

REVIEW ARTICLE

Roles for lysophospholipid S1P receptors in multiple sclerosis

Kyoko Noguchi, and Jerold Chun

Department of Molecular Biology, Dorris Neuroscience Center, The Scripps Research Institute, La Jolla, CA, USA

Abstract

Sphingosine 1-phosphate (S1P) signaling in the treatment of multiple sclerosis (MS) has been highlighted by the efficacy of FTY720 (fingolimod), which upon phosphorylation can modulate S1P receptor activities. FTY720 has become the first oral treatment for relapsing MS that was approved by the FDA in September 2010. Phosphorylated FTY720 modulates four of the five known S1P receptors (S1P₁, S1P₃, S1P₄, and S1P₅) at high affinity. Studies in human MS and its animal model, experimental autoimmune encephalomyelitis (EAE), have revealed that FTY720 exposure alters lymphocyte trafficking via sequestration of auto-aggressive lymphocytes within lymphoid organs, representing the current understanding of its mechanism of action. These effects primarily involve S1P₁, which is thought to attenuate inflammatory insults in the central nervous system (CNS). In addition, FTY720's actions may involve direct effects on S1P receptor-mediated signaling in CNS cells, based upon the known expression of S1P receptors in CNS cell types relevant to MS, access to the CNS through the blood–brain barrier (BBB), and *in vitro* studies. These data implicate lysophospholipid signaling – via S1P₁ and perhaps other lysophospholipid receptors – in therapeutic approaches to MS and potentially other diseases with immunological and/or neurological components.

Keywords: sphingosine 1-phosphate; FTY720; fingolimod; GPCR; experimental autoimmune encephalitis

Introduction

Sphingosine 1-phosphate (S1P) is a lysophospholipid, which exerts diverse biological activities under physiological and pathological conditions through cell surface G protein-coupled receptors (GPCRs), named S1P₁, S1P₂, S1P₃, S1P₄, and S1P₅ (Ishii *et al.*, 2004). S1P is present at submicromolar concentrations in various biological fluids and tissues (Ishii *et al.*, 2004) and is produced intracellularly by a series of enzymatic reactions involving membrane-derived sphingolipids that ultimately provide sphingosine as a substrate for sphingosine kinase type 1 (SphK1) and type 2 (SphK2), to produce S1P, which can then act in both autocrine and paracrine fashions (Alvarez *et al.*, 2007).

This receptor-mediated signaling system, along with its sphingolipid metabolic pathway, is central to the actions of a compound known as FTY720 (fingolimod) that was recently approved by the FDA as a treatment for

relapsing multiple sclerosis (MS). FTY720 was initially reported as a myriocin derivative identified through studies of fungal metabolites, which possessed immunosuppressive properties by selectively depleting mature T cells in skin allograft models (Adachi *et al.*, 1995). FTY720 is a sphingosine analog that is also phosphorylated *in vivo* by SphKs, particularly SphK2, to produce FTY720-phosphate (FTY720-P) (Paugh *et al.*, 2003; Billich *et al.*, 2003; Zemmann *et al.*, 2006), whereupon it can activate four subtypes of S1P receptors, S1P_{1/3/4/5}, with single-digit or lower nanomolar affinities (Brinkmann *et al.*, 2002; Mandala *et al.*, 2002). FTY720 was evaluated in humans as an agent to prevent renal transplantation rejection, although these studies were ultimately terminated for lack of efficacy (Budde *et al.*, 2006). However, studies of FTY720 in experimental autoimmune encephalitis (EAE), an animal model of MS, supported therapeutic relevance to this disease (Brinkmann *et al.*, 2002; Fujino *et al.*, 2003; Webb *et al.*, 2004; Kataoka *et al.*, 2005;

Address for Correspondence: Jerold Chun, Department of Molecular Biology, The Scripps Research Institute, 10550 N. Torrey Pines Rd., La Jolla, CA 92037, USA. Tel: +1 858 784 8410. E-mail: jchun@scripps.edu

(Received 09 July 2010; revised 27 August 2010; accepted 08 September 2010)

ISSN 1040-9238 print/ISSN 1549-7798 online © 2011 Informa Healthcare USA, Inc.
DOI: 10.3109/10409238.2010.522975

<http://www.informahealthcare.com/bmg>

RIGHTS LINK
Copyright Clearance Center

Papadopoulos *et al.*, 2010), warranting further clinical evaluation.

MS is a chronic inflammatory demyelinating disease of the central nervous system (CNS) and can be associated with irreversible progression of neurological disability (Frohman *et al.*, 2006). Currently, there is no cure for MS. Until the recent approval of FTY720 as an orally bioavailable treatment for relapsing MS, FDA-approved disease modifying therapies only consisted of injectable, immunosuppressive drugs such as interferons (IFNs) β -1a and -1b, glatiramer acetate and natalizumab (Martin, 2010). FTY720 has been assessed in extensive clinical trials for relapsing-remitting MS (Kappos *et al.*, 2010; Cohen *et al.*, 2010), where it has shown efficacy along with an acceptable safety profile, and has received an approval from FDA in September 2010. The side-effect profile consists of a range of generally rare events (Cohen *et al.*, 2010; Kappos *et al.*, 2010), the most common of which is a transient bradycardia with initial treatment, resolving with continued exposure. Rarer events include nasopharyngitis, slightly reduced pulmonary function, and reversible macular edema (reviewed in Chun and Hartung, 2010). FTY720 recently received approval from the FDA as the first oral treatment for relapsing MS.

The current view on the mechanism of action of FTY720 is that it improves MS signs and symptoms by altering immune responses, particularly through effects on lymphocyte trafficking. In addition, the fact that relevant S1P receptors are expressed within the brain, and that FTY720 can penetrate the blood-brain barrier (BBB), raises the possibility that FTY720 may have direct effects on the CNS cells as well. This review will focus on S1P receptor mechanisms and relevant cell types that could contribute to the efficacy of FTY720 in MS to produce its demonstrated *in vivo* effects on the immune system, along with possible contributions of direct CNS influences.

S1P signaling and FTY720 efficacy in the immune system

T cells

MS is thought to be caused, at least in part, by an autoimmune attack of the CNS by myelin-specific CD4-positive T cells. The pathogenesis of MS is characterized by demyelination associated with infiltration of inflammatory cells and release of various cytokines and chemokines in the CNS (Frohman *et al.*, 2006). In EAE animal models, therapeutic or prophylactic administration of FTY720 reduces the infiltration of lymphocytes into the spinal cord with a rapid reduction in lymphocyte numbers in the peripheral blood produced by sequestration of lymphocytes within

primary and secondary lymphoid organs. This is thought to be the central mechanism of action of FTY720 for disease attenuation (Brinkmann *et al.*, 2002; Fujino *et al.*, 2003; Webb *et al.*, 2004; Kataoka *et al.*, 2005; Mehling *et al.*, 2008). In addition, FTY720 reduces levels of proinflammatory products, such as interleukin (IL)-17, IFN- γ , and inducible nitric oxide synthase (iNOS), in the spinal cord of EAE animals, which also may contribute to attenuating the disease state (Fujino *et al.*, 2003; Webb *et al.*, 2004; Kataoka *et al.*, 2005; Papadopoulos *et al.*, 2010).

As just noted, FTY720 reduces lymphocyte numbers in the blood and lymph by sequestering them in the thymus and the secondary lymphoid organs such as lymph nodes and Peyer's patches (Chiba *et al.*, 1998; Pinschewer *et al.*, 2000; Mandala *et al.*, 2002). Histology has shown that FTY720 treatment induces emptying of lymphoid sinuses, suggesting that lymphocytes cannot access egress structures and cannot egress into lymph (Mandala *et al.*, 2002). The effect is reversible (Pinschewer *et al.*, 2000) and observed in both naïve and activated T cells (Xie *et al.*, 2003). Thus, the mechanism of action of FTY720 in MS is believed to be a blockage of the inflammatory cell infiltration into the lesion site, resulting from sequestration of lymphocytes in the thymus and secondary lymphoid organs and a subsequent depletion of circulating autoaggressive lymphocytes.

The regulation of lymphocyte egress involves the S1P receptor subtype S1P₁, which allows lymphocytes to sense an S1P concentration gradient existing between blood/lymph and lymphoid tissues. This process regulates lymphocyte recirculation from within lymphoid organs back to the blood. Several lines of evidence support this model. In the normal condition, S1P concentrations are high in blood and lymph, and low in lymphoid organs (Schwab and Cyster, 2007). When S1P in lymph is lost by genetic deletion of the S1P producing enzymes SphK1/2 from lymphatic endothelial cells, lymphocytes cannot egress from lymph nodes into lymph circulation (Pappu *et al.*, 2007; Pham *et al.*, 2010). Expression level of S1P₁ in thymocytes increases during their maturation, and CD4 or CD8 single-positive mature T cells acquire the ability to migrate towards increasing S1P concentrations (Matloubian *et al.*, 2004). S1P₁ deletion from lymphocytes results in an inhibition of lymphocyte egress from the thymus and peripheral lymphoid organs. This has been shown in studies of conditional S1P₁ deletion from lymphocytes using a *Lck* promoter-driven Cre or transplantation of S1P₁-null hematopoietic cells into irradiated wild-type animals (Allende *et al.*, 2004; Matloubian *et al.*, 2004). These studies indicate that lymphocyte egress is dependent on lymphocyte expression of S1P₁ and requires an S1P concentration gradient. Since FTY720 treatment mimics the effect of S1P₁ deletion from lymphocytes, FTY720, via its active phosphorylated metabolite, may act predominantly as a functional antagonist of

lymphatic S1P₁ under therapeutic conditions despite its agonist properties under acute exposure conditions. In contrast to the dramatic effect on lymphocyte egress, FTY720 does not appear to affect significantly the activation, proliferation, or effector functions of T and B cells (Pinschewer *et al.*, 2000; Brinkmann *et al.*, 2001).

B cells

Although investigations into MS pathophysiology have focused mainly on T cells, growing evidence suggests a contribution of B cells which act as antigen presenting cells to T cells and secrete proinflammatory cytokines, chemokines and autoantibodies targeting structures on the myelin sheath and the axon (McLaughlin and Wucherpfennig, 2008). Rituximab (rituxan), a monoclonal antibody against CD20, has provided direct evidence of B cell involvement in MS pathology. Rituximab inhibits MS-related inflammation by specific depletion of B cells and B cell-producing autoantibodies (Hauser *et al.*, 2008). In addition, several studies have demonstrated that S1P₁-mediated signaling regulates trafficking of B cells. Deletion or downregulation of S1P₁ in developing bone marrow B cells inhibits the release of newly generated immature B cells from the bone marrow into the blood (Allende *et al.*, 2010). Either FTY720 treatment or S1P₁-deletion reduces the number of IgG- and IgA-secreting mature B cells in blood and bone marrow by sequestration in secondary lymphoid organs (Kabashima *et al.*, 2006; Kunisawa *et al.*, 2007). Indeed, B cell numbers in the blood of EAE animals decrease following FTY720 treatment (Kataoka *et al.*, 2005). Thus, it is likely that S1P signaling in B cells, as well as T cells, is altered during FTY720 exposure by regulating the distribution of B cells, and possibly altering the release of cytokines, chemokines and autoantibodies. Other S1P receptor subtypes may be involved in this process based on a report identifying S1P₃ as contributing to B cell positioning (Cinamon *et al.*, 2008).

Other immune cells

Natural killer (NK) cells have been shown to play a role in MS, but controversy exists as to whether they are protective or pathogenic (Morandi *et al.*, 2008). S1P₅ is highly expressed in NK cells and is required for NK cell egress from bone marrow and lymph nodes (Walzer *et al.*, 2007; Jenne *et al.*, 2009). In addition, S1P₁ is expressed in NK cells and may be involved in the regulation of NK cell egress (Jenne *et al.*, 2009). S1P₅ deficiency severely blocks NK cell egress, whereas S1P₁ deficiency does not, indicating that NK cell egress is regulated mostly by S1P₅. Thus, targeting S1P receptors in NK cells may influence the pathogenesis of MS by altering their tissue distribution, although the outcome could be either protective or

pathogenic. Whether FTY720 efficacy involves alterations of NK cells through S1P₅ remains unclear.

Antigen presenting cells, such as dendritic cells (DCs), macrophage/microglia, and astrocytes, are also involved in MS pathology (Slavin *et al.*, 2010). FTY720 treatment affects DC features such as migration and cytokine production *in vitro*, which are essential as antigen presenting cells (Muller *et al.*, 2005), and modulates DC trafficking *in vivo* (Czeloth *et al.*, 2005; Lan *et al.*, 2005). It is possible that S1P signaling in DCs may be involved in MS pathogenesis and could therefore be a therapeutic target.

S1P signaling and FTY720 efficacy in the CNS

S1P receptors are expressed in the CNS. FTY720 can penetrate the BBB and enter the CNS where it can be phosphorylated to its bioactive form, FTY720-P (Meno-Tetang *et al.*, 2006; Foster *et al.*, 2007). Brain levels of FTY720 and FTY720-P increase dose-dependently, and over time, exceed levels present in blood by several fold (Meno-Tetang *et al.*, 2006; Foster *et al.*, 2007). In addition, studies have demonstrated effects of FTY720 on CNS cell types as described below, consistent with their expression of S1P receptors. These observations raise the possibility that FTY720 efficacy for MS may involve direct actions on CNS cell types, in addition to effects on the immune system.

Astrocytes

Astrocytes are glial cells involved in the maintenance of the BBB, CNS metabolism, and synaptic functioning, as well as responding to pathological insults in the CNS. Recent evidence suggests a dual role of astrocytes in CNS inflammatory diseases such as MS. Astrocytes not only have the ability to enhance immune responses and inhibit myelin repair by forming a glial scar and preventing migration and maturation of oligodendrocyte progenitor cells, but can also be protective and limit CNS inflammation while supporting oligodendrocyte and axonal regeneration in some experimental systems (Williams *et al.*, 2007; Nair *et al.*, 2008).

There is evidence for the involvement of S1P signaling in astrocytes relevant to the pathogenesis of MS. Activation of S1P signaling induces astrogliosis *in vivo*, a prominent feature of CNS injury and neurodegenerative diseases, including MS (Sorensen *et al.*, 2003), and promotes proliferation of astrocytes *in vitro* (Pebay *et al.*, 2001; Sorensen *et al.*, 2003; Yamagata *et al.*, 2003; Bassi *et al.*, 2006). An animal model of Sandhoff disease, another neurodegenerative disease associated with astrogliosis, can be attenuated by genetic deletion of either SphK1 or S1P₃ (Wu *et al.*, 2008). S1P is released from astrocytes in an SphK dependent manner and acts

in both autocrine and paracrine manners (Riboni *et al.*, 2000; Anelli *et al.*, 2005). Upon injury, S1P production is locally increased and is associated with reactive astrocytes and microglia, suggesting S1P production from these cell types at the injury site (Kimura *et al.*, 2007). In cultured astrocytes, FTY720 exposure activates G_i -mediated signaling cascades, such as decreases in cyclic AMP, inositol phosphate formation and extracellular signal-regulated kinase (ERK) 1/2 phosphorylation, and stimulates migration (Mullershausen *et al.*, 2007; Osinde *et al.*, 2007). Many of these cell culture effects are mimicked by S1P₁ agonists (S1P, SEW2871 and AUY954) and are attenuated by S1P₁ antagonists (VPC23019, W123 and W146) or genetic deletion of S1P₁ (Mullershausen *et al.*, 2007; Osinde *et al.*, 2007; Dev *et al.*, 2008), suggesting the involvement of S1P₁. Astrocytes in culture preferentially express S1P₁ and S1P₃, and a low level of S1P₂ (Pebay *et al.*, 2001; Rao *et al.*, 2003; Anelli *et al.*, 2005). By comparison, S1P₅ expression appears to be below detectable limits under basal conditions, but can be upregulated in culture when cells are exposed to growth factors (Rao *et al.*, 2004). *In vivo* effects in EAE models as well as direct and indirect actions of signaling on astrocytes remain to be determined.

Oligodendrocytes

Oligodendrocytes are myelin-forming glial cells of the CNS. Loss of CNS myelin and a failure of remyelination by oligodendrocytes are a characteristic of the disease and likely contribute to subsequent irreversible disability in MS (Miller and Mi, 2007). Thus, overcoming remyelination failure could be a therapeutic strategy in MS. Remyelination requires proliferation and migration of oligodendrocyte progenitor cells into demyelinated lesion sites and subsequent differentiation into mature myelin-forming cells (Miller and Mi, 2007).

In vivo, therapeutic administration of FTY720 reduces the area of demyelination in the spinal cord of animals with EAE (Kataoka *et al.*, 2005; Papadopoulos *et al.*, 2010). In organotypic culture where the systemic immune system is absent, FTY720 treatment enhances remyelination following lyssolecithin-induced demyelination. This includes an increase in the number of oligodendrocyte progenitor cells, membrane outgrowth and elaboration of processes, as well as increases in microglia number and immunoreactivity for the astrocyte marker glial fibrillary acidic protein (GFAP). Both microglia and astrocytes can create an environment permissive for remyelination. Enhanced remyelination and associated astrogliosis are thought to be mediated through S1P_{3/5}, whereas microgliosis may occur through S1P_{1/5}, based upon *in vitro* experimental studies (Miron *et al.*, 2010). Other *in vitro* studies have demonstrated direct effects of FTY720 on oligodendrocytes and progenitor cells that include

survival, proliferation, migration, and differentiation, all of which are involved in the process of remyelination. In cultured oligodendrocytes and progenitor cells, FTY720 exposure activates ERK1/2 and Akt, which are involved in cell survival signals (Coelho *et al.*, 2007; Jung *et al.*, 2007). Indeed, exposure to FTY720 or FTY720-P protects these cells from apoptosis induced by deprivation of serum/growth factor, as well as apoptosis induced by inflammatory cytokines (e.g. tumor necrosis factor (TNF)- α and IFN- γ) and microglial activation, which have all been implicated in the pathogenesis of MS (Coelho *et al.*, 2007; Jung *et al.*, 2007; Miron *et al.*, 2008). A study using primary cells prepared from S1P₅-null animals has shown that S1P₅ is required for survival of mature, but not immature, oligodendrocytes (Jaillard *et al.*, 2005). In addition, FTY720 can synergistically increase platelet derived growth factor-dependent cell cycle progression of oligodendrocyte progenitor cells (Jung *et al.*, 2007), inhibit migration of oligodendrocyte progenitor cells through S1P₅ (Novgorodov *et al.*, 2007), and induce process retraction in oligodendrocytes, although the effect is transient and followed by subsequent re-extension (Jaillard *et al.*, 2005; Miron *et al.*, 2008). FTY720 can either promote or inhibit differentiation of oligodendrocyte progenitor cells into oligodendrocytes depending on its dose (Coelho *et al.*, 2007; Jung *et al.*, 2007). Thus, direct action of FTY720 exposure on oligodendroglial lineage cells can be both beneficial (promotion of survival, proliferation, and differentiation) and detrimental (inhibition of migration and differentiation) for remyelination. However, FTY720 seems to enhance remyelination in conjunction with other CNS cells combined with altering immune system influences.

Gene expression studies have identified S1P receptors, along with other lysophospholipid receptors, on oligodendrocytes and/or their precursor cells (Weiner *et al.*, 1998; McGiffert *et al.*, 2002), and oligodendroglial lineage cells preferentially express S1P₅, with lower levels of S1P₁, S1P₂, and S1P₃ (Terai *et al.*, 2003; Yu *et al.*, 2004; Miron *et al.*, 2008). Overall, S1P₅ gene expression is prominent in oligodendroglial lineages, but it is still unclear if the FTY720-S1P₅ signaling axis is actually involved in remyelination of MS lesions. It is of note that S1P₅-deficient mice do not show deficits in myelination (Jaillard *et al.*, 2005).

Microglia

Microglia, brain-resident, non-neural cells, play a role in MS throughout the disease process. They are rapidly activated and recruited to inflammatory sites within the CNS, and function as antigen-presenting cells, initiating and propagating immune responses, phagocytosing damaged tissues and debris, and producing various factors that are both tissue-toxic and protective (Jack *et al.*, 2005).

S1P signaling in microglia may be involved in migration and enhancement of the inflammatory response, but its *in vivo* role for MS remains unclear. *In vitro*, S1P treatment increases the expression of proinflammatory cytokines such as TNF- α and IL-1 β and nitric oxide in lipopolysaccharide (LPS)-activated microglia (Tham *et al.*, 2003; Nayak *et al.*, 2010). *In vivo*, FTY720 treatment attenuates the infiltration of reactive macrophages/microglia into lesion sites produced by traumatic brain injury (Zhang *et al.*, 2007). Gene expression levels of S1P receptors in microglia vary depending on their activation state. Microglia in inactive states express S1P₁ and S1P₃, with little S1P₂, and very low S1P₅ (Tham *et al.*, 2003). Upon activation, downregulation of S1P₁ and S1P₃ and upregulation of S1P₂ occur (Tham *et al.*, 2003). S1P may be produced from activated microglia, as described above (Kimura *et al.*, 2007). The effects of *in vivo* FTY720 exposure on microglial responses, as mediated by identified receptors, remain to be established, particularly with respect to MS therapeutic effects.

Neurons

S1P receptors S1P₁₋₃ are expressed in the developing brain (McGiffert *et al.*, 2002), and can influence neurogenesis (Mizugishi *et al.*, 2005). Mice with constitutive deletion of either SphK1/SphK2 or S1P₁ show neurogenic defects (Mizugishi *et al.*, 2005). In primary cultures of neural progenitor cells, S1P treatment induces survival, proliferation, and morphological changes, and enhances nerve growth factor (NGF)-induced neurite extension (Edsall *et al.*, 1997; Harada *et al.*, 2004; Toman *et al.*, 2004). In primary dorsal root ganglion neurons, S1P treatment affects NGF-induced neurite extension and enhances NGF-induced neuronal excitability (Toman *et al.*, 2004; Zhang *et al.*, 2006). In *Xenopus*, S1P signaling can influence axon guidance (Strochlic *et al.*, 2008). In addition, S1P signaling may promote neuronal repair after injury: neural stem/progenitor cells transplanted into the injured spinal cord migrate toward injured sites in an S1P₁-dependent manner (Kimura *et al.*, 2007).

FTY720 has been reported to have neuroprotective effects. Treatment with FTY720 may reduce sequelae in an ischemic stroke rat model (Hasegawa *et al.*, 2010) and may reduce inflammation and promote functional recovery after spinal cord injury (Lee, KD *et al.*, 2009). However, it is unclear whether these effects involve direct actions on neurons or secondary effects of immunosuppression. Uncertainties inherent to these models, particularly their inability to predict efficacy in humans, support further studies to ascertain both possible neuroprotective functions, as well as direct versus indirect mechanisms.

Blood-brain barrier (BBB)

The pathogenesis of MS includes the penetration of inflammatory cells across the BBB into the CNS parenchyma (Correale and Villa, 2007). The penetration occurs through (a) adherence of activated T cells and other lymphocytes to endothelial cells; (b) subsequent degradation of endothelial basement membrane; and (c) migration through the endothelium into the CNS parenchyma (Correale and Villa, 2007). S1P receptors are expressed on the endothelium and could therefore participate in aspects of the BBB since vascular endothelial cells express S1P₁ and S1P₃ (Lee, MJ *et al.*, 1999). S1P₁ expression within endothelial cells is essential for embryonic blood vessel development, which was shown by a study using conditional mutants with specific deletion of S1P₁ from endothelial cells (Allende *et al.*, 2003). Furthermore, S1P enhances physical barrier properties of endothelial cells by inducing adherens junction assembly and tight junction formation (Lee, MJ *et al.*, 1999; Sanchez *et al.*, 2003; Lee, JF *et al.*, 2006). It also attenuates vascular permeability induced by thrombin, vascular endothelial cell growth factor (VEGF) or LPS-mediated acute lung/renal injury (Sanchez *et al.*, 2003; Schaphorst *et al.*, 2003; Peng *et al.*, 2004). Like S1P, FTY720 exposure can also induce adherens junction assembly and attenuate vascular leakage induced by VEGF or in LPS-mediated acute lung injury (Sanchez *et al.*, 2003; Peng *et al.*, 2004). In addition, both S1P and FTY720 can activate G_i/Akt/ERK cell survival signals in endothelial cells, and can protect endothelial cells from apoptosis induced by serum deprivation or C₂-ceramide (Lee, MJ *et al.*, 1999; Sanchez *et al.*, 2003). Thus, unlike lymphocyte trafficking in which FTY720 exposure may result in functional antagonistic activities, FTY720's effects on endothelial cell functions seem to be agonistic. Interestingly, S1P-induced barrier enhancement and survival of endothelial cells appear to be mediated through S1P₁ and S1P₃ (Lee, MJ *et al.*, 1999; Schaphorst *et al.*, 2003; Dudek *et al.*, 2007), while FTY720-induced barrier enhancement is likely through non-S1P₁, G_i-coupled receptor(s) (Dudek *et al.*, 2007). Additionally, an integral cellular element of the BBB is the astrocyte through its documented interactions with endothelial cells (Abbott *et al.*, 2006), which may have particular relevance to FTY720's effects in view of the aforementioned S1P receptor-mediated activities influenced by FTY720 exposure.

In MS patients and EAE animals, lesion sites, cerebrospinal fluid and/or serum exhibit evidence of upregulation for vascular cell adhesion molecules (i.e. ICAM-1, P-selectin, and VCAM-1) and matrix metalloproteinases (i.e. MMP-2, -3, -7, and -9), the former facilitating cell adhesion and the latter, basement membrane degradation (Cuzner and Opdenakker, 1999; Waubant *et al.*, 1999; Foster *et al.*, 2009). FTY720

treatment could conceivably reduce or reverse the BBB breakdown that occurs in MS/EAE, as evidenced by therapeutic FTY720 treatment on EAE animals that displayed a reduction in immunoglobulin precipitation that reflects BBB damage in the spinal cord (Foster *et al.*, 2009). Moreover, both prophylactic and therapeutic treatment of FTY720 normalized upregulated gene expression of vascular cell adhesion molecules (ICAM-1, P-selectin, and VCAM-1) and MMP-9 in the spinal cord of EAE animals, suggesting at least a partial recovery of the BBB (Foster *et al.*, 2009). A recent study using a BBB model with isolated human brain endothelial cells has suggested the involvement of S1P₁ in protection from oxygen/glucose deprivation (Zhu *et al.*, 2010). Additional evidence is needed to establish effects of FTY720 on BBB regulation.

Receptor mechanisms

Several studies have shown that FTY720 can inhibit S1P signaling by inducing prolonged receptor internalization and degradation (Matloubian *et al.*, 2004; Graler and Goetzl, 2004). These effects can be attributed to the irreversible internalization of bound FTY720-P that results in ubiquitination and proteosomal degradation of at least S1P₁ (Oo *et al.*, 2007). As a result of this irreversible internalization, S1P₁ is unavailable to sense the S1P gradient that is necessary for lymphocytes to egress out of the immune compartment via the efferent lymph (e.g. within lymph nodes) (Schwab and Cyster, 2007). This mechanism, referred to as functional antagonism as noted above, may be more complex based on a report of persistent intracellular signaling from internalized S1P₁ by FTY720-P (Mullershausen *et al.*, 2009), although the biological significance of such signaling on lymphocyte trafficking remains to be determined. The endogenous levels of FTY720 within tissues may dictate the actual modulatory effects observed within lymphoid organs (Sensken *et al.*, 2009). These data indicate that the precise definition of whether FTY720-P functions as an agonist or an antagonist in an experimental disease setting may vary depending on experimental conditions. In humans, reductions of peripheral blood lymphocytes, indicative of reduced lymphocyte egress, clearly follow a dose-response (higher FTY720 concentrations are proportional to a reduction in peripheral blood lymphocytes) (Tedesco-Silva *et al.*, 2005), indicating that the dominant effect of FTY720 on lymphocytes is likely to be through functional antagonism of S1P₁ and possibly other S1P receptors, at least with respect to lymphocyte trafficking. In contrast, FTY720's effects on endothelial cells are most consistent with agonism of non-S1P₁, G_i-coupled receptor(s).

Conclusion

The discovery of FTY720 and establishment of its efficacy in humans for the treatment of relapsing-remitting MS have revealed the relevance of receptor-mediated S1P signaling to MS. A majority of *in vivo* functional studies have demonstrated that FTY720, through identified S1P receptors, affects lymphocyte trafficking, which in turn has been inferred as the major mechanism for ameliorating MS signs and symptoms. In addition, experimental data support the actions of FTY720 exposure on CNS components that could theoretically contribute to efficacy in MS. However, CNS functional *in vivo* data relevant to MS remain to be established. Further studies will elucidate the mechanism of action of FTY720 in MS, including a more complete view of affected cell types, S1P receptor subtypes, downstream signaling pathways, and interactions between the immune system and the CNS. With the recent FDA approval of FTY720 (fingolimod) as the first orally bioavailable therapy for relapsing MS, a new chapter in the treatment of MS could be opening, based upon S1P lysophospholipid receptor signaling.

Acknowledgements

The authors thank Yasuyuki Fujii and Ji Woong Choi for vital discussions and Danielle Letourneau for editorial assistance.

Declaration of interest

This work was supported by NIH grants NS048478 and DA019674 to JC. KN is a recipient of a postdoctoral fellowship from Novartis Pharma, AG. JC is a consultant for Novartis Pharmaceutical Corp.

References

- Abbott NJ, Ronnback L and Hansson E. 2006. Astrocyte-endothelial interactions at the blood-brain barrier. *Nat Rev Neurosci* 7:41–53.
- Adachi K, Kohara T, Nakao N, Arita M, Chiba K, Mishina T, Sasaki S and Fujita T. 1995. Design, synthesis, and structure-activity relationships of 2-substituted-2-amino-1,3-propanediols: discovery of a novel immunosuppressant, FTY720. *Bioorg Med Chem Lett* 5:853–856.
- Allende ML, Yamashita T and Proia RL. 2003. G-protein-coupled receptor S1P1 acts within endothelial cells to regulate vascular maturation. *Blood* 102:3665–3667.
- Allende ML, Dreier JL, Mandala S and Proia RL. 2004. Expression of the sphingosine 1-phosphate receptor, S1P1, on T-cells controls thymic emigration. *J Biol Chem* 279:15396–15401.
- Allende ML, Tuymetova G, Lee BG, Bonifacio E, Wu YP and Proia RL. 2010. S1P1 receptor directs the release of immature B cells from bone marrow into blood. *J Exp Med* 207:1113–1124.

- Alvarez SE, Milstien S and Spiegel S. 2007. Autocrine and paracrine roles of sphingosine-1-phosphate. *Trends Endocrinol Metab* 18:300–307.
- Anelli V, Bassi R, Tettamanti G, Viani P and Riboni L. 2005. Extracellular release of newly synthesized sphingosine-1-phosphate by cerebellar granule cells and astrocytes. *J Neurochem* 92:1204–1215.
- Bassi R, Anelli V, Giussani P, Tettamanti G, Viani P and Riboni L. 2006. Sphingosine-1-phosphate is released by cerebellar astrocytes in response to bFGF and induces astrocyte proliferation through Gi-protein-coupled receptors. *Glia* 53:621–630.
- Billich A, Bornancin F, Devay P, Mechtcheriakova D, Urtz N and Baumrucker T. 2003. Phosphorylation of the immunomodulatory drug FTY720 by sphingosine kinases. *J Biol Chem* 278:47408–47415.
- Brinkmann V, Chen S, Feng L, Pinschewer D, Nikolova Z and Hof R. 2001. FTY720 alters lymphocyte homing and protects allografts without inducing general immunosuppression. *Transplant Proc* 33:530–531.
- Brinkmann V, Davis MD, Heise CE, Albert R, Cottens S, Hof R, Bruns C, Prieschl E, Baumrucker T, Hiestand P, Foster CA, Zollinger M, Lynch KR. 2002. The immune modulator FTY720 targets sphingosine 1-phosphate receptors. *J Biol Chem* 277:21453–21457.
- Budde K, Schutz M, Glander P, Peters H, Waiser J, Liefeldt L, Neumayer HH and Bohler T. 2006. FTY720 (fingolimod) in renal transplantation. *Clin Transplant* 20 Suppl 17:17–24.
- Chiba K, Yanagawa Y, Masubuchi Y, Kataoka H, Kawaguchi T, Ohtsuki M and Hoshino Y. 1998. FTY720, a novel immunosuppressant, induces sequestration of circulating mature lymphocytes by acceleration of lymphocyte homing in rats. I. FTY720 selectively decreases the number of circulating mature lymphocytes by acceleration of lymphocyte homing. *J Immunol* 160:5037–5044.
- Chun J and Hartung HP. 2010. Mechanism of action of oral fingolimod (FTY720) in multiple sclerosis. *Clin Neuropharmacol* 33:91–101.
- Cinamon G, Zachariah MA, Lam OM, Foss FW Jr and Cyster JG. 2008. Follicular shuttling of marginal zone B cells facilitates antigen transport. *Nat Immunol* 9:54–62.
- Coelho RP, Payne SG, Bittman R, Spiegel S and Sato-Bigbee C. 2007. The immunomodulator FTY720 has a direct cytoprotective effect in oligodendrocyte progenitors. *J Pharmacol Exp Ther* 323:626–635.
- Cohen JA, Barkhof F, Comi G, Hartung HP, Khatri BO, Montalban X, Pelletier J, Capra R, Gallo P, Izquierdo G, Tiel-Wilck K, de Vera A, Jin J, Stites T, Wu S, Aradhye S, Kappos L; TRANSFORMS Study Group. 2010. Oral fingolimod or intramuscular interferon for relapsing multiple sclerosis. *N Engl J Med* 362:402–415.
- Correale J and Villa A. 2007. The blood-brain-barrier in multiple sclerosis: functional roles and therapeutic targeting. *Autoimmunity* 40:148–160.
- Cuzner ML and Opdenakker G. 1999. Plasminogen activators and matrix metalloproteases, mediators of extracellular proteolysis in inflammatory demyelination of the central nervous system. *J Neuroimmunol* 94:1–14.
- Czeloth N, Bernhardt G, Hofmann F, Genth H and Forster R. 2005. Sphingosine-1-phosphate mediates migration of mature dendritic cells. *J Immunol* 175:2960–2967.
- Dev KK, Mullershausen F, Mattes H, Kuhn RR, Bilbe G, Hoyer D and Mir A. 2008. Brain sphingosine-1-phosphate receptors: implication for FTY720 in the treatment of multiple sclerosis. *Pharmacol Ther* 117:77–93.
- Dudek SM, Camp SM, Chiang ET, Singleton PA, Usatyuk PV, Zhao Y, Natarajan V and Garcia JG. 2007. Pulmonary endothelial cell barrier enhancement by FTY720 does not require the S1P1 receptor. *Cell Signal* 19:1754–1764.
- Edsall LC, Pirianov GG and Spiegel S. 1997. Involvement of sphingosine 1-phosphate in nerve growth factor-mediated neuronal survival and differentiation. *J Neurosci* 17:6952–6960.
- Foster CA, Howard LM, Schweitzer A, Persohn E, Hiestand PC, Balatoni B, Reuschel R, Beerli C, Schwartz M and Billich A. 2007. Brain penetration of the oral immunomodulatory drug FTY720 and its phosphorylation in the central nervous system during experimental autoimmune encephalomyelitis: consequences for mode of action in multiple sclerosis. *J Pharmacol Exp Ther* 323:469–475.
- Foster CA, Mechtcheriakova D, Storch MK, Balatoni B, Howard LM, Bornancin F, Wlachos A, Sobanov J, Kinnunen A and Baumrucker T. 2009. FTY720 rescue therapy in the dark agouti rat model of experimental autoimmune encephalomyelitis: expression of central nervous system genes and reversal of blood-brain-barrier damage. *Brain Pathol* 19:254–266.
- Frohman EM, Racke MK and Raine CS. 2006. Multiple sclerosis – the plaque and its pathogenesis. *N Engl J Med* 354:942–955.
- Fujino M, Funeshima N, Kitazawa Y, Kimura H, Amemiya H, Suzuki S and Li XK. 2003. Amelioration of experimental autoimmune encephalomyelitis in Lewis rats by FTY720 treatment. *J Pharmacol Exp Ther* 305:70–77.
- Graler MH and Goetzl EJ. 2004. The immunosuppressant FTY720 down-regulates sphingosine 1-phosphate G-protein-coupled receptors. *FASEB J* 18:551–553.
- Harada J, Foley M, Moskowitz MA and Waeber C. 2004. Sphingosine-1-phosphate induces proliferation and morphological changes of neural progenitor cells. *J Neurochem* 88:1026–1039.
- Hasegawa Y, Suzuki H, Sozen T, Rolland W and Zhang JH. 2010. Activation of sphingosine 1-phosphate receptor-1 by FTY720 is neuroprotective after ischemic stroke in rats. *Stroke* 41:368–374.
- Hauser SL, Waubant E, Arnold DL, Vollmer T, Antel J, Fox RJ, Bar-Or A, Panzara M, Sarkar N, Agarwal S, Langer-Gould A, Smith CH; HERMES Trial Group. 2008. B-cell depletion with rituximab in relapsing-remitting multiple sclerosis. *N Engl J Med* 358:676–688.
- Ishii I, Fukushima N, Ye X and Chun J. 2004. Lysophospholipid receptors: signaling and biology. *Annu Rev Biochem* 73:321–354.
- Jack C, Ruffini F, Bar-Or A and Antel JP. 2005. Microglia and multiple sclerosis. *J Neurosci Res* 81:363–373.
- Jaillard C, Harrison S, Stankoff B, Aigrot MS, Calver AR, Duddy G, Walsh FS, Pangalos MN, Arimura N, Kaibuchi K, Zalc B, Lubetzki C. 2005. Edg8/S1P5: an oligodendroglial receptor with dual function on process retraction and cell survival. *J Neurosci* 25:1459–1469.
- Jenne CN, Enders A, Rivera R, Watson SR, Bankovich AJ, Pereira JP, Xu Y, Roots CM, Beilke JN, Banerjee A, Reiner SL, Miller SA, Weinmann AS, Goodnow CC, Lanier LL, Cyster JG, Chun J. 2009. T-bet-dependent S1P5 expression in NK cells promotes egress from lymph nodes and bone marrow. *J Exp Med* 206:2469–2481.
- Jung CG, Kim HJ, Miron VE, Cook S, Kennedy TE, Foster CA, Antel JP and Soliven B. 2007. Functional consequences of S1P receptor modulation in rat oligodendroglial lineage cells. *Glia* 55:1656–1667.
- Kabashima K, Haynes NM, Xu Y, Nutt SL, Allende ML, Proia RL and Cyster JG. 2006. Plasma cell S1P1 expression determines secondary lymphoid organ retention versus bone marrow tropism. *J Exp Med* 203:2683–2690.
- Kappos L, Radue EW, O'Connor P, Polman C, Hohlfeld R, Calabresi P, Selmaj K, Agoropoulou C, Leyk M, Zhang-Auberson L, Burtin P. FREEDOMS Study Group. 2010. A placebo-controlled trial of oral fingolimod in relapsing multiple sclerosis. *N Engl J Med* 362:387–401.
- Kataoka H, Sugahara K, Shimano K, Teshima K, Koyama M, Fukunari A and Chiba K. 2005. FTY720, sphingosine 1-phosphate receptor modulator, ameliorates experimental autoimmune encephalomyelitis by inhibition of T cell infiltration. *Cell Mol Immunol* 2:439–448.
- Kimura A, Ohmori T, Ohkawa R, Madoiwa S, Mimuro J, Murakami T, Kobayashi E, Hoshino Y, Yatomi Y and Sakata Y. 2007. Essential roles of sphingosine 1-phosphate/S1P1 receptor axis in the migration of neural stem cells toward a site of spinal cord injury. *Stem Cells* 25:115–124.
- Kunisawa J, Kurashima Y, Gohda M, Higuchi M, Ishikawa I, Miura F, Ogahara I and Kiyono H. 2007. Sphingosine 1-phosphate regulates peritoneal B-cell trafficking for subsequent intestinal IgA production. *Blood* 109:3749–3756.
- Lan YY, De Creus A, Colvin BL, Abe M, Brinkmann V, Coates PT and Thomson AW. 2005. The sphingosine-1-phosphate receptor agonist FTY720 modulates dendritic cell trafficking *in vivo*. *Am J Transplant* 5:2649–2659.

- Lee JF, Zeng Q, Ozaki H, Wang L, Hand AR, Hla T, Wang E and Lee MJ. 2006. Dual roles of tight junction-associated protein, zonula occludens-1, in sphingosine 1-phosphate-mediated endothelial chemotaxis and barrier integrity. *J Biol Chem* 281:29190-29200.
- Lee KD, Chow WN, Sato-Bigbee C, Graf MR, Graham RS, Colello RJ, Young HF and Mathern BE. 2009. FTY720 reduces inflammation and promotes functional recovery after spinal cord injury. *J Neurotrauma* 26:2335-2344.
- Lee MJ, Thangada S, Claffey KP, Ancellin N, Liu CH, Kluk M, Volpi M, Sha'afi RI and Hla T. 1999. Vascular endothelial cell adherens junction assembly and morphogenesis induced by sphingosine-1-phosphate. *Cell* 99:301-312.
- Mandala S, Hajdu R, Bergstrom J, Quackenbush E, Xie J, Milligan J, Thornton R, Shei GJ, Card D, Keohane C, Rosenbach M, Hale J, Lynch CL, Rupprecht K, Parsons W, Rosen H. 2002. Alteration of lymphocyte trafficking by sphingosine-1-phosphate receptor agonists. *Science* 296:346-349.
- Martin R. 2010. Multiple sclerosis: closing in on an oral treatment. *Nature* 464:360-362.
- Matloubian M, Lo CG, Cinamon G, Lesneski MJ, Xu Y, Brinkmann V, Allende ML, Proia RL and Cyster JG. 2004. Lymphocyte egress from thymus and peripheral lymphoid organs is dependent on S1P receptor 1. *Nature* 427:355-360.
- McGiffert C, Contos JJ, Friedman B and Chun J. 2002. Embryonic brain expression analysis of lysophospholipid receptor genes suggests roles for s1p(1) in neurogenesis and s1p(1-3) in angiogenesis. *FEBS Lett* 531:103-108.
- McLaughlin KA and Wucherpfennig KW. 2008. B cells and autoantibodies in the pathogenesis of multiple sclerosis and related inflammatory demyelinating diseases. *Adv Immunol* 98:121-149.
- Mehling M, Brinkmann V, Antel J, Bar-Or A, Goebels N, Vedrine C, Kristofic C, Kuhle J, Lindberg RL and Kappos L. 2008. FTY720 therapy exerts differential effects on T cell subsets in multiple sclerosis. *Neurology* 71:1261-1267.
- Meno-Tetang GM, Li H, Mis S, Pyszczyński N, Heining P, Lowe P and Jusko WJ. 2006. Physiologically based pharmacokinetic modeling of FTY720 (2-amino-2[2-(-4-octylphenyl)ethyl]propane-1,3-diol hydrochloride) in rats after oral and intravenous doses. *Drug Metab Dispos* 34:1480-1487.
- Miller RH and Mi S. 2007. Dissecting demyelination. *Nat Neurosci* 10:1351-1354.
- Miron VE, Hall JA, Kennedy TE, Soliven B and Antel JP. 2008. Cyclical and dose-dependent responses of adult human mature oligodendrocytes to fingolimod. *Am J Pathol* 173:1143-1152.
- Miron VE, Ludwin SK, Darlington PJ, Jarjour AA, Soliven B, Kennedy TE and Antel JP. 2010. Fingolimod (FTY720) enhances remyelination following demyelination of organotypic cerebellar slices. *Am J Pathol* 176:2682-2694.
- Mizugishi K, Yamashita T, Olivera A, Miller GF, Spiegel S and Proia RL. 2005. Essential role for sphingosine kinases in neural and vascular development. *Mol Cell Biol* 25:11113-11121.
- Morandi B, Bramanti P, Bonaccorsi I, Montalto E, Oliveri D, Pezzino G, Navarra M and Ferlazzo G. 2008. Role of natural killer cells in the pathogenesis and progression of multiple sclerosis. *Pharmacol Res* 57:1-5.
- Muller H, Hofer S, Kaneider N, Neuwirt H, Mosheimer B, Mayer G, Konwalinka G, Heufler C and Tiefenthaler M. 2005. The immunomodulator FTY720 interferes with effector functions of human monocyte-derived dendritic cells. *Eur J Immunol* 35:533-545.
- Mullershausen F, Craveiro LM, Shin Y, Cortes-Cros M, Bassilana F, Osinde M, Wishart WL, Guerini D, Thallmair M, Schwab ME, Sivasankaran R, Seuwen K, Dev KK. 2007. Phosphorylated FTY720 promotes astrocyte migration through sphingosine-1-phosphate receptors. *J Neurochem* 102:1151-1161.
- Mullershausen F, Zecri F, Cetin C, Billich A, Guerini D and Seuwen K. 2009. Persistent signaling induced by FTY720-phosphate is mediated by internalized S1P1 receptors. *Nat Chem Biol* 5:428-434.
- Nair A, Frederick TJ and Miller SD. 2008. Astrocytes in multiple sclerosis: a product of their environment. *Cell Mol Life Sci* 65:2702-2720.
- Nayak D, Huo Y, Kwang WX, Pushparaj PN, Kumar SD, Ling EA and Dheen ST. 2010. Sphingosine kinase 1 regulates the expression of proinflammatory cytokines and nitric oxide in activated microglia. *Neuroscience* 166:132-144.
- Novgorodov AS, El-Alwani M, Bielawski J, Obeid LM and Gudiz TI. 2007. Activation of sphingosine-1-phosphate receptor S1P5 inhibits oligodendrocyte progenitor migration. *FASEB J* 21:1503-1514.
- Oo ML, Thangada S, Wu MT, Liu CH, Macdonald TL, Lynch KR, Lin CY and Hla T. 2007. Immunosuppressive and anti-angiogenic sphingosine 1-phosphate receptor-1 agonists induce ubiquitinylation and proteasomal degradation of the receptor. *J Biol Chem* 282:9082-9089.
- Osinde M, Mullershausen F and Dev KK. 2007. Phosphorylated FTY720 stimulates ERK phosphorylation in astrocytes via S1P receptors. *Neuropharmacology* 52:1210-1218.
- Papadopoulos D, Rundle J, Patel R, Marshall I, Stretton J, Eaton R, Richardson JC, Gonzalez MI, Philpott KL and Reynolds R. 2010. FTY720 ameliorates MOG-induced experimental autoimmune encephalomyelitis by suppressing both cellular and humoral immune responses. *J Neurosci Res* 88:346-359.
- Pappu R, Schwab SR, Cornelissen I, Pereira JP, Regard JB, Xu Y, Camerer E, Zheng YW, Huang Y, Cyster JG, Coughlin SR. 2007. Promotion of lymphocyte egress into blood and lymph by distinct sources of sphingosine-1-phosphate. *Science* 316:295-298.
- Paugh SW, Payne SG, Barbour SE, Milstien S and Spiegel S. 2003. The immunosuppressant FTY720 is phosphorylated by sphingosine kinase type 2. *FEBS Lett* 554:189-193.
- Pebay A, Toutant M, Premont J, Calvo CF, Venance L, Cordier J, Glowinski J and Tence M. 2001. Sphingosine-1-phosphate induces proliferation of astrocytes: regulation by intracellular signalling cascades. *Eur J Neurosci* 13:2067-2076.
- Peng X, Hassoun PM, Sammani S, McVerry BJ, Burne MJ, Rabb H, Pearce D, Tuder RM and Garcia JG. 2004. Protective effects of sphingosine 1-phosphate in murine endotoxin-induced inflammatory lung injury. *Am J Respir Crit Care Med* 169:1245-1251.
- Pham TH, Baluk P, Xu Y, Grigorova I, Bankovich AJ, Pappu R, Coughlin SR, McDonald DM, Schwab SR and Cyster JG. 2010. Lymphatic endothelial cell sphingosine kinase activity is required for lymphocyte egress and lymphatic patterning. *J Exp Med* 207:17-27.
- Pinschewer DD, Ochsenbein AF, Odermatt B, Brinkmann V, Hengartner H and Zinkernagel RM. 2000. FTY720 immunosuppression impairs effector T cell peripheral homing without affecting induction, expansion, and memory. *J Immunol* 164:5761-5770.
- Rao TS, Lariosa-Willingham KD, Lin FF, Palfreyman EL, Yu N, Chun J and Webb M. 2003. Pharmacological characterization of lysophospholipid receptor signal transduction pathways in rat cerebrocortical astrocytes. *Brain Res* 990:182-194.
- Rao TS, Lariosa-Willingham KD, Lin FF, Yu N, Tham CS, Chun J and Webb M. 2004. Growth factor pre-treatment differentially regulates phosphoinositide turnover downstream of lysophospholipid receptor and metabotropic glutamate receptors in cultured rat cerebrocortical astrocytes. *Int J Dev Neurosci* 22:131-135.
- Riboni L, Viani P, Bassi R, Giussani P and Tettamanti G. 2000. Cultured granule cells and astrocytes from cerebellum differ in metabolizing sphingosine. *J Neurochem* 75:503-510.
- Sanchez T, Estrada-Hernandez T, Paik JH, Wu MT, Venkataraman K, Brinkmann V, Claffey K and Hla T. 2003. Phosphorylation and action of the immunomodulator FTY720 inhibits vascular endothelial cell growth factor-induced vascular permeability. *J Biol Chem* 278:47281-47290.
- Schaphorst KL, Chiang E, Jacobs KN, Zaiman A, Natarajan V, Wigley F and Garcia JG. 2003. Role of sphingosine-1 phosphate in the enhancement of endothelial barrier integrity by platelet-released products. *Am J Physiol Lung Cell Mol Physiol* 285:L258-267.
- Schwab SR and Cyster JG. 2007. Finding a way out: lymphocyte egress from lymphoid organs. *Nat Immunol* 8:1295-1301.
- Sensken SC, Bode C and Graler MH. 2009. Accumulation of fingolimod (FTY720) in lymphoid tissues contributes to prolonged efficacy. *J Pharmacol Exp Ther* 328:963-969.
- Slavin A, Kelly-Modis L, Labadia M, Ryan K and Brown ML. 2010. Pathogenic mechanisms and experimental models of multiple sclerosis. *Autoimmunity* 47(7):1-10.
- Sorensen SD, Nicole O, Peavy RD, Montoya LM, Lee CJ, Murphy TJ, Traynelis SF and Hepler JR. 2003. Common signaling pathways

- link activation of murine PAR-1, LPA, and S1P receptors to proliferation of astrocytes. *Mol Pharmacol* 64:1199-1209.
- Strochlic L, Dwivedy A, van Horck FP, Falk J and Holt CE. 2008. A role for S1P signalling in axon guidance in the *Xenopus* visual system. *Development* 135:333-342.
- Tedesco-Silva H, Mourad G, Kahan BD, Boira JG, Weimar W, Mulgaonkar S, Nashan B, Madsen S, Charpentier B, Pellet P, Vanrenterghem Y. 2005. FTY720, a novel immunomodulator: efficacy and safety results from the first phase 2A study in de novo renal transplantation. *Transplantation* 79:1553-1560.
- Terai K, Soga T, Takahashi M, Kamohara M, Ohno K, Yatsugi S, Okada M and Yamaguchi T. 2003. Edg-8 receptors are preferentially expressed in oligodendrocyte lineage cells of the rat CNS. *Neuroscience* 116:1053-1062.
- Tham CS, Lin FF, Rao TS, Yu N and Webb M. 2003. Microglial activation state and lysophospholipid acid receptor expression. *Int J Dev Neurosci* 21:431-443.
- Toman RE, Payne SG, Watterson KR, Maceyka M, Lee NH, Milstien S, Bigbee JW and Spiegel S. 2004. Differential transactivation of sphingosine-1-phosphate receptors modulates NGF-induced neurite extension. *J Cell Biol* 166:381-392.
- Walzer T, Chiossone L, Chaix J, Calver A, Carozzo C, Garrigue-Antar L, Jacques Y, Baratin M, Tomasello E and Vivier E. 2007. Natural killer cell trafficking *in vivo* requires a dedicated sphingosine 1-phosphate receptor. *Nat Immunol* 8:1337-1344.
- Waubant E, Goodkin DE, Gee L, Bacchetti P, Sloan R, Stewart T, Andersson PB, Stabler G and Miller K. 1999. Serum MMP-9 and TIMP-1 levels are related to MRI activity in relapsing multiple sclerosis. *Neurology* 53:1397-1401.
- Webb M, Tham CS, Lin FF, Lariosa-Willingham K, Yu N, Hale J, Mandala S, Chun J and Rao TS. 2004. Sphingosine 1-phosphate receptor agonists attenuate relapsing-remitting experimental autoimmune encephalitis in SJL mice. *J Neuroimmunol* 153:108-121.
- Weiner JA, Hecht JH and Chun J. 1998. Lysophosphatidic acid receptor gene vzg-1/lpA1/edg-2 is expressed by mature oligodendrocytes during myelination in the postnatal murine brain. *J Comp Neurol* 398:587-598.
- Williams A, Piaton G and Lubetzki C. 2007. Astrocytes - friends or foes in multiple sclerosis? *Glia* 55:1300-1312.
- Wu YP, Mizugishi K, Bektas M, Sandhoff R and Proia RL. 2008. Sphingosine kinase 1/S1P receptor signaling axis controls glial proliferation in mice with Sandhoff disease. *Hum Mol Genet* 17:2257-2264.
- Xie JH, Nomura N, Koprak SL, Quackenbush EJ, Forrest MJ and Rosen H. 2003. Sphingosine-1-phosphate receptor agonism impairs the efficiency of the local immune response by altering trafficking of naive and antigen-activated CD4+ T cells. *J Immunol* 170:3662-3670.
- Yamagata K, Tagami M, Torii Y, Takenaga F, Tsumagari S, Itoh S, Yamori Y and Nara Y. 2003. Sphingosine 1-phosphate induces the production of glial cell line-derived neurotrophic factor and cellular proliferation in astrocytes. *Glia* 41:199-206.
- Yu N, Lariosa-Willingham KD, Lin FF, Webb M and Rao TS. 2004. Characterization of lysophosphatidic acid and sphingosine-1-phosphate-mediated signal transduction in rat cortical oligodendrocytes. *Glia* 45:17-27.
- Zemann B, Kinzel B, Muller M, Reuschel R, Mechtcheriakova D, Urtz N, Bornancin F, Baumruker T and Billich A. 2006. Sphingosine kinase type 2 is essential for lymphopenia induced by the immunomodulatory drug FTY720. *Blood* 107:1454-1458.
- Zhang YH, Vasko MR and Nicol GD. 2006. Intracellular sphingosine 1-phosphate mediates the increased excitability produced by nerve growth factor in rat sensory neurons. *J Physiol* 575:101-113.
- Zhang Z, Fauser U, Artelt M, Burnet M and Schluesener HJ. 2007. FTY720 attenuates accumulation of EMAP-II+ and MHC-II+ monocytes in early lesions of rat traumatic brain injury. *J Cell Mol Med* 11:307-314.
- Zhu D, Wang Y, Singh I, Bell RD, Deane R, Zhong Z, Sagare A, Winkler EA and Zlokovic BV. 2010. Protein S controls hypoxic/ischemic blood-brain barrier disruption through the TAM receptor Tyro3 and sphingosine 1-phosphate receptor. *Blood* 115:4963-4972.

Editor: Michael M. Cox