Fingolimod Impedes Schwann Cell–Mediated Myelination

Implications for the Treatment of Immune Neuropathies?

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Background: Fingolimod (FTY720), a first-in-class sphingosine-1-phosphate (S1P) receptor agonist, is a recently approved drug for treating relapsing multiple sclerosis. Experimental evidence suggests that FTY720 not only exhibits anti-inflammatory properties but also promotes myelination in the central nervous system by direct interaction with oligodendrocytes.

Objective: To assess the effects of FTY720 on Schwann cells (SCs) and peripheral nerve myelination.

Design: Receptor expression studies and myelination were investigated in primary rat SCs and rat neuronal/SC cocultures. Cells were treated with physiologically relevant concentrations of the active phosphorylated form of FTY720 (FTY720P). In addition, S1P receptor expression was corroborated in human and rat peripheral nerve tissue sections.

Results: Schwann cells express all known S1P receptors on the RNA level, not altered by FTY720P. In the myelination model, treatment with FTY720P resulted in a significant reduction of quantitative myelin formation. FTY720P induced reactive oxygen species in SCs associated with apoptosis of these cells, as demonstrated by the detection of cysteine aspartic acid–specific protease 3 and 7, as well as terminal deoxynucleotidyl transferase dUTP nick-end labeling. This effect was dependent of S1P signaling because the blocking of S1P receptors ameliorated reactive oxygen species production, SC apoptosis, and myelin loss.

Conclusions: FTY720P at greater concentrations induces apoptosis in SCs and may interfere with peripheral nerve myelination.


GUILLAIN-BARRÉ SYNDROME (GBS) and chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) are prototypic immune-mediated neuropathies. Even with improved therapeutic options available, both diseases carry a grave prognosis because they are associated with sustained disability and even significant mortality.1,2 Understanding of the underlying pathomechanisms is still incomplete. There is consensus that both GBS and CIDP result from aberrant cellular and humoral immune responses directed to peripheral nerve antigens resulting in demyelination and/or axonal damage of the peripheral nerve.3 The histopathologic analysis of classic GBS and CIDP is characterized by mononuclear cellular infiltration and multifocal demyelination and various degrees of admixed axonal degeneration.4 The latter in large part determines the clinical outcome in terms of permanent disability. In CIDP, another typical morphologic feature is Schwann cell (SC) proliferation, with onion-bulb formation pointing to the capacity of the peripheral nervous system (PNS) to (re-)myelinate. However, this process is often rather incomplete, specifically in the context of ongoing chronic inflammation.3 All currently available therapeutic strategies in GBS as well as CIDP target inflammation that results in damage to the peripheral nerve.6 To date, no treatment is available to intervene at the level of myelination in immune neuropathies. Thus, novel treatments that enhance the endogenous capacity of SCs to remyelinate axons are of great interest.

FTY720 (also known as fingolimod) is a synthetic drug developed by chemical modification of ISP-1 (myriocin), a sphingosine-1-phosphate (S1P) receptor agonist, which is a recently approved drug for treating relapsing multiple sclerosis.
gosine-like metabolite produced by the fungus *Isaria sinalii*. FTY720 has been demonstrated to prolong allograft survival in different animal models of transplantation and exert a protective effect in various animal models of autoimmune disorders. In experimental autoimmune encephalomyelitis (EAE), an animal model mimicking certain immunopathogenic aspects of multiple sclerosis (MS), an autoimmune disease of the central nervous system (CNS), showed that FTY720 ameliorated clinical symptoms and pathology in both rats and mice. Also in experimental autoimmune neuritis (EAN), a model system for human GBS, as well as in the B7-2–deficient nonobese diabetic mouse, a model used to study human CIDP, FTY720 attenuated the clinical course of both diseases. Based on the positive results of 2 phase 3 clinical trials, FTY720 was approved for treating patients with relapsing forms of MS. The clinical effects seen in EAN, EAE, and MS have been attributed to its capacity to sequester circulating lymphocytes, especially T lymphocytes, into secondary lymphoid tissues. However, a growing body of evidence points to additional direct effects of FTY720 on cells of the nervous system. Recent studies suggest that FTY720 has a cytoprotective effect on oligodendrocyte progenitors and enhances CNS remyelination. To our knowledge, the impact of FTY720 on glial cells of the peripheral nerve, specifically SCs, has not been studied so far.

Given the unmet need for more efficacious treatments specifically for CIDP, FTY720 could conceptionally not only provide strong anti-inflammatory effects but also promote myelination in the damaged PNS. Thus in our study, we assessed the effect of the activated phosphorylated form of fingolimod (FTY720P) on SCs and dorsal root ganglion (DRG) cells. We specifically evaluated treatment dose–dependent and duration-dependent effects of FTY720P on myelination in the DRG-based peripheral nerve model system.

### METHODS

FTY720 and its active phosphorylated form (FTY720P) were purchased from Cayman and dissolved in dimethyl sulfoxide and 50 µM of hydrochloric acid and diluted in culture media before each experiment. Cells were treated with different doses of FTY720P (10nM, 100nM, and 1 µM). In long-term cultures, this dose was maintained. The appropriate vehicles served as controls. For blocking experiments, the S1P receptor antagonist W123 (Cayman) was used.

### PREPARATION OF PURE SC CULTURES

Schwann cells were prepared by a modified Brocks method. Briefly, anesthetized neonatal rats were killed by decapitation and sciatic nerves were dissected. After digestion with 0.1% collagenase (Worthington) and 0.25% trypsin, cells were plated in Dulbecco Modified Eagle Medium with 10% fetal bovine serum. To eliminate fibroblasts, cells were treated with 2 cycles of cytosine arabinoside (10 µM) followed by complement lysis with antithymidine 1.1 antibody (Serotec), achieving a purity of SCs greater than 95%. The resulting cultures were expanded by medium with forskolin (Sigma-Aldrich).

All experiments were conducted according to state regulations for animal experimentation and were approved by the responsible local authorities.

### MYELINATING CULTURES AND QUANTITATIVE PCR

Dissociated and undissociated neuronal/SC cultures were prepared from embryonic rat (E15) DRGs as described previously. Entire ganglia were plated on collagen-coated 35-mm plastic dishes (Greiner Bio-One). Cell cultures were maintained for 2 days in neurobasal medium (Invitrogen) supplemented with 1% fetal bovine serum (HyClone), 2M of L-glutamine, 2% B-27 serum–free supplement (Invitrogen), and 100 ng/mL of nerve growth factor (Sigma-Aldrich). Myelination was initiated by switching from neurobasal medium to Eagle medium with Earle salts containing 15% fetal bovine serum, 10 ng/mL of nerve growth factor, and 50 µg/mL of ascorbic acid (Sigma-Aldrich).

Polymerase chain reaction (PCR) was conducted to determine expression and alteration of S1P receptor messenger RNA levels in SCs as well as DRG cocultures. Cells were detached with trypsin (Invitrogen) and washed with phosphate-buffered saline, and total cellular RNA was extracted with a RNeasy Mini Kit (Qiagen) according to the manufacturer’s instructions. Also, 200 ng of total RNA were photometrically determined by Nanodrop and then used for complementary DNA synthesis and subsequent real-time PCR, as described previously (Table 1). Data were analyzed with the ABI PRISM Detection System using the comparative Ct (threshold cycle) method. Samples were normalized to the housekeeping genes GAPDH and 18S to account for the variability in the initial concentration of the total RNA and the conversion efficiency of the reverse transcription. Normalization of samples was performed by dividing the copies of the gene of interest by copies of the reference gene.

### Table 1. PCR Primer Pairs Used for Quantitative PCR

<table>
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<th>Gene</th>
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<th>Reverse 5’-3’</th>
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<td>TGC AGT AGA GGA TGA CGA</td>
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<td>GTG CTT G TG GGG GTC TAG C</td>
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<td>GCC ATG GAC TGG CAA CAT GA</td>
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Abbreviations: PCR, polymerase chain reaction; S1PR, sphingosin-1-phosphate receptor.
IMMUNOCYTOCHEMISTRY AND IMMUNOHISTOCHEMISTRY

Sphingosine-1-phosphate receptor expression was analyzed by immunocytochemistry in dissociated DRG cultures or SCs fixed with 4% paraformaldehyde for 45 minutes. After washing, cell cultures were blocked with 10% normal horse serum in 0.1% TritonX for 1 hour at room temperature. Different polyclonal anti–S1P receptor antibodies or blocking solution without primary antibody as control were added for 12 hours at 4°C (Table 2). A fluorescence-labeled antirabbit IgG (Vector Laboratories) was used as secondary antibody.

To confirm S1P receptor expression in the PNS, immunohistochemistry was performed on sciatic nerve sections from Lewis rats as well as on sural nerve biopsies from patients. The rat samples were taken from animals induced with EAN at peak of clinical disease severity as well as healthy control samples, as published previously.23 Sural nerve biopsies were taken from 3 patients with CIDP prior to initiation of immunotherapy and 1 patient with a hereditary neuropathy. All biopsies were obtained and the mean length and total length of all Sudan black–stained internodal segments were measured using a Genios-Pro reader.

ASSESSMENT OF MYELINATION

Myelin segments were visualized by Sudan black staining followed by a morphologic and quantitative myelin analysis of the prepared cell cultures, as described previously.24 Briefly, whole culture dishes were fixed with 4% paraformaldehyde and postfixed with 0.1% osmium tetroxide for 43 minutes. Cultures were then dehydrated and stained with 0.5% Sudan black in 70% ethanol for 1 hour, washed with ethanol, and mounted in glycerine jelly. For myelin quantification, pictures in low magnification (×20) were obtained and the mean length and total length of all Sudan black–stained internodal segments were measured. Data are expressed as total length of myelinated internodes per well and the mean length of myelinated internodal segments.

PROLIFERATION AND VIABILITY ASSAY AND ASSESSMENT OF APOPTOSIS

The CellTiter-Blue assay (Promega) was used to assess proliferation and viability of cells. The reagent was added directly to cell cultures in serum-supplemented medium and results were recorded using a Genios-Pro reader (LI-COR Biosciences). Cells treated with 10 µM of deoxyribonuclease I, an endonuclease that nonspecifically cleaves DNA to release di-nucleotide, trinucleotide, and oligonucleotide products with 5’-phosphorylated and 3’-hydroxylated ends, served as positive control cells.

Members of the cysteine aspartic acid–specific protease (caspase) family play key effector roles in apoptosis in mammalian cells. The Caspase-Glo 3/7 Assay (Promega) was used according to the manufacturer’s instructions to determine caspase 3 and caspase 7 activity in SCs treated with FTY720P. Luminescence was detected with a Genios-Pro reader.

In addition, terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) was assessed with an in situ cell detection kit (Promega) according to standard protocols, quantified densitometrically (fluorescence per area), and correlated with the background. Altogether, 3 independent experiments were performed, containing 12 measurements each.

DETECTION OF REACTIVE OXYGEN SPECIES

FTY720P is known to stimulate reactive oxygen species (ROS) production, which culminate in protein kinase C activation and subsequent caspase 3–dependent apoptosis.24 Thus, pure SCs were treated with the nicotinamide adenine dinucleotide phosphate oxidase inhibitor diphenyleneiodonium (10 µmol/L), which is able to block ROS production, for 30 minutes followed by cotreatment with 10 nM to 1 µM of FTY720P for 24 hours. Afterward, cell viability was assessed by the CellTiter-Blue assay, as just described.

STATISTICAL ANALYSIS

One-way analysis of variance and Newman-Keuls test multiple comparisons were used as principal statistical tests. P < .05 was considered significant. Data are given as means and standard errors.

RESULTS

SCs EXPRESS S1P RECEPTORS

Sphingosine-1-phosphate receptor RNA expression was assessed in DRG cocultures during a period of 6 weeks as well as in pure SC cultures. The housekeeping genes GAPDH and 18S did not differ in their expression levels (data not shown). Five of 5 known receptors (S1P1, S1P2, S1P3, S1P4, and S1P5) receptors) were found to be expressed by SCs (Figure 1A), as well as by DRG cocultures from day 1 to day 28 of culture age on the RNA level (Figure 1B-E). Expression levels did not change during the process of myelination (Figure 1B-E).

Table 2. Antibodies Used for Immunohistochemistry

<table>
<thead>
<tr>
<th>Specificity</th>
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<th>Dilution</th>
<th>Source</th>
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</tr>
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<td>Cayman</td>
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<td>1:100</td>
<td>Acris</td>
</tr>
<tr>
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<td>Schwann cells</td>
<td>Rabbit</td>
<td>1:100</td>
<td>Invitrogen</td>
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<tr>
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<td>T cells</td>
<td>Rat</td>
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<td>Novo Nordisk</td>
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<td>Rat IgG Alexa Fluor 488</td>
<td>Rat</td>
<td>1:500</td>
<td>Invitrogen</td>
</tr>
</tbody>
</table>

Abbreviation: S1PR, sphingosin-1-phosphate receptor.
To corroborate our findings on the protein level, immunocytochemistry was performed on SCs and DRG cocultures in vitro. All SCs stained positive for S100 and by costaining positive immunoreactivity was depictable for the receptor S1P1 (Figure 2A-C). For S1P2, S1P3, S1P4, and S1P5 receptors, we were not able to identify appropriate antibodies useful for immunocytochemistry. In the DRG cocultures, positive immunoreactivity was exclusively localized to S100-positive SCs but not to sensory neurons or accompanying fibroblasts.

Treatment of pure SC cultures and DRG cocultures with FTY720P at different concentrations (10nM, 100nM, and 1 µM) and different exposure rates (6, 12, 24, 48, and 72 hours during 6 weeks) did not alter the expression levels of any S1P receptor studied, neither in pure SCs or in DRG cocultures (Figure 1). Pretreatment and coincubation with a proinflammatory cytokine, inter-

**Figure 1.** Graphs of RNA expression. A, RNA expression of all 5 known sphingosine-1-phosphate receptors (S1P1-S1P5 receptors) in rat Schwann cells. All receptors were found to be expressed by Schwann cells (light gray). Treatment with FTY720P did not change the expression on the RNA level (dark gray). In DRG cocultures from day 1 to day 21 of culture age, S1P1 to S1P5 receptors were found to be expressed on the RNA level (light gray; B-F). During the course of myelination, receptor-expression levels did not change. Treatment with FTY720P at different concentrations (here 1 µM of FTY720P) did not alter expression levels of any S1P receptor studied (dark gray). RNA levels expressed as a fraction of the housekeeping gene GAPDH, expressed as ΔΔCt (A) or ΔΔCt (B-F). NS indicates not significant. The error bars indicate standard deviation.
feron-γ, which is known to increase expression of various surface molecules on SCs at a concentration of 500 U/mL,\textsuperscript{25} did not alter the expression on RNA and protein levels (data not shown).

To underline the clinical relevance of our observations, tissue sections from sciatic nerves of Lewis rats and tissue samples from human sural nerve biopsies were investigated histologically. In rat and human nerves, we were able to stain for the S1P\textsubscript{1} receptor. Immunoreactivity was primarily detected within the endoneurium and localized to SCs (Figure 2D-I). Few perivascular mononuclear cells in the perineurium and epineurium were also found to express the S1P\textsubscript{1} receptor, most likely lymphocytes invading the inflamed peripheral nerve in biopsies obtained from patients with CIDP (Figure 2I). On serial sections, appearance and distribution of the staining pattern was similar to the immunoreactivity observed with an anti-CD3 antibody (data not shown). There was no difference in the number of S1P\textsubscript{1} receptor-immunoreactive SCs in samples from animals with acute EAN compared with control samples. Similarly, the number of S1P\textsubscript{1} receptor-positive SCs was in the same range when comparing sections from patients with CIDP with a noninflammatory control (data not shown). We were unable to demonstrate the specificity of commercially available human reactive S1P receptor antibodies apart from the one used against the S1P\textsubscript{1} receptor.

**INFLUENCE OF FTY720P ON MYELINATION IN DRG COCULTURES**

The effect of FTY720P on peripheral nerve myelination was assessed in an in vitro model. Continuous treatment with FTY720P at 3 different concentrations (10nM, 100nM, and 1 μM) resulted in a dose-dependent decrease in the total amount of myelin per well studied. This effect was statistically significant for the treatment with 100nM and 1 μM of FTY720P when compared with control subjects (Figure 3A, C, and D). The addition of the competitive S1P receptor antagonist W123 in a dose of 1 μM to DRG cocultures treated with FTY720P (100nM and 1 μM) prevented this dose-dependent decrease of myelination (Figure 3B).
To further explore the biological effect of FTY720P on myelinated cells, cultures with completed myelination (21 days of age) were treated with FTY720P using the same dosages as before. In this experimental setting, FTY720P did not exhibit any effects, neither on total amount of myelin or on measures for myelination (data not shown).

FTY720P AFFECTS CELLULAR VIABILITY AND INDUCES APOPTOSIS

To evaluate potential cellular toxicity of FTY720P on myelinating cells, SCs were treated with different concentrations of this drug. Lower concentrations (10nM and 100nM) exhibited no effect on proliferation or cell viability, whereas treatment with 1 µM of FTY720P resulted in a significant decrease in proliferation and cellular viability \( (P < .05; \text{Figure 4A}). \) To further clarify this effect, caspase 3 and caspase 7 were assessed in supernatants: SCs treated with different concentrations of FTY720P exhibited increased activation of caspase 3 and caspase 7 in a dose-dependent manner (Figure 4B). Both markers of apoptosis were significantly increased compared with control samples at 100nM and 1 µM of FTY720P. This effect on proliferation and cellular viability was blocked when adding the competitive S1P receptor antagonist W123 in a dose of 1 µM, indicating that the effect seen was mediated by the interaction between FTY720P and corresponding S1P receptors (Figure 4B). These results were corroborated and quantified by TUNEL staining (Figure 4C-F).

**INHIBITION OF ROS MITIGATES THE EFFECT OF FTY720P ON APOPTOSIS**

Because evidence is provided that FTY720P can activate the production of ROS, pure SCs were pretreated with the ROS inhibitor diphenyleneiodonium followed by co-incubation with FTY720P at different concentrations. Analysis of cellular viability after 24 hours revealed that the inhibition of ROS production by a nicotinamide adenine dinucleotide phosphate oxidase inhibitor significantly protects SCs from FTY720P-induced apoptosis (Figure 5).
COMMENT

Immune-mediated demyelinating polyneuropathies represent a group of disorders with an unmet need for more efficacious treatment options, especially therapies that promote myelination. An accumulating body of experimental evidence suggests that FTY720, apart from its anti-inflammatory properties, exhibits direct effects on the CNS by promoting myelin formation by oligodendrocytes and as such, represents an interesting drug for exploration in inflammatory and demyelinating diseases of the PNS.

Prerequisite for a direct interaction with FTY720 is the presence of S1P receptors, which have been demonstrated to be expressed on CNS glial cells such as astrocytes and oligodendrocytes. In contrast, data on the expression of S1P receptors in the PNS are sparse. Here we show that SCs express all 5 known receptors at least on the RNA level with similar magnitude for each of these receptors. This differs from patterns seen in cultured rat oligodendroglial cells and in oligodendroglial progenitor cells. By immunocytochemistry, the expression of S1P receptor could be validated on the protein level as well.

When comparing pure SCs with DRG cultures, no differences in the quantitative amount of receptor transcripts were detectable. Thus, it can be assumed that SCs represent the major cellular source of S1P receptors in the model system of myelination used here. Sphingosine-1-phosphate–receptor expression levels were independent of the presence of the proinflammatory cytokine interferon-γ and the state of myelination, indicating that SCs retain a potential susceptibility to S1P receptor ligands during the whole process of myelination.

We were able to corroborate our in vitro findings in rat and human peripheral nerve samples ex vivo by immunohistochemistry, where the S1P1 receptor could be localized to SCs as well. The number of immunoreactive SCs also did not differ between tissue samples obtained during acute inflammation compared with non-inflammatory conditions, whether studied in rat or human nerves. In addition, some S1P1 receptor immunoreactivity could also be localized to perivascular T cells in the perineurium and epineurium, a finding not unexpected because lymphocytes are known to display this receptor. In human MS lesions, the S1P1 receptor has been reported to also be expressed on reactive astrocytes. The main anti-inflammatory effect of fingolimod is mediated via the S1P1 receptor. Owing to the lack of commercially available reliable specific human-reactive antibodies, we were not able to define the overall expression pattern of S1P receptors in the human PNS in greater detail. Thus at present, the entire expression of this receptor class on the protein level in the human peripheral nerve cannot be assessed until better antibodies are developed.

We could demonstrate the presence of the S1P receptor in SCs. In contrast to other studies in different cell types, treatment of SCs or DRG cocultures with different physi-
ologically relevant concentrations of FTY720P did not change the expression of S1P receptor transcripts.

Recently, S1P receptor at a concentration of 1 µM has been reported to cause rearrangements to the cytoskeleton in primary rat SCs and stimulate SC migration. In our cell culture model, FTY720P did not exert any measurable effect on already myelinated culture. In contrast, during the establishment of myelination, exposure of FTY720P inhibited myelination in a dose-dependent fashion, an effect that could be overcome by coinoculation with the competitive S1P receptor antagonist W123. Potential explanations for this include differences in cell culture methods and the fact that our cell culture model selects SCs with myelinating phenotype, which may behave differently compared with nonmyelinating SCs used in previous studies. We observed that FTY720P induces the production of ROS in pure SC culture, which may cause apoptosis of these cells, as demonstrated by the detection of caspase 3 and caspase 7, as well as TUNEL staining. We were able to prevent this effect by coinoculation with a nicotinamide adenine dinucleotide phosphate oxidase inhibitor protecting SCs. Therefore, the reduced myelin synthesis in our model system seems to be mediated by FTY720P-induced apoptosis. Our findings are in line with observations reported by others. A large body of evidence suggests that FTY720P is not only involved in mechanisms that reduce inflammation and facilitate differentiation, survival, and repair, but that it also plays an important role in apoptosis of various human tumor cell lines. This antitumor effect is noteworthy because of an apparent involvement of S1P receptor-independent mechanisms. A recent study demonstrated that FTY720P suppresses the proliferation of hepatocellular carcinoma cells by stimulating ROS production, leading to caspase 3-dependent apoptosis. Moreover, other signaling pathways have been proposed to be responsible for the ability of FTY720P to facilitate apoptosis including mitogen-activated protein kinases, protein kinase B, focal adhesion kinase, and Rho-GTPase. Most of these reports rely on high concentrations of FTY720P (>10 µM). At such high concentrations, apoptosis and necrosis have been demonstrated in various tissues.

However, in our study, the dosages applied are in the range considered to be physiological and are identical to those that have been applied in various studies demonstrating FTY720P-induced myelination by oligodendrocytes. At low concentrations of 100nM we could detect increased levels of caspase 3 and caspase 7 in SCs, which excludes any toxicity evoked by overdosing our model system. Our present data suggest that, at least in large part, apoptosis observed in SCs is eventuated via S1P receptor-mediated pathways. Clearly, further studies are warranted to dissect this question in greater detail. Our findings clearly differ from the reports on the effect of FTY720P on oligodendroglial cells and oligodendroglial progenitor cells. For these cells, FTY720P has been reported to reduce apoptosis and promote myelination. It remains elusive at present which differential regulatory events during diverse biological situations may account for these discrepancies, but also technical issues cannot be excluded. The S1P/S1P receptor system is exquisitely suitable for fine tuning owing to the panel of S1P receptors expressed by different cell types in varying extent and composition. Therefore, it can be envisioned that subtle changes in this composition may provoke striking differences in the behavior of various cell types.

The clinical relevance of our findings is difficult to assess. In view of the unmet need for therapies that suppress inflammation and promote myelination, FTY720P appears to be a very promising therapy not only for MS, but also for disorders of the PNS, specifically CIDP. FTY720P is very lipophilic and has been shown to cross the blood-brain barrier, sequestering in the CNS tissue relative to the blood, where it could exert various effects. Although not demonstrated yet and given the high content of lipids in the peripheral nerve, sequestration of FTY720P in the peripheral nerve appears plausible. Therefore, our data would suggest that FTY720P may not promote PNS repair, as might have been speculated. In addition, its anti-inflammatory actions demonstrated in animal models of PNS inflammation may be executed outside the PNS where FTY720P does not accumulate. It will be tedious to dissect anti-inflammatory from reparative properties of FTY720P in animal models especially because EAN is predominantly mirroring the inflammatory cascade mounted against the peripheral nerve. Nevertheless, further experimental studies are needed to better understand the direct effects of FTY720P in the peripheral nerve, in particular because plans to embark on a clinical trial of this drug in CIDP are well under way.

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**REFERENCES**


