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CXCR4 Signaling Regulates Remyelination by Endogenous Oligodendrocyte Progenitor Cells in a Viral Model of Demyelination

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Abstract

Following intracranial infection with the neurotropic JHM strain of mouse hepatitis virus (JHMV), susceptible mice will develop widespread myelin destruction that results in pathological and clinical outcomes similar to those seen in humans with the demyelinating disease Multiple Sclerosis (MS). Partial remyelination and clinical recovery occurs during the chronic phase following control of viral replication yet the signaling mechanisms regulating these events remain enigmatic. Here we report the kinetics of proliferation and maturation of oligodendrocyte progenitor cells (OPCs) within the spinal cord following JHMV-induced demyelination and that CXCR4 signaling contributes to the maturation state of OPCs. Following treatment with AMD3100, a specific inhibitor of CXCR4, mice recovering from widespread demyelination exhibit a significant ($P < 0.01$) increase in the number of OPCs and fewer ($P < 0.05$) mature oligodendrocytes compared with HBSS-treated animals. These results suggest that CXCR4 signaling is required for OPCs to mature and contribute to remyelination in response to JHMV-induced demyelination. To assess if this effect is reversible and has potential therapeutic benefit, we pulsed mice with AMD3100 and then allowed them to recover. This treatment strategy resulted in increased numbers of mature oligodendrocytes, enhanced remyelination, and improved clinical outcome. These findings highlight the possibility to manipulate OPCs in order to increase the pool of remyelination-competent cells that can participate in recovery.

Keywords

oligodendrocytes; remyelination; chemokines; chemokine receptors

INTRODUCTION

Remyelination failure in patients with the demyelinating disease multiple sclerosis (MS) results in progressive neurodegeneration (Trapp et al., 1998). In the early stages of MS,

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resident oligodendrocyte progenitor cells (OPCs) are thought to replenish myelinating cells that subsequently repair demyelinated lesions. However, as the disease progresses, the ability of OPCs to participate in repair is diminished. Previous studies in diverse experimental models of CNS disease have given insight into the kinetics of OPC proliferation and maturation following neurologic infection or insult. Indeed, these findings indicate that OPC proliferation rates increase dramatically from baseline levels during disease or injury to restore mature oligodendrocyte numbers lost during toxin-induced damage (Aharoni et al., 2008; Armstrong et al., 2006; Blakemore and Keirstead, 1999; McTigue and Tripathi, 2008; Penkowa and Hidalgo, 2003). Furthermore, higher levels of myelin debris found during active disease have been reported to restrict OPC maturation, thereby preventing damage of new oligodendrocytes while at the same time possibly keeping OPCs in a proliferative state (Kotter et al., 2006). However, the molecular and cellular mechanisms that contribute to OPC proliferation/maturation are complex and additional insight into factors associated with these processes is needed. Understanding the events and signaling involved in how quiescent OPCs respond to inflammation-induced demyelination is essential to any effort seeking to treat demyelinating diseases.

We have used a well-established model of demyelination triggered by intracranial (i.c.) infection with the JHM strain of mouse hepatitis virus (JHMV). Susceptible mice inoculated i.c. with JHMV develop a disease with both clinical and histologic features similar to MS (Cheever et al., 1949). During the acute stage of the infection, extensive oligodendrocyte damage results from direct infection as well as from the indirect effects of the inflammatory environment (Lampert et al., 1973; Wang et al., 1990). Although viral replication is controlled, sterile immunity is not achieved and virus will persist primarily in white matter tracts. Demyelination ensues as activated immune cells, e.g., T cells and macrophages accumulate in the CNS primarily within areas of viral persistence. Eventually, remyelination of axons will occur although not immediately, indicating that microenvironmental signals may suppress myelin repair mediated by this resident cell population. Thus, the JHMV model provides a relevant model system to interrogate mechanisms of inadequate remyelination by cells of an oligodendrocyte lineage during periods of immune-mediated demyelination (Bergmann et al., 2006; Cheever et al., 1949; Wang et al., 1990). Here, we report the kinetics of OPC proliferation and their subsequent maturation following JHMV infection. We also provide evidence that CXCR4 signaling contributes to OPC differentiation into mature oligodendrocytes and show that it is possible to harness this mechanism to improve remyelination.

MATERIALS AND METHODS

Animals and Virus

Five-week-old C56BL/6 male mice were used for all studies (National Cancer Institute, Bethesda, MD) and injected intracranially (i.c.) with 200 PFU of JHMV (strain V2.2-1) as previously described (Lane et al., 1998). Mice were euthanized at 3, 6, 10, 14, 35, and 49 days post-infection at which point brains and/or spinal cords were removed for analysis. Viral titers were assayed as previously described (Hirano et al., 1978; Lane et al., 1998).

***In Vivo* Chemokine Receptor Blocking and BrdU Treatment**

For CXCR4-blocking experiments, continuous AMD3100 (Sigma-Aldrich, St. Louis, MO) or HBSS treatment was carried out by osmotic pumps (Durect, Cupertino, CA) that were implanted subcutaneously 14 days p.i. as per manufacturer's instructions. AMD3100 was delivered at a rate of $130 \mu\text{g day}^{-1}$ (Carbajal et al., 2010; McCandless et al., 2006; Patel et al., 2010). Pumps were extracted 35 days p.i. to stop treatment and their contents measured to verify proper delivery. For BrdU (Sigma) treatments, mice were treated i.p. twice daily with 50 mg kg^{-1} of BrdU in saline or saline alone.

Histology

Animals were euthanized by inhalation of isoflurane (Piramal Healthcare, India) at defined times following infection and fixed by cardiac perfusion with 4% paraformaldehyde (Fisher Scientific, Pittsburgh, PA) in 0.1 M PBS, pH 7.4. The spinal cord was extracted and processed for resin or cryostat sectioning as previously described (Totoiu et al., 2004). Demyelinated, remyelinated, and normally myelinated axons were counted within $4 \times 3,750 \mu\text{m}^2$ areas, totaling $15,000 \mu\text{m}^2$, of each tissue section using a $60\times$ objective with a $2\times$ optical zoom representing $\sim 10\%$ of the total area of remyelination within transplanted animals as previously determined (Hardison et al., 2006).

Immunocytochemistry and TUNEL Staining

For immunofluorescent staining, the following reagents were used: rabbit anti-GST- π (MBL International, Japan), rabbit anti-CXCR4 pS339 [recognizes the ligand-bound, activated form of the receptor (Woerner et al., 2005)], rat anti-MBP, rabbit anti-NG2 (Chemicon, Billerica, MA), rabbit anti-Ki67 (Abcam, San Francisco, CA), rat-anti BrdU (Accurate Chemical Scientific, Westbury, NY), rat anti-PDGFR α , and rabbit-antiCXCL12 (eBiosciences, San Diego, CA). Secondary antibodies used for visualization were Alexa 488 and 594 goat anti-rabbit and goat anti-rat (Invitrogen). Vectashield with DAPI (Vector, Burlingame, CA) was used to visualize nuclei. Fluorescence intensity was calculated using ImageJ software. Images of the same sections were then compared with each other to correlate the location of PDGFR α - and GST π -positive cells with respect to myelin staining. Positive cells were counted on the entire white matter of coronal sections from rostral, medial, and caudal regions of the spinal cord. For detection of *in situ* apoptosis, a TMR-red TUNEL kit (Roche, Germany) was used according to the manufacturer's instructions.

Cell Isolation and Flow Cytometry

Isolation of infiltrating cells present within the spinal cords of mice was accomplished by Percoll (GE Healthcare, Sweden) extraction as previously described (Walsh et al., 2007). Cells were blocked with anti-CD16/CD32 and stained with antibodies against CD4 (allophycocyanin-conjugated rat anti-mouse, BD Pharmingen) and CD8b.2 (PE-conjugated rat anti-mouse, BD Pharmingen). As controls, isotype- and conjugate-matched antibodies were used.

Statistical Analysis

Two-tailed student's *t* test was carried out with most data sets. With clinical scores, a repeated measures analysis using PROC MIXED in SAS version 8.2 was employed.

RESULTS

JHMV Infection of Mice Results in Demyelination and Partial Remyelination

Intracranial (i.c.) infection of C57BL/6 mice with JHMV results in an acute encephalitis characterized by viral titers that peak at ~3–5 days p.i. within the brain but ultimately decline below the level of detection (~100 PFU g⁻¹) by 12 days p.i. (Fig. 1A). However, sterile immunity is not achieved and viral RNA and protein remain detectable in the CNS (Adami et al., 1995; Stohlman et al., 1998). Importantly, virus persists primarily within white matter tracts and is associated with robust demyelination mediated, in part, by accumulation of activated T cells and macrophages (Lane et al., 2000; Williamson and Stohlman, 1990; Wu et al., 2000). Extensive demyelination is apparent in the ventral funiculus and in lateral white matter tracts as evidenced by luxol fast blue (LFB) staining (Fig. 1B). Although the majority of axons within these affected areas are stripped of their myelin sheath, some endogenous remyelination is observable by toluidine blue staining by 35 days p.i. (Fig. 1C).

OPC Numbers Increase Dramatically and Mature Oligodendrocyte Numbers Rebound Following JHMV Infection

To better understand mechanisms associated with remyelination in response to JHMV-induced demyelination, we first examined how CNS infection influences survival of cells of the oligodendrocyte lineage. Coronal spinal cord sections were stained for oligodendrocytes (GST π -positive) and OPCs (PDGFR α -positive) (Fig. 2A,B) and positive cells enumerated. GST π -positive cell numbers dropped precipitously from ~500 cells mm⁻² of white matter in healthy animals to ~250 cells mm⁻² by 10 days p.i. These cells then rebounded to ~600 cells mm⁻² by 49 days p.i. (Fig. 2C). The decline in numbers of oligodendrocytes coincided with increased resident OPC proliferation and numbers. Prior to infection, mice have ~20 PDGFR α -positive cells mm⁻² of white matter and about 3% of these are expressing the proliferative marker Ki67. By 6 days p.i. ~25% of PDGFR α -positive cells are expressing Ki67 and by Day 14 p.i., OPC proliferation rates have decreased to ~13% and cell numbers have reached ~125 cells mm⁻² (Fig. 2D,E). A similar rebound in oligodendrocyte numbers following viral control as well as a proliferative burst of OPCs following infection with a related neurotropic strain of mouse hepatitis virus have been previously reported (Armstrong et al., 2002; Redwine and Armstrong, 1998). These data suggest that endogenous OPCs are proliferating and replenishing mature oligodendrocyte pools. Furthermore, we re-stained sections from Day 14 p.i. with LFB to correlate the location of OPCs with regard to myelinated and demyelinated areas. We found that OPC densities were enriched within areas cleared of myelin by 14 days p.i. (Fig. 2F). These findings indicate that OPCs can reside and proliferate in areas of active demyelination and that OPC migration into areas of demyelination is carried out by Day 14 p.i.

CXCL12 is Expressed in Demyelinated Areas in the Spinal Cords of JHMV-Infected Mice

The CXCR4/CXCL12 signaling axis has been shown to play important roles in oligodendrocyte biology both in development and during disease (Li and Ransohoff, 2008). We have previously shown that CXCL12 expression is increased within the spinal cords of JHMV-infected mice and that astrocytes are an important cellular source of this chemokine (Carbajal et al., 2010). Immunostaining confirmed that CXCL12 is enriched in areas of demyelination within spinal cord white matter tracts (Fig. 3A). Staining intensities for myelin basic protein (MBP) and CXCL12 correlate negatively, indicating that areas of demyelination have high levels of CXCL12 (Fig. 3B,C). Furthermore, OPCs express CXCR4, the chemokine receptor for CXCL12 (Fig. 3D).

CXCR4 Signaling Modulates OPC Maturation

To provide further insight into the relationship between CXCR4 signaling and OPC-mediated remyelination, JHMV-infected mice were treated with AMD3100, a small molecule that specifically inhibits CXCL12 binding to CXCR4 and has been broadly used in basic and clinical studies for evaluating CXCR4 function (De Clercq, 2003; Donzella et al., 1998). Continuous delivery of AMD3100 or HBSS was achieved with s.c.-implanted osmotic pumps in JHMV-infected mice on Day 14 p.i. At this time, demyelination is established in surviving mice and demyelinated areas have high concentrations of OPCs. Treatment with AMD3100 did not dampen T cell infiltration into the CNS at any time following treatment (Fig. 4A,B). Moreover, no evidence of increased inflammatory leukocyte migration from the microvasculature into the parenchyma was observed in response to AMD3100 treatment (Fig. 4C) as has been previously reported during autoimmune neuroinflammation (McCandless et al., 2006).

Following 3 weeks of continuous AMD3100 treatment, we stained spinal cord sections for GST π and PDGFR α to detect mature oligodendrocytes and OPCs and assess the role of CXCR4 in OPC-mediated recovery of mice with JHMV-induced demyelination. Furthermore, we correlated the location of immunopositive cells with LFB staining to identify if labeled cells were in myelinated or demyelinated areas. The morphology of PDGFR α -positive cells within AMD3100-treated animals revealed characteristic morphology of OPCs, e.g., predominantly bi-polar cells and this was in marked contrast to a more arborized appearance of PDGFR α -positive cells in response to HBSS treatment, suggesting more mature OPCs (Fig. 5A). Quantification of PDGFR α -positive cells within the spinal cords of AMD3100-treated mice revealed a significant ($P < 0.05$) increase of these cells in total white matter and particularly so in demyelinated ($P < 0.001$) white matter when compared with HBSS-treated mice (Fig. 5B). The overall increase in PDGFR α staining in response to AMD3100 was associated with increased proliferation of these cells as determined by Ki67 staining (Fig. 5C). Correspondingly, there was a significant reduction ($P < 0.05$) in numbers of GST π -positive cells in demyelinated areas in response to AMD3100 treatment when compared with HBSS-treated mice (Fig. 5D). TUNEL staining indicated that CXCR4 antagonism did not affect survival of GST π -positive cells (Fig. 5E) as very few TUNEL-positive cells are present 35 days p.i. in controls or AMD3100-treated mice. There were no differences in the number of TUNEL/GST π double-positive cells between groups following 3 weeks of treatment. Collectively, these data suggest that CXCR4 antagonism

impedes the normal OPC response to JHMV infection, i.e., by hindering maturation and augmenting proliferation. Surprisingly, we did not observe a significant difference in remyelination (Fig. 5F) or clinical scores (Fig. 5G) between these treatment groups.

Release from AMD3100 Treatment Results in Improved Motor Skills and Increased Remyelination

JHMV-infected mice were treated 21 days with AMD3100 beginning on Day 14 p.i. Subsequently, animals were released from treatment and allowed 14 days for recovery to assess clinical disease. During this time, animals also received i.p. injections with BrdU in order to label cells generated during the treatment window. Two weeks after AMD3100 treatment was stopped, spinal cord sections from control (Fig. 6A) and treatment (Fig. 6B) groups stained for GST π and BrdU revealed a marked difference in numbers of GST π -positive and GST π /BrdU double-positive cells. When quantified, significantly ($P < 0.05$) more GST π -positive cells were detected within the white matter in animals receiving the AMD3100 treatment regimen than in those receiving HBSS alone (Fig. 6C). When compared with the numbers of GST π -positive cells mm⁻² observed in animals sacrificed immediately after treatment (Fig. 5B), mice receiving the HBSS treatment regimen had ~200 more cells mm⁻² whereas mice in the AMD3100 group had ~400 more cells mm⁻². Accordingly, similar numbers of GST π /BrdU double-positive cells mm⁻² were observed in these mice, indicating that the increase in GST π -positive cells in AMD3100 treated animals originated from cells incorporating BrdU during the treatment window (Fig. 6D). Numbers of PDGFR α -positive cells mm⁻² of white matter were not different between groups following this treatment strategy (Fig. 6E). To assess if the resulting increase in mature oligodendrocytes had a physiological impact, we monitored clinical improvement and quantified the amount of remyelination present in the spinal cords of control and treated mice. Mice receiving AMD3100 during the treatment window had improved clinical recovery when compared with controls ($P < 0.05$) (Fig. 6F). Furthermore, these mice also had a significant increase in remyelination ($P < 0.05$) (Fig. 6G,H).

DISCUSSION

It has long been accepted that OPCs will proliferate in response to active demyelination and will subsequently mature into myelinating cells to initiate repair (Aharoni et al., 2008; Armstrong et al., 2006; Blakemore and Keirstead, 1999; Bunge et al., 1961; Keirstead and Blakemore, 1999; Liu et al., 2010; Ludwin, 1979; McTigue and Tripathi, 2008; Murtie et al., 2005; Penkowa and Hidalgo, 2003). However, increased understanding of the kinetics of OPC proliferation and differentiation during the course of inflammation-mediated demyelination is necessary. We used a model of demyelination triggered by viral infection to better understand signals controlling OPC maturation and remyelination within the context of immune-mediated white matter damage. Following intracranial infection with JHMV, PDGFR α -positive cell replication increases and this coincides with the peak levels of infiltrating, activated immune cells into the CNS seen around Day 6 p.i. (Bergmann et al., 2006). This increase in proliferation correlates with neuroinflammation and onset of damage to mature oligodendrocytes. Numbers of PDGFR α -positive cells increase sixfold between Days 6 and 14 p.i., yet by 35 days p.i., the frequency of PDGFR α -positive cells expressing

Ki67 has dropped to levels statistically indistinguishable to those seen in uninfected mice. GST π -positive cells, in contrast, rebound to pre-infection levels by 49 days p.i. This rebound is presumably due to maturing OPCs that aid in remyelinating axons and is not associated with complete clinical recovery and remyelination.

The signaling cues that govern OPC response to injury must be better understood in order to develop therapeutic strategies for demyelinating diseases. To this end, extensive studies *in vivo* have shed light on the roles of several cytokines, growth factors and transcription factors on influencing OPC biology (Imitola et al., 2003). For example, signaling through the CXC chemokine receptor 2 (CXCR2) signaling has been shown to be involved in OPC proliferation and differentiation as its inhibition resulted in reduced proliferation and increased maturation in an autoimmune model of demyelination as well as in toxin-induced lesions (Kerstetter et al., 2009). Huang et al. (2010) used RNA interference knockdown of retinoid acid receptor RXR- γ to identify it as an important positive regulator of OPC differentiation into mature oligodendrocytes. Furthermore, Armstrong and colleagues have used the JHMV-model to extensively characterize PDGF and FGF2 as regulators of proliferation and differentiation of OPCs. Their studies have shown that these growth factors stimulate OPC proliferation and inhibit differentiation and, more specifically, that FGF2 interacts with notch signaling to inhibit OPC maturation (Armstrong et al., 2002; Frost et al., 2003; Murtie et al., 2005).

Though CXCL12 is expressed constitutively at low levels within the normal CNS (Lu et al., 2002; McCandless et al., 2006; Stumm et al., 2002), inflammatory pathological conditions result in an increase in CXCL12 production with activated astrocytes being identified as a primary cellular source (Calderon et al., 2006; McCandless et al., 2008a; Salmaggi et al., 2009; Zhang et al., 2003). Indeed, astrocytes are a source for CXCL12 following JHMV infection of the CNS (Carbajal et al., 2010). In MS patients, both activated and silent lesions have activated astrocytes expressing CXCL12 (Moll et al., 2009). Accordingly, this chemokine has been shown to have an important role in CNS biology. Cultured OPC migration, proliferation, and maturation have been shown to be associated with CXCL12 signaling through CXCR4 (Dziembowska et al., 2005; Kadi et al., 2006; Maysami et al., 2006). We have previously shown *in vivo* that transplanted neural stem cells rely on CXCL12/CXCR4 signaling to migrate away from engraftment site to areas of demyelination following JHMV infection (Carbajal et al., 2010). Therefore, CXCL12 signaling via CXCR4 is associated with a variety of glial responses and appears important during both development and disease.

To determine the role of CXCR4 signaling in the proliferation and maturation of OPCs within an inflammatory model of demyelination, we treated mice with the specific CXCR4 inhibitor AMD3100 during the chronic (after Day 14 p.i.) phase of the JHMV model. Treatment was restricted to the chronic stage as blocking CXCR4 signaling has been shown to modulate T-cell infiltration into the CNS by facilitating their exit from the perivascular space into the parenchyma during acute viral encephalitis (McCandless et al., 2008b). Importantly, we did not observe any effect on either T cell migration to the CNS nor leukocyte entry into the parenchyma following AMD3100 treatment during chronic JHMV-induced disease. More importantly, our strategy for AMD3100 treatment was to concentrate

on determining how CXCR4 signaling impacted function of cells of the oligodendrocyte lineage following establishment of demyelination. Our findings indicate that CXCR4 antagonism mutes OPC maturation to oligodendrocytes and results in higher rates of proliferation. Importantly, these findings support and extend earlier work by Patel and colleagues (Patel et al., 2010) using a toxin-induced model of oligodendrocyte death in the corpus callosum to reveal that OPCs with impaired CXCR4 signaling had impaired maturation and increased rates of proliferation. Allowing mice to recover following the removal of AMD3100 treatment from JHMV-infected mice was associated with increased numbers of mature oligodendrocytes that correlated with improved motor skills and enhanced remyelination. These data suggest that release from blocking CXCR4 signaling allowed OPCs to mature and ultimately lead to increased numbers of mature oligodendrocytes capable of contributing to remyelination.

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Fig. 1.

Immune mediated demyelination induced by intracranial infection with JHMV. (A) Following intracranial (i.c.) infection of C57BL/6 mice with JHMV, viral titers within the brain will peak between 3 and 5 days p.i. and subsequently viral replication will be reduced below levels of detection (~ 100 pfu g^{-1} tissue) ($n = 5$ mice per time point; data is presented as average $\pm$ SD). (B) Representative coronal spinal cord section stained with luxol fast blue (LFB) and H&E depicts the characteristic ventral and lateral white matter demyelination in animals infected with JHMV at Day 35 p.i. (C) A representative light microscopy image of a toluidine blue-stained coronal section showing remyelinated (*) and demyelinated (#) axons at 35 days p.i., (scale bar = 5 μ m).

Fig. 2.

OPCs found throughout the spinal cord proliferate and replenish mature oligodendrocyte numbers following JHMV infection. Representative immunofluorescence staining showing GST π -positive cells (A) in the spinal cord 35 days p.i. and a PDGFR α -positive cell (B) expressing the proliferative marker Ki67 on Day 6 p.i. Spinal cords from JHMV-infected mice were removed at defined times p.i. and (C) GST- π , (D) PDGFR α /Ki67, and (E) PDGFR α -positive cell numbers in spinal cord white matter quantified. (F) PDGFR α -positive cells were enumerated within total white matter tracts (total WM—both demyelinated and normal), normal appearing white matter (LFB-positive), and demyelinated (LFB-negative) tracts at Day 14 p.i. as described in Materials and Methods. For Panels A and B, scale bars = 20 μ m. Data in C, D, E, and F represent a minimum of four mice/time point; data is presented as average $\pm$ SD; ** $P < 0.01$.

Fig. 3.

CXCL12 is expressed in areas of demyelination and OPCs express CXCR4. (A) Representative immunofluorescent image of spinal cord white matter from a mouse 35 days p.i. stained for MBP (green) and CXCL12 (red). (B) Immunofluorescence intensity plot indicating the relationship between MBP staining (green) and CXCL12 (red). (C) Regression analysis reveals increased staining for CXCL12 is associated with diminished MBP staining. Data were collected from over 50 images from three mice (Day 35 p.i.). (D) Representative confocal image of sections stained for PDGFR α and CXCR4-pS339 reveals OPCs in white matter of JHMV-infected mice at Day 35 p.i. stain positive for the active form of this receptor (>95% in all sections stained). Scale bar = 10 μ m.

Fig. 4.

Treatment with AMD3100, a CXCR4 small molecule antagonist, in JHMV-infected mice does not affect T cell infiltration into the CNS. JHMV-infected mice were treated with either HBSS or AMD3100 for 2 weeks beginning on Day 14 p.i. and the effects on neuroinflammation evaluated after 2 weeks of treatment. (A) Representative flow dot plots of cells stained for CD4 and CD8 antigens; numbers represent frequency of positive cells within the gated population. (B) Quantitative analysis of CD4+ and CD8+ T cell infiltration into the CNS following treatment with either HBSS or AMD3100; data is representative of three independent experiments with $n = 3$ mice/group. (C) Representative spinal cord sections from experimental mice at Day 35 p.i. stained with LFB/H&E from Day 35 p.i. revealed similar parenchymal infiltration by inflammatory cells.

Fig. 5.

AMD3100 treatment and OPC maturation *in vivo*. Mice were treated with either HBSS or AMD3100 between Days 14 and 35 p.i. At the end of this treatment, mice were sacrificed and spinal cords analyzed by immunofluorescent staining. **(A)** Representative images of spinal cords show increased numbers of PDGFRα-positive cells in AMD3100-treated mice (left panel) compared with HBSS-treated mice (right panel). **(B)** Quantification of PDGFRα-positive cells present in total WM (normal appearing and demyelinated), myelinated, and demyelinated at Day 35 p.i. **(C)** Quantification of the frequency of PDGFRα and Ki67 dual-positive cells in mice treated with either HBSS or AMD3100 at Day 35 p.i. **(D)** Numbers of GST-π-positive cells present in total WM (normal appearing and demyelinated), myelinated, and demyelinated at Day 35 p.i. **(E)** Representative image of a TUNEL-stained section from Day 35 p.i. GST-π, TUNEL, and DAPI stains are shown individually for the area in the dotted box. **(F)** Remyelination across the length of the cord was not significantly different between treatment groups. **(G)** Clinical scores of mice in the two groups after 3 weeks of treatment did not reach a significant difference. For Panels A and E, scale bar = 20 μm. For panels B, C, and D, data represents two independent experiments with $n = 4$ per group; data is presented as average $\pm$ SD; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Fig. 6.

Release from AMD3100 treatment increases numbers of mature oligodendrocytes. JHMV-infected mice were pulsed with BrdU and either AMD3100 or HBSS for 3 weeks beginning on Day 14 p.i. Osmotic pumps were excised and mice allowed to recover for 2 weeks at which point animals were sacrificed and spinal cords removed. Representative images of coronal sections (ventral white matter) stained for DAPI, BrdU, and GST π from mice receiving HBSS (**A**) or AMD3100 (**B**) treatment. (**C**) Quantification of GST π -positive cells in total WM (normal appearing and demyelinated), myelinated, and demyelinated at 2 weeks following release from either HBSS or AMD3100 treatment. (**D**) Numbers of GST π -BrdU-positive cells present in total WM (normal appearing and demyelinated) following release from either HBSS or AMD3100 treatment. (**E**) Numbers of PDGFR α -positive cells present in total WM (normal appearing and demyelinated), myelinated, and demyelinated following release from either HBSS or AMD3100 treatment. (**F**) Release from AMD3100 treatment is associated with improved motor skills compared with mice that had been treated with HBSS that is associated with an increase in the frequency of remyelinated axons in spinal cord (**G**). (**H**) Representative toluidine blue sections revealed increased numbers of remyelinated axons (*) and fewer demyelinated axons (#) in AMD3100 treated mice compared with HBSS-treated mice. Data for all figures was derived from two independent experiments with a minimum of eight mice/experiment. For Panels A and B, scale bar = 20 μ m and Panel H, scale bar = 5 μ m; * P 0.05, ** P 0.01.