

June 2014

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Recommended Citation

Crawford, Madelyn Elizabeth (2014) "FTY720 (Fingolimod) Provides Insight into the Molecular Mechanisms of Multiple Sclerosis," *Pursuit - The Journal of Undergraduate Research at The University of Tennessee*: Vol. 5 : Iss. 1 , Article 7.

<https://doi.org/10.7290/pur5cnkv>

Available at: <https://trace.tennessee.edu/pursuit/vol5/iss1/7>

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FTY720 (Fingolimod) Provides Insight into the Molecular Mechanisms of Multiple Sclerosis

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Multiple sclerosis (MS) is a neurodegenerative disorder caused by a prolonged immune-mediated inflammatory response that targets myelin. Nearly all of the drugs approved for the treatment of MS are general immunosuppressants or only function in symptom management. The oral medication fingolimod, however, is reported to have direct therapeutic effects on cells of the central nervous system in addition to immunomodulatory functions. Fingolimod is known to interact with sphingosine-1-phosphate (S1P) receptors, and the most widely-accepted theory for its mechanism of action is functional antagonism of the receptor. This review examines significant neuromodulatory effects achieved by functional antagonism of the receptors S1P₁ and S1P₃ on astrocytes and speculates on the potential role of S1P receptors in the pathogenesis of MS.

Introduction

INTRODUCTION

Multiple Sclerosis (MS) is an immune-mediated inflammatory neurological disorder that affects approximately 1 in every 1000 people.¹ The disease is characterized by recurring damage to the myelin sheath that surrounds the axons of nerve cells.^{2,3} The myelin sheath is composed of layers of cellular membrane generated by specialized myelin-forming cells – Schwann cells in the peripheral nervous system (PNS) and oligodendrocytes in the central nervous system (CNS).² Multiple layers of this membrane envelope axons, forming a thick, protective covering.^{4,5} The myelin sheath serves as an insulator that increases the speed and efficiency of ion-mediated nerve impulses.⁵ When it is damaged by the inflammatory factors associated with MS, the ability of nerve cells to conduct impulses is impaired.²

MS exhibits a fluctuating pattern of disease severity in which remission periods with reduced symptoms alternate with periods of heightened disease activity.² During relapses, regions of active demyelination develop in the brain and central nervous system (CNS), forming the characteristic MS lesions.^{6,3,2,7} Over time, the duration and the severity of attacks increase, and the disease transitions from the relapsing-remitting (RR) stage to the progressive stage in which continual axonal damage occurs.^{2,7}

While the clinical manifestations of MS have been documented since the nineteenth century, the basic molecular pathology of the disease continues to be debated.^{6,4} Multiple sclerosis is a complex disorder that involves interactions among several different body systems and numerous molecular components. Additionally, it is a heterogeneous disease that appears to

have different pathological characteristics in distinct subsets of patients.^{6,3} While much research has been conducted on physiological elements that contribute to the MS disease mechanism, many details still remain unclear.

No cure has been developed for MS, but a variety of treatments have been approved by the FDA.^{2,3} The drug known as fingolimod (Gilenya), developed by Novartis and approved for the treatment of multiple sclerosis in September 2010, has been the subject of particularly intensive study.⁸ There is evidence that fingolimod's mechanism of action may include direct CNS neuromodulatory effects, presenting the possibility of a pharmacological MS research approach.^{9,8} Examining the molecular interactions and responses that contribute to this drug's function has the potential to provide valuable insight into the disease mechanisms associated with multiple sclerosis.

Molecular Pathology of MS

The prevailing theory states that multiple sclerosis results from an autoimmune reaction that causes perivascular inflammation and demyelination as well as other related forms of nerve damage.^{6,10} The inflammatory response begins when major histocompatibility complex class II (MHCII) molecules on the surface of macrophages and B-cells present a myelin antigen to CD4+ T-cells and render them myelin-reactive.^{11,6,3} The exact nature of the myelin antigen is unknown, but studies have found that immune cells can be triggered to produce antibodies targeting different component proteins of myelin including myelin basic protein, myelin proteolipid protein, and myelin/oligodendrocyte glycoprotein.^{12,10}

Myelin-reactive CD4+ T-cells differentiate into a pathogenic T-cell subtype that produces high levels of inflammatory cytokines and inhibits the functioning of regulatory T-cells.³ Perhaps because of this altered T-cell phenotype or as a result of an existing genetic mutation, MS is characterized by decreased function of regulatory T-cells of the type CD4+CD25+FoxP3+.^{3,13} These normally suppress T-cell immune reactions after an infection has been cleared, and they are also responsible for inhibiting auto-reactive T-cells that are not tolerant of "self" antigens.¹⁴ One study has found that MS patients have an abnormal version of the FoxP3 transcription factor that normally mediates these regulatory T-cell functions.¹³ It has been demonstrated that the addition of functional CD4+CD25+ T-cells can prevent the development of experimental autoimmune encephalomyelitis (EAE), a disease model used to study multiple sclerosis in laboratory animals, and that the removal of these regulatory T-cells is sufficient to cause certain other autoimmune disorders.^{13,14} Thus, the reduced function of these cells is a key factor in the pathology of MS, allowing for the proliferation of activated T-cells that lack immunological tolerance.

These reactive T-cells stimulate B-cells to produce myelin-specific antibodies. They recruit cytotoxic CD8+ T-cells and produce adhesion molecules to facilitate interaction with the cerebral endothelial cells (CECs) that are a key component of the blood-brain barrier (BBB).⁶

^{3,11} Activated T-cells, B cells, and macrophages then cross the BBB by integrin-mediated transendothelial migration.^{8,11} In the CNS, T-cells destroy the myelin layer, and invading macrophages as well as the resident immunoreactive cells in the CNS known as microglia release signaling molecules that activate Fas cell death signal receptors on oligodendrocytes.⁸ The signaling cascades induce lysis, further reducing the myelin sheath and triggering the release of additional myelin antigens.^{6,3} The continued presentation of these antigens on CECs, microglia, and astrocytes results in the persistent recruitment of inflammatory agents.¹¹ As the disease progresses, regions of demyelination accumulate around cerebral blood vessels, forming lesions or plaques.⁶ It has been suggested that the locations of these plaques in the CNS determine the symptoms that individual patients experience, but this correlation is not precise.³

^{15,6}

Immunoreactive cells in the CNS can also contribute to other aspects of MS-related neuropathy.³ Macrophages produce oxidative free radicals, proteases, and toxic compounds that cause tissue damage.³ Microglia and astrocytes proliferate at the sites of CNS damage, causing a condition known as gliosis. Reactive astrogliosis in particular is a common feature in the pathology of MS.^{17 10 16} While this biological response can have a beneficial function in preventing the spread of inflammatory agents into healthy CNS tissue, the astrogial scarring that results prevents axonal regeneration following nerve damage.¹⁶⁻¹⁷ Additionally, certain signaling pathways stimulate reactive astrocytes to produce pro-inflammatory cytokines, neurotoxic reactive oxygen species, and cytotoxic signaling molecules that aggravate the inflammatory reaction and contribute to the breakdown of the BBB.^{16 18}

As with other autoimmune disorders, the initial trigger that stimulates the immune system to react to a “self” antigen and cause MS has not been elucidated. Several causative factors for MS have been proposed, however, on the basis of trends in patient demographics. Research has confirmed a genetic component associated with multiple sclerosis, but genes are only 20-35% predictive of disease development.^{2 3} The genetic component most likely reflects a predisposition to exaggerated or inappropriate inflammatory response due to a polymorphism in the promoter region of an unidentified immune system reactivity gene.³ Additional genetic factors might pertain to an individual’s natural hormone levels, which can affect the abundance of pro- or anti-inflammatory molecules in the body.¹⁹

The molecular mimicry hypothesis suggests that T-cells may in fact be activated by an external antigen and then induced to attack myelin because its component proteins are chemically similar to the original antigen.³ A related hypothesis suggests that a viral infection is responsible for activating T-cells and directing them to attack myelin.^{2 3} One of the most convincing correlations between virus infection and MS is the incidence of the Epstein-Barr virus (EBV). The connection between EPV and MS has long been reported, and recent studies have demonstrated significant increases in the risk of MS after infection with EPV, and correspondingly a very low incidence of MS among individuals that have not contracted EPV.²⁰ Some component of the viral infection or the immune response to it seem to prime the body for later development of MS.

Environmental factors have also been implicated in the onset of multiple sclerosis.^{3 2} The disease exhibits a clear geographical distribution; it is most prevalent in individuals that spend the early years of their lives in the northernmost and southernmost regions of the world, and it is significantly less common in equatorial regions. One explanation for the geographical correlation involves the effect of sunlight on vitamin D levels in the human body. UV radiation is necessary for the endogenous synthesis of vitamin D, so in general, people living in regions of the world that receive more sunlight – equatorial regions – have higher vitamin D levels. Vitamin D inhibits the production of pro-inflammatory cytokines and increases the production of anti-inflammatory cytokines, contributing, perhaps, to reduced frequency of inflammation-triggered disorders such as MS.³

In recent years, a new causative theory of MS has evoked widespread interest and criticism. The theory originated with a controversial study that reported the presence of chronic cerebrospinal venous insufficiency (CCSVI) in a significant percentage of MS patients. This condition is characterized by a narrowing of the veins that drain blood from the brain and spinal cord, resulting in circulation problems and shunting of blood to other vessels.²¹ This was publicized as a causative factor of multiple sclerosis, and therapeutic surgeries to increase blood flow in the CNS were prescribed. The fact that increased perivascular iron levels have been observed in the CNS of a number of MS patients, an effect often associated with restricted circulation, has lent support to the CCSVI hypothesis.²² Subsequent research on the subject has produced variable findings, however, and the methodology employed in the original CCSVI study has been severely criticized. Recent meta-analysis of applicable studies on CCSVI suggests that it is more common in individuals with MS than in the general population, but that there is not a sufficient correlation to label it as a causative factor at this time.²³

Integration of these proposed causative factors suggests that the development of MS is likely the result of an external trigger acting on a genetically and physiologically predisposed individual.

Molecular Mechanisms of FTY720 (Fingolimod) Efficacy

Because there is no known cure for MS, the goals of pharmaceutical treatment are primarily to slow the progression of the disease and to improve patients' quality of life.² Consistent with this philosophy, nearly all of the drugs approved for the treatment of multiple sclerosis are general immunosuppressants, anti-inflammatory steroids, or drugs that function only in treating common symptoms such as fatigue.^{5,8,24} There are numerous disadvantages associated with this limited therapeutic approach. In addition to potentially serious side effects, immunomodulatory drugs have limited efficacy during progressive stage MS when reactive immune cells are no longer the primary cause of neurodegeneration.^{7,12} Furthermore, general immunosuppressive drugs are not the ideal treatment for a heterogeneous, multifocal disease like multiple sclerosis.⁶ The prodrug FTY720, approved by the FDA in September 2010 and marketed as fingolimod or Gilenya, may be the first drug to address this deficit in the pharmaceutical treatment of MS.⁸ Clinical trials have demonstrated its efficacy in reducing the frequency and severity of MS relapses, decreasing the size and number of active MS lesions, and preventing brain volume loss, suggesting a role in tissue preservation and potentially repair.^{7,8} Patients treated with fingolimod also retain functioning naïve T-cells, minimizing the risk of opportunistic infection.⁸ Furthermore, this is the first MS medication that can be taken orally, making it a more desirable treatment option for patients, and studies have demonstrated that fingolimod is in fact more effective than immunomodulatory interferons in treating multiple sclerosis.^{25,26}

While not fully characterized, much research has been conducted regarding the mechanism of action of fingolimod. FTY720 is phosphorylated *in vivo* by sphingosine kinase 2 (SK2), and the phosphorylated analogue acts as a nonselective ligand of four of the five sphingosine-1-phosphate (S1P) G-protein coupled receptors.^{8,27} Until recently, the majority of research has focused on the interactions of FTY720-P with sphingosine-1-phosphate receptor 1 (S1P₁). FTY720 was originally thought to function purely as an S1P₁ agonist.²⁸ Studies had demonstrated that FTY720-P bound competitively at the ligand binding site, activated the signaling pathway, and triggered endocytosis of the activated receptor.^{29,27} More recent research suggests that FTY720-P operates instead as a functional antagonist.^{9,8,30} Initially, FTY720-P activates the S1P₁ receptor and triggers its endocytosis. Following internalization, however, the drug maintains the receptor in an active conformation, allowing agonist effects to persist for at least 24 hours.²⁹ FTY720-P binding causes an alteration in phosphorylation patterns of the internalized receptor as compared to the natural ligand binding, and this differential modification prevents the receptor from being recycled to the plasma membrane as it normally would.⁸ Instead, the receptor is degraded, preventing subsequent activation of the signaling pathway by the natural ligand S1P.^{9,8}

Immunomodulatory Function

When FTY720 was initially developed, it was thought to function immunologically by inhibiting lymphocyte trafficking.^{9,5,8,7} T-cells, B-cells, and natural killer (NK) cells normally migrate from lymphoid organs in response to the concentration gradient of the signaling molecule S1P. If lymphocytes are activated by an antigen, they are initially retained in the lymphoid organs where they proliferate and differentiate in preparation for the attack on the antigen source. Subsequent upregulation of S1P₁ on lymphocytes allows S1P to bind the receptor. Activation of the associated signaling pathway triggers lymphocyte migration from the lymphoid organs to

the source of the antigen.⁸

Functional antagonism of lymphocytic S1P₁ receptors by FTY720-P inhibits the cyclic upregulation of S1P₁ and, as a result, diminishes the ability of the receptor to interact with the natural ligand. In multiple sclerosis patients, this prevents egress of myelin antigen-activated lymphocytes that would otherwise be involved in the inflammatory autoimmune response.²⁶

⁸ This molecular mechanism has received extensive experimental validation. Fingolimod has been shown to significantly reduce blood lymphocyte counts, and the lymphopenia is reversed upon discontinuation of therapy.^{26, 8, 14} Fingolimod's lymphocyte sequestration capacities do not affect all types of immune cells. Notably, the migration of regulatory T-cells is not inhibited by fingolimod, and there is some evidence that it enhances their function, further countering MS pathology.^{14, 31} Additionally, FTY720 has been shown to inhibit the production of certain inflammatory cytokines produced by activated immune cells that escape sequestration in the lymphoid organs, providing further attenuation of the inflammatory autoimmune response.³²

While the immunomodulatory effects of fingolimod continue to be reported, a growing body of research has begun to question whether this is the drug's primary mechanism of action. Studies have demonstrated that blood lymphocyte counts are not consistently dose-dependent and do not correlate well with the clinical efficacy of the drug.^{9, 8, 14} Conflicting data have been reported on the potency of fingolimod as an immunosuppressant.^{14, 8} Furthermore, FTY720 readily crosses the BBB and concentrates in the brain and specifically localizes to white matter containing myelin.^{5, 14, 8, 33} In fact, both the drug and its phosphorylated analog are present in significantly higher quantities in the brain than in the blood or lymphoid organs.^{14, 33} These and other findings have promoted a more thorough investigation of the potential effects of fingolimod on components of the CNS.

Neuromodulatory Function

It has been suggested that fingolimod may have direct neuromodulatory effects that attenuate MS activity independently of its immunological functions.^{9, 8, 5} This review addresses these effects with regard to the interactions of FTY720 and S1P with S1P receptors found on astrocytes. Astrocytes are the most abundant cells in the CNS and in active MS lesions, and the efficacy of fingolimod has been linked to S1P receptors on astrocytes rather than on microglia or neurons.^{8, 9, 34} Specifically, a recent study demonstrated that the selective deletion of S1P₁ in astrocytes of EAE mice eliminated any observable response to fingolimod.⁹

Studies like this have primarily focused on S1P₁ as the key mediator of S1P and FTY720 effects. Some reports have shown that S1P₁ is responsible for 40-75% of S1P receptor activity, depending on the region of the brain assessed.³⁵ Some studies have suggested that FTY720-P is a potent agonist of S1P₁ but only a partial agonist of S1P₃,^{36, 7} while others' findings characterize the drug as a potent agonist of all S1P receptors except S1P₂.^{8, 9} In support of the latter characterization, the interactions of FTY720 with multiple S1P receptors including S1P₃ outside the CNS have been implicated in side effects associated with fingolimod treatment.^{27, 8} The therapeutic effects of fingolimod, however, have been mimicked by S1P₁-selective agonists and inhibited by S1P₁-selective antagonists.^{36, 37} Taken together, research seems to indicate that the efficacy of fingolimod is achieved primarily through S1P₁, but it is reasonable to expect that its interactions with other S1P receptors also contribute to its observed effects. This review addresses the potential contributions of both astrocytic S1P₁ and S1P₃ interactions in the efficacy of fingolimod and the molecular pathology of MS with the understanding that S1P₁-mediated effects are likely dominant.

These S1P receptors were selected for analysis because S1P₁ and S1P₃ are the most abundant S1P receptors on astrocytes.^{36, 17, 8} The expression of both receptors is significantly increased in reactive astrocytes, and both receptors are found in active MS lesions.^{5, 17, 38} Furthermore,

both receptors contribute to the development of reactive astrogliosis. S1P has been found to induce astrogliosis both in vitro and in vivo via the S1P₁ and S1P₃ pathways,^{36,39,8} and FTY720 exposure or deletion of S1P₁ or S1P₃ has been shown to inhibit astrogliosis.^{9,8,33} As previously described, astrogliosis contributes to the pathology of MS by prolonging the CNS inflammatory reaction in MS lesions and preventing remyelination.^{17,16} Fingolimod treatment has been shown to moderate astrogliosis.⁵

The mechanism of action of fingolimod is likely to be similar in S1P₁ and S1P₃ receptors. There is a high degree of structural homology among S1P receptors, particularly with regard to their ligand binding pocket and residues associated with the activating conformational change of the receptor.²⁷ The surface expression of both receptors is dependent on similar phosphorylation patterns,³⁶ suggesting that the alterations in phosphorylation that affect internalization and subsequent degradation of S1P₁ after FTY720-P binding will similarly affect S1P₃. Additionally, physiological effects associated with S1P₃ activation have been observed at the initiation of treatment with fingolimod,²⁷ but they are only transient,⁸ suggesting activation followed by functional antagonism in the same manner associated with fingolimod activity at S1P₁.

Astrocytic S1P receptors function in regulating permeability of the blood-brain barrier

Studies have shown that the integrity of the blood-brain barrier is compromised at the sites of active multiple sclerosis lesions.⁴⁰ Enhanced permeability of the BBB facilitates increased transendothelial migration of activated immune system cells, increasing the inflammatory response and demyelination in the CNS. Treatment with fingolimod has been shown to reduce this breakdown of the BBB.^{14,8}

Regulation of the BBB phenotype is a cooperative effort involving bidirectional crosstalk between cerebral endothelial cells and astrocytes.^{40,12,36,34} CECs make up the walls of blood vessels and form the primary barrier separating materials in the blood from the CNS. Astrocytes form a secondary barrier component. These cells cover 90% of the surface area of CECs and serve as key regulators of their function. Communication between the two cell types is essential for BBB integrity.⁴⁰

The combined pertinence of astrocytes in maintaining the BBB and the efficacy of fingolimod in reducing MS effects on its integrity demonstrate a role for astrocytic S1P receptors in regulating the permeability of the BBB. Indeed, activity of the S1P₁ and S1P₃ receptors has been shown to modulate BBB integrity directly by affecting astrocytic gap junctions.^{41,42} Gap junctions on astrocytes are essential for communication among groups of astrocytes and among astrocytes and their neighboring cells, including CECs.⁴² Heightened S1P₁ and S1P₃ activity associated with reactive astrogliosis significantly inhibits the formation and function of these gap junctions.⁴¹⁻⁴² This impairs astrocyte communication with the CECs and severely compromises the BBB.⁴² This general weakening of the BBB would permit significant lymphocyte migration into the CNS. It has been established that frequent penetration by invading cells can cause lasting damage to the affected blood vessels,⁴⁰ causing the BBB disruption proximal to MS lesions and contributing, perhaps, to the chronic cerebrospinal venous insufficiency that has been documented in some MS patients. Functional antagonism of S1P₁ and S1P₃ receptors by fingolimod would restore astrocyte communication with CECs, resulting in the observed improvements in BBB phenotype and contributing to the reduction of MS symptoms associated with fingolimod treatment.

S1P receptors mediate inflammatory cytokine and chemokine secretion

When CNS damage occurs or the BBB is penetrated, astrocytes are activated and begin to secrete pro-inflammatory Th1 cytokines.⁴⁰ Some of these cytokines including interferon gamma (IFN- γ), tumor necrosis factor alpha (TNF- α), and interleukins (IL) -1 β , -6, and -17, have been directly associated with MS pathology.⁴⁰⁻⁹⁻⁴³ The production of many of these cytokines has been attributed to S1P receptor signaling. Expression of IFN- γ , IL-1 β , IL-6, and IL-17 has been significantly reduced by both fingolimod treatment and conditional deletion of astrocytic S1P₁.⁹⁻³²⁻⁴³ Less is known about the specific effects of S1P₃ on cytokine production, but its activity has been correlated with increased production of several inflammatory cytokines and chemokines, among them several that have been implicated in MS pathology.⁴⁴⁻⁴⁵ Additionally, the presence of TNF- α has been shown to substantially increase the expression of S1P₃ and to moderately increase the expression of S1P₁ on astrocytes, indicating a role for the receptors in this cytokine signaling pathway.⁴³⁻³⁸ It has been proposed that S1P₃ signaling in particular stimulates the pro-inflammatory effects of TNF- α and that the cytokine acts as a synergist on S1P₃, enhancing the vascular disruption and other effects associated with S1P₃ signaling.¹¹

Astrocyte production of proinflammatory cytokines helps to initiate and sustain the CNS inflammatory response in multiple sclerosis.⁸⁻⁴⁰⁻⁴³ Th1 cytokines are the initial stimuli that activate astrocytes and microglia, causing them to become immunoreactive and proliferate.⁴⁰⁻⁴³ Astrocytic cytokine production induces the expression of adhesion molecules such as ICAM-1, ECAM-1, PECAM-1, and E-selectin on CECs that guide activated lymphocytes to the CNS and facilitate their migration across the BBB.⁴⁰ To better facilitate this migration, proinflammatory cytokine signaling can alter tight junctions and adherens junctions to increase BBB permeability.⁴⁰

The cytokines IFN- γ , IL-1 β , and TNF- α also stimulate the production of chemokines. These chemokines direct lymphocyte movement and adhesion to the BBB. They also facilitate the transition from selectin-mediated adhesion to integrin-mediated interactions that enable lymphocytes to cross the BBB.⁴⁰ Unsurprisingly, a number of chemokines have been associated with MS activity.⁴⁰⁻³⁸ The expressions of several of these appear to be dependent on S1P receptor signaling. CCL2, a chemokine that contributes to demyelination in multiple sclerosis, is inhibited by fingolimod, making interaction with S1P₁ and/or S1P₃ receptors probable.⁴³ Both S1P₁ and S1P₃ have been definitively implicated in triggering the production of the chemokine MCP-1.⁴⁵ The expression of MCP-1 has also been shown to decrease following treatment with fingolimod.³⁸ Functional antagonism of astrocytic S1P₁ and S1P₃ receptors prevents the receptors from inducing proinflammatory cytokine production, lessening the astrocytic inflammatory response and inhibiting further proliferation of reactive astrocytes.

The interactions of S1P receptor signaling, cytokine and chemokine production, and CNS inflammation are complex. In addition to inhibiting the production of numerous proinflammatory cytokines, functional antagonism of S1P₁ would also decrease the production of some anti-inflammatory cytokines such as INF- β and chemokines such as CXCL10. S1P₁ also inhibits some components of TNF- α -mediated signaling, and its inactivation would result in the loss of a negative regulator of proinflammatory signaling.⁴⁴ It is possible that fingolimod may have different effects on S1P receptors in different tissue types, particularly in CECs,⁴² that contribute to its efficacy in spite of these complications. It has also been suggested that fingolimod treatment in fact facilitates a transition in cytokine production from the Th1 type immune response to a Th2 type in which more anti-inflammatory molecules are secreted.⁴⁶⁻²⁴ While this hypothesis requires further substantiation, it can be reasonably concluded at this time that inhibition of S1P₁ and S1P₃ receptors contribute to an overall reduction in the astrocytic inflammatory response that is associated with cytokine and chemokine production.

Positive feedback between S1P receptor and growth factor receptor signaling pathways contribute to MS pathology

S1P, the natural ligand of S1P receptors, is formed by the phosphorylation of sphingosine molecules by sphingosine kinase 1 (SphK1). Growth factor receptor signaling increases the production of S1P by activating SphK1. Consequently, an increase in growth factor concentration often results in the transactivation of astrocytic S1P receptors.²⁴ S1P receptor signaling frequently stimulates the production of growth factors by astrocytes, creating a series of positive feedback loops that result from crosstalk between growth factor (GF) and S1P signaling pathways.^{24 36 47 48} The amplification of both signaling pathways can increase disease activity associated with multiple sclerosis.

In addition to the consequences of enhanced S1P receptor signaling previously discussed in this review, heightened SphK1 activity and S1P generation can independently aggravate the MS condition. SphK1 is upregulated in reactive astrocytes and contributes to disease activity in MS lesions.¹⁷ In addition to generating S1P, the kinase triggers the production of proinflammatory molecules¹⁷ and enhances vascular permeability.⁴⁹ This helps to prolong the CNS inflammatory response and to increase its severity by permitting additional lymphocytes to cross the BBB. The high levels of S1P that can result from S1P-GF positive feedback loops can also contribute to CNS damage independent of a signaling pathway. While S1P normally promotes cell survival, prolonged exposure to high levels of S1P has been shown to trigger cell apoptosis.³⁶ Treatment with fingolimod can reduce these adverse effects. The drug has proven effective in inhibiting the biosynthesis of S1P and S1P precursors including SphK1.^{30 17}

Growth factors can also have individual effects on the CNS that contribute to the pathology of MS. Excessive production of vascular endothelial growth factor (VEGF) is typically observed in MS. Increased concentrations of this growth factor alter intercellular junctions and increase BBB permeability⁴⁰ and at the same time promote lymphocyte migration.⁴⁷ VEGF also triggers the proliferation of reactive astrocytes, contributing to the many proinflammatory effects of astrogliosis.¹⁶ Significant crosstalk occurs between the VEGF and S1P₁ pathways.⁴⁸ S1P₁ activity stimulates astrocytic secretion of VEGF, and VEGF increases the expression of S1P₁.⁴⁷ The desensitization of S1P₁ following fingolimod treatment has been shown to protect against vascular permeability induced by VEGF,⁴² and the removal of this essential component of the feedback loop likely contributes to the reduction in astrogliosis associated with the drug.⁵ The same feedback loop mechanism exists between S1P₁ and the fibroblast growth factor (FGF).^{36 8} FGF also stimulates reactive astrocyte proliferation,¹⁶ and this activity is blocked by fingolimod's inhibition of S1P₁ and SphK1.³⁶

Both S1P₁ and S1P₃ receptors on astrocytes have been found to increase signaling by epidermal growth factor receptor (EGFR), insulin-like growth factor receptor (IGFR),⁵⁰ and platelet-derived growth factor receptor (PDGFR).^{36 24} Astrocytes produce EGFR in response to neural injury like that caused by multiple sclerosis. This growth factor stimulates astrocytes to release proinflammatory substances such as S1P at the site of injury in an effort to confine and eliminate the antigen source.⁵¹ IGF-1 is produced primarily by astrocytes.⁴³ It stimulates the cells' proliferation and survival,⁵⁰ and it has been associated with demyelination in MS and related diseases.⁴³ Both EGFR and IGFR are unusually active in MS lesions, and this potentiation of their signaling has been causally linked to the activity of the S1P₁ and S1P₃ receptors.⁵⁰ This provides strong evidence for the integral role of reactive astrocytes and their S1P receptors in MS pathology. PDGFR activity is associated with cell migration,²⁴ and it has also been linked to reactive astrocyte proliferation and to astrocytic secretion of IL-1 β and MCP-1.⁵² PDGFR signaling contributes to the most critical features of reactive astrogliosis, and its activity as well as certain aspects of S1P signaling are mutually dependent on positive feedback between the two pathways.^{53 36 24} By desensitizing S1P₁ and S1P₃ receptors, inhibiting the activity of SphK1 and

the biosynthesis of S1P, and limiting the proliferation of reactive astrocytes, fingolimod will significantly reduce the expression of growth factors that interact with the S1P pathway.⁴³

Conclusions: Role of Astrocytic S1P Receptors in MS Pathology

Reactive astrogliosis has long been recognized as a feature of multiple sclerosis. In a typical response to nerve damage, reactive astrocytes proliferate around the locations at which myelin-reactive lymphocytes have crossed the BBB and begun degrading myelin sheaths. Reactive astrocytes then contribute to the inflammatory response by producing high concentrations of S1P in addition to proinflammatory cytokines, chemokines, and growth factors.^{43 40 51} Signaling through the astrocytic S1P₁ and S1P₃ pathways is essential for the initiation and potentiation of all of the key features of this inflammatory response including the weakening of the BBB, as well as for the proliferation, migration, and survival of the reactive astrocytes. This response can be adaptive in many cases of CNS injury, but in an autoimmune disorder such as MS in which the target antigen is a normal and abundant component of the CNS, the antigen source cannot be easily confined and eliminated. Thus, the normal function of reactive astrocytes and particularly of the S1P₁ and S1P₃ receptors likely serves to aggravate and prolong the initial MS autoimmune response, contributing to the progressive demyelination and neurodegeneration associated with the disease.⁴³

Increasingly, however, research is beginning to suggest a more direct role for S1P receptors in the pathology of MS. Both S1P₁ and S1P₃ are expressed at abnormally high levels in MS lesions, and the expression of S1P₁ has been directly correlated with the degree of demyelination.⁴³ The conditional deletion of astrocytic S1P₁ reduced levels of demyelination and lessened the severity of all symptoms associated with EAE.⁹ Most significantly, treatment with fingolimod prior to challenge with myelin antigens has been shown repeatedly to prevent the development of EAE.⁸ In conjunction with the data identifying astrocytic S1P₁ as the primary mediator of fingolimod's therapeutic effects,⁹ these findings indicate that the activity of astrocytic S1P₁ is an essential component in the pathogenesis of EAE.

While astrocytic S1P₁ may mediate the primary CNS activity in demyelinating disorders, lymphocytic S1P₁ may contribute to another aspect of the autoimmune reaction. A recent study discovered that this receptor was responsible for regulating T-cell differentiation. S1P₁ signaling stimulates the development of CD4+ TH1 cells – the reactive T-cells associated with the initial autoimmune reaction to myelin – and inhibits the formation of regulatory CD4+CD25+FoxP3+ T-cells.⁵⁴ Hyperactive signaling or significantly-increased expression of S1P₁ could thus explain the deficiency in regulatory T-cells observed in individuals with MS. A systematic increase in S1P receptor activity would also help to explain the unusually high levels of S1P and growth factors present in the cerebrospinal fluid of MS patients.¹⁷ This abnormal receptor activity would serve as the initial stimulus for the potentiation of the S1P-GF feedback loop systems.

Further research is needed to determine the precise roles of the S1P₁ and S1P₃ receptors in the mechanism of action of fingolimod and in the pathology and pathogenesis of multiple sclerosis. It is apparent, however, that this novel MS drug achieves its therapeutic effects by regulating signaling pathways that are critical components in the function of the immune system. A growing body of research is generating information implicating S1P₁ and other S1P receptors in the pathology of other diseases including systematic lupus erythematosus (SLE), myocarditis, type-I diabetes, arthritis,⁴² colitis,³¹ atherosclerosis,⁴⁵ SIV encephalitis,⁵² and even cancer.⁴⁷ It seems likely that the S1P receptors will soon be recognized as key elements of the body's inflammatory and immune responses. They may also become key targets for drug design, especially since the crystal structure of S1P₁ has now been obtained, with structures of the other S1P receptors likely to follow.⁵⁵ The new structural data will enable scientists to identify the precise interactions of FTY720-P in the binding pocket of S1P₁ and the other S1P

receptors and to settle the remaining debates regarding the drug's mechanism of action. The growing understanding of the SIP receptors may provide new insight into the pathology of multiple sclerosis and perhaps other autoimmune disorders as well as the opportunity to develop more specific and more effective drugs to treat them.

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