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Sphingosine-1-Phosphate Receptor Antagonism Enhances Proliferation and Migration of Engrafted Neural Progenitor Cells in a Model of Viral-Induced Demyelination

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The oral drug FTY720 affects sphingosine-1-phosphate (S1P) signaling on targeted cells that bear the S1P receptors S1P1, S1P3, S1P4, and S1P5. We examined the effect of FTY720 treatment on the biology of mouse neural progenitor cells (NPCs) after transplantation in a viral model of demyelination. Intracerebral infection with the neurotropic JHM strain of mouse hepatitis virus (JHMV) resulted in an acute encephalomyelitis, followed by demyelination similar in pathology to the human demyelinating disease, multiple sclerosis. We have previously reported that intraspinal transplantation of mouse NPCs into JHMV-infected animals resulted in selective colonization of demyelinated lesions, preferential differentiation into oligodendroglia accompanied by axonal preservation, and increased remyelination. Cultured NPCs expressed transcripts for S1P receptors S1P1, S1P2, S1P3, S1P4, and S1P5. FTY720 treatment of cultured NPCs resulted in increased mitogen-activated protein kinase phosphorylation and migration after exposure to the chemokine CXCL12. Administration of FTY720 to JHMV-infected mice resulted in enhanced migration and increased proliferation of transplanted NPCs after spinal cord engraftment. FTY720 treatment did not improve clinical disease, diminish neuroinflammation or the severity of demyelination, nor increase remyelination. These findings argue that FTY720 treatment selectively increases NPC proliferation and migration but does not either improve clinical outcome or enhance remyelination after transplantation into animals in which immune-mediated demyelination is initiated by the viral infection of the central nervous system. *(Am J Pathol 2015, 185: 2819–2832; http://dx.doi.org/10.1016/j.ajpath.2015.06.009)*

Intracranial infection with the neurotropic JHM strain of mouse hepatitis virus (JHMV) results in an acute encephalomyelitis, followed by chronic demyelination characterized by viral persistence within the central nervous system (CNS), axonal damage, and demyelination.[1–7] Previous studies from our laboratory have used the JHMV model of neuroinflammation-mediated demyelination to evaluate the therapeutic benefit of mouse neural progenitor cell (NPC) engraftment on remyelination.[8–10] Transplantation of mouse NPCs into the spinal cords of JHMV-infected mice results in extensive migration and colonization of areas of white matter damage and preferential differentiation into oligodendroglia.[8–10] Engrafted NPCs physically engage damaged axons, and this ultimately leads to increased axonal integrity that correlates with remyelination.[8,11] These findings, along with others,[12–14] argue that engraftment of NPCs may provide an important unmet clinical need for treatment of human demyelinating

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diseases, including multiple sclerosis (MS), by facilitating sustained remyelination that can restore motor function and ameliorate clinical symptoms.

After engraftment of NPCs into the spinal cords of JHMV-infected mice, transplanted cells migrate both rostral and caudal from the implantation site.[8,9] The chemokine ligand CXCL12 is enriched within areas of demyelination, and transplanted NPCs express the signaling receptor CXCR4, resulting in colonization of areas of white matter damage. Blocking CXCR4 signaling on NPC transplantation impaired NPC migration, arguing for an important role for this chemokine signaling pathway in contributing to repair by mediating trafficking to sites of myelin damage.[9] However, the molecular mechanisms governing positional migration of NPCs are likely complex and consist of additional soluble factors that affect the ability of NPCs to effectively congregate within areas of white matter pathology.

Among potential molecules that may influence migration is the lysophospholipid sphingosine-1-phosphate (S1P) that is well documented in controlling proliferation and migration of numerous cell types.[15–18] Although the importance of S1P signaling in controlling lymphocyte homing and egress from lymphatic tissues is well documented,[19–21] increasing evidence indicates a functional role within the CNS as glia and neurons express different combinations of specific signaling receptors S1P1, S1P2, S1P3, S1P4, and S1P5.[22,23] Activation of these receptors yields different effects on migration and survival of astrocytes, microglia, and oligodendrocytes.[24–26] In addition, NPCs express S1P receptors, and signaling has previously been reported to influence *in vitro* differentiation.[27] Moreover, Kimura et al[28] demonstrated an important role for S1P signaling in controlling migration of transplanted NPCs to an injury site in a model of spinal cord injury.

We examined the functional role of S1P signaling after NPC transplantation into the spinal cords of JHMV-infected mice. FTY720 is a U.S. Food and Drug Administration–approved oral drug for treatment of patients with relapsing MS.[22,23,29–31] FTY720 exerts immunomodulatory effects that reduce acute relapses, new lesion formation, and disability progression and brain volume loss in MS patients.[32] The mechanism(s) behind FTY720 functions are not yet defined; however, the phosphorylated active form of FTY720 (FTY720P) is an S1P receptor modulator that inhibits egress of lymphocytes from lymph nodes. FTY720 is a functional antagonist of S1P1 on lymphocytes,[20] yet also can act as a nonselective agonist of S1P1, S1P3, S1P4, and S1P5.[33] Therefore, the available evidence suggests that cellular source and receptor expression profile are critical in terms of how FTY720 affects S1P signaling, and likely lead to a dampening of autoreactive T cells specific for myelin antigens infiltrating into the CNS. More important, FTY720, because of its lipophilic nature, penetrates the blood-brain barrier and readily enters the CNS parenchyma. Furthermore, FTY720P is detected *in situ*, suggesting that it may influence the biology of resident cells of the CNS. Our findings reveal that treatment of cultured NPCs with FTY720P led to an active signaling response, as determined by phosphorylation of mitogen-activated protein (MAP) kinase, yet did not influence lineage fate commitment. FTY720 treatment of JHMV-infected mice, transplanted with NPCs, demonstrated enhanced migration associated with increased numbers of NPCs compared with vehicle-treated control animals. FTY720 treatment did not affect the accumulation of T cells or macrophages within the CNS. Finally, after treatment in animals in which demyelination is established, FTY720 did not augment the effects of NPCs on influencing remyelination, indicating a selective effect on migration/proliferation on spinal cord engraftment into JHMV-infected mice.

Materials and Methods

Mice and Virus

Age-matched (5 to 7 weeks) S1P1 enhanced green fluorescent protein (eGFP) knock-in mice (C57BL/6 background)[34] and C57BL/6 mice were anesthetized with an i.p. injection of 150 μL of a mixture of ketamine (Western Medical Supply, Arcadia, CA) and xylazine (Phoenix Pharmaceutical, Saint Joseph, MO) in Hanks' balanced salt solution. Mice were injected intracranially with 150 plaque-forming units of JHMV (strain V2.2-1) suspended in 30 μL saline.[9] Clinical severity was assessed by blinded investigators (T.E.L. and C.A.B.) using a previously described four-point scoring scale.[35] FTY720 (2-amino-2-[2-(4-octylphenyl) ethyl]-1,3-propanediol, hydrochloride) and FTY720P (2-amino-2 [2-(4-octylphenyl) ethyl]-1,3-propanediol, mono dihydrogen phosphate ester) were purchased from Cayman Chemical Co (Ann Arbor, MI). FTY720 or the vehicle was administered by daily i.p. injections of 100 μL, starting at day 13 postinfection (p.i.). Experiments for all animal studies were reviewed and approved by the University of Utah (Salt Lake City) and the University of California (Irvine) Institutional Animal Care and Use Committees.

NPC Isolation and Culture

Neurosphere cultures were prepared from brains of perinatal *S1P1* eGFP knock-in mice, as previously described.[8,36] Briefly, dissected striata were razor minced and triturated in 0.05% trypsin for 10 minutes, followed by anti-trypsin to inactivate the digestion. Single cells were resuspended in Dulbecco's modified Eagle's medium:F12 (Invitrogen, Carlsbad, CA) supplemented with B27 (Invitrogen), insulin-transferrin-selenium-X (Invitrogen), penicillin-streptomycin (Invitrogen), 40 ng/mL T3 (T67407; Sigma, St. Louis, MO), and 20 ng/mL human recombinant epidermal growth factor (E9644; Sigma). Cells were cultured for 6 days with replacement of media every other day, at which point