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REVIEW

FTY720 (fingolimod) in Multiple Sclerosis: therapeutic effects in the immune and the central nervous system

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FTY720 (fingolimod) is a first-in-class sphingosine 1-phosphate (S1P) receptor modulator that was highly effective in Phase II clinical trials for Multiple Sclerosis (MS). FTY720 is phosphorylated *in vivo* by sphingosine kinase-2 to form the active moiety FTY720-phosphate that binds to four of the five G protein-coupled S1P receptor subtypes. Studies using conditional S1P1 receptor-deficient and sphingosine kinase-deficient mice showed that the egress of lymphocytes from lymph nodes requires signalling of lymphocytic S1P1 receptors by the endogenous ligand S1P. The S1P mimetic FTY720-phosphate causes internalization and degradation of cell membrane-expressed S1P1, thereby antagonizing S1P action at the receptor. In models of human MS and demyelinating polyneuropathies, functional antagonism of lymphocytic S1P1 slows S1P-driven egress of lymphocytes from lymph nodes, thereby reducing the numbers of autoaggressive TH17 cells that recirculate via lymph and blood to the central nervous system and the sciatic/ischiatic nerves. Based on its lipophilic nature, FTY720 crosses the blood–brain barrier, and ongoing experiments suggest that the drug also down-modulates S1P1 in neural cells/astrocytes to reduce astrogliosis, a phenomenon associated with neurodegeneration in MS. This may help restore gap-junctional communication of astrocytes with neurons and cells of the blood–brain barrier. Additional effects may result from (down-) modulation of S1P3 in astrocytes and of S1P1 and S1P5 in oligodendrocytes. In conclusion, FTY720 may act through immune-based and central mechanisms to reduce inflammation and support structural restoration of the central nervous system parenchyma. Beyond the autoimmune indications, very recent studies suggest that short-term, low-dose administration of FTY720 could help treat chronic (viral) infections. Differential effects of the drug on the trafficking of naïve, central memory and effector memory T cell subsets are discussed.
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Abbreviations: BBB, blood–brain barrier; CNS, central nervous system; DC, dendritic cell; EAE, experimental autoimmune encephalomyelitis; EAN, experimental autoimmune neuritis; FTY720-P, FTY720-phosphate; LCMV, lymphocytic choriomeningitis virus; Lm, Listeria monocytogenes; LN, lymph node; MS, Multiple Sclerosis; OGC, oligodendrocyte; S1P, sphingosine 1-phosphate; SphK, sphingosine kinase

Introduction

Since 2002, a large number of studies reported efficacy of FTY720 in models of autoimmune diseases, particularly experimental autoimmune encephalomyelitis (EAE), a model of human Multiple Sclerosis (MS) (Brinkmann *et al.*, 2002; Fujino *et al.*, 2003; Webb *et al.*, 2004; Kataoka *et al.*, 2005; Balatoni *et al.*, 2007; Brinkmann, 2007; Foster *et al.*, 2009). More recent data showed that the drug was also highly effective in rat experimental autoimmune neuritis (EAN), a model of human demyelinating polyneuropathies (Zhang *et al.*, 2009). Meanwhile, clinical Phase II studies suggest that the drug may provide an effective treatment for human relapsing remitting MS (Kappos *et al.*, 2006; O'Connor *et al.*, 2009).

In vivo, FTY720 is phosphorylated by sphingosine kinase (SphK)-2 (Brinkmann *et al.*, 2002; Zemann *et al.*, 2006) to yield the biologically active (*S*)-configured FTY720-phosphate (FTY720-P) (Albert *et al.*, 2005). FTY720-P represents a close structural analogue of sphingosine 1-phosphate (S1P), a sphingolipid mediator that regulates multiple biological processes through binding to five G protein-coupled S1P

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The drug/molecular target nomenclature conforms to BJP's Guide to Receptors and Channels (Alexander *et al.*, 2008).
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receptors (Chun *et al.*, 2002; Hla, 2004; Brinkmann, 2007). The biological activity of FTY720 and its phosphates was first assessed in S1P receptor assays, which detect agonist-induced GTP[γ-^{35}S] binding as functional readout of receptor activation. It was found that the (*S*)-configured FTY720-P [but not the (*R*)-FTY720-P or parent FTY720] acts as a full agonist at S1P1 (0.3 nM), S1P4 (0.6 nM) and S1P5 (0.3 nM) and with approximately 10-fold lower potency at S1P3 (3.1 nM), but has no activity at S1P2 (>10 000 nM) (Brinkmann *et al.*, 2002; Mandala *et al.*, 2002; Albert *et al.*, 2005). Comparable low-nM affinities to the receptors were reported using competitive receptor binding assays (Mandala *et al.*, 2002). The differing receptor affinities and potencies between FTY720-P and S1P suggest the possibility of inducing distinct responses in target cells *in vivo*, either via agonistic signalling or via functional antagonism/internalization of S1P receptors.

As described below, conditional deletion of S1P1 receptors from lymphocytes (Matloubian *et al.*, 2004) or neural cells/astrocytes (Choi *et al.*, 2008) mimicked the effects of FTY720 *in vivo*. This suggests that the drug acts as a functional antagonist of S1P1 to interrupt the pro-inflammatory S1P-S1P1 axis, modulating MS pathology on immune and central levels.

Sphingosine 1-phosphate

S1P is generated from intracellular sphingosine, a break-down product of the cell membrane constituent sphingomyelin, and all cells are thought to be able to generate S1P in the process of normal sphingolipid turnover (Hla, 2004). During this process, sphingomyelin is degraded via ceramide to sphingosine, which is then phosphorylated by SphK1 and SphK2 to yield S1P. Platelets had long been considered to be the major source of plasma S1P; however, recent studies revealed the importance of erythrocytes as a major supply (Pappu *et al.*, 2007). Although both erythrocytes and platelets can produce S1P, only platelets synthesize and release FTY720-P (Kihara and Igarashi, 2008). The release of S1P from cells may involve the ATP-binding cassette family of transporters that catalyse the transport of lipids from the inner to the outer leaflet of the plasma membrane (Van Meer and Lisman, 2002), and FTY720/FTY720-P may also use this transport system (Honig *et al.*, 2003).

During embryogenesis, S1P is critically involved in the development of the cardiovascular (Allende *et al.*, 2003) and the central nervous system (CNS) (Mizugishi *et al.*, 2005). In the adult, plasma S1P is tightly associated with albumin and lipoproteins, particularly high-density lipoprotein (Okajima, 2002), and a significant concentration gradient of S1P exists between plasma and interstitial fluids. Tissue levels of S1P are generally low, whereas plasma S1P levels are high and many fold above the dissociation constant K_d for S1P receptors (Toman and Spiegel, 2002; Schwab and Cyster, 2007; Kim *et al.*, 2009). Excessive production of S1P can occur at inflammatory sites as a result of cell activation by pro-inflammatory stimuli, including interleukin-1, tumour necrosis factor and vascular endothelial growth factor (Alvarez *et al.*, 2007; Brinkmann, 2007), leading to activation of S1P receptors. The different S1P levels in tissues/body fluids at steady state, as well as its local production at inflammatory sites, appears to be crucial to the maintenance of directed immune cell trafficking (Matloubian *et al.*, 2004), the maintenance of the vascular tone and endothelial barriers (McVerry and Garcia, 2004) and the gap-junctional communication of neural cells in the CNS (Rouach *et al.*, 2006; Brinkmann, 2007).

S1P receptors

S1P binds to five related G protein-coupled receptors (GPCRs), termed S1P1–5 (formerly Edg-1, -5, -3, -6 and -8 respectively) (Chun *et al.*, 2002; Hla, 2004). S1P1, S1P2 and S1P3 receptors are widely expressed in the immune, cardiovascular and central nervous systems, with S1P1 being the dominant receptor also on lymphocytes/leukocytes (Chae *et al.*, 2004). S1P4 is specifically expressed in lymphoid tissue (Graeler and Goetzl, 2002), and S1P5 is present in spleen and white matter tracts of the CNS [primarily on oligodendrocytes (OGC)] (Jaillard *et al.*, 2005). To date, S1P receptor expression in tissues has primarily been studied at the mRNA level, due to a lack of specific monoclonal antibodies. The reported mRNA levels may not always match receptor protein expression, and they do not unravel the distribution in intracellular compartments versus the cell membrane, the latter being critical for S1P-dependent cell migration (Matloubian *et al.*, 2004; Pappu *et al.*, 2007). Furthermore, S1P receptor expression patterns significantly change with the activation status of cells (Matloubian *et al.*, 2004; Miron *et al.*, 2008), and receptor expression in cell membranes is modulated by S1P levels present in body fluids/tissues (Shiow *et al.*, 2006). Finally, S1P receptors cross-talk to various growth factor receptors that are also regulated by cell activation processes and modulated by their specific ligands (Igarashi *et al.*, 2003; Pyne *et al.*, 2003; Alvarez *et al.*, 2007). It is therefore difficult to model S1P/S1P receptor homeostasis and modulation that occurs *in vivo* by using *in vitro* cultures of serum/S1P-starved cells. Thus, the mechanistic data discussed below refer primarily to *in vivo* analysis of S1P receptor-deficient mice or reverse pharmacology approaches using *in vivo* treatment with S1P or functional antagonists of S1P receptors.

Regulation of egress from lymph nodes by lymphocytic S1P1

Lymphocyte egress from lymphoid organs must be controlled during a normal immune response. Within the first hours after an antigenic stimulus (i.e. an infection), exit from the draining lymph nodes (LNs) is blocked to increase the number of antigen-specific T cells in the node, a phenomenon known since 50 years as 'shutdown' (Schwab and Cyster, 2007). Only recently it was found that 'shutdown' is associated with a striking down-modulation of S1P1 mRNA expression in LN T cells (Matloubian *et al.*, 2004). A key role of S1P and S1P1 receptors in LN egress was finally demonstrated by using foetal liver chimeric mice with specific deletion of the receptor from haematopoietic cells (Matloubian *et al.*, 2004). In such mice, S1P1-deficient thymocytes and T cells were unable to egress from thymus and LNs respectively. Further, elimination of the receptor ligand S1P (via genetic deletion of

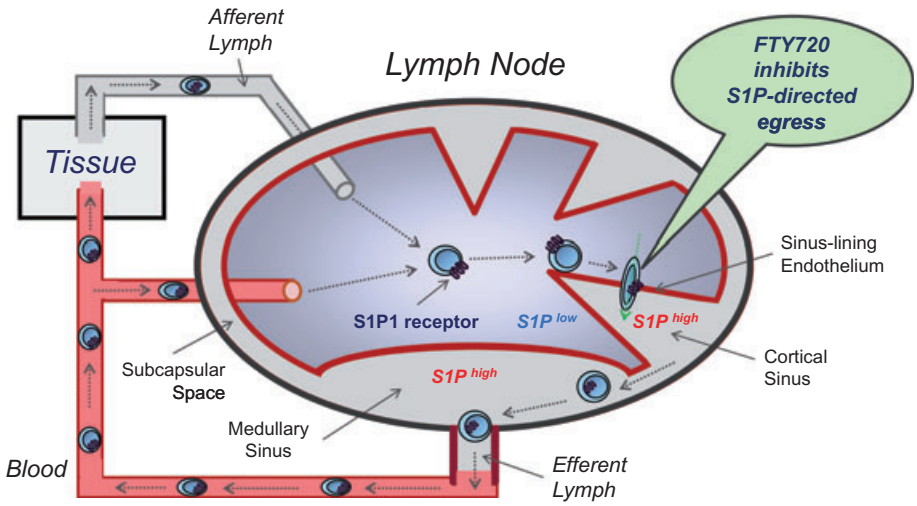

Figure 1 FTY720 inhibits the egress of lymphoytes from lymph nodes. T cells recirculate between blood and lymph nodes (LNs) in search for foreign antigen, entering LNs either from blood via high endothelial venules or from tissues via afferent lymphatics. Once in the LNs, the cells up-regulate sphingosine 1-phosphate (S1P)1 on cell membranes as a consequence of the $S1P^{low}$ environment. To egress from LNs, T cells transmigrate through the sinus-lining endothelium into the $S1P^{high}$ cortical sinus. Imaging studies showed that this process depends on lymphocytic S1P1, as only wild type but not S1P1-deficient T cells transmigrate through the endothelium. In the cortical sinuses, T cells are captured in a region of flow, passaged to medullary sinuses and flushed into the subcapsular space and the efferent lymph. Treatment with FTY720 causes internalization of lymphocytic S1P1 on T cells, thereby inhibiting the egress from LNs.

SphKs) also blunted lymphocyte egress, suggesting that signalling of S1P at lymphocytic S1P1 receptors was promoting egress. Accordingly, restoration of S1P to plasma of SphK-deficient mice rescued egress of wild type but not S1P1-deficient lymphocytes (Pappu *et al.*, 2007). Visualization of the branched organization of LN cortical sinuses showed that S1P1-deficient T cells probed the (LYVE-1+) lymphatic vascular endothelial surface of cortical sinuses but failed to enter. In contrast, wild-type T cells probed and entered the cortical sinuses, were then captured in a region of flow, passaged to medullary sinuses and were flushed into the subcapsular space and the efferent lymph (Grigorova *et al.*, 2009) (Figure 1).

Treatment of wild-type mice with FTY720 mimicked the effects of S1P1 deletion from haematopoietic cells, strongly suggesting that the drug acts as a functional antagonist of S1P1 receptors to prevent egress (Matloubian *et al.*, 2004). Accordingly, FTY720 treatment caused an internalization of membrane-expressed S1P1 in LN T cells *in vivo* (Pham *et al.*, 2008), and the drug could promote ubiquitinylation and proteasomal degradation of the receptor in endothelial cells *in vitro* (Oo *et al.*, 2007). Western blot analysis further revealed that treatment of mice with FTY720 dose-dependently reduced S1P1 receptor protein in cytosolic and membrane fractions of organ tissues (unpublished), suggesting that receptor degradation also occurs *in vivo*. It is likely that the 10-fold higher concentrations of FTY720 and its phosphate in LNs compared with blood (Sensken *et al.*, 2008) provide some tissue specificity of S1P1 modulation.

All together, the data propose an obligatory role of lymphocytic S1P1 receptors in the egress from lymphoid organs, and suggest that FTY720 down-modulates lymphocytic S1P1 to slow S1P-S1P1-dependent egress into cortical sinuses of LNs. As a consequence, autoaggressive T cells remain in the antigen-draining LNs, and this reduces their recirculation via blood and lymph to the CNS and abrogates EAE/MS relapses and central inflammation (Fujino *et al.*, 2003). Importantly, FTY720 neither affected the functionality of T cells (Pinschewer *et al.*, 2000; Brinkmann *et al.*, 2001; Kursar *et al.*, 2008) nor the motility of CNS-resident T cells (Bartholomäus *et al.*, 2008), and this could be important for the maintenance of central immunesurveillance under FTY720 therapy.

Experiments using green-fluorescent protein-transgenic bone marrow chimeric mice showed that the development of single positive thymocytes in the thymus was normal. Furthermore, mature T cells developed and recirculated to blood and lymphoid organs. Similar maximal levels of T cell chimerizm were achieved in FTY720-treated and untreated mice, but the egress of T cells from thymus and the repopulation of lymphoid organs was delayed by FTY720 (Metzler *et al.*, 2008). These data suggest that in FTY720-treated animals, the trafficking of T cell precursors from bone marrow to the thymus is grossly normal, and that a repopulation of lymphoid and peripheral organs with T cells occurs, however, with delayed kinetics.

Does endothelial S1P1 contribute to inhibition of T cell egress?

Initial reports suggested that S1P receptor drugs like FTY720 may act as pure agonists (rather than functional antagonists) and activate endothelial S1P1 to increase barrier function in LNs and prevent egress (Mandala *et al.*, 2002; Sanchez *et al.*, 2003). This concept was further supported by: (i) short-term *in vitro* studies with serum/S1P-deprived cells that show activation if re-exposed to S1P receptor ligands (Mullershausen

et al., 2009); (ii) T cell motility studies in explanted LNs (Wei *et al.*, 2005); and (iii) the finding that novel direct competitive S1P1 antagonists did not block lymphocyte egress *in vivo* (Sanna *et al.*, 2006). However, in contrast to this concept, administration of the natural S1P receptor agonist S1P promoted, rather than inhibited, egress in SphK-deficient mice (Pappu *et al.*, 2007). Furthermore, studies with explanted LNs may not appreciate S1P gradients and physiological flow that exist between LNs and efferent lymph *in vivo*, and the ineffective S1P1 antagonists may not be present at sufficiently high concentrations in LNs to mask the endogenous S1P gradient. Accordingly, mice with as little as 3–5% of plasma S1P concentrations of wild-type mice showed normal egress and, thus, synthetic antagonists would have to be exceedingly efficient (Pappu *et al.*, 2007). Indeed, in contrast to the ineffective S1P1 antagonists, S1P1-blocking antibodies with high effective avidity for the receptor were able to inhibit egress, suppressed T cell chemotaxis to S1P *in vivo* and reduced the severity of colitis (Liao *et al.*, 2009). These considerations further support the concept that natural S1P acts as an agonist at lymphocytic S1P1 receptors to promote egress, and that FTY720 slows egress by down-modulating/internalizing lymphocytic S1P1 (Brinkmann *et al.*, 2004; Matloubian *et al.*, 2004).

Internalization of GPCRs occurs via endocytosis after ligand binding and provides a general mechanism to terminate receptor signalling and regulate receptor degradation and recycling (Marchese *et al.*, 2008). Specific sorting signals, including intracytosolic domains of GPCRs and endocytic adaptor proteins, regulate trafficking of the internalized receptor-ligand complexes through the intracellular endosomal-lysosomal system. The internalized receptor-ligand complexes may continue to signal from endosomes for several hours (Mullershausen *et al.*, 2009), until the ligand is finally shed and the receptor either recycled to the cell membrane or degraded (Marchese *et al.*, 2008). In case of S1P binding to S1P1, internalized receptors are rapidly recycled, whereas S1P1-FTY720-P complexes are sorted from endosomes to lysosomes and fed into the proteasomal degradation pathway (Oo *et al.*, 2007).

Differential trapping of naive and memory T cell subsets

FTY720 differentially affects the recirculation of naive and memory T cells subsets between LNs and the blood (Figure 2). In MS patients, FTY720 primarily reduced the numbers of CCR7$^+$ CD45RA$^+$ naive T cells and of CCR7$^+$ CD45RA$^-$ central memory T cells in blood, presumably because these cells express the homing receptor CCR7, recirculate through LNs on a regular basis and, thus, can be trapped by FTY720 (Mehling *et al.*, 2008a). In contrast, CCR7$^-$ CD45RA$^-$ and CCR7$^-$ CD45RA$^+$ effector memory T cell subsets remained in blood. Upon restimulation *in vitro*, these blood T cells displayed a reduced potential to secrete IL-2 and to proliferate but rapidly produced IFNγ. FTY720 did not directly affect proliferation and cytokine expression (Brinkmann *et al.*, 2001; Mehling *et al.*, 2008a), suggesting that the altered IL-2/IFNγ ratios in the remaining blood T cells reflected a functional effector memory phenotype. Earlier reports had already shown that antigen-specific, long-lived memory T cells preferentially home to non-lymphoid tissue where they can proliferate in response to IL-7 and IL-15 (Sallusto *et al.*, 1999; Masopust *et al.*, 2001; Tan *et al.*, 2002), and that CD8 cells isolated from such tissues exhibit direct cytolytic activity *ex vivo* (Masopust *et al.*, 2001; Sallusto *et al.*, 2004). Thus, effector memory T cells constitute a reservoir of tissue-resident cells

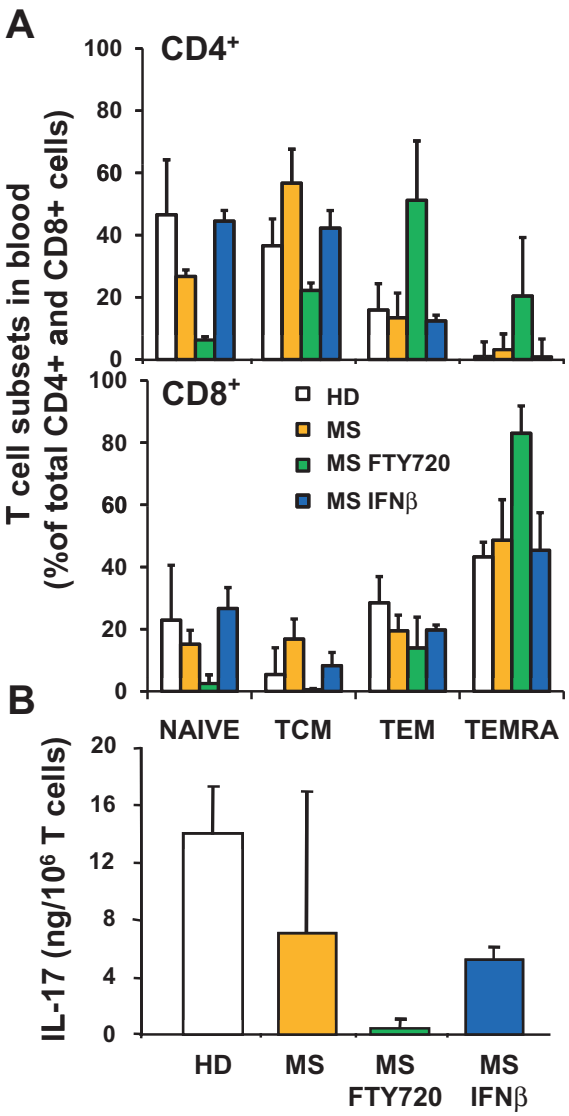

Figure 2 Redistribution of naive, memory and TH17 T cell subsets in FTY720-treated Multiple Sclerosis (MS) patients. (A) The percentage of naive and memory T cell subsets within blood CD4$^+$ and CD8$^+$ populations was determined by flow cytometry. The characterized subsets are CCR7$^+$ CD45RA$^+$ naive T cells (naive), CCR7$^+$ CD45RA$^-$ central memory T cells (TCM), CCR7$^-$ CD45RA$^-$ effector memory T cells (TEM) and CCR7$^-$ CD45RA$^+$ effector memory T cells (TEMRA). The subsets were determined in healthy controls (HD, $n = 7$), drug-untreated MS patients (MS, $n = 3$), IFNβ-treated MS patients (MS-IFNβ, $n = 3$) and FTY720-treated MS patients (MS-FTY720, $n = 5$). (B) The production of IL-17 by equal numbers of anti-CD3/anti-CD28-stimulated CD3$^+$ T cells isolated from indicated donor populations was determined by ELISA (HD, $n = 5$; MS, $n = 4$; MS FTY720, $n = 6$; MS-IFNβ, $n = 6$). Data represent means ± SD.

whose function is likely not to be affected by FTY720. In immune-experienced humans, FTY720 preferentially traps CD4 T cells in LNs since they contain predominantly CCR7$^+$ naive and central memory T cells, whereas the blood CD8 T cell pool consists primarily of CCR7$^-$ effector memory T cells that are not trapped in LNs (Figure 2).

Trapping of TH17 cells

There is now evidence that the T cells trapped in LNs by FTY720 contain the pro-inflammatory CD4$^+$ TH17 subset (Mehling *et al.*, 2008b). Th17 cells produce IL-17 and IL-22, thereby inducing a massive tissue reaction owing to the broad distribution of the IL-17 and IL-22 receptors. TH17 cells transmigrate efficiently across the blood–brain barrier (BBB) and disrupt tight junctions, highly express granzyme B and kill human neurons, and promote CNS inflammation through additional CD4 T cell recruitment (Kebir *et al.*, 2007). Accordingly, large numbers of TH17 cells are found in brain tissues from MS patients, particularly at the borders of acute and chronic active lesions (Tzartos *et al.*, 2008). In FTY720-treated MS patients, the numbers of circulating CD4$^+$ TH17-like cells (which produced large amounts of IL-17 upon restimulation) were reduced by >95%, and this related to the preferential trapping of CD4$^+$ (compared with CD8$^+$) T cells in LNs. In line with the human data, FTY720 attenuated lesional TH17 cell accumulation in rat EAN, a model of human demyelinating polyneuropathies (Zhang *et al.*, 2009). In sciatic nerves of EAN rats, TH17 cells were found around blood vessels and correlated with severity of neurological signs. FTY720 strikingly reduced TH17 cells in sciatic nerves and this correlated to trapping of these cells in LNs. The data are consistent with the possibility that TH17 cells contribute to the pathogenesis of MS and polyneuropathies, and that FTY720 prevents trafficking of such cells to the CNS and the sciatic nerves (Figure 3).

S1P receptors and dendritic cells

Dendritic cells (DCs) represent the major antigen-presenting cell population that is crucial to the induction of primary immune responses. Depending on their maturation stage and tissue origin, DCs can express mRNA for all S1P receptors (Lan *et al.*, 2005; Idzko *et al.*, 2006), and skin DCs showed activation of S1P1 and S1P3 genes after stimulation by antigen and migration into the LNs (Czeloth *et al.*, 2005). Treatment of mice with FTY720 or the S1P1-selective agent SEW2871 slightly increased the numbers of CD11c$^+$ DCs in blood, while decreasing blood lymphocyte counts (Lan *et al.*, 2005). Later

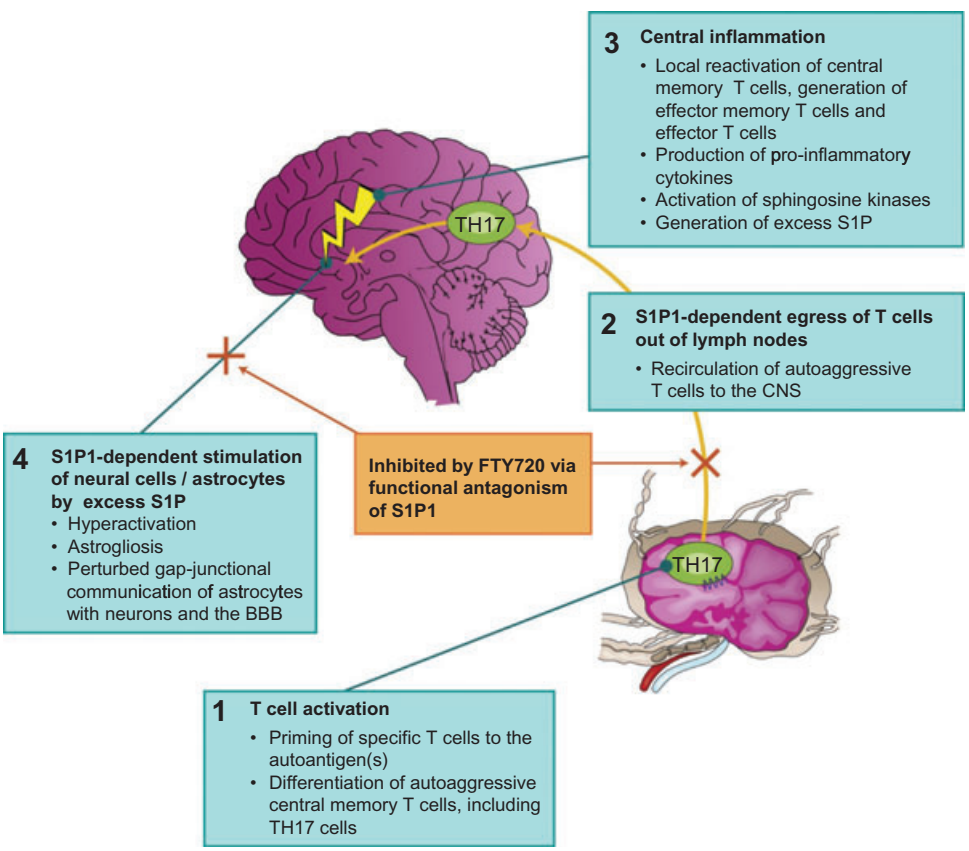

Figure 3 Proposed mode of action of FTY720 in Multiple Sclerosis. The model is based on experiments using conditional sphingosine 1-phosphate (S1P)1 receptor-deficient and sphingosine kinase-deficient mice, and *in vivo* analysis of FTY720 and S1P. The data suggest that FTY720 functionally antagonizes S1P-S1P1-dependent immune and central processes. This reduces: (i) the egress of TH17 cells from lymphoid organs and their recirculation to the central nervous system (CNS); and (ii) the astrogliosis associated with Multiple Sclerosis, to restore gap-junctional communication between CNS cells. It remains to be determined whether (down-) modulation of S1P3 in astrocytes and of S1P1 and/or S1P5 in oligodendrocytes further contributes to the therapeutic effects.

studies proposed that the anti-inflammatory effects of FTY720 in models of asthma (Idzko *et al.*, 2006), skin transplantation (Lan *et al.*, 2008) and allergic contact dermatitis (Reines *et al.*, 2009) may relate, at least in part, to a reduction of DC numbers in the antigen-draining LNs. However, the FTY720-induced redistribution of lymphocytes from blood to LNs may have caused relative rather than absolute changes in DC counts in the analysed tissues, and the anti-inflammatory effect of the drug could relate solely to T cell trapping in LNs. Indeed, general effects of FTY720 on DC function *in vivo* are unlikely, given the unimpaired antigen-specific T and B cell activation and proliferation observed in models of vaccination and viral/bacterial infections (Pinschewer *et al.*, 2000; Xie *et al.*, 2003; Kursar *et al.*, 2008; Marsolais *et al.*, 2009). Conditional deletion of S1P receptors in macrophage/DC lineages may be needed to determine in more detail the immunological consequences of S1P receptor modulation in DCs.

S1P1 receptor modulation and infection

Effects of FTY720 on anti-viral and anti-bacterial immune responses were studied in models of lymphocytic choriomeningitis virus (LCMV), vesicular stomatitis virus (Pinschewer *et al.*, 2000) and Listeria monocytogenes (Lm) infection (Kursar *et al.*, 2008). Administration of FTY720 at EAE-therapeutic doses of 0.3 $mg \cdot kg \cdot day^{-1}$ did not impair the generation of specific cytotoxic CD8 T cells in either model, and did not affect the induction of anti-viral humoral immunity that requires functional DCs, $CD4^+$ T cells and B cells (Pinschewer *et al.*, 2000). However, the drug reduced the numbers of antigen-specific naive T cells being recruited from other sites/LNs to the antigen-draining LNs (Xie *et al.*, 2003), and slowed the egress kinetics of antigen-activated T cells from the LNs (Pinschewer *et al.*, 2000; Xie *et al.*, 2003; Kursar *et al.*, 2008). Together these effects may be relevant particularly to primary infections where immune responses develop in the absence of preformed memory T cells present in non-lymphoid tissues and bone marrow.

Interestingly, FTY720 treatment did not affect Lm-specific memory responses in wild-type mice and in lymphotoxin-β receptor-deficient mice that lack functional LNs, and the drug only marginally changed the frequencies and numbers of Lm-specific T cells in LNs, spleen and liver, allowing clearance of the bacteria (Kursar *et al.*, 2008). However, a recall response was profoundly inhibited if intravenously infected mice were splenectomized subsequently to recovery from a primary infection, suggesting a key role of the spleen in generating the observed recall response. It has been suggested that FTY720 may not trap T cells in the spleen because, unlike in LNs, lymphocytes do not egress from the spleen via lymphatics (Mandala *et al.*, 2002; Hofmann *et al.*, 2006). The spleen is specialized to present blood-borne antigens, whereas LNs filter lymph draining from skin or mucosal surfaces, and Peyer's patches obtain antigen by transepithelial transport from the intestinal lumen (Schwab and Cyster, 2007). Taken together, potential effects of S1P receptor drugs on anti-infective immunity may depend on the invasion route of the infectious agent and the numbers of preformed memory T cells in the infected non-lymphoid tissues and the bone marrow.

Notably, FTY720 completely cleared a severe chronic LCMV infection if administered only transiently for 3 days and at very low dosing of 0.004 $mg \cdot kg^{-1}$ (Premenko-Lanier *et al.*, 2008). Under these conditions, FTY720 treatment augmented LCMV-specific CD4 and CD8 T cell responses, particularly in LNs that are also a significant site of LCMV virus production and pathology. It has been shown earlier that optimal activation of LN T cells by antigen (in the absence of S1P receptor drugs) required T cell receptor-induced down-modulation of S1P1 to delay T cell egress (Matloubian *et al.*, 2004). In situations of chronic infection, suboptimal down-modulation of S1P1 may occur (as a result of low-antigen exposure), and this could be compensated by FTY720. After withdrawal of the drug, the fully activated T cells could now recirculate and fight the virus in the periphery. The demonstration of anti-viral activity of FTY720 is so far limited to the single example of the murine LCMV model, and it remains to be determined whether the effect could translate to the successful treatment of other chronic infections, including those that afflict humans.

A more recent study evaluated effects of a transient, low-dose FTY720 regimen in simian human immunodeficiency virus ($SHIV_{SF162P3}$)-infected rhesus macaques (Kersh *et al.*, 2009). Under the applied conditions, FTY720 did not induce significant deviations from the natural pattern of viral control and did not change T cell activity throughout the drug course. It is possible that in this model, $CD8^+$ T cells were already controlling the infection to a maximal extent, without FTY720 administration, which could not be improved by the drug. More work is needed to reveal whether FTY720 could be beneficial in more pathogenic SHIV, simian immunodeficiency virus or HIV.

Down-modulation of S1P1 receptors on neural cells

Besides their role in the immune system, S1P receptors may also be critical to the regulation of neural cell migration/function, particularly during central inflammation in MS. In the CNS, S1P receptors are expressed in astrocytes (Sorensen *et al.*, 2003; Wu *et al.*, 2008), OGCs (Jaillard *et al.*, 2005), neurons (Kimura *et al.*, 2006) and microglia/macrophages (Kimura *et al.*, 2006). Furthermore, increased production of S1P has been detected locally in the spinal cord after contusion injury (Kimura *et al.*, 2006), and injection of natural S1P directly into the striata of mice produced astrogliosis (Sorensen *et al.*, 2003), a phenotype also seen in human MS. A recent study demonstrated a functional role of S1P synthesis and S1P receptor expression, particularly S1P3, in astrocyte proliferation leading to astrogliosis during the terminal stages of neurodegeneration in Sandhoff disease, a prototypical neuronopathic lysosomal storage disorder (Wu *et al.*, 2008). Because astrocyte responses are involved in many types of neurodegeneration, the SphK/S1P receptor signalling axis may be generally important during the pathogenesis of neurodegenerative diseases. Interestingly, an abstract reporting preliminary results using a conditional deletion of S1P1 supports a role for astrocytic S1P1 during the progression of EAE (Choi *et al.*, 2008). In this model, astrogliosis was a histopathological feature of EAE, but was attenuated by either conditional S1P1

deficiency in astrocytes or by FTY720 treatment. Taken together, the above data may indicate a role for both S1P1 and S1P3 in promoting astrogliosis, perhaps with differential involvement in early acute and later chronic disease.

FTY720 readily crosses the BBB (Meno-Tetang *et al.*, 2006), resulting in high pM concentrations of free FTY720-P in the cerebrospinal fluid (Foster *et al.*, 2007). At 100 pM concentration, the bioactive *S*-configured FTY720-P internalizes S1P1 and S1P3 receptors in transfected cell lines by 50–80% (unpublished). Thus, the drug may also down-modulate and functionally antagonize these receptors in astrocytes *in vivo* to modulate their functional properties, without changing astrocyte numbers, and this could reduce the reported negative effects of increased S1P levels and astrocytes on gap junctions between neural cells (Rouach *et al.*, 2006; Choi *et al.*, 2008). This may help restore effective communication of astrocytes with neurons and with endothelial cells in the BBB, as gap junction channels connect the cytoplasm of contacting cells and coordinate electric and metabolic activity (Rouach *et al.*, 2006) (Figure 3).

It remains to be determined whether FTY720 affects other cells of the CNS. In OGCs, FTY720 could produce process extension or retraction *in vitro*, depending on culture conditions, and induced reciprocal and cyclic modulation of S1P1 and S1P5 mRNA levels (Antel and Miron, 2008; Miron *et al.*, 2008). OGCs expressed particularly high levels of S1P5 mRNA; however, genetic deletion of S1P5 *in vivo* did not affect the myelination process (Jaillard *et al.*, 2005). *In vitro*, activation of S1P receptors and down-stream signalling pathways by FTY720 is often observed if cell cultures are performed with serum-starved cells in the absence of S1P (Chen *et al.*, 2001; Osinde *et al.*, 2007). In contrast, the drug down-modulates and functionally antagonizes the very same receptors *in vivo* to suppress for example S1P-S1P1-dependent egress of T cells from LNs (Matloubian *et al.*, 2004; Grigorova *et al.*, 2009). The data imply that conditional S1P receptor knock-out approaches in specific CNS cell lineages *in vivo* are needed to unravel the role of individual S1P receptor subtypes in the pathophysiology of EAE/MS.

In neurons, transactivation of distinct S1P receptors by neurite growth factor *in vitro* modulated neuronal development in a reciprocal manner, whereby S1P1 acted in opposition to S1P2 and S1P5 to coordinate neurite extension (Milstien *et al.*, 2007). Neurons express S1P1 (Chae *et al.*, 2004); however, preliminary data suggest that conditional deletion of S1P1 in neuronal cell lineages may not alter EAE scores and FTY720 efficacy (Choi *et al.*, 2008). In contrast, the sub-cellular distribution of S1P and SphKs in neurons may be critical to their survival (Maceyka *et al.*, 2005), as S1P induced neuronal apoptosis when accumulating above a certain threshold in the endoplasmatic reticulum (Hagen *et al.*, 2009). It remains to be determined whether FTY720 could directly affect neurodegeneration by modulating the intracellular SphK/S1P metabolism to alter S1P-sphingosine-ceramide rheostats.

Experimental autoimmune encephalomyelitis

FTY720 is highly effective as prophylactic and therapeutic treatment in EAE, the rodent model of human MS (Brinkmann *et al.*, 2002; Fujino *et al.*, 2003; Webb *et al.*, 2004; Chiba, 2005; Kataoka *et al.*, 2005; Brinkmann, 2007). As discussed above, the therapeutic effects of FTY720 in all autoimmune models may relate primarily to a reduced recruitment of autoaggressive T cells to the disease-relevant tissues and, in MS, to a direct reduction of astrogliosis.

Recent studies analysed in more detail the effects of FTY720 on inflammation, BBB leakiness, demyelination and nerve conductance in a rat model of EAE (Balatoni *et al.*, 2007; Foster *et al.*, 2009). Therapeutic treatment reversed central inflammation by blocking T cell infiltration, and this was associated with a down-regulation of inflammatory genes and vascular adhesion molecules. Drug treatment further decreased expression of matrix metalloproteinase gene MMP-9 and increased its counter-regulator, the tissue inhibitor of metalloproteinases TIMP-1, resulting in a proteolytic balance that favours preservation of BBB integrity. Accordingly, there was no evidence for immunoglobulin precipitation in the CNS of FTY720-treated animals. Most importantly, late-stage rescue therapy started up to 1 month after EAE onset also reversed inflammatory infiltrates and demyelination, and normalized disturbances to visual and somatosensory evoked action potentials. None of the FTY720-treated animals showed active demyelinating lesions, but they showed Schwann cell remyelinated areas in the spinal cord, whereas vehicle controls exhibited actively demyelinating and inactive demyelinated lesions. In conclusion, the data indicate a rapid blockade of ongoing disease by FTY720, and structural restoration of the CNS parenchyma, which is likely due to inhibition of autoimmune T cell infiltration and direct modulation of neural cells/astrocytes.

Clinical trials

FTY720 was highly effective in Phase II clinical trials involving 255 patients with relapsing remitting MS (Kappos *et al.*, 2006). The median total number of gadolinium-enhanced lesions on magnetic resonance imaging was lower with 1.25 mg of FTY720 (1 lesion, $P < 0.001$) and 5.0 mg (3 lesions, $P = 0.006$) than with placebo (5 lesions). The annualized relapse rate was 0.77 in the placebo group, as compared with 0.35 in the group given 1.25 mg of FTY720 ($P = 0.009$) and 0.36 in the group given 5.0 mg ($P = 0.01$). During the 2 year extension phase, patients who switched from placebo to FTY720 also showed clear reductions in annualized relapse rates and lesion counts compared with the placebo phase (O'Connor *et al.*, 2009).

FTY720 was generally well tolerated and the safety profile was in line with previous experience (Kappos *et al.*, 2006; O'Connor *et al.*, 2009). The transient reduction of heart rate observed in all FTY720 clinical trials may relate to a short, S1P1-dependent activation of the G protein-gated potassium channel IK_{Ach} in atrial myocytes, prior to internalization and/or desensitization of the S1P1 receptors by the drug (Mazurais *et al.*, 2002; Koyrakh *et al.*, 2005; Brinkmann, 2007). A reduction of heart rate comparable to FTY720 was also reported in a Phase I clinical trial exploring the novel S1P1-selective agonist ACT-128800 (http://www1.actelion.com/en/investors/events/actelion-day-2009.page). Earlier studies in mice suggested a preferential role for S1P3 in heart

rate regulation (Sanna *et al.*, 2004); however, species differences may exist. Indeed, S1P1 mRNA and protein are strongly expressed in human ventricular, septal and atrial cardiomyocytes, whereas S1P3 is only weakly expressed in cardiomyocytes from both atria and ventricles (Mazurais *et al.*, 2002). The mild increase in blood pressure observed in the FTY720 clinical trials may relate to a down-modulation of S1P1 in vascular endothelium (Oo *et al.*, 2007), which would reduce activation of the vasodilatory endothelial nitic oxide synthase pathway by endogenous S1P (Igarashi *et al.*, 2003; Brinkmann, 2007).

An abstract summarizing results from the 1 year Phase III TRANSFORMS study of FTY720 in relapsing remitting MS has recently been published, and the data suggest superior efficacy of oral FTY720 at 0.5 and 1.25 mg doses compared with a standard of care, the injectable interferon-β 1a (IFNβ) (Avonex®) (Cohen *et al.*, 2009 and Trial watch: Phase III promise for oral MS therapy. *Nat Rev Drug Discov* 2009; 8: 98–99). Two other ongoing studies – FREEDOMS and FREEDOMS II – are 2 year placebo-controlled Phase III studies to assess the impact of FTY720 in reducing the frequency of relapses and slowing the progression of disability. In depth analyses of all Phase III studies will soon provide a comprehensive assessment of FTY720's benefit–risk profile.

Conclusions

Current data suggest that FTY720 acts as a functional antagonist of S1P1 receptors. Down-modulation of S1P1 on lymphocytes slows egress kinetics of pro-inflammatory T(H17) cells from LNs, preventing their recirculation to the CNS to reduce central inflammation. Functional antagonism of S1P1 in astrocytes may directly reduce MS-associated astrogliosis to improve gap-junctional communication between cells and allow structural restoration of the CNS parenchyma. Recent clinical trials indicate that FTY720 could provide an effective treatment for human relapsing remitting MS. Further studies are needed to confirm whether short-term, low-dose FTY720 treatment regimens could translate into effective treatments for chronic infections, including those that afflict humans.

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Statement of conflict of interest

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References

Albert R, Hinterding K, Brinkmann V, Guerini D, Mueller-Hartwieg C, Knecht H *et al.* (2005). Novel immunomodulator FTY720 is phosphorylated in rats and humans to form a single stereoisomer. identification, chemical proof, and biological characterization of the biologically active species and its inactive enantiomer. *J Med Chem* **48**: 5373–77.

Alexander SP, Mathie A, Peters JA (2008). Guide to Receptors and Channels (GRAC). *Br J Pharmacol* **153** (Suppl. 2): S1–S209.

Allende ML, Yamashita T, Proia RL (2003). G-protein-coupled receptor S1P1 acts within endothelial cells to regulate vascular maturation. *Blood* **102**: 3665–3667.

Alvarez SE, Milstien S, Spiegel S (2007). Autocrine and paracrine roles of sphingosine-1-phosphate. *Trends Endocrinol Metab* **18**: 300–307.

Antel J, Miron V (2008). Central nervous system effects of current and emerging multiple sclerosis-directed immuno-therapies. *Clin Neurol Neurosurg* **110**: 951–957.

Balatoni B, Storch MK, Swoboda EM, Schönborn V, Koziel A, Lambrou GN *et al.* (2007). FTY720 sustains and restores neuronal function in the DA rat model of MOG-induced experimental autoimmune encephalomyelitis. *Brain Res Bull* **74**: 307–316.

Bartholomäus I, Schläger C, Brinkmann V, Wekerle H, Flügel A (2008). Intravital 2-photon imaging of encephalitogenic effector cells during fingolimod (FTY720) treatment of experimental autoimmune encephalomyelitis. World Congress on Treatment and Research in Multiple Sclerosis (ECTRIMS), Montreal, Canada. Poster P7.

Brinkmann V (2007). Sphingosine 1-phosphate receptors in health and disease: mechanistic insights from gene deletion studies and reverse pharmacology. *Pharmacol Ther* **115**: 84–105.

Brinkmann V, Chen S, Feng L, Pinschewer D, Nikolova Z, 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 *et al.* (2002). The immune modulator FTY720 targets sphingosine 1-phosphate receptors. *J Biol Chem* **277**: 21453–21457.

Brinkmann V, Cyster JG, Hla T (2004). FTY720: sphingosine 1-phosphate receptor-1 in the control of lymphocyte egress and endothelial barrier function. *Am J Transplant* **4**: 1019–1025.

Chae SS, Proia RL, Hla T (2004). Constitutive expression of the S1P1 receptor in adult tissues. *Prostaglandins Other Lipid Mediat* **73**: 141–150.

Chen S, Bacon KB, Garcia G, Liao R, Pan ZK, Sullivan SK *et al.* (2001). FTY720, a novel transplantation drug, modulates lymphocyte migratory responses to chemokines. *Transplant Proc* **33**: 3057–3063.

Chiba K (2005). FTY720, a new class of immunomodulator, inhibits lymphocyte egress from secondary lymphoid tissues and thymus by agonistic activity at sphingosine 1-phosphate receptors. *Pharmacol Ther* **108**: 308–319.

Choi JW, Herr D, Lee C-W, Teo S, Kennedy G, Chun J (2008). S1P1 receptor signalling on cells of astrocytic lineages in experimental autoimmune encephalomyelitis: a role in disease progression and the efficacy of fingolimod (FTY720). World Congress on Treatment and Research in Multiple Sclerosis (ECTRIMS) 2008, Montreal, Canada. Poster P29.

Chun J, Goetzl EJ, Hla T, Igarashi Y, Lynch KR, Moolenaar W *et al.* (2002). International union of pharmacology. XXXIV. Lysophospholipid receptor nomenclature. *Pharmacol Rev* **54**: 265–269.

Cohen J, Pelletier J, Kappos L, Montalban X, Hartung HP, Comi G *et al.* (2009). Oral fingolimod (FTY720) versus Interferon beta-1a in relapsing remitting Multiple Sclerosis: Results from a Phase III study (TRANSFORMS). 61st Annual Meeting of the American Academy of Neurology, Seattle, Washigton, U.S.A. [S21.004].

Czeloth N, Bernhardt G, Hofmann F, Genth H, Foerster R (2005). Sphingosine-1-phosphate mediates migration of mature dendritic cells. *J Immunol* **175**: 2960–2967.

Foster CA, Howard LM, Schweitzer A, Persohn E, Hiestand PC, Balatoni B *et al.* (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 *et al*. (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* **9**: 254–266.

Fujino M, Funeshima N, Kitazawa Y, Kimura H, Amemiya H, Suzuki S *et al*. (2003). Amelioration of experimental autoimmune encephalomyelitis in Lewis rats by FTY720 treatment. *J Pharmacol Exp Ther* **305**: 70–77.

Graeler M, Goetzl EJ (2002). Activation-regulated expression and chemotactic function of sphingosine 1-phosphate receptors in mouse splenic T cells. *FASEB J* **16**: 1874–1878.

Grigorova IL, Schwab SR, Phan TG, Pham TH, Okada T, Cyster JG (2009). Cortical sinus probing, S1P1-dependent entry and flow-based capture of egressing T cells. *Nat Immunol* **10**: 58–65.

Hagen N, Van Veldhoven PP, Proia RL, Park H, Merrill AH Jr, Van Echten-Deckert G (2009). Subcellular origin of sphingosine-1-phosphate is essental for its toxic effect in lyase deficient neurons. *J Biol Chem* **284**: 11346–11353.

Hla T (2004). Physiological and pathological actions of sphingosine 1-phosphate. *Semin Cell Dev Biol* **15**: 513–520.

Hofmann M, Brinkmann V, Zerwes HG (2006). FTY720 preferentially depletes naive T cells from peripheral and lymphoid organs. *Int Immunopharmacol* **6**: 1902–1910.

Honig SM, Fu S, Mao X, Yopp A, Gunn MD, Randolph GJ *et al*. (2003). FTY720 stimulates multidrug transporter- and cysteinyl leukotriene-dependent T cell chemotaxis to lymph nodes. *J Clin Invest* **111**: 627–637.

Idzko M, Hammad H, van Nimwegen M, Kool M, Müller T, Soullié T *et al*. (2006). Local application of FTY720 to the lung abrogates experimental asthma by altering dendritic cell function. *J Clin Invest* **116**: 2935–2944.

Igarashi J, Erwin PA, Dantas AP, Chen H, Michel T (2003). VEGF induces S1P1 receptors in endothelial cells: implications for cross-talk between sphingolipid and growth factors receptors. *Proc Natl Acad Sci USA* **100**: 10664–10669.

Jaillard C, Harrison S, Stankoff B, Aigrot MS, Calver AR, Duddy G *et al*. (2005). Edg8/S1P5: an oligodendroglial receptor with dual function on process retraction and cell survival. *J Neurosci* **25**: 1459–1469.

Kappos L, Antel J, Comi G, Montalban X, O'Connor P, Polman CH *et al*. (2006). Oral fingolimod (FTY720) for relapsing multiple sclerosis. *N Engl J Med* **355**: 1124–1140.

Kataoka H, Sugahara K, Shimano K, Teshima K, Koyama M, Fukunari A *et al*. (2005). FTY720, sphingosine 1-phosphate receptor modulator, ameliorates experimental autoimmune encephalomyelitis by inhibition of T cell infiltration. *Cell Mol Immunol* **2**: 439–448.

Kebir H, Kreymborg K, Ifergan I, Dodelet-Devillers A, Cayrol R, Bernard M *et al*. (2007). Human TH17 lymphocytes promote blood-brain barrier disruption and central nervous system inflammation. *Nat Med* **13**: 1173–1175.

Kersh EN, Luo W, Adams DR, Mitchell J, Garcia-Lerma JG, Butera S (2009). Evaluation of the lymphocyte trafficking drug FTY720 in $SHIV_{SF162P3}$-infected rhesus macaques. *J Antimicrob Chemother* **63**: 758–762.

Kihara A, Igarashi Y (2008). Production and release of sphingosine 1-phosphate and the phosphorylated form of the immunomodulator FTY720. *Biochim Biophys Acta* **1781**: 496–502.

Kim RH, Takabe K, Milstien S, Spiegel S (2009). Export and functions of sphingosine-1-phosphate. *Biochim Biophys Acta* **1791**: 692–696.

Kimura A, Ohmori T, Ohkawa R, Madoiwa S, Mimuro J, Murakami T *et al*. (2006). 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.

Koyrakh L, Roman MI, Brinkmann V, Wickman K (2005). The heart rate decrease caused by the sphingosine 1-phosphate receptor agonist FTY720 results from activation of the G protein-gated potassium channel IKACh. *Am J Transplant* **5**: 529–536.

Kursar M, Jänner N, Pfeffer K, Brinkmann V, Kaufmann SH, Mittrücker HW (2008). Requirement of secondary lymphoid tissues for the induction of primary and secondary T cell responses against Listeria monocytogenes. *Eur J Immunol* **38**: 127–138.

Lan YY, De Creus A, Colvin BL, Abe M, Brinkmann V, Coates PT *et al*. (2005). The sphingosine-1-phosphate receptor agonist FTY720 modulates dendritic cell trafficking in vivo. *Am J Transplant* **5**: 2649–2659.

Lan YY, Tokita D, Wang Z, Wang HC, Zhan J, Brinkmann V *et al*. (2008). Sphingosine 1-phosphate receptor agonism impairs skin dendritic cell migration and homing to secondary lymphoid tissue: association with prolonged allograft survival. *Transpl Immunol*. **20**: 88–94.

Liao JJ, Huang MC, Fast K, Gundling K, Yadav M, Van Brocklyn JR *et al*. (2009). Immunosuppressive human anti-lymphocyte autoantibodies specific for the type 1 sphingosine 1-phosphate receptor. *FASEB J* **23**: 1786–1796.

Maceyka M, Sankala H, Hait NC, Le Stunff H, Liu H, Toman R *et al*. (2005). SphK1 and SphK2, sphingosine kinase isoenzymes with opposing functions in sphingolipid metabolism. *J Biol Chem* **280**: 37118–37129.

McVerry BJ, Garcia JG (2004). *In vitro* and *in vivo* modulation of vascular barrier integrity by sphingosine 1-phosphate: mechanistic insights. *Cell Signal* **17**: 131–139.

Mandala S, Hajdu R, Bergstrom J, Quackenbush E, Xie J, Milligan J *et al*. (2002). Alteration of lymphocyte trafficking by sphingosine 1-phosphate receptor agonists. *Science* **296**: 346–349.

Marchese A, Paing MM, Temple BR, Trejo J (2008). G protein-coupled receptor sorting to endosomes and lysosomes. *Annu Rev Pharmacol Toxicol* **48**: 601–629.

Marsolais D, Hahm B, Walsh KB, Edelmann KH, McGavern D, Hatta Y *et al*. (2009). A critical role for the sphingosine analog AAL-R in dampening the cytokine response during influenza virus infection. *Proc Natl Acad Sci USA* **106**: 1560–1565.

Masopust D, Vezys V, Marzo AL, Lefrançois L (2001). Preferential localization of effector memory cells in nonlymphoid tissue. *Science* **291**: 2413–2417.

Matloubian M, Lo CG, Cinamon G, Lesneski MJ, Xu Y, Brinkmann V *et al*. (2004). Lymphocyte egress from thymus and peripheral lymphoid organs is dependent on S1P receptor 1. *Nature* **427**: 355–360.

Mazurais D, Robert P, Gout B, Berrebi-Bertrand I, Laville MP, Calmels T (2002). Cell type-specific localization of human cardiac S1P receptors. *J Histochem Cytochem* **50**: 661–670.

Mehling M, Brinkmann V, Antel J, Bar-Or A, Goebels N, Vedrine C *et al*. (2008a). FTY720 therapy exerts differential effects on T cell subsets in multiple sclerosis. *Neurology* **71**: 1261–1267.

Mehling M, Lindberg RL, Kuhle J, Vedrine C, Kappos L, Brinkmann V (2008b). Oral fingolimod (FTY720) treatment reduces peripheral IL-17-producing TH17 cells in patients with multiple sclerosis. ECTRIMS 2008, Montreal, Canada. Poster P697.

Meno-Tetang GM, Li H, Mis S, Pyszczynski N, Heining P, Lowe P *et al*. (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.

Metzler B, Gfeller P, Wieczorek G, Li J, Nuesslein-Hildesheim B, Brinkmann V (2008). Modulation of T cell homeostasis and alloreactivity under continuous FTY720 exposure. *Int Immunol* **20**: 633–644.

Milstien S, Gude D, Spiegel S (2007). Sphingosine 1-phosphate in neural signalling and function. *Acta Paediatr Suppl* **96**: 40–43.

Miron VE, Jung CG, Kim HJ, Kennedy TE, Soliven B, Antel JP (2008). FTY720 modulates human oligodendrocyte progenitor process extension and survival. *Ann Neurol* **63**: 61–71.

Mizugishi K, Yamashita T, Olivera A, Miller GF, Spiegel S, Proia RL (2005). Essential role for sphingosine kinases in neural and vascular development. *Mol Cell Biol* **25**: 11113–11121.

Mullershausen F, Zecri F, Cetin C, Billich A, Guerini D, Seuwen K (2009). Persistent signaling induced by FTY720-phosphate is mediated by internalized S1P1 receptors. *Nat Chem Biol* **6**: 377–378.

O'Connor P, Comi G, Montalban X, Antel J, Radue EW, de Vera A *et al* (2009). Oral fingolimod (FTY720) in multiple sclerosis: two-year results of a phase II extension study. *Neurology* **72**: 73–79.

Okajima S (2002). Plasma lipoproteins behave as carriers of extracellular sphingosine 1-phosphate: is this an atherogenic mediator or an anti-atherogenic mediator? *Biochim Biophys Acta* **1582**: 132–137.

Osinde M, Mullershausen F, Dev KK (2007). Phosphorylated FTY720 stimulates ERK phosphorylation in astrocytes via S1P receptors. *Neuropharmacology* **52**: 1210–1218.

Oo ML, Thangada S, Wu MT, Liu CH, Macdonald TL, Lynch KR *et al*. (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.

Pappu R, Schwab SR, Cornelissen I, Pereira JP, Regard JB, Xu Y *et al*. (2007). Promotion of lymphocyte egress into blood and lymph by distinct sources of sphingosine-1-phosphate. *Science* **316**: 295–298.

Pham TH, Okada T, Matloubian M, Lo CG, Cyster JG (2008). S1P1 receptor signaling overrides retention mediated by G alpha i-coupled receptors to promote T cell egress. *Immunity* **28**: 122–133.

Pyne NJ, Waters C, Moughal NA, Sambi BS, Pyne S (2003). Receptor tyrosine kinase-GPCR signal complexes. *Biochem Soc Trans* **31**: 1220–1225.

Pinschewer DD, Ochsenbein AF, Odermatt B, Brinkmann V, Hengartner H, Zinkernagel RM (2000). FTY720 immunosuppression impairs effector T-cell peripheral homing without affecting induction, expansion, and memory. *J Immunol* **164**: 5761–5770.

Premenko-Lanier M, Moseley NB, Pruett ST, Romagnoli PA, Altman JD (2008). FTY720 treatment promotes immune-mediated clearance of a chronic viral infection. *Nature* **454**: 894–898.

Reines I, Kietzmann M, Mischke R, Tschernig T, Lüth A, Kleuser B *et al*. (2009). Topical application of sphingosine-1-phosphate and FTY720 attenuate allergic contact dermatitis reaction through inhibition of dendritic cell migration. *J Invest Dermatol* **129**: 852–853.

Rouach N, Pébay A, Même W, Cordier J, Ezan P, Etienne E *et al*. (2006). S1P inhibits gap junctions in astrocytes: involvement of G and Rho GTPase/ROCK. *Eur J Neurosci* **23**: 1453–1464.

Sallusto F, Lenig D, Förster R, Lipp M, Lanzavecchia A (1999). Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature* **401**: 708–712.

Sallusto F, Geginat J, Lanzavecchia A (2004). Central memory and effector memory T cell subsets: function, generation, and maintenance. *Annu Rev Immunol* **22**: 745–763.

Sanchez T, Estrada-Hernandez T, Paik JH, Wu MT, Venkataraman K, Brinkmann V *et al*. (2003). Phosphorylation and action of the immunomodulator FTY720 inhibits vascular endothelial cell growth factor-induced vascular permeability. *J Biol Chem* **278**: 47281–47290.

Sanna MG, Liao J, Jo E, Alfonso C, Ahn MY, Peterson MS *et al*. (2004). Sphingosine 1-phosphate (S1P) receptor subtypes S1P1 and S1P3, respectively, regulate lymphocyte recirculation and heart rate. *J Biol Chem* **279**: 13839–13848.

Sanna MG, Wang SK, Gonzalez-Cabrera PJ, Don A, Marsolais D, Matheu MP *et al*. (2006). Enhancement of capillary leakage and restoration of lymphocyte egress by a chiral S1P1 antagonist in vivo. *Nat Chem Biol* **2**: 396–398.

Schwab SR, Cyster JG (2007). Finding a way out: lymphocyte egress from lymphoid organs. *Nat Immunol* **8**: 1295–1301.

Sensken SC, Bode C, Graler MH (2008). Accumulation of FTY720 in lymphoid tissues contributes to prolonged efficacy. *J Pharmacol Exp Ther* **328**: 963–969.

Shiow LR, Rosen DB, Brdicková N, Xu Y, An J, Lanier LL *et al*. (2006). CD69 acts downstream of interferon-alpha/beta to inhibit S1P1 and lymphocyte egress from lymphoid organs. *Nature* **440**: 540–544.

Sorensen SD, Nicole O, Peavy RD, Montoya LM, Lee CJ, Murphy TJ *et al*. (2003). Common signaling pathways link activation of murine PAR-1, LPA, and S1P receptors to proliferation of astrocytes. *Mol Pharmacol* **64**: 1199–1209.

Tan JT, Ernst B, Kieper WC, LeRoy E, Sprent J, Surh CD (2002). Interleukin (IL)-15 and IL-7 jointly regulate homeostatic proliferation of memory phenotype CD8+ cells but are not required for memory phenotype CD4+ cells. *J Exp Med* **195**: 49–52.

Toman RE, Spiegel S (2002). Lysophospholipid receptors in the nervous system. *Neurochem Res* **27**: 619–627.

Tzartos JS, Friese MA, Craner MJ, Palace J, Newcombe J, Esiri MM *et al*. (2008). Interleukin-17 production in central nervous system-infiltrating T cells and glial cells is associated with active disease in multiple sclerosis. *Am J Pathol* **172**: 146–155.

Van Meer G, Lisman Q (2002). Sphingolipid transport: rafts and translocators. *J Biol Chem* **277**: 25855–25858.

Webb M, Tham CS, Lin FF, Lariosa-Willingham K, Yu N, Hale J *et al*. (2004). Sphingosine 1-phosphate receptor agonists attenuate relapsing–remitting experimental autoimmune encephalitis in SJL mice. *J Neuroimmunology* **153**: 108–121.

Wei SH, Rosen H, Matheu MP, Sanna MG, Wang SK, Jo E *et al*. (2005). Sphingosine 1-phosphate type 1 receptor agonism inhibits transendothelial migration of medullary T cells to lymphatic sinuses. *Nat Immunol* **6**: 1228–1235.

Wu YP, Mizugishi K, Bektas M, Sandhoff R, 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, 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.

Zemann B, Kinzel B, Müller M, Reuschel R, Mechtcheriakova D, Urtz N *et al*. (2006). Sphingosine kinase type 2 is essential for lymphopenia induced by the immunomodulatory drug FTY720. *Blood* **107**: 1454–1458.

Zhang ZY, Zhang Z, Schluesener HJ (2009). FTY720 attenuates lesional interleukin-17 cell accumulation in rat experimental autoimmune neuritis. *Neuropathol Appl Neurobiol* [Epub ahead of print].