

Oligodendrocytes in HIV-associated pain pathogenesis

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Abstract

Background: Although the contributions of microglia and astrocytes to chronic pain pathogenesis have been a focal point of investigation in recent years, the potential role of oligodendrocytes, another major type of glial cells in the CNS that generates myelin, remains largely unknown.

Results: We report here that cell markers of the oligodendrocyte lineage, including NG2, PDGFR α , and Olig2, are significantly increased in the spinal dorsal horn of HIV patients who developed chronic pain. The levels of myelin proteins myelin basic protein and proteolipid protein are also aberrant in the spinal dorsal horn of “pain-positive” HIV patients. Similarly, the oligodendrocyte and myelin markers are up-regulated in the spinal dorsal horn of a mouse model of HIV-1 gp120-induced pain. Surprisingly, the expression of gp120-induced mechanical allodynia appears intact up to 4 h after myelin basic protein is knocked down or knocked out.

Conclusion: These findings suggest that oligodendrocytes are reactive during the pathogenesis of HIV-associated pain. However, interfering with myelination does not alter the induction of gp120-induced pain.

Keywords

Oligodendrocyte, myelin, spinal dorsal horn, pain, HIV-1 gp120

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Background

There are around 35 million HIV/AIDS patients worldwide (WHO: <http://www.who.int/gho/hiv/en/>). Between 30% and 40% of them develop chronic pain,¹ and the prevalence increases up to 75% as the disease progresses.^{2–4} Pathological pain is the most prevalent neurological disorder among HIV patients. Various types of pain, including neuropathic pain, headaches, chest pain, and gastrointestinal pain, have been reported in HIV patients.^{2,3,5,6} Although it is widely thought that HIV infection, HIV-related opportunistic infections, and antiretroviral treatment contribute to pain pathogenesis,^{7,8} the underlying mechanisms are still poorly understood.

Analyses of the spinal dorsal horn (SDH) of postmortem HIV-1 patient tissues reveal specific molecular pathways that are implicated in HIV-1-associated pain. These include MAPK signaling, cytokine signaling, and Wnt signaling cascades.^{9,10} Similarly, activation of MAPK and Wnt signaling pathways^{11,12} and pro-inflammatory cytokine cascades is observed in the SDH of HIV-1 gp120-induced pain models.^{12–14} The contribution of

JNKs (MAPK) and cytokines to gp120-induced pain has been suggested by recent studies.^{15–17} Furthermore, the Wnt5a/JNK signaling pathway was found to regulate the expression of pro-inflammatory cytokines during gp120-induced pain pathogenesis.¹²

Converging evidence suggests critical roles of reactive glia in the pathogenesis of chronic pain in various animal models.^{18–21} We found that astrocytes are specifically activated in the SDH of HIV-1 patients who have chronic pain, indicating an important contribution of astrocyte activation to HIV-associated pain.⁹ In rodent

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models of HIV-related chronic pain, both microglia and astrocytes are activated in the SDH.^{13,22–24} Reactive microglia and astrocytes may release viral proteins (gp120, Tat, and Vpr), chemokines, pro-inflammatory cytokines, and other regulatory molecules to cause neuronal damage, including pain-related peripheral neuropathy.^{25,26} In contrast to the ongoing research focus on microglia and astrocytes, little is known about the involvement of oligodendrocytes in HIV-associated pain.

Here, we report on the reaction of oligodendrocytes and the dysregulation of myelination in the SDH of “pain-positive” HIV/AIDS patients and HIV-1 gp120 pain models. These findings provide initial evidence for a potential role of reactive oligodendrocytes in the pathogenesis of HIV-associated pain.

Materials and methods

Animals

Young wide-type (WT) C57BL/6 mice (18–23 g) and myelin-deficient mutant mice *Mbp*^{shi27–29} were purchased from Jackson Laboratory (Bar Harbor, ME, USA). Animal procedures were performed following protocols approved by the University of Texas Medical Branch Animal Care and Use Committee.

Materials

HIV-1 gp120-IIIB (Cat # 11784) was provided by the NIH AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH. For immunoblotting, we used the following antibodies: anti-NG2 (1:1000, Millipore: AB5320), anti-PDGFR α (1:1000, Santa Cruz: sc-338), anti-Olig2 (1:2000, Millipore: MABN50), anti-MBP (1:5000, Covance: SMI 94 and SMI 99), anti-proteolipid protein (PLP) (1:1000, Thermo: PA3-150), and anti- β -actin (1:1000, Santa Cruz: sc-1616-R).

Human postmortem tissues

Fifteen subjects were selected from the autopsy archive of the Texas NeuroAIDS Research Center, a unit of The National NeuroAIDS Tissue Consortium (NNTC).³⁰ Based on their clinical manifestations,³¹ the patients were divided into three groups. Group 1 (#1–5) consisted of HIV-1-negative subjects with no known history of peripheral neuropathy, myelopathy, or chronic pain (HIV⁻ Pain⁻). Group 2 (#6–10) was HIV-positive subjects without HIV-associated distal sensory polyneuropathy (HDSPN) and clinical pain syndrome (“pain-negative” HIV patients; HIV⁺ Pain⁻). Group 3 (#11–15) was HIV-positive subjects with HDSPN and clinically documented pain syndrome (pain-positive HIV patients; HIV⁺ Pain⁺). The postmortem spinal

cord specimens were sectioned into 1.0 mm slices and stored at -80°C for immunoblotting analysis.

HIV-gp120 pain model

HIV-gp120 intrathecal (i.t.) injection was performed as previously described.¹³ Briefly, mice were anesthetized under 2.5% isoflurane, and 7 μl of 15 ng/ μl gp120 solution was injected into the subarachnoid space between L5 and L6 vertebrae using a 30 $\frac{1}{2}$ -gauge stainless steel needle attached to a Hamilton Syringe. For inducing short-term pain that lasts for four to five days, gp120 was administered only once. For longer lasting pain (three weeks), gp120 was injected three times at days zero, three, and six.

siRNA administration

The preparation and delivery of siRNA were performed as previously described with slight modification.³² Briefly, Scramble RNA (Qiagen, 1022076) or myelin basic protein (MBP) siRNA (Qiagen, SI01302035) was diluted to 200 μM with RNase-free water, aliquoted, and stored at -80°C . Prior to administration, RNA/lipid complexes were prepared by mixing RNA (2 μg) with 8 μl i-FectTM reagent (NeuroMics). After incubating for 10 min at room temperature, 5 μL of the RNA/lipid complex was delivered into the lumbar region of the spinal cord by intrathecal injection. The injection was given daily for three consecutive days.

Measurement of mechanical allodynia

Allodynia measurement was performed as described.^{33,34} A set of calibrated von Frey filaments (Stoelting, Wood Dale, IL) was applied to the plantar surface of the mouse hind paw. Mechanical sensitivity changes were assessed by alteration of paw withdrawal thresholds in response to von Frey stimuli. The testing was carried out in a quiet environment with mice in a resting state. The experimenter did not know the experimental and control groups in all behavioral tests.

Western blotting analysis

Lumbar SDHs of human autopsy specimens and mouse spinal cords were dissected and homogenized in RIPA lysis buffer (1% Nonidet P-40, 50 mM Tris-HCl, 0.5% sodium deoxycholate, 150 mM NaCl, 1 mM EDTA, pH 8.0) with a protease inhibitor cocktail (Sigma). After centrifugation (12,000 $\times g$; 10 min) at 4°C , the supernatant was collected. Then, the protein concentration was determined using the BCA Protein Assay Kit (Thermo). Equal amounts of protein (50 μg) were electrophoretically separated on 10%–12% sodium dodecyl

sulfate–polyacrylamide electrophoresis gels (SDS-PAGE) and transferred to polyvinylidene fluoride membranes. The membranes were then blocked in 5% nonfat milk in TBST buffer (50 mM Tris, 150 mM NaCl, 0.05% Tween 20, pH 8.0) for 1 h at RT, followed by incubation with primary antibodies in TBST buffer (50 mM Tris, 150 mM NaCl, 0.05% Tween 20, pH 7.6) overnight at 4°C. After washing with TBST (pH 7.6) four times (4 × 5 min), HRP-conjugated secondary antibody was applied. The protein bands were visualized with the Enhanced Chemiluminescence kit (Thermo) and quantified using NIH ImageJ software. β -actin was used as a loading control.

Statistical analysis

All quantitative data were expressed by mean \pm SEM. Statistical analysis was performed using Prism 5 (GraphPad) software. One-way ANOVA (using the Western blotting data) or two-way ANOVA with a Bonferroni post hoc test (using the pain behavior data) was performed ($p < 0.05$ was considered significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Results

Dysregulation of oligodendrocyte and myelin markers in the SDH of pain-positive HIV patients

Oligodendrocytes and myelination are disturbed in the CNS under various disease conditions,^{35–37} but their involvement in pathological pain in human patients has not been examined. Using cohorts of postmortem HIV patient biopsies,^{9,10} we measured the protein levels of multiple cell markers of the oligodendrocyte lineage by semi-quantitative Western blot analysis. The results showed that NG2 and PDGFR α , markers of oligodendrocyte precursors, were significantly increased in the SDH of HIV⁺ Pain⁺ patients, compared with their levels in HIV⁻ Pain⁻ patients (NG2: 2.1 fold, $p < 0.05$; PDGFR α : 1.1 fold, $p < 0.05$) or HIV⁺ Pain⁻ patients (NG2: 2.1 fold, $p < 0.05$; PDGFR α : 1.4 fold, $p < 0.01$) (Figure 1). Similarly, Olig2, which is specifically expressed in both oligodendrocyte precursors and mature oligodendrocytes, was also markedly increased in the HIV⁺ Pain⁺ patients, as compared with that in HIV⁻ Pain⁻ (0.6 fold, $p < 0.05$) and HIV⁺ Pain⁻ patients (1.5 fold, $p < 0.01$, Figure 1). These observations indicate that oligodendrocyte proliferation and differentiation are stimulated in the SDH of HIV⁺ Pain⁺ patients. Since terminally differentiated oligodendrocytes form myelin,³⁸ we also measured the protein levels of myelin markers. We observed that MBP, a major protein component of myelin sheaths, was significantly up-regulated in the HIV⁺ Pain⁺ patients ($p < 0.05$), indicating the

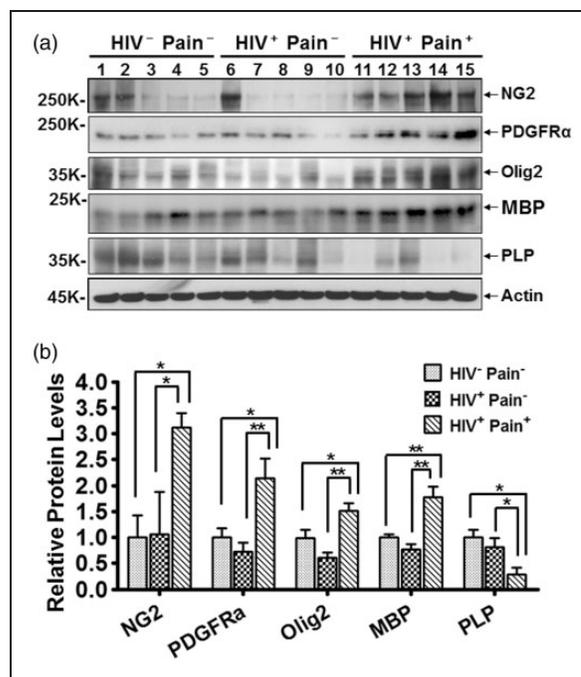


Figure 1. Protein levels of oligodendrocyte and myelin markers are increased in the SDH of human patients. (a) Immunoblots of NG2, PDGFR α , Olig2, MBP, and PLP in the SDH of postmortem tissues. (b) Quantitative summary of (a) most protein markers were significantly increased in the SDH of pain-positive HIV patients, with the exception of PLP (* $p < 0.05$; ** $p < 0.01$; one-way ANOVA with Tukey's Post Test).

formation of myelin. Interestingly, the other major myelin protein, PLP, was decreased in the SDH of HIV⁺ Pain⁺ patients by 71% (vs. HIV⁻ Pain⁻ patients, $p < 0.05$) and 65% (vs. HIV⁺ Pain⁻ patients, $p < 0.05$). PLP was hardly detectable in some HIV⁺ Pain⁺ patients (Figure 1). Since a main biological function of PLP is to establish and maintain the multi-lamellar structure of myelin,³⁸ the finding that PLP decreases suggests that the assembly of myelin sheaths might be defective in the SDH of HIV⁺ Pain⁺ patients.

HIV-1 gp120-induced up-regulation of oligodendrocyte and myelin markers in the mouse spinal cord

Next, we sought to test if oligodendrocytes and myelination in the spinal cord are stimulated in animal models of HIV-associated pain. To this end, we used the mouse model generated by intrathecal injection (i.t.) of gp120 because this model extensively phenocopied the pain-related pathologies manifested in HIV⁺ Pain⁺ patients.¹³ We first investigated the acute effect of gp120 administration. A single i.t. injection of gp120 was sufficient to induce the up-regulation of oligodendrocyte and myelin markers. The levels of NG2 and PDGFR α proteins increased soon after gp120 injection and peaked

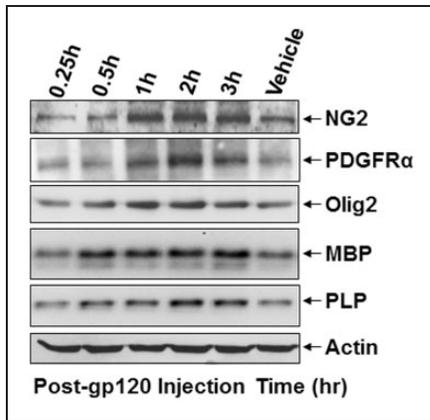


Figure 2. gp120 induces rapid up-regulation of oligodendrocyte and myelin markers in the mouse spinal cord.

by 2–3 h. Similarly, temporal profiles were observed with Olig2 and MBP proteins (Figure 2). The upregulation of these proteins coincided with the development of gp120-induced mechanical allodynia, which was observed at 1 h post gp120 injection and fully developed by 2–5 h.¹¹ In contrast to HIV⁺ Pain⁺ patients, the model also manifested pain with increased PLP (Figure 2).

To better model the chronic pain in HIV-1 patients, we also administered gp120 to mice for three times (at days zero, three, and six) to induce long-lasting pain.¹³ SDH tissues were collected at days 14 and 21 for Western blotting analysis. We observed that NG2, PDGFR α , Olig2, MBP, and PLP proteins were all up-regulated at days 14 and 21 post-injection (Figure 3). Thus, oligodendrocytes and myelination are stimulated in the SDH of the mouse models of both acute and chronic pain induced by gp120.

MBP was dispensable for gp120 to induce mechanical allodynia

Myelin is a multi-layered membrane sheath that wraps axons to facilitate impulse conduction, and MBP is essential for myelin formation.³⁹ Our findings of upregulation of the oligodendrocyte markers and MBP in the SDH of HIV⁺ Pain⁺ patients and in the gp120 pain mouse models indicate that the development of HIV-associated pain is associated with ongoing myelination. Hence, we wanted to test if myelination is a critical process during the pathogenesis of gp120-induced pain. Toward this task, we used siRNA-mediated MBP knock-down approaches. MBP-siRNA (i.t.) was able to drastically block the expression of MBP expression (Figure 4(a)). However, the mice that had MBP knock-down still expressed i.t.-gp120-induced mechanical allodynia like the sham siRNA controls, with similar magnitudes and temporal profiles (Figure 4(b)).

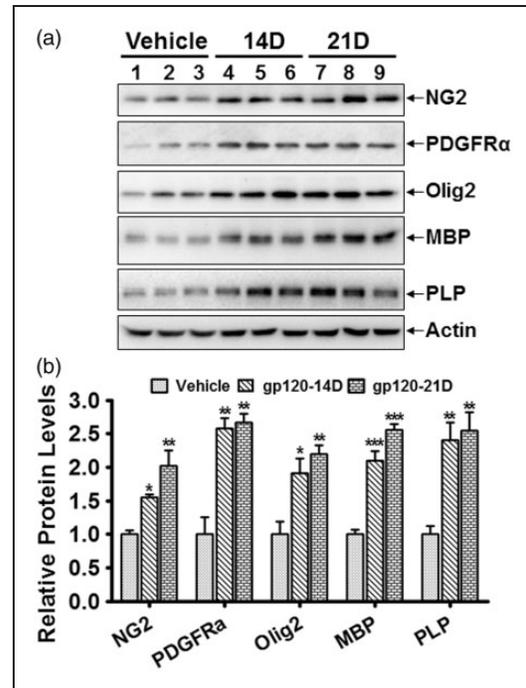


Figure 3. Long-lasting up-regulation of oligodendrocyte and myelin markers in the gp120 chronic pain model. gp120 was injected (i.t.) at days 0, 3, and 6. (a) Immunoblots of NG2, PDGFR α , Olig2, MBP, and PLP in the spinal cord at day 14 (14D) or 21 (21D). (b) Quantitative summary of a (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; one-way ANOVA with Tukey's Post Test).

Mechanical allodynia in both control and MBP-siRNA-treated mice was observed at 2 h post-gp120 injection and maintained for at least 4 h. To rule out the possibility that a low level of MBP after siRNA knockdown was sufficient to support the induction of gp120-induced allodynia, we further tested the expression of mechanical allodynia in MBP-knockout mice (MBP-KO; myelin-deficient mutant mice *Mbp*^{sh1}), whose myelin formation is severely blocked.^{27–29} Again, gp120 (i.t.) was able to induce mechanical allodynia in the MBP-KO mice to similar magnitude and temporal dynamics as the WT controls (Figure 4(b)). These data suggest that MBP up-regulation is not necessary for the induction of gp120-induced mechanical allodynia.

Discussion

We report in this paper that oligodendrocyte biomarkers (including NG2 and PDGFR α for oligodendrocyte precursors, Olig2 for oligodendrocytes and MBP for myelin) are up-regulated in the SDH of pain-positive patients and i.t. gp120 mouse models. These data indicate that the pathogenesis of HIV-associated pain is linked to oligodendrocytes and myelination.

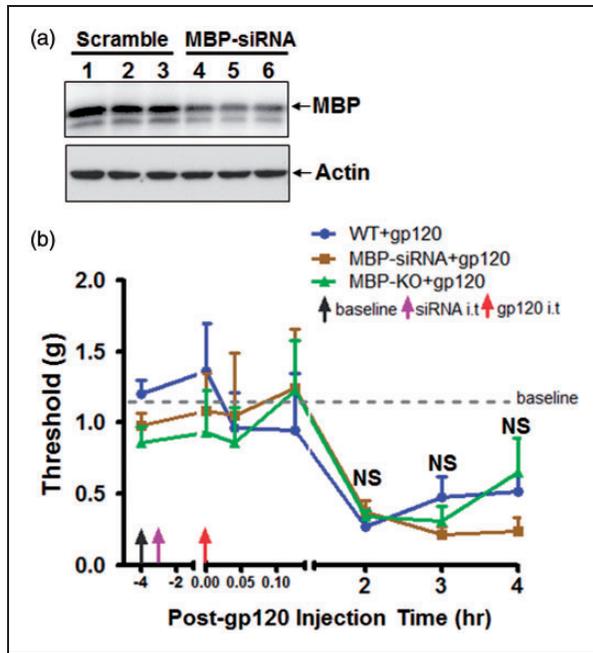


Figure 4. MBP knockdown or knockout does not impair the early expression of gp120-induced mechanical allodynia. (a) Immunoblots of MBP in the spinal cord of mice i.t.-injected with scramble RNA or MBP siRNA. (b) Measurement of mechanical allodynia by von Frey testing. No significant difference (NS) of gp120-induced allodynia was observed between wide-type (WT) and MBP-siRNA-treated (MBP-siRNA) or MBP knockout (MBP-KO) mice.

Oligodendrocytes in the CNS generate myelin to maintain axonal insulation and integrity. Loss or functional impairment of oligodendrocytes leads to myelin defects implicated in specific neurological conditions such as multiple sclerosis (MS)⁴⁰ and spinal cord injury (SCI).^{41,42} In the pain transmission neural pathway, oligodendrocytes are critical for maintaining normal sensitivity to somatosensory stimuli, and ablation of oligodendrocytes causes sensory changes and triggers central neuropathic pain.⁴³ Dysfunction of oligodendrocytes is observed in neuropathic pain models.^{36,44} For instance, it was reported that oligodendrocyte dysregulation may facilitate the development of neuropathic pain by releasing pro-inflammatory cytokine IL-33.⁴⁴ In support of a role of oligodendrocytes in pain pathogenesis, we found that oligodendrocytes are reactive in the SDH of pain-positive HIV-1 patients and the gp120 mouse model. However, in contrast to microglia and astrocytes, the mechanistic understanding of oligodendrocytes in pain is still primitive, and much is to be learned by future studies.

Demyelination is a pathological hallmark of peripheral neuropathy, which develops in over 30% of HIV/AIDS patients and is commonly concomitant with HIV-associated chronic pain.^{1,45,46} Our previous studies

revealed that HIV patients with chronic pain showed severe loss of myelinated nerve fibers in the distal sural nerve.⁹ In the CNS, severe demyelinating leukoencephalopathy is also observed in HIV/AIDS patients.^{47,48} Inflammatory neuropathies, induced by macrophages, lymphocytes perivascular infiltration, and gliosis, are closely associated with the HIV-related demyelination in the CNS and PNS.^{47,49} These findings suggest that myelin injuries can be induced by HIV-1 infection. In this context, the up-regulation of oligodendrocyte markers revealed in this study may indicate an ongoing attempt to re-myelinate during HIV-associated pain pathogenesis. Interestingly, PLP, a protein that is crucial for assembly of myelin sheaths,³⁸ is markedly decreased in the SDH of pain-positive HIV-1 patients (Figure 1). This finding indicates that the attempt at re-myelination that is related to HIV-associated pain is probably impaired in patients at the step of myelin sheath assembly. In this context, the remyelination processes indicated by the up-regulation of oligodendrocyte markers and MBP may not sufficient to block myelination damage-induced pain. Our data indicate that HIV-1 gp120 elicits a reaction by oligodendrocytes in the mouse SDH. Similarly, Zhang et al.⁵⁰ reported that gp120 induces axonal injury of rat corpus callosum by binding to the CXCR4 receptor on both oligodendrocytes and neurons. In rat cerebral cortex cultures, gp120 can cause dysfunction of oligodendrocytes and loss of myelin sheaths.⁵¹ These data together suggest that gp120 can directly regulate oligodendrocyte biology and myelin integrity.

Interestingly, gp120 can still induce allodynia when myelination is blocked after MBP knockdown or knockout. These results suggest that MBP and myelination might be not critical for the induction of gp120-induced allodynia, especially at its early phase of expression. However, these observations do not exclude possible contribution of oligodendrocytes in the pathogenesis of pain. Oligodendrocytes are able to support the physiological functions and integrity of axons by providing energy and neurotrophic factors independent of myelination.⁵²⁻⁵⁴ Ablation of oligodendrocytes elicits neuropathic pain without evident demyelination.⁴³ In addition, recent studies uncover that oligodendrocytes facilitate the development of neuropathic pain by releasing pro-inflammatory cytokine IL-33.⁴⁴ Along this line, we hypothesize that the reaction of oligodendrocytes to gp120 could regulate pain pathogenesis independent of myelination.

Conclusion

Results from this study reveal for the first time that oligodendrocytes are specifically reactive in the SDH in the HIV-infected human patients who developed chronic pain, and that HIV-1 gp120 induces spinal

oligodendrocyte reaction in the mouse model. These findings implicate a role of oligodendrocytes in the development of HIV-associated pathological pain.

Authors' contributions

YS, JS, ZL, and SY carried out experiments and data analyses. SJ designed the study. YS and ST prepared the manuscript. All authors read and approved the final manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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