Neuroinflammation and Virus Replication in the Spinal Cord of Simian Immunodeficiency Virus–Infected Macaques

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Abstract
Studies of neurologic diseases induced by simian immunodeficiency virus (SIV) in Asian macaques have contributed greatly to the current understanding of human immunodeficiency virus pathogenesis in the brain and peripheral nervous system. Detailed investigations into SIV-induced alterations in the spinal cord, a critical sensorimotor relay point between the brain and the peripheral nervous system, have yet to be reported. In this study, lumbar spinal cords from SIV-infected pigtailed macaques were examined to quantify SIV replication and as-yet-to-be reported. In this study, lumbar spinal cords from SIV-infected pigtailed macaques were examined to quantify SIV replication and associated neuroinflammation. In untreated SIV-infected animals, there was a strong correlation between amount of SIV RNA in the spinal cord and expression of the macrophage marker CD68 and the key proinflammatory mediators tumor necrosis factor and CCL2. We also found a significant correlation between SIV-induced alterations in the spinal cord and the degree of distal epidermal nerve fiber loss among untreated animals. Spinal cord changes (including elevated glial fibrillary acidic protein immunostaining and enhanced CCL2 gene expression) were also present in SIV-infected antiretroviral drug–treated animals despite SIV suppression. A fuller understanding of the complex virus and host factor dynamics in the spinal cord during human immunodeficiency virus infection will be critical in the development of new treatments for human immunodeficiency virus–associated sensory neuropathies and studies aimed at eradicating the virus from the central nervous system.

Key Words: Gial activation, HIV-AIDS, Macaque, Neuroinflammation, Neuropathy, Simian immunodeficiency virus (SIV), Spinal cord.

INTRODUCTION
Although a multitude of studies have thoroughly described the morphologic and molecular alterations developing in the brain and peripheral nerves of human immunodeficiency virus (HIV)–infected patients and simian immunodeficiency virus (SIV)–infected macaques (1–8), there have been few detailed analyses of the effects of HIV/SIV infection on the spinal cord. Most studies that addressed HIV-associated spinal cord disease were published during the pre–combination antiretroviral therapy (cART) era, when opportunistic central nervous system (CNS) infections, fulminant HIV encephalomyelitis, and an enigmatic degenerative condition known as vacuolar myelopathy were common findings at autopsy (9–14). Although the frequency of serious complications directly attributable to spinal cord disease has waned since the deployment of modern cART, investigators have recently begun to consider the role of spinal cord alterations in the pathogenesis of what is currently the most common neurologic complication of HIV infection, HIV-associated sensory neuropathy (HIV-SN). Human immunodeficiency virus–associated sensory neuropathy has been reported to affect up to 60% of individuals living with HIV infection and to cause a range of uncomfortable, difficult-to-manage symptoms, including “painful numbness” or burning sensation, hyperalgesia, and allodynia (15, 16). Although the pathogenesis of this condition is incompletely understood, the development of chronic neuropathic pain likely involves sensitization peripherally (at the level of nerves and ganglia) and centrally (within the spinal cord) (17). Notably, recent studies by Shi et al (18, 19) and Yuan et al (20) demonstrated increased glial activation, enhanced proinflammatory signaling, and elevated concentrations of the HIV envelope glycoprotein gp120 in the spinal cords of patients with a history of HIV-associated pain versus “pain-negative” HIV patients at autopsy.

In-depth analysis of HIV/SIV infection in the spinal cord is also highly relevant to ongoing investigations of the CNS as a reservoir for residual viral replication and latent integrated proviruses in patients receiving cART. It is widely recognized that macrophages/microglia (and potentially astrocytes) in the brain serve as reservoirs for viral replication and reactivation, and that neuroimmune activation often persists in the brain despite systemic viral suppression (21–23). There are, however, currently no reports addressing whether a similar situation exists in the spinal cord. With its positioning behind the blood-CNS-barrier and a full complement of potentially infectable and immunoreactive glial cells, residual HIV infection of the spinal cord could significantly contribute to viral persistence in the CNS and impact therapeutic strategies aimed at viral eradication.

In the present study, we performed morphologic and molecular analyses of the spinal cords of SIV-infected pigtailed macaques (Macaca nemestrina). This well-established animal model has been shown to recapitulate HIV-induced...
lesions in the brain and peripheral nervous system (PNS) within a consistent time course of 12 weeks (24, 25). In contrast to human studies, use of macaques allows for a comprehensive sampling of key CNS and PNS components at progressive time points throughout the course of infection, with and without concurrent cART, and in the absence of potentially confounding comorbid conditions. Because symptomatic HIV-SN occurs predominantly in patients with advanced disease and in patients on cART (26, 27), we focused on groups of animals killed during the terminal stage of SIV infection (84 days postinfection) and a group of infected animals that received virally suppressive cART for several months. Individual animal’s spinal cord alterations were compared with PNS changes, including loss of epidermal nerve fiber (ENF) density, a commonly used measure of peripheral nerve injury. We hypothesized that spinal cords of infected animals would show evidence of neuroinflammation (such as glial cell activation; i.e. of microglia and astrocytes) and induction of soluble proinflammatory mediators that have been associated with pain-facilitating signaling pathways, specifically tumor necrosis factor (TNF) and the chemokine CCL2. Furthermore, we postulated that certain neuroinflammatory parameters would remain elevated in the spinal cords of macaques receiving long-term cART, as compared with uninfected control animals, similar to persistent neuroinflammation found in the brains of cART-treated SIV-infected macaques.

MATERIALS AND METHODS

Animal Studies

Twenty male pigtailed macaques were intravenously inoculated with both the neurovirulent clone SIV/17E-Fr and the immunosuppressive swarm SIV/DeltaB670, as previously described (28). Fifteen animals did not receive antiretroviral treatment (untreated SIV-infected group) and were killed at 12 weeks postinfection. Five animals were treated with antiretroviral medications beginning on day 12 postinfection and were killed at approximately 170 days postinfection (long-term cART group). The 4-drug combination therapy consisted of the nucleotide reverse transcriptase inhibitor tenofovir (Gilead, Foster City, CA), the protease inhibitors saquinavir (Roche, Basel, Switzerland) and atazanavir (Bristol-Myers Squibb, New York, NY), and the integrase inhibitor L-870812 (Merck, White House Station, NJ) (29). Doses and routes of administration had been previously detailed (23). Five additional uninfected pigtailed macaques served as untreated virus-negative controls.

At necropsy, the animals were saline-perfused, and collected tissues were either immersion-fixed in Streck tissue fixative (Streck Laboratories, Omaha, NE) and paraffin-embedded, or snap-frozen in liquid N2 and stored at −80°C. The animal procedures in this study were in accordance with the principles set forth by the Institutional Animal Care and Use Committee at Johns Hopkins University and the National Research Council’s Guide for the Care and Use of Laboratory Animals (Eight Edition).

Immunohistochemistry

Immunohistochemistry was performed on Streck-fixed paraffin-embedded sections of the lumbar spinal cord. Tissues were deparaffinized in Histo-Clear (National Diagnostics, Atlanta, GA) and rehydrated in a gradient series of alcohols. After antigen retrieval in sodium citrate buffer with 8-minute microwave treatment, sections were washed and blocked before incubation in the appropriate primary antibody dilution—anti–CD68, 1:2000; clone KP1 and anti–glial fibrillary acidic protein [GFAP], 1:4000 (DAKO, Carpinteria, CA); and anti–SIVmac251 transmembrane glycoprotein 41 [gp41], KK41, 1:4000 (NIH AIDS Research and Reagent Reference Program, Bethesda, MD)—for 1 hour at room temperature. Sections were incubated sequentially in biotinylated secondary multilink antibody and horseradish peroxidase–labeled streptavidin (Biogenex, San Ramon, CA). Chromogen detection was performed by incubating the sections in the substrate 3,3′-diaminobenzidine. The sections were washed, cleared, and coverslipped with Permount mounting medium (Fisher Scientific, Pittsburgh, PA). Image acquisition and quantification of positive CD68 and GFAP immunostaining were performed using a Nikon DS-Ri1 color camera mounted on a Nikon Eclipse 90i microscope using NIS Elements software version AR710. A composite image of an entire transverse section of the lumbar spinal cord was created by aligning serial images of contiguous 100× fields. Regions of interest were traced manually, and the percentage of total area occupied by positively stained cells was determined for the entire transverse section of the spinal cord and for the white matter and gray matter compartments separately.

Immunofluorescent Staining and Confocal Microscopy

To demonstrate active SIV infection of macrophages/microglia in the lumbar spinal cord, we performed fluorescent double labeling for SIV gp41 and the polyclonal macrophage/microglia marker Iba-1 followed by confocal laser microscopy. The staining protocol was similar to that described for immunohistochemistry, except that the primary antibodies and concentrations were KK41 (1:100) and Iba-1 (1:100; WAKO Lab Chemicals, Richmond, VA) and secondary antibodies were fluorescent-tagged AF488 goat anti-mouse and AF546 goat anti-rabbit (both 1:100; Invitrogen, Carlsbad, CA). Sections were mounted using Prolong Gold Antifade reagent (Invitrogen) and sealed with clear nail polish. Colocalization of the resulting green (KK41) and red (Iba-1) fluorescent labeling was visualized using a Nikon C1 confocal laser microscope system mounted on a Nikon Eclipse TE2000-E microscope.

Quantification of SIV RNA and Cytokine Messenger RNA in Spinal Cord Tissue

Total RNA was isolated from 25 mg of snap-frozen tissue from the lumbar spinal cord by extraction with RNA-Stat 60 (Tel-Test Inc, Friendswood, TX) and chloroform, followed by purification using the MirVana kit (Invitrogen). Genomic DNA was removed from the samples using either RQ1 DNase (Promega, Fitchburg, WI) or Turbo DNAse (Invitrogen) according to the manufacturer’s protocols. All tissue samples for RNA isolation were consistently taken from the dorsal half of the spinal cord (dorsal to the central canal) and included both white and gray matter. Purified RNA was analyzed by real-time polymerase chain reaction using...
specific primers and probes for SIV gag (28), TNF, and CCL2 (23). Simian immunodeficiency virus RNA copy number was determined by comparison to a standard curve. TNF and CCL2 gene expression was determined using the ΔΔCt (cycle threshold) method (30) with normalization of cellular messenger RNA to 18S ribosomal RNA levels. Gene expression data are reported as fold change relative to that of control animals.

Measurement of ENF Density

Full-thickness skin samples from the plantar footpad were collected from control and infected animals at necropsy using a 3-mm punch biopsy tool. Sections were obtained from an identical location in all animals. Skin sections were fixed and cryoprotected, as previously described (5). Cryoprotected samples were sectioned to 50 μm thickness with a freezing-sliding microtome and immunostained for the panaxonal marker PGP9.5 (1:10,000; ABD Serotec, Oxford, UK), as previously described (31). Epidermal nerve fiber densities were measured using a modification of the method described by Kennedy et al (32) and McCarthy et al (31). Briefly, 15 adjacent, nonoverlapping, collapsed Z-stack images were obtained for each immunostained skin section. Serial Z-stack images for each microscopic field were collected at 0.5-μm intervals using a Zeiss microscope equipped with a z-motor at 400× magnification (Carl Zeiss, Oberkochen, Germany). PGP9.5 immunoreactivity was measured by digital image analysis using iVision software version 4.0.14 (BioVision Technologies, Exton, PA). To control for variations in thickness among sections, we normalized the results to the thickness of each skin sample.

Statistics

All statistical inferences were calculated using nonparametric methods and GraphPad Prism software version 5.0d. Group comparisons were performed using Mann-Whitney U test. Relationships between variables were determined using Spearman rank correlation. For all analyses, statistical significance was assumed when the p value was less than 0.05.

RESULTS

SIV Infection Induces Morphologic Changes in the Lumbar Spinal Cord

Histopathologic lesions observed in the lumbar spinal cord of untreated SIV-infected macaques were predominantly mild and consisted of modest perivascular infiltrates of lymphocytes and macrophages, most notably in the gray matter and meninges (mild lesions in 6 [40%] of 15 animals). A subset of animals exhibited more severe myelitis with lesions similar to those seen in SIV encephalitis, including glial nodules (n = 4), pronounced perivascular cuffing (n = 4; Fig. 1A), and multinucleated giant cells (n = 3; Fig. 1B). Interestingly, all animals in which giant cells were observed in the lumbar spinal cord also had severe encephalitis. Immunostaining for the macrophage marker CD68 and SIV gp41 confirmed that foci of inflammation included variable numbers of activated macrophages/microglia often harboring SIV (Figs. 1C, D).

Confocal laser scanning microscopy of double-stained spinal cord sections showed clear colocalization of the macrophage/microglia marker Iba-1 and SIV gp41, demonstrating active SIV infection of macrophage lineage cells. Histologic lesions in the lumbar spinal cords of cART-treated animals were limited to minimal lymphohistiocytic infiltrates in the meninges. However, in 1 cART-treated animal, there was mild, bilaterally symmetric vacuolization of the lateral white matter tracts characterized by frequent dilated myelin sheaths, similar to changes described in mild cases of HIV-associated vacuolar myelopathy (9).

Viral Load Is Directly Associated With Increasing CD68, but not GFAP Expression, in the Lumbar Spinal Cord of Untreated SIV-Infected Macaques

Simian immunodeficiency virus and HIV infections elicit immune activation of resident glial cells in the brain and PNS and recruitment of infiltrating macrophages (13, 25, 26, 33). Because the extent of glial cell activation is often not appreciated on routine histopathologic review, we used immunohistochemical staining to assess macrophage/microglia and astrocyte activation in the spinal cord. In untreated SIV-infected animals, there was a significant increase in the amount of CD68 immunostaining, reflecting macrophage/microglia activation in the gray matter, but not white matter (p = 0.29; data not shown), of the lumbar spinal cord compared with control animals (p = 0.045; Fig. 2A). Furthermore, there was a direct correlation between SIV RNA levels and CD68 expression in both gray matter (p = 0.027, r = 0.59; Fig. 2C) and white matter (p = 0.047, r = 0.54; data not shown). In contrast, there was no significant difference in the level of astrocyte activation, as measured by GFAP expression, between control and untreated SIV-infected animals (gray matter: p = 0.089, Fig. 2B; white matter: p = 1.0, data not shown), nor was there an apparent relationship between viral load and astrocyte activation (gray matter: p = 0.90, Fig. 2D; white matter: p = 0.20, data not shown).

SIV Induces Expression of Soluble Proinflammatory Mediators, TNF and CCL2, in the Lumbar Spinal Cord of Untreated SIV-Infected Macaques

The proinflammatory cytokine TNF and the monocyte-attracting chemokine CCL2 (also known as monocyte chemoattractant protein-1) have previously been shown to be elevated in the brain tissue of SIV-infected macaques (23, 34). In addition, enhanced expression of these molecules in the spinal cord has been implicated as a key factor in the genesis of neuropathic pain in a number of rodent models (35–37). Gene expression analysis by quantitative reverse transcription polymerase chain reaction revealed marked induction of these soluble mediators in the lumbar spinal cords of untreated SIV-infected macaques. The degrees of both TNF and CCL2 gene expression showed a strong direct correlation with SIV RNA levels (TNF: p = 0.0011, r = 0.78, Fig. 3A; CCL2: p < 0.0001, r = 0.86, Fig. 3B) and CD68 expression in the lumbar spinal cord (TNF: p = 0.0064, r = 0.67, Fig. 3C; CCL2: p = 0.0066, r = 0.67, Fig 3D). There was no significant association between TNF or CCL2 gene expression and GFAP in untreated animals (p = 0.42 and p = 0.58, respectively; data not shown).
FIGURE 1. Morphologic changes in the lumbar spinal cord of untreated SIV-infected macaques. (A, B) Although histologic lesions observed in hematoxylin and eosin-stained sections of the lumbar spinal cord were typically mild, a subset of SIV-infected animals showed evidence of more severe myelitis, including pronounced perivascular cuffing (A) and multinucleated giant cells (B). (C, D) Immunostaining for the macrophage marker CD68 and SIV gp41 demonstrated that inflammatory foci included numerous activated macrophages/microglia (C) that were often infected by SIV (D). (E-G) Confocal laser scanning microscopy was performed on sections of the lumbar spinal cord that were double-stained for the macrophage marker Iba-1 (D; red) and for SIV gp41 (E; green). A composite image (G) showed clear colocalization of Iba-1 and SIV gp41, confirming that macrophages in the lumbar spinal cord are target cells for SIV infection. Scale bars = (A-D) 100 μm. (E-G) Original magnification: 400×.
Loss of ENF Is Associated With Increasing Viral Load, CD68, TNF, and CCL2 Expression in the Lumbar Spinal Cord of Untreated SIV-Infected Macaques

Numerous studies have demonstrated the importance of glial activation and enhanced cytokine and chemokine signaling within the spinal cord during neuropathic pain syndromes (18, 38–40). To investigate the association between proinflammatory alterations in the lumbar spinal cord during SIV infection and pathologic changes in the PNS, we compared the results of each animal’s spinal cord analyses to its degree of ENF loss, which in HIV patients has been shown to correlate with the development of neuropathic symptoms (41). Striking loss of ENF density has also been previously reported in the accelerated SIV/pigtailed macaque model, with a significant decline in epidermal innervation by 8 weeks post-infection. (5). In untreated SIV-infected macaques, there was a significant inverse correlation between ENF density and spinal cord viral load \( p = 0.010, r = -0.66 \); Fig. 4A), CD68 immunostaining \( (p = 0.014, r = -0.62; \text{Fig. 4B}) \), TNF gene expression \( (p = 0.016, r = -0.61; \text{Fig. 4C}) \), and CCL2 gene expression \( (p = 0.0091, r = -0.65; \text{Fig. 4D}) \).

Long-Term cART-Treated Macaques Show Elevated GFAP and CCL2 Expression in the Spinal Cord Without Detectable Viral Replication

A previous study using the accelerated SIV/pigtailed macaque model (23) demonstrated that long-term cART effectively suppressed viral replication in the brain and normalized some inflammatory parameters, including GFAP and major histocompatibility complex class II expression. However, evidence of persistent inflammation was detected in the brains of treated animals in the form of elevated CD68 immunostaining and expression of interleukin (IL) 6, TNF, and...
CCL2, all of which failed to return to normal levels with treatment. Viral DNA was also detected in the brain tissue of these cART-treated animals, suggestive of latent integrated proviruses. Persistent immune activation has also been observed in the brains of virally suppressed HIV patients in the absence of detectable viral replication and is thought to play a critical role in the progressive neuronal degeneration seen in HIV-associated neurocognitive dysfunction (2, 7, 42, 43). As in the brain, SIV RNA was below the level of detection (<100 copies per microgram of RNA) in the lumbar spinal cord of cART-treated animals on quantitative reverse transcription polymerase chain reaction, but there was no significant difference in the levels of spinal cord CD68 immunostaining or TNF expression between cART-treated animals and controls (p = 0.11 and p = 0.79, respectively; data not shown). Conversely, there were significantly elevated levels of GFAP immunostaining in the gray matter and white matter of cART-treated animals (p = 0.016 and p = 0.021, respectively; Figs. 5A-D) and a modest elevation in spinal cord CCL2 expression (p = 0.071; data not shown), indicating that these parameters remain elevated above control values despite long-term suppressive cART.

Similar to untreated SIV-infected macaques, animals treated with long-term cART had markedly decreased ENF density at the time of necropsy. We did not find a significant correlation between GFAP and ENF loss among cART-treated animals (p = 0.92, r = 0.20; data not shown); however, this may be related to the small number of cART-treated animals for which paraffin-embedded lumbar spinal cord was available (n = 4). In addition, there was no significant association between spinal cord CCL2 expression and ENF density among cART-treated animals (p = 0.083, r = 0.90; data not shown).

DISCUSSION

For more than 2 decades, SIV-infected macaques have served as the preeminent animal model for studying HIV...
pathogenesis; however, spinal cord neuropathology has not been reported in detail in the SIV/macaque model. Together, our findings demonstrate that the spinal cord, like the brain, is an important CNS site of SIV viral replication in untreated animals and that a subset of neuroinflammatory changes persists in the spinal cord despite virally suppressive cART. In untreated SIV-infected animals, there were significant inverse correlations between ENF density and (A) SIV viral load (p = 0.010, r = -0.66), (B) CD68 immunostaining (p = 0.014, r = -0.62), (C) TNF expression (p = 0.016, r = -0.61), and (D) CCL2 expression (p = 0.0091, r = -0.65) in the lumbar spinal cord. All statistical inferences were based on Spearman rank correlation.

The lesions present in the PNS of HIV patients with sensory neuropathy and SIV-infected pigtailed macaques have previously been described in detail (1, 5, 24). It is well recognized that these patients and macaques show evidence of morphologic and functional abnormalities of small-diameter nerve fibers in the periphery (which detect and transduce noxious stimuli and conduct nociceptive signals to the CNS) and inflammatory changes in the dorsal root ganglia (which house the cell bodies of primary afferent sensory neurons). It has also been proposed that the development of chronic
neuropathic pain in HIV patients also involves pathologic alterations in the spinal cord (17), where primary afferent neurons synapse with second-order afferents and interneurons in the dorsal horns and where nociceptive signals are processed and modulated before transmission to the brain (44). Indeed, many studies using rodent models of nociception have clearly established that spinal glial cell activation is a critical and necessary factor in the generation of acute and chronic pain (45–47). Furthermore, soluble inflammatory mediators released by activated glial cells, such as cytokines TNF, IL-1, and IL-6, and chemokines CCL2 and CX3CL1 (fractalkine), have been shown to modulate synaptic transmission of sensory signals in the spinal cord (38–40, 48). Although the physiologic functions of glial activation and enhanced immune signaling may be evolutionarily protective in the setting of acute pain and important in the clearance of injurious agents and damaged neurons, prolonged immune stimulation and dysregulation also may contribute to pathologic pain states.

Human immunodeficiency virus infection induces long-term systemic immune activation that often involves the nervous system and is not fully normalized by the use of virally suppressive cART. Thus, it is not surprising that a large proportion of HIV-infected patients are currently living with symptoms of chronic pathologic pain (49). To investigate this challenging phenomenon, Shi et al (18, 19) and Yuan et al (20) recently published a series of reports outlining changes present in the spinal cord dorsal horns of HIV patients with painful symptoms of sensory neuropathy but absent in HIV-infected individuals without pain. Alterations in glial activation profile that were specific for “pain-positive” HIV patients...
included elevated GFAP expression indicative of astrocyte activation, increased production of cytokines TNF and IL-1β, and upregulation of proteins involved in mitogen-activated protein kinase signaling. In addition, dorsal horns of HIV patients with pain showed evidence of synaptic loss, along with increases in markers of neuronal activity and plasticity, consistent with the process of central sensitization. Interestingly, quantification of HIV viral proteins revealed that patients with pain, compared with pain-negative patients, had significantly higher levels of gp120 in the spinal cord dorsal horns but relatively lower levels of Tat, suggesting differential viral gene expression and the potential importance of gp120 specifically in the development of painful symptoms. Intrathecal injection of gp120 in the spinal canal of mice resulted in molecular alterations that closely mirrored those seen in HIV patients with pain (20). Mice also developed marked spinal macrophage/microglia activation, which was not evident in HIV patients, along with mechanical hypersensitivity and progressive decline in ENF density in the hind paws.

The reported findings in the spinal cords of HIV patients with pain reflect a combination of changes present in the spinal cords of untreated macaques killed during terminal disease and cART-treated animals. This is not surprising because the HIV patients in these studies died of clinical acquired immune deficiency syndrome but also had received various antiretroviral drugs throughout the course of their disease. It is widely recognized that sensory neuropathy can arise from the neurotoxic effects of certain antiretroviral drugs, resulting in a syndrome known as antiretroviral toxic neuropathy, which is clinically indistinguishable and likely often concomitant with HIV-SN (50). The predominance of astrocytic reaction in the spinal cords of HIV patients with pain closely resembles that seen in cART-treated macaques, whereas upregulation of proinflammatory mediators is more in line with untreated macaques that had advanced disease. It is possible that astrocyte activation in the spinal cord is associated with toxic neuronal injury rather than a virus-induced inflammatory response. Another explanation would be that GFAP expression was elevated in untreated animals at earlier time points during the course of disease but declined during the terminal stage. A distinct pattern has previously been reported in the dorsal root ganglia of SIV-infected pigtailed macaques, in which there is an early increase in GFAP expression that is sustained throughout asymptomatic infection but wanes by the 84-day postinfection time point (5), possibly reflecting progression from a state of sustained glial activation to eventual degeneration and loss. Furthermore, although GFAP immunostaining is widely used as a marker of astrocyte activation, GFAP expression does not necessarily correlate with functional changes (such as altered expression of surface receptors and ion channels or production of cytokines and chemokines), which more directly influence pain signaling (38).

Significant correlations between ENF loss and severity of spinal cord inflammation and viral load in cART-naive macaques are compelling and, along with the findings of Shi et al (18, 19) and Yuan et al (20) in mice, suggest that long-term stimulation and perhaps injury of the central terminus of sensory nerves can contribute to pathologic changes in peripheral nerve fibers. In cART-treated animals, which also exhibited significant ENF loss, peripheral nerve damage may be attributable to antiretroviral toxicity rather than a virus-induced injury to the sensory pathway. Future studies with uninfected cART-treated macaques will be instrumental in dissecting this complex pathogenesis.

Study of the spinal cord is also germane to another critically important topic in contemporary HIV research—viral eradication at the CNS. Several studies have demonstrated the presence of low-level viral RNA, integrated viral DNA, and persistent immune activation in the brains of HIV patients and SIV-infected primates on suppressive cART; some authors have hypothesized that microglia and infiltrating macrophages serve as reservoirs for residual viral replication and reactivation in the CNS (2, 21–23, 51–53). However, there are currently no published studies addressing whether the spinal cord, a major component of the CNS, is also a potential viral reservoir for patients on modern cART. Our results show that the spinal cord is an active site of viral infection and replication in some cART-naive animals. Because microglia and infiltrating macrophages are also abundant in the spinal cord, studies investigating CNS viral latency and persistent neuroinflammation should consider evaluating multiple anatomic locations in the spinal cord and the brain. Furthermore, rodent models of acute CNS inflammation have revealed major differences in immune responses, microglia activation, and blood-CNS-barrier breakdown when comparing the brain and the spinal cord (54–58). This dichotomy has yet to be investigated in a primate model of chronic CNS inflammation and could have substantial impact on the pathogenesis of HIV infection and persistence in the CNS. Studies based on the SIV/pigtailed macaque model could investigate the presence of residual low-level viral replication and latent integrated proviruses in the macrophages/microglia of the spinal cord.

Although widespread use of cART has drastically reduced morbidity and mortality caused by HIV infection, the continued high frequency of long-term neurologic complications and the pursuit of systemic viral eradication are enduring challenges. This investigation into the neuropathology of the spinal cords of SIV-infected macaques demonstrates the importance of considering this key CNS compartment in future studies aimed at elucidating the mechanisms underlying painful HIV-SN and at developing novel treatment strategies for this debilitating condition. Furthermore, careful examination of the spinal cord is warranted in the continuing effort to understand CNS viral reservoirs and to achieve sterilizing cure for HIV infection.

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