Fingolimod Immune Effects Beyond Its Sequestration Ability

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ABSTRACT

Fingolimod is the first orally administered drug approved for the treatment of relapsing–remitting multiple sclerosis (MS). This drug, modulating sphingosine receptors, regulates the trafficking of lymphocytes between primary and secondary lymphoid organs, trapping naive T cells and central memory T cells in secondary lymphoid organs, without affecting effector memory T cells and therefore without compromising immunosurveillance. Additionally, fingolimod inhibits expression of Th1 and Th17 cytokines and enhances regulatory T-cell differentiation. It also acts on the B arm of immunity through an increased ratio of naive to memory B cells, higher percentage of plasma cells, and highly increased proportion of transitional B cells as well as additional regulatory subsets. Fingolimod treatment enhances the capacity of regulatory B cells to transmigrate across brain endothelial cells. In fact, patients treated with fingolimod have increased regulatory B-cell frequency in the cerebrospinal fluid. These findings suggest a novel role for fingolimod in MS, by both direct effects and indirect partitioning effects on lymphocytes.

Keywords: B lymphocytes; Fingolimod; T lymphocytes; Mechanism of action

Key Summary Points

This paper shows new effects of fingolimod on the immune system beyond its sequestration ability.

Fingolimod is an effective drug in adults with relapsing–remitting MS and in pediatric forms of the disease.

Fingolimod negatively regulates Th1 and Th17 differentiation while promoting Th2 differentiation.

Fingolimod exerts its effects through action on B lymphocytes.

Fingolimod not only modulates lymphocyte trafficking but is able to modulate the composition of B- and T-cell subsets.
INTRODUCTION

Fingolimod is the first orally administered drug approved for the treatment of relapsing–remitting multiple sclerosis (MS). Fingolimod derives from myriocin, a metabolite of the fungus Isaria sinclairii [35]. Unlike myriocin, fingolimod does not affect the palmitoyltransferase and does not inhibit the activation and proliferation of T and B cells [11]. It is structurally similar to endogenous sphingosine and takes part in physiological sphingolipid signaling [31]. Because of its structural similarity to sphingosine, it is phosphorylated to fingolimod phosphate by two protein kinases, type-1 and type-2 sphingosine kinase (SphK1 and -2), enabling it to interact with sphingosine receptors.

Sphingosine-1-phosphate (S1P) is a lipid that interacts with G protein-coupled receptors expressed by various cell types in both the central nervous system (CNS) and the periphery. These receptors perform a variety of biological functions, including CNS homeostasis, vascular development, and circulation of T cells [41]. There are five types of sphingosine receptors, namely S1P1, S1P2, S1P3, S1P4, and S1P5. Fingolimod interacts with all these receptors except S1P2 [31]. It plays the role of superagonist of S1P1Rs, implying its overactivation and subsequent internalization, therefore working as a functional antagonist [2].

To date, the cause of MS is still unknown, but the activation of potentially autoreactive myelin-specific CD4+ T lymphocytes is considered the initial event in its pathogenesis. Activated autoreactive T lymphocytes express on their surface adhesive molecules that allow them to bind to the endothelial cells of the blood–brain barrier (BBB). These cells produce matrix metalloproteinase enzymes that disrupt the BBB, allowing activated T lymphocytes to reach the cerebral parenchyma. Following entry into the CNS, T cells accumulate within perivascular spaces, where they encounter myelin antigens presented by the major histocompatibility complex class II (MHC II) molecules expressed by the antigen-presenting cells [23]. This results in reactivation of autoreactive T cells, secretion of proinflammatory cytokines, and activation of local microglia, leading to progressive damage to cerebral tissue [22].

The resulting CNS damage is a trio of inflammation, demyelination, and neurodegeneration [36]. This article is based on previously conducted studies and does not contain any studies with human participants or animals performed by any of the authors.

Effects of Fingolimod on T Cells

Physiologically, after antigenic stimulation, lymphocytes can differentiate into two types of memory T lymphocytes in the lymph nodes: central memory T cells (TCMs) and effector memory T cells (TEMs) [6].

The type of T-cell differentiation depends on the intensity of antigenic stimulation of naïve T cells in the lymph nodes. High-intensity antigenic stimulation of naïve T cells generates C-C chemokine receptor 7 (CCR7)+ effector memory T cells, which recirculate to infected tissues and display effector functions. Weaker-intensity stimulation generates CCR7+ central memory T cells, which are retained in secondary lymphoid organs. CCR7 is a chemokine involved in T-cell retention in the lymph node.

TEMs, once out of the lymph node, reach the peripheral circulation and perform an immunosurveillance function. TCMs, on the other hand, require further antigenic stimulation to differentiate into TEMs in a specific tissue. Most of the pathogenetic TEMs in MS are derived from TCMs that interact once again with myelin antigens in the CNS. This interaction leads to damage to the nerve structures [12].

TCMs recirculate between the blood and secondary lymphoid organs in a CCR7-dependent manner.

They require activation of the S1P1 receptor to egress from lymph nodes, overriding CCR7-mediated retention in lymph nodes. TEM cells irreversibly lose CCR7, becoming independent of sphingosine signaling for egress from lymph nodes. The balance between CCR7 retention signals and S1P1 egress signals determines the T-cell egress from lymph node to the blood [21].
Fingolimod, after phosphorylation, binds to S1P1 receptors on T cells and causes internalization of the receptor. This reduces the responsiveness of T cells to the egress signal S1P and favors CCR7-mediated retention in lymph nodes [48].

Fingolimod blocks only the central memory T cells in the lymph nodes, without compromising the circulation of effector memory T cells. This mechanism limits the formation of new self-reactive lymphocyte clones supporting the disease, without compromising the defense against pathogens [3].

In humans, a preferential retention of CD4 + compared with CD8 + T cells occurs, and this is related to the higher content of CCR7 + naïve cells in the CD4 + compartment [56].

Central memory lymphocyte subsets are believed to be important for inducing the neurological damage associated with multiple sclerosis; their containment in lymphoid tissues is therefore expected to have beneficial effects in MS patients. As a result of cell retention within lymphoid tissues, peripheral blood lymphocyte counts are reduced during treatment with fingolimod. This effect is readily reversed when treatment is stopped, because lymphocytes are redistributed and not destroyed. Intrinsic lymphocyte functions are not affected by treatment with fingolimod [14]. Moreover, much evidence indicates that fingolimod may also exert direct effects within the CNS. Following oral administration, it is found in the CNS where S1P receptors are expressed on most neural lineages and resident cells, particularly glia and neurons. By modulating the S1P receptors expressed on CNS cells [9, 29], fingolimod may have a direct impact on neuropathological processes such as neurodegeneration, gliosis, and endogenous repair mechanisms [7].

The efficacy of fingolimod in MS patients has been confirmed in phase 3 clinical trials. In placebo-controlled trials, fingolimod reduced the relapse rate by approximately 50%, MRI activity by over 70%, and brain volume loss by 34%. Confirmed Expanded Disability Status Scale (EDSS) progression was significantly reduced in only one study [33].

The efficacy of fingolimod in pediatric MS was also recently demonstrated, proving more effective than interferon β-1a in controlling disease activity measured by relapse rate and MRI parameters. Moreover, pediatric MS patients treated with fingolimod experienced less disability progression for up to 2 years [17].

It is a matter of fact that fingolimod works by modulating lymphocyte trafficking. However, in recent years, scientific evidence has shown other mechanisms of action potentially involved in the immunomodulatory effects of this drug. Th1 and Th17 cells play important roles in MS development [39], and accordingly, both cell types are increased in frequency in the circulating blood in MS patients [13]. These cells produce proinflammatory cytokines such as IL1β and IL17, and contribute to disease development and activity [47]. Ex vivo studies showed that fingolimod affects the phenotype and function of circulating T cells. Dominguez-Villar et al. examined the in vivo effects of fingolimod on T cells and regulatory T cells (Treg) among relapsing–remitting MS patients during a 12-month treatment period. Consistent with previous observations, these authors found a clear reduction in the number of total lymphocytes, CD3 + T cells, CD4 + T cells, CD8 + T cells, and B cells after the third month of treatment, with no effects on monocyte and natural killer (NK) cell counts. The level of circulating proinflammatory cytokines was also reduced. TNF and IL27, Th1-related cytokines, were significantly decreased after 3 months of fingolimod therapy. Effector CD4 T cells significantly downregulated the transcription factors RORC and TBX21 (main regulators of Th1 and Th17 cells) and significantly upregulated the exhaustion marker Tim-3. Consequently, ex vivo stimulation of effector T cells exposed to fingolimod showed reduced production of IL17 and increased IL10. It remains to be clarified whether the effect observed in T cells is due to direct signaling of fingolimod, or whether the change in T helper phenotypes is due to an indirect effect through antigen-presenting cell modulation [18].

A recently discovered mechanism of action of fingolimod is the modulation of T-cell factor 1 (TCF-1) in CD4 cells. TCF-1 is a transcription factor involved in T-cell development; it negatively regulates Th1 and Th17 differentiation while
promoting Th2 differentiation via stimulation of Th2-specific transcription factor (GATA3). In mice, TCF-1 expression was found to be involved in the regulation of inflammatory Th1 and Th17 differentiation and the development of experimental autoimmune encephalomyelitis (EAE), the most commonly used animal model for MS. Mazzola and colleagues observed that fingolimod treatment was able to restore the reduced TCF-1 expression in T cells from relapsing–remitting patients, inducing a less inflammatory phenotype with reduced production of IFN\(\gamma\) and granzyme B [43]. A similar effect has been found in the CD8\(^+\) compartment. The levels of circulating CD8\(^+\) T cells producing IFN\(\gamma\) and IL17 appear to be decreased after only a month of fingolimod therapy [54].

The fingolimod mechanism of action negatively affects the migration of new T cells from the thymus, resulting in a reduction in naïve T cells and an increase in memory phenotypes. This effect might potentiate the shift toward an older immune system, detected in MS patients, and at the same time may reduce regulatory T-cell function. Haas et al. showed that while the number of naïve and recent thymic emigrants in the peripheral T cells of MS patients was reduced, exposure to fingolimod did not deteriorate this imbalance. The number of Treg expressing two TCR-V\(\alpha\) chains and the prevalence of conventional CD4\(^+\) T cells expressing two TCR-V\(\alpha\) chains, both surrogate markers of thymic T-cell development, did not change after 3 and 6 months of fingolimod therapy. Taken together, these observations seem to exclude an effect of fingolimod on the release of T cells from the thymus [26].

The ability of fingolimod to polarize T cells toward an anti-inflammatory phenotype is probably related to its effects on antigen-presenting cells such as dendritic cells (DCs). CD11c\(^+\) dendritic cells from fingolimod-treated patients produce lower levels of proinflammatory cytokines, such as IL1\(\beta\) and IL6, after lipopolysaccharide (LPS) stimulation. Such a different pattern of cytokine production by dendritic cells affects their ability to stimulate T cells. Pretreatment of DCs with fingolimod leads to reduced proliferation and significantly less production of TNF\(\alpha\) and IFN\(\gamma\) by T cells [40].

Fingolimod also affects innate immunity by influencing monocyte function. Supernatants of cultivated monocytes from fingolimod-treated patients show altered secretion of IL1\(\beta\) and TNF\(\alpha\) after LPS stimulation. This result confirms a reduced proinflammatory response by monocytes during treatment with fingolimod [40].

In addition to an increase in myelin-reactive cells, MS is characterized by a deficit of peripheral tolerance due to a reduction in regulatory T cells [25]. Fingolimod in vivo was found to significantly increase the proportion of Treg cells CD4\(^+\)CD25\(^{hi}\)CD127\(^{lo}\) from the third month of therapy. This increase leads to a normalization of Treg cells comparable to those in healthy subjects.

All these results support the idea that fingolimod controls MS not only by reducing proinflammatory cell levels but also by increasing immune tolerance [18, 54].

**Effects of Fingolimod on B Cells**

MS has traditionally been considered to be mediated by CNS reactive CD4\(^+\) T cells. However, the finding of B-cell depletion by anti-CD20 antibodies in MS, leading to a substantial reduction in the development of new brain lesions and relapses [4, 32], indicates an important role for B cells as mediators of MS disease activity. This role may include the production of autoreactive antibodies, abnormal antigen-presenting capacity, altered cytokine response, and possibly impaired number or function of regulatory B cells (Breg), all leading to increased activation of autoreactive CD4 T cells [5, 38, 42]. B cells in the CNS may also enhance the generation of ectopic lymphoid follicles, which have recently been described in the meninges of patients with progressive MS and are considered to be related to cortical neuronal damage [28, 53]. It was shown that memory B cells, which are increased in MS patients, produce proinflammatory cytokines [19] and can efficiently present myelin antigens to T cells [27]. By contrast, transitional B cells possess anti-inflammatory properties in humans [8] and mice [20]. The percentage of regulatory B cells is significantly increased in MS patients
treated with fingolimod; thus the increased proportion of transitional cells during treatment may suggest B cells remaining in the periphery [24]. The differential reduction of immune cells in the periphery has been attributed to the cellular level of CCR7 [30, 45].

In addition, B cells in the cerebrospinal fluid of MS patients are reported to exclusively express the memory B cell phenotype [15], suggesting that these cells are preferentially recruited to the CNS of patients with MS. The observed reduction in memory B cells in MS patients treated with fingolimod is thought to be due to the suppression of memory B cells migrating to the CNS [46].

It is interesting to note that an increased proportion of transitional B cells has been demonstrated in association with the beneficial effects of B-cell depletion by anti-CD20 antibody in lupus [1, 51] and by alemtuzumab in MS [34], suggesting that transitional B cells are associated with the benefit of treatments that remove B cells from the circulation.

Only recently, the capacity of B cells to control T-cell responses was demonstrated—for instance the ability of regulatory B cells to suppress Th1 and Th17 differentiation while favoring the generation of Treg [24]. Thus, the observed reduction in the proportion of circulating B cells with suppressed proinflammatory (TNF) and enhanced anti-inflammatory (IL-10) cytokine profiles among the remaining B cells in patients receiving fingolimod should contribute to suppressing proinflammatory B cells entering the CNS, leading to a reduction in disease activity [10].

IL10 downregulates the immune response by reducing production of proinflammatory cytokines, expression of co-stimulatory molecules, and antigen presentation [49]. The increase in IL10+ cells is most likely due to the higher proportion of IL10-producing transitional cells and of plasmablasts and plasma cells, the main B-cell producers of both IL10 and IL35 regulatory cytokines [16].

Circulating B cells after 3 months of fingolimod treatment were found to produce increased levels of both anti-inflammatory and proinflammatory cytokines, despite the relative reduction in memory cells [10], which in general produce more cytokines than naïve cells [16, 19]. This may be due to the unchanged and elevated proportions of plasmablasts and plasma cells, respectively, and indicates that the remaining B cells are in a more activated state. Breg are thought to suppress the pathogenic T cells throughout the production of IL10, IL35, and TGFβ [50, 52]. The ratios of TGFβ to either LTα or TNF and the ratio of IL4 to LTα were elevated after 3 months therapy with fingolimod, suggesting that the overall cytokine profile becomes more anti-inflammatory. TGFβ can trigger a vast array of regulatory responses including inhibition of antigen presentation and induction of Treg [37, 50]. However stimulation of TGFβ together with pro-inflammatory IL6 has been shown in myelin-reactive T cells to abrogate their pathogenic function and increase IL10 production, despite also increasing IL17 [44]. Moreover, the reduced expression level of CD80 in fingolimod-treated patients suggests that their B cells have suppressed antigen-presenting capacity. The drug also reduces the percentage of ICAM-1 cells, confirming that fingolimod may reduce antigen-presenting capacity [55].

Considering the effects of fingolimod treatment on lymphocyte migration in the periphery, Grutzke and colleagues asked whether it also influences lymphocyte transmigration across the blood–brain barrier. They therefore investigated the migratory properties of peripheral blood mononuclear cell compartment in an in vitro model of the BBB. Fingolimod treatment demonstrated no affect on the migration of T cells under either non-inflamed or inflamed conditions. In contrast to T cells, B cells derived from fingolimod display significantly enhanced migratory activity, which could be attributed to a selective increase in the migration of regulatory B cells. This difference was more pronounced during transmigration across a non-inflamed endothelial cell layer but could be observed under inflamed conditions as well. While there was no difference in the transmigratory capacity of naïve and memory B cells between the groups, a profound increase in transmigration of regulatory B cells derived from fingolimod-treated patients was observed compared with healthy.
controls and untreated MS patients. Moreover, after ≥ 12 months of treatment, the group of “stable” patients exhibited a significantly higher percentage of regulatory B cells in the peripheral blood than the group of “active” patients, whereas before initiation of treatment there was no difference in this respect between the groups. Although fingolimod significantly reduces the absolute numbers of B-cell subpopulations in the periphery, the CSF in patients receiving fingolimod exhibits naïve and memory B-cell counts comparable to those of control individuals, and even increased numbers of regulatory B cells. These findings support the perception that fingolimod treatment enhances the migration of B cells across the BBB [24].

DISCUSSION

Fingolimod, interacting with sphingosine receptors, leads to quantitative and qualitative changes in a wide range of immune cells. This drug, after phosphorylation, binds to S1P1 receptors on T cells and causes internalization of the receptor, reducing the responsiveness of T cells to the egress sphingosine signal and favoring CCR7-mediated retention in lymph nodes.

To date, the block of the central memory T cells in the lymph node, without compromising the circulation of effector memory T cells, is the best known fingolimod mechanism of action. This mechanism limits the formation of new self-reactive lymphocyte clones supporting the disease, without compromising the defense against pathogens.

However, recent evidence shows that the drug performs different actions on T and B lymphocytes.

Fingolimod therapy significantly reduces Th1-related cytokines, such as TNFα and IL27, on CD4+ T cells in vivo, while at the same time increasing the expression of exhaustion markers. A similar effect has been found in the CD8+ compartment, with decreased levels of circulating CD8+ T cells producing IFNγ and IL17 appearing already after only a month of fingolimod therapy.

The ability of fingolimod to induce a shift in T cells toward an anti-inflammatory phenotype is probably related to its effect on dendritic cells. CD1c+ dendritic cells from fingolimid-treated patients produce lower levels of proinflammatory cytokines after lipopolysaccharide stimulation.

Recent evidence confirms that fingolimod affects B cells as well. The percentage of regulatory and transitional B cells in treated MS patients is significantly increased, thus inducing an anti-inflammatory shift. Fingolimod may also reduce the antigen-presenting capacity of B cells; the reduced expression of CD80 and ICAM-1 cells seems to confirm this possibility.

CONCLUSION

In summary, fingolimod is found to play a novel and as yet unrecognized role, modulating the composition of B- and T-cell subsets by increasing the ratio of naïve cells to memory cells and significantly increasing the proportion of transitional cells and several other regulatory subsets, including IL10+ cells.

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