

In this study, we show that immune conditioning induced by repeated LPS exposures during lentivirus infections prevented the development of neurovirulence in both in vitro and in vivo models in conjunction with suppression of CXCL10, which was in part modulated by IL-10.
induction. In vivo LPS-mediated neuroprotection was associated with reduced T cell infiltration of the brain, reduced viral burden, and suppression of CXCL10. Based on the present in vitro studies, IL-10 prevented the induction of CXCL10; it is likely that reduced CXCL10 in vivo prevented chemotaxis of T cells and monocytic cells into the brain (43), thereby reducing neuroinflammation and ensuing neurodegeneration. Indeed, infiltrating leukocytes might express proteases (i.e., granzyme A), which have the capacity to kill neurons (44); this is congruent with our findings of reduced neuronal counts in the parietal cortex of FIV-infected animals. CXCL10 also exerts direct neurotoxic effects, which also underlies its neuropathogenic effects (36). Collectively, the present findings underline the importance of chemokine expression in the brain, in terms of influencing leukocyte migration in lentivirus neurovirulence, but also point to a role for regulation of innate immunity in a structured and chronic manner as a plausible neuroprotective strategy.

Induction of select innate immune mechanisms represent the hallmark of several neurodegenerative diseases, which are age-dependent, sex-dependent, and host genetic background-dependent and are frequently apparent as a fine balance between beneficial and adverse effects, depending on the immune molecules implicated (45, 46). In fact, limited neuroinflammation was evident in the FIV model at week 6 postinfection (Supplemental Fig. 2). Failure to regulate interferon-α production in the context of chronic HIV infection has compelling pathogenic consequences for immune status (47) and likely neurologic disease (48, 49). Alternatively, sequential activation of TLR4 initiates different outcomes depending on the model; repeated LPS exposures of mice carrying a mutant SOD1 accelerated the disease course leading to death (50). In contrast, LPS exposure before the induction of cerebral ischemia ameliorated the size of the ensuing lesion, although these effects might be related to age and the species used in the respective studies (51–53). LPS also shows diverse effects on the outcomes of experimental autoimmune encephalomyelitis, which might depend on the phenotype of the dendritic cells under different experimental conditions (15). In humans with chronic sepsis, the risk of septic encephalopathy is greater (54) and might also contribute to the emergence of HIV-associated dementia (14). In the present model, the bacterial rRNA levels in the brain did not differ among the different experimental groups, thereby excluding this effect as a determinant of neuropathogenesis in the present context.

The underlying neuroprotective defense mechanism in the present studies appeared to be dependent on CXCL10 suppression, perhaps by IL-10, which is known to occur as a consequence of LPS exposure (55, 56), but other pathways including differential expression of PGE2 and other host cytokines are also regulated by chronic LPS exposure (57). Importantly, IL-10 is an anti-inflammatory cytokine that exerts effects on both systemic inflammation and neuroinflammation (58). However, it is likely that the effects of LPS were confined to cells in the circulation with consequent neuroprotective outcomes in the present in vivo model, because LPS would have been rapidly degraded after i.p. injection. Indeed, LPS is unlikely to cross the blood–brain barrier to any extent, except at sites devoid of a blood–brain barrier (i.e., the circumventricular organs), all remote

![FIGURE 5. Altered host gene expression in brains of FIV+ animals. CD3e (A), F4/80 (B), GFAP (C), and CXCL10 (D), and granzyme A (E) transcript levels were increased in FIV+ animals in the CTX and BG, but these gene inductions were suppressed by repeated LPS exposures. IL-10 transcript levels were reduced in brains of FIV+ animals receiving PBS in the CTX, but this finding was reversed in the LPS-treated FIV+ animals (F, mean ± SD; ANOVA with post hoc Bonferroni testing; *p < 0.05).](http://www.jimmunol.org/content/jimmunol/191/12/1572/F5.large.jpg)
from the cortex or basal ganglia. Given the in vitro findings of LPS-mediated induction of viral replication in macrophages, the benefits of LPS exposure likely represent a more protracted tolerogenic or deactivating effect on circulating leukocytes, eventually reducing their egress from the blood into the brain with accompanying re-duction in brain and plasma viral load.

In the current studies, CXCL10 was principally expressed in neurons, and clinical studies, depending on the model and the experimental circumstances (59). However, CXCL10 has multiple actions, including chemotaxis as well as neurotoxicity, regulated through its cognate receptor, CXCR3 (60). CXCR3 is also expressed by neurons; therefore, an autocrine neurotoxic pathway is plausible, although its chemotactic effects are more widely recognized and understood (61). Previous studies have shown that both the expression and function of CXCL10 and CXCR3 are influenced by IL-10, likely through the receptor of IL-10, which is expressed on both glia and neurons (62, 63). In contrast, chronic septicemia does not appear to affect the expression of CXCL10 in the nervous system, precluding a role for microbial translocation in the present set of experiments. Nonetheless, persistent microbial translocation during HIV infection owing to increased gut permeability could exercise unfavorable effects on the disease course through multiple mechanisms (12). The effects of microbial translocation on the nervous system are unappreciated to date in diseases—such as inflammatory and altered permeability bowel diseases (64), gout-versus host disease (65), and lymphangiectasia (66)—in which it is posited to be a pathogenic factor.

Assuming that the receptors for LPS, CD14, and TLR4 are largely expressed on cells of myeloid lineage (monocytes and macrophages), conditioning of innate immune cells by structured activation of TLR4 offers an appealing approach to modulating the outcomes of both acute and chronic neurologic disorders. Moreover, IL-10 is also chiefly derived from myeloid cells, further limiting the off-target effects of LPS; a conceivable explanation for the reduced viral load in the current studies is that repeated activation of myeloid cells by LPS contributed to a tolerogenic state in myeloid (macrophages and monocytes) and T cells, leading to diminished activation with ensuing reduced ability to cross the blood brain-barrier or support viral replication. The recent availability of new TLR ligands with high specificity poses an exciting opportunity for testing these agents as therapeutics in neurologic diseases involving the pathogenic activation of innate immunity.

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FIGURE 6. FIV-induced neuronal loss, glial activation, and lymphocyte infiltration are diminished by LPS exposures. Analysis of FIV+ parietal lobe brain sections (A) stained with Iba-1 revealed lower levels of staining (A and B) than in FIV+ sections (C and D), but Iba-1 immunostaining was reduced in the FIV+LPS-exposed brains (D). Low levels of GFAP immunoreactivity were evident in FIV− sections (E and F) compared with FIV+ sections (G, H), revealing increased levels of astrocyte activation with hypertrophy that were again diminished in the FIV+LPS-exposed brains (H). Occasional CD3 immunopositive cells were present in FIV− brain sections (A, inset), but were more apparent in FIV+ sections (C, inset). FIV− brain sections (D) showed abundant levels of NeuN immunopositive neuronal nuclei (I and J), whereas FIV+LPS-exposed brain sections (H) revealed a loss of neurons, which was not evident in the FIV+LPS-exposed animals (L). NeuN-positive cells were counted in five to eight randomly selected fields in four cats and revealed a loss of neurons in the CTX among FIV+LPS-exposed animals (M). The loss of NeuN-positive cells in the CTX of FIV+ animals was restored by LPS exposures in FIV+. FIV infection caused worsened neurobehavioral performance, whereas FIV+ animals exposed to LPS showed no differences compared with FIV− animals (N; mean ± SD; ANOVA with post hoc Bonferroni testing, *p < 0.05). A–L, original magnification ×400; inset, ×630.


