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ORIGINAL ARTICLE

Siponimod (Mayzent) Downregulates RhoA and Cell Surface Expression of the S1P1 and CX3CR1 Receptors in Mouse RAW 264.7 Macrophages

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Abstract

The Siponimod (Mayzent) is a newly developed drug, similar to Fingolimod (FTY720) but with fewer side effects, approved by the Food and Drug Administration for the treatment of multiple sclerosis (MS). The therapeutic effect of siponimod and FTY720 in MS relies on their inhibitory effect on the sphingosine 1-phosphate (S1P) signaling. These drugs bind to the S1P receptors and block the CCL2 chemokine pathway that is responsible for the exit of the immune cells from the lymphoid organs, and circulation, thus preventing immune cell-dependent injury to the nervous system. We recently found that FTY720 beside its effect on the S1P pathway also blocks the RhoA pathway, which is involved in the actin cytoskeleton-related function of macrophages, such as expression/recycling of fractalkine (CX3CL1) receptors (CX3CR1), which direct macrophages to the transplanted organs during the development of the long-term (chronic) rejection. Here we tested the effects of siponimod on the RhoA pathway and the expression of the S1P1 and CX3CR1 receptors in mouse RAW 264.7 macrophages. We found that siponimod downregulates the expression of RhoA protein and decreases the cell surface expression of S1P1 and CX3CR1 receptors. This newly discovered crosstalk between S1P and RhoA/CX3CR1 pathways may help in the development of novel anti-chronic rejection therapies in clinical transplantation.

Keywords Siponimod · FTY720 · S1P1 · CX3CR1 · RhoA · Macrophage · Actin

Abbreviations

CCL2	C–C motif chemokine 2
CCR2	C–C motif chemokine 2 receptor
CX3CL1	C–X3–C motif chemokine ligand 1, fractalkine
CX3CR1	C–X3–C motif chemokine receptor 1, fractalkine receptor
FTY720	Fingolimod
GAP	GTPase-activating protein
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GEF	Guanine nucleotide exchange factor
HRP	Horseradish peroxidase
MS	Multiple sclerosis
Rac1	Rac family small GTPase 1
RAW 264.7	Abelson murine leukemia transformed mouse macrophages
RhoA	Ras homolog family member A
ROCK	Rho-associated protein kinase
S1P	Sphingosine 1-phosphate
S1P1	Sphingosine 1-phosphate receptor 1

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Introduction

The fingolimod (FTY720) and similar drug but with fewer side effects, the siponimod, are Food and Drug Administration approved for the treatment of multiple sclerosis (MS) (Anastasiadou and Knöll 2016; Behrangi et al. 2019; Chiba

et al. 2006, 2011; Huwiler and Zangemeister-Wittke 2018; Kremer et al. 2019; Pan et al. 2013). The therapeutic function of finglolimod and siponimod in MS is based on the ability of their phosphorylated forms to bind sphingosine 1-phosphate (S1P) receptors and inhibit the C–C motif chemokine 2 (CCL2), and its receptor (CCR2) pathway (Li et al. 2014) that regulates the egress of immune cells from the lymphatic system, and circulation; thus, preventing the immune cell-dependent damage of the central nervous system (Anastasiadou and Knöll 2016; Behrangi et al. 2019; Cannavo et al. 2017; Chiba et al. 2006, 2011; Huwiler and Zangemeister-Wittke 2018; Kremer et al. 2019; Pan et al. 2013). Recent studies showed that the S1P also affects the RhoA pathway (Quarta et al. 2017; Sun et al. 2018) and that the FTY720 beside inhibiting the S1P pathway also inhibits the RhoA pathway (Aydin et al. 2010; Chen et al. 2018b; Zhou et al. 2006). The small GTPase RhoA and its downstream effector the p160 kinase ROCK regulate the actin cytoskeleton and actin-related structures and functions in all eukaryotic cells (Chen et al. 2018a; Haga and Ridley 2016; Kloc et al. 2014). The activation of RhoA depends on guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs). The GEFs catalyze the exchange from G-protein-bound GDP to GTP, and GAPs induce GTP hydrolysis (Bos et al. 2007; Chen et al. 2017; Mahajan-Thakur et al. 2017). Several studies show that the RhoA pathway is reciprocally regulated by the small GTPase Rac1 (Chen et al. 2018a; Kloc et al. 2014; Parri and Chiarugi 2010;). Our previous studies in the rodent transplantation models showed that the inhibition of RhoA/ROCK pathway or the macrophage-specific deletion of RhoA affected macrophage actin cytoskeleton and reduced macrophage movement to the transplanted hearts inhibiting the long-term (chronic) rejection in rodent transplantation model (Kloc and Ghobrial 2014; Kloc et al. 2014; Liu et al. 2016a, b, c, d, 2017; Zhang et al. 2012). Also, beside our studies, the studies of Ohki et al (2001) showed that the ROCK inhibitor Y27632 prolongs cardiac allograft survival in the mouse transplantation model. The macrophage movement into the graft and their targeting to the graft's blood vessels depend mainly on the fractalkine (CX3CL1/CX3CR1) pathway. The chemokine fractalkine (CX3CL1) is produced by the blood vessel endothelium and the fractalkine receptors (CX3CR1) are expressed on the macrophage surface. In addition, the CX3CL1/CX3CR1 pathway plays an important role in the osteoarthritis (Wojdasiewicz et al. 2014) and brain and spinal cord injury (Poniatowski et al. 2017). We showed that the deletion of RhoA decreased the expression of CX3CR1 receptors in the mouse macrophages (Liu et al. 2017). It is known that the expression of the receptors on the cell surface depends on the actin-dependent vesicular trafficking and recycling. We showed previously that the downregulation of CX3CR1 receptors in RhoA-deleted mouse macrophages was caused by a faulty receptor recycling, which depends on the Golgi apparatus and the Golgi-dependent vesicular transport (Liu et al. 2017). Because the RhoA inhibition/deletion disrupts CX3CR1 receptors and the CX3CL1/CX3CR1 pathway, and the FTY720, a parental drug of siponimod also inhibits RhoA pathway and S1P pathway, here we studied if/how the siponimod affects the expression of RhoA and the surface expression of macrophage receptors CX3CR1 and S1P1. In the long term, the results of this study should be relevant to the development of the novel therapies, which will prevent the chronic rejection of transplanted organs.

Materials and Methods

Cells and Siponimod Treatment

Siponimod (cat # M15683-5MG) was from Sigma Aldrich (St. Louis, MO 63178, USA). All experiments were performed on the Abelson murine leukemia transformed mouse macrophages RAW 264.7 (ATCC TIB-71) from the ATCC.

Western Blot and Immunostaining

We used the following antibodies: S1P1 (cat # PA-20610) was from Invitrogen (St. Louis, MO, USA). The anti-RhoA (cat # 2117S) and anti-rabbit IgG HRP-conjugated (cat #7074P2) antibodies were from Cell Signaling Technology (Danvers, MA 01923 USA). Rac1 (cat # PA1-091) antibody and GAPDH-HRP-conjugated antibody (cat # PA-987-HRP) were from Thermo Fisher Scientific (Houston, TX, USA) and CX3CR1 (cat # ab8021) was from Abcam (Cambridge, MA, USA). The Western blot analysis and immunostaining were performed as described in Chen et al. (2018b). The experiments were repeated 3–9 times and the results are expressed as mean ± SEM. Statistical significance ($p < 0.05$) was determined by Student's t test.

Flow Cytometry

The following antibodies were used for the flow cytometry analysis: Anti-mouse PE-conjugated CX3CR1 antibody (cat # FAB5825P) from R&D Systems (Minneapolis, MN 55413, USA), RhoA rabbit monoclonal antibody (cat # SN0612), and S1P1 rabbit monoclonal antibody (cat # JM10-66) from Thermo Fisher Scientific (Houston, TX, USA). For the surface (receptor) staining, the cells were incubated with Live/Dead Fixable Violet Dead Cell Stain (Invitrogen, St. Louis, MO, USA) to exclude dead cells. Cells were blocked with Fc blocker (antimouse CD16/CD32, cat # 14-0161-86, from eBioscience/Thermo Fisher Scientific, Houston, TX, USA) and 5% goat serum. Cells were then stained with appropriate primary antibodies. If primary antibodies were

unconjugated, cells were then stained with appropriate fluorophore-conjugated secondary antibodies. All incubations were performed for 15 min on ice. For the intracellular protein staining, following the surface staining, cells were fixed with 1X fixation buffer (cat # 00-5123-43 from eBioscience/Thermo Fisher Scientific, Houston, TX, USA). Cells were re-blocked in 1X permeabilization buffer (cat # 00-8333-56 from eBioscience/Thermo Fisher Scientific, Houston, TX, USA) containing Fc blocker and 5% goat serum. Cells were then stained with appropriate primary antibodies, followed by labeling with fluorophore-conjugated secondary antibodies if necessary. All incubations were performed for 15 min on ice. Samples were acquired on an LSR-II flow cytometer (BD Biosciences, San Jose, CA, USA).

Results

Siponimod Effect on RhoA and Rac1

We incubated the mouse RAW 264.7 macrophages in the presence of 300 nM siponimod for 24 h and tested the expression of RhoA protein by Western blot and flow cytometry analysis. We also tested the effect of siponimod on the expression of Rac1, which has been shown to reciprocally regulate the RhoA. The expression levels of RhoA and Rac1 proteins in the Western blots were normalized to the level of expression of GAPDH marker. The experiments were repeated four times, and the results were expressed as mean ± SEM. Statistical significance was determined by Student's *t* test, and the $p < 0.05$ was considered as a statistically significant. Western blot and flow cytometry analyses, which were gated for live cells, showed that, in comparison to the untreated controls, the treatment with siponimod caused a statistically significant decrease in the expression of RhoA protein (Fig. 1a, b). However, there was no statistically

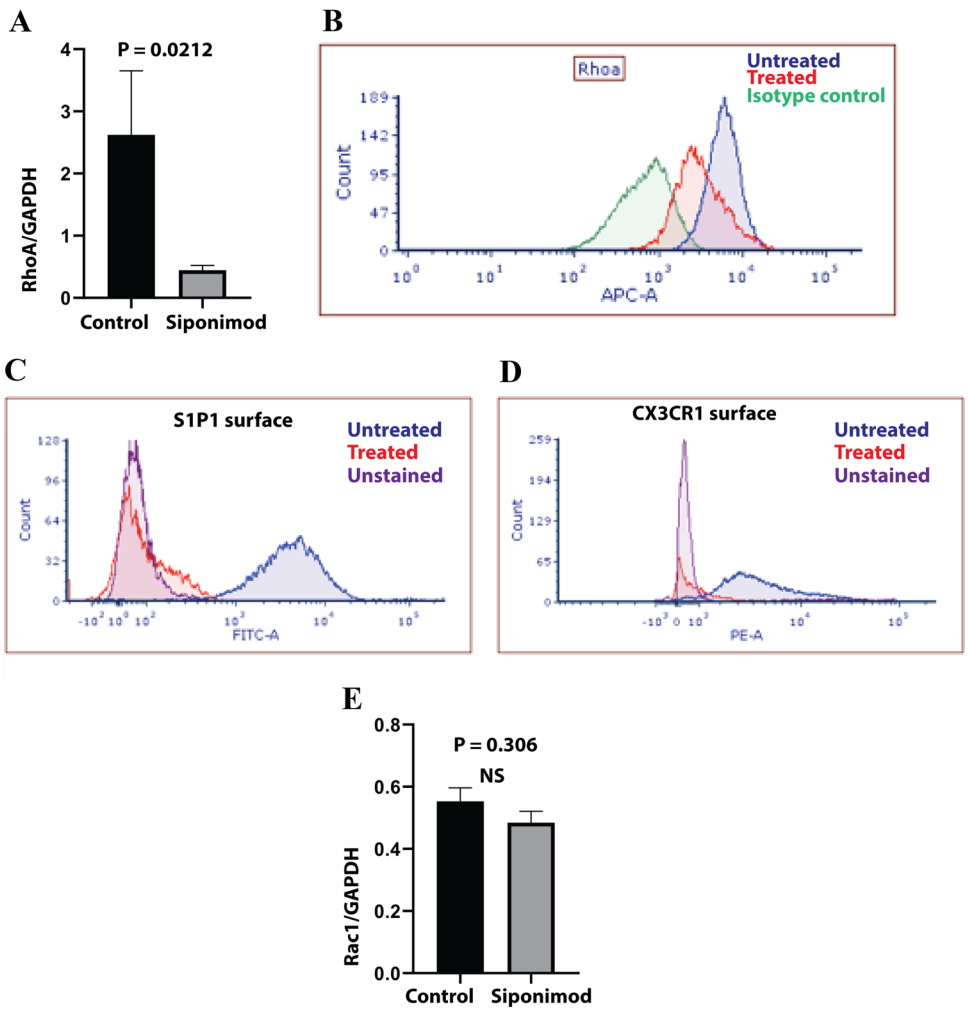

Fig. 1 Expression of RhoA, Rac1, and S1P1 and CX3CR1 receptors. The Western blot graph (**a**) and flow cytometry (**b**) show the statistically significant decrease in the expression of RhoA protein in the siponimod-treated cells in comparison to the untreated cells. The flow cytometry analysis shows a decrease in the surface expression of S1P1 (**c**) and CX3CR1 (**d**) receptors in the siponimod-treated cells. **e** The Western blot graph shows that there is no statistically significant difference in the expression of Rac1 protein between the siponimod-treated and -untreated cells

significant effect on the expression of Rac1 protein (Fig. 1e). This confirms that siponimod, similar to its parental drug FTY720, inhibits the expression of RhoA.

Siponimod effect on the CX3CR1 and S1P1 receptors

We analyzed the effect of siponimod on the cell surface expression of CX3CR1 and S1P1 receptors using flow cytometry (Fig. 1c, d) and immunostaining (Fig. 2). These analyses showed that the treatment with Siponimod reduced the surface expression of CX3CR1 and S1P1 receptors (Figs. 1c, d, 2). This indicates that siponimod, not only downregulates the S1P/S1P1 pathway, which is critical for the development of MS but also inhibits the expression of the CX3CR1 receptors, which promote macrophage movement into the transplanted organ during the development of chronic rejection. Our previous studies on the mouse-derived macrophages showed that the inhibition or deletion of RhoA causes the change of macrophage shape and polarity (Liu et al. 2016a, b, c, 2017). In presented here study we used RAW 264.7 cells that are virally transformed/immortalized, which makes them extremely variable in size and shape. Thus, it is impossible to assess if the siponimod treatment changes the macrophage polarity and/or shape. In the future, we are planning to repeat these experiments on the macrophages isolated from mice, such mouse-derived macrophages are much more uniform in shape and any changes in cell shape or polarity should be readily visible.

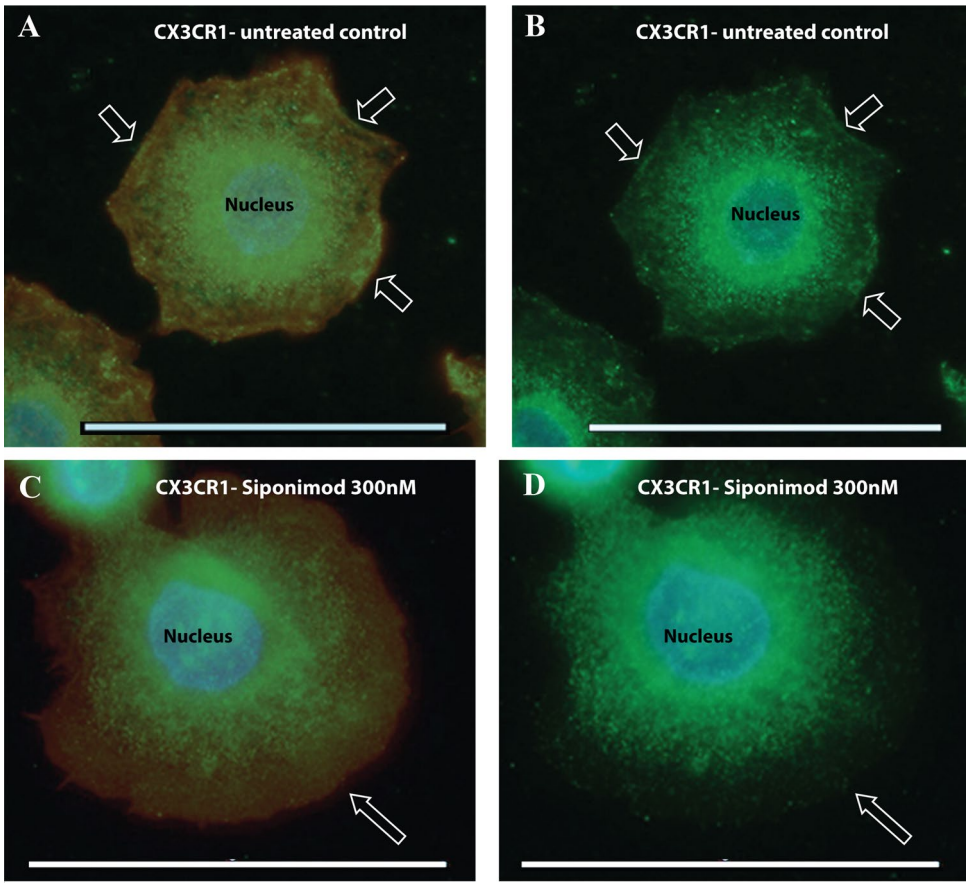

Fig. 2 Siponimod treatment internalizes CX3CR1 receptors. The immunostaining of RAW 264.7 macrophages with the anti-CX3CR1 antibody (green). The actin was stained with Rhodamine-Phalloidin (red) and the nuclei were stained with Dapi (blue). The RAW 264.7 macrophages are extremely variable in the shape (from round to highly elongated), and the size. For the comparison, we have chosen the similarly shaped (roundish) cells. **a**, **b** In the untreated cells, the CX3CR1 receptor is present around the nucleus, at the cell periphery, and the cell membrane (short arrows). The image in **a** is a merged CX3CR1, actin, and Dapi staining. The image in **b** is a merged CX3CR1 and Dapi staining. **c**, **d** The treatment with 300 nM siponimod for 24 h causes disappearance of CX3CR1 from the cell periphery and cell membrane. The long arrows point to the cell boundary. The image in **b** is a merged CX3CR1, actin, and Dapi staining. The image in **d** is a merged CX3CR1 and Dapi staining. The bar is equal to 50 µm

Discussion

Our previous study showed that FTY720 downregulated the expression of RhoA in mouse peritoneal macrophages (Chen et al. 2018b). Here we showed that similar to the FTY720, the siponimod also inhibits the expression of RhoA protein in mouse RAW 264.7 macrophages. We also showed that siponimod decreased the cell surface expression of CX3CR1 and S1P1 receptors.

Numerous studies showed that the therapeutical effect of FTY720 and siponimod in MS is based on their inhibitory effect on the S1P receptors and the downstream CCL2 pathway, which directs an egress of immune cells from the blood vessels, and lymphatic system, to the nervous system, where they impose the damage (Fig. 3a, b) (Chiba et al. 2006, 2011; Huwiler and Zangemeister-Wittke 2018; Li et al. 2014). It has been shown that the FTY720 causes a downregulation of the S1P1 receptors (Chen et al. 2018b; Huwiler and Zangemeister-Wittke 2018), probably through the inhibition of receptor recycling (Fig. 4). However, very little attention has been paid to the effect of these drugs and S1P/S1P1 pathway on the RhoA pathway, and RhoA/actin-dependent functions of immune cells, and their possible therapeutical effects on the chronic rejection of transplanted organs. The chronic rejection depends mainly on the macrophages, which infiltrate the transplant and cause vessel occlusion and tissue fibrosis (Kloc and Ghobrial 2014; Kloc et al. 2014). The macrophage infiltration of the graft depends on RhoA pathway and CX3CR1 (fractalkine) pathway (Liu et al. 2017). We showed previously that the inhibition of RhoA pathway or macrophage-specific deletion of RhoA inhibited the CX3CR1 receptors, decreased macrophage number in the graft, and abrogated

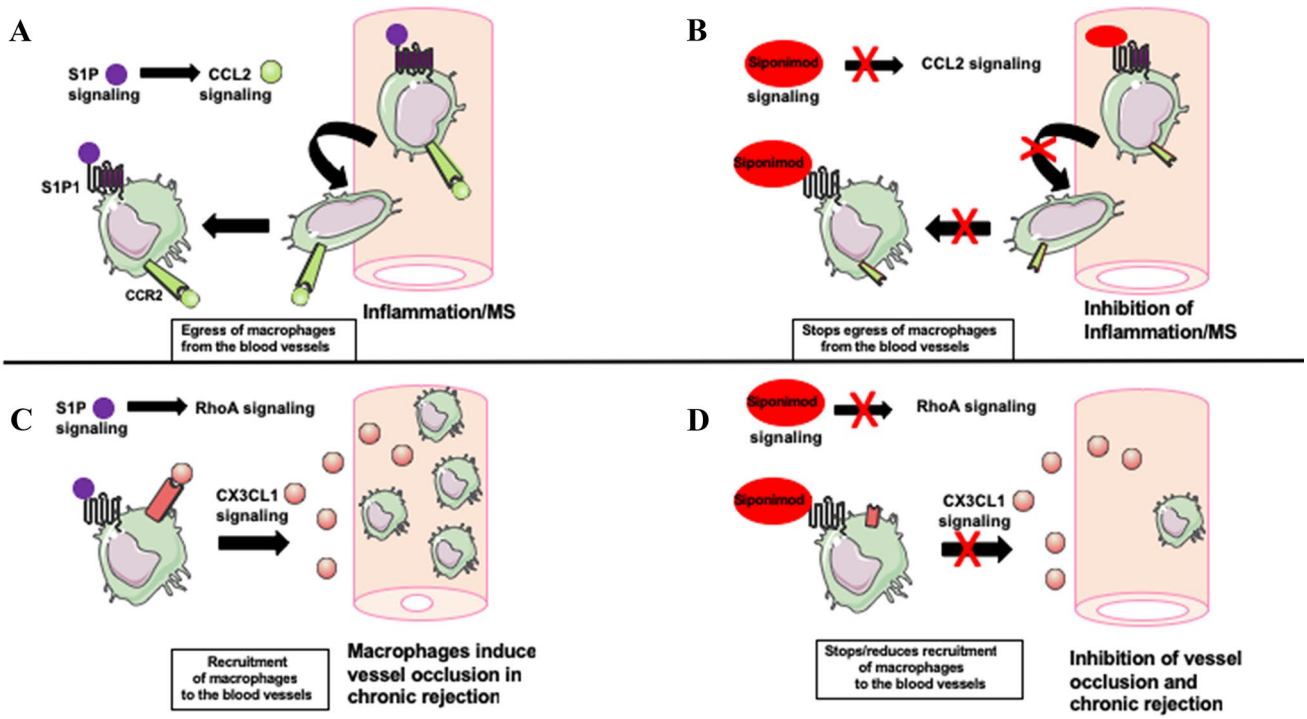

Fig. 3 The effects of S1P and siponimod on the CCL2/CCR2 and CX3CL1/CX3CR1 signaling. The effects of S1P and siponimod on CCL2/CC2 and CX3CL1/CX3CR1 pathway are highly intertwined, however, for better clarity, the diagrams depict the proven (in multiple sclerosis) and hypothetical (in transplant chronic rejection) effects on these two pathways as the separate events. The immune cells, such as macrophages contain S1P1, CX3CR1, and CCR2 receptors which bind to their respective ligands: S1P, CX3CL1 (fractalkine), and CCL2. **a** During Inflammation and multiple sclerosis (MS), the S1P binding to the S1P1 receptors increases the production of the CCL2 chemokine. This, in turn, stimulates egress of immune cells, such as monocytes/macrophages from the blood vessels and increases inflammation/MS. **b** Binding of the siponimod to the S1P1 receptors downregulates CCL2 signaling, reduces the surface expression of S1P1 receptors, prevents immune cells egress from the blood vessels, and decreases inflammation/MS. **c** We had previously shown that during chronic rejection of transplanted organs the macrophage migration into the graft and their accumulation in the vicinity of the blood vessels depend mainly on the CX3CL1 (produced by the vessel endothelium) and CX3CR1 receptors. The binding of the S1P to its receptors stimulates the RhoA pathway, which through regulation of the actin cytoskeleton upregulates CX3CR1/CX3CL1 pathway and macrophage accumulation within the graft. **d** Because, as shown in this study, the binding of siponimod downregulates the surface expression of CX3CR1 receptors (and also S1P1 receptors), we hypothesize that this will prevent macrophage movement to the graft resulting in the inhibition of chronic rejection

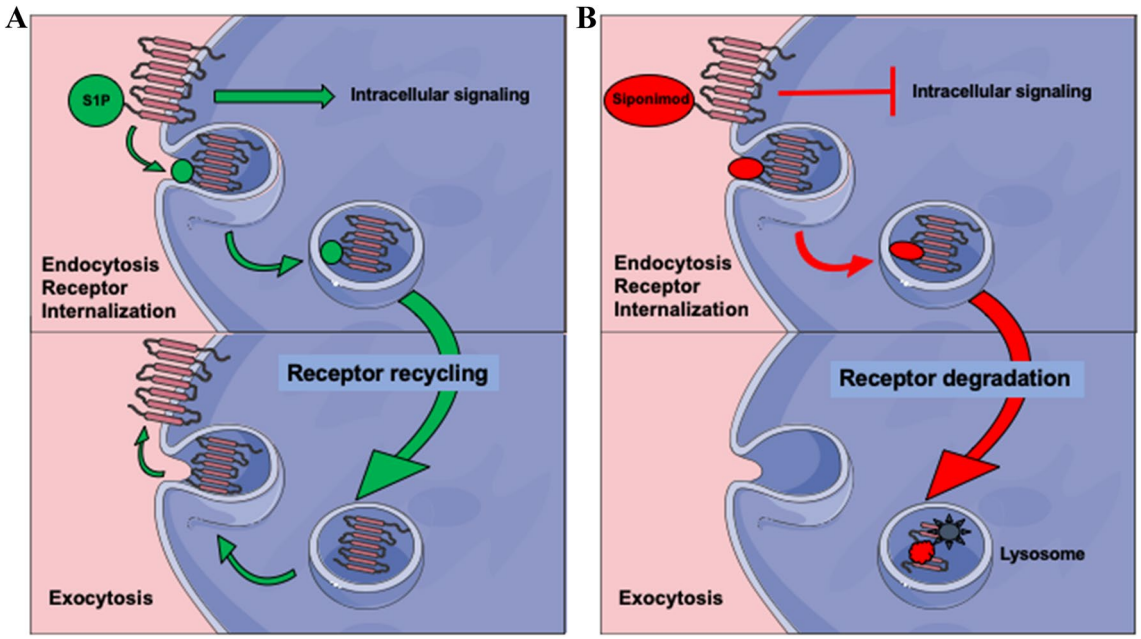

Fig. 4 The effect of S1P and siponimod on the recycling of the S1P1 receptor. **a** Binding of the S1P to S1P1 receptors induces intracellular signaling. The S1P-bound receptors present at the cell membrane are internalized through the endocytic vesicles. After the dissociation of the ligand, the restored/ functional receptors are being recycled to the cell surface by the exocytic vesicles. **b** Binding of the siponimod to the S1P1 receptors impairs the intracellular signaling and the siponimod-bound internalized receptors are directed for degradation through the lysosomal pathway. This incapacitates receptor recycling and lowers the number of the receptors at the cell surface

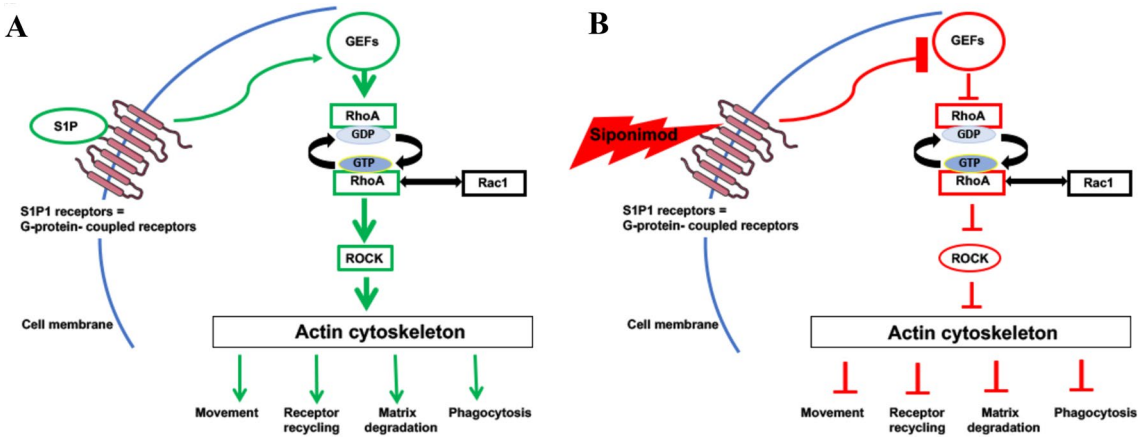

Fig. 5 The effect of S1P/S1P1 receptor and siponimod on the RhoA pathway and actin-related functions of macrophages. The diagram summarizes the effect of S1P /S1P1 and siponimod on the RhoA pathway and RhoA pathway-regulated actin cytoskeleton-dependent functions. **a** The S1P1 receptor belongs to the family of the G-protein-coupled receptors. After binding its ligand (S1P), the S1P1 signals the guanine nucleotide exchange factors (GEFs), which activate the RhoA. The RhoA, through its effector kinase ROCK, affects the polymerization and assembly of actin filaments. This, in turn, regulates all actin-dependent cell functions of macrophages, such as cell movement, vesicular trafficking/receptor recycling, matrix degradation, and phagocytosis. The RhoA pathway is also reciprocally regulated by the Rac1 pathway. **b** The siponimod is an antagonist od S1P and a selective modulator of S1P1. Binding of the siponimod to the S1P1 inhibits GEFs-dependent activation of the RhoA pathway. This, in turn, disrupts actin cytoskeleton and actin-dependent cell functions

chronic rejection in the rodent transplantation model (Chen et al. 2018a; Kloc and Ghobrial 2014; Kloc et al. 2014; Liu et al. 2016a, b, c, d, 2017; Zhang et al. 2012). The activation of the RhoA pathway depends on its upstream regulators the guanine nucleotide exchange factors (GEFs) proteins (Cannavo et al. 2017; Chen et al. 2017). It has been shown that the S1P receptors, including the S1P1 receptor, belong to the family of the G-protein-coupled

receptors (Mahajan-Thakur et al. 2017). After binding its ligand (S1P) the S1P1 signals the GEFs, which activate the RhoA. The RhoA, through its effector kinase ROCK, affects the polymerization, and assembly of actin filaments (Fig. 5). This, in turn, regulates all actin-dependent cell functions of macrophages, such as cell movement, vesicular trafficking/receptor recycling, matrix degradation, and phagocytosis (Fig. 5) (Kloc et al. 2014). The siponimod, as it is parental drug FTY720, is the antagonist od S1P and a selective modulator of S1P1. Our data suggest that the binding of the siponimod to the S1P1 not only inhibits CCL2 pathway but also inhibits GEFs-dependent activation of the RhoA pathway. This, in turn, disrupts actin cytoskeleton and actin-dependent cell functions, such as the recycling of CX3CR1 receptors, which target the macrophages into the transplanted organs (Figs. 3c, d, 5). These data suggest that the effects of siponimod on the RhoA and CX3CR1 pathway might be relevant to the development of the novel antirejection therapies in clinical transplantation. Our recent (unpublished) studies showed that the FTY720 inhibits chronic rejection in the mouse and rat cardiac transplantation model. On the basis of this studies, we are starting the clinical trials, in which FTY720, added to the routine immunosuppression protocol, will be tested for its effectiveness in the inhibition of chronic rejection in the transplanted kidney patients. In addition, the expression level of RhoA and the level of the surface expression of the CX3CR1 receptors in the macrophages isolated from the transplanted patients' blood, and/or assessed in the transplanted organ biopsies could be very useful as the potential markers for the prediction of the transplant survival and the progress/inhibition of the chronic rejection.

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Compliance with ethical standards

Conflict of interest None of the authors has a conflict of interests.

References

Anastasiadou S, Knöll B (2016) The multiple sclerosis drug fingolimod (FTY720) stimulates neuronal gene expression, axonal growth and regeneration. Exp Neurol 279:243–260

Aydin M, Downing K, Villegas G et al (2010) The sphingosine-1-phosphate pathway is upregulated in response to partial urethral obstruction in male rats and activates RhoA/Rho-kinase signalling. BJU Int 106:562–571

Behrangi N, Fischbach F, Kipp M (2019) Mechanism of siponimod: anti-inflammatory and neuroprotective mode of action. Cells 8:E24

Bos JL, Rehmann H, Wittinghofer A (2007) GEFs and GAPs: critical elements in the control of small g proteins. Cell 129:865–877

Cannavo A, Liccardo D, Komici K et al (2017) Sphingosine kinases and sphingosine 1-phosphate receptors: signaling and actions in the cardiovascular system. Front Pharmacol 8:556

Chen W, Li XC, Kubiak JZ et al (2017) Rho-specific Guanine nucleotide exchange factors (Rho-GEFs) inhibition affects macrophage phenotype and disrupts Golgi complex. Int J Biochem Cell Biol 93:12–24

Chen W, Chen S, Chen W et al (2018a) Screening RhoA/ROCK inhibitors for the ability to prevent chronic rejection of mouse cardiac allografts. Transpl Immunol 50:15–25

Chen W, Ghobrial RM, Li XC et al (2018b) Inhibition of RhoA and mTORC2/Rictor by Fingolimod (FTY720) induces p21-activated kinase 1, PAK-1 and amplifies podosomes in mouse peritoneal macrophages. Immunobiology 223:634–647

Chiba K, Matsuyuki H, Maeda Y et al (2006) Role of sphingosine 1-phosphate receptor type 1 in lymphocyte egress from secondary lymphoid tissues and thymus. Cell Mol Immunol 3:11–19

Chiba K, Kataoka H, Seki N et al (2011) Fingolimod (FTY720), the sphingosine 1-phosphate receptor modulator, as a new therapeutic drug in multiple sclerosis. Inflamm Regen 31:167–173

Haga RB, Ridley AJ (2016) Rho GTPases: Regulation and roles in cancer cell biology. Small GTPases 7:207–221

Huwiler A, Zangemeister-Wittke U (2018) The sphingosine 1-phosphate receptor modulator fingolimod as a therapeutic agent: recent findings and new perspectives. Pharmacol Ther 185:34–49

Kloc M, Ghobrial RM (2014) Chronic allograft rejection: a significant hurdle to transplant success. Burns Trauma 2:3–10

Kloc M, Li XC, Ghobrial RM (2014) RhoA cytoskeletal pathway to transplantation. J Immunol Clin Res 2:1012

Kremer D, Akkermann R, Kury P et al (2019) Current advancements in promoting remyelination in multiple sclerosis. Mult Scler 25:7–14

Li MH, Harel M, Hla T et al (2014) Induction of chemokine (C–C motif) ligand 2 by sphingosine-1-phosphate signaling in neuroblastoma. J Pediatr Surg 49:1286–1291

Liu Y, Tejpal N, You J et al (2016a) ROCK inhibition impedes macrophage polarity and functions. Cell Immunol 300:54–62

Liu Y, Kloc M, Li XC (2016b) Macrophages as effectors of acute and chronic allograft injury. Curr Transplant Rep 3:303–312

Liu Y, Chen W, Minze LJ et al (2016c) Dissonant response of M0/M2 and M1 bone marrow derived macrophages to RhoA pathway interference. Cell Tissue Res 366:707–720

Liu Y, Minze LJ, Mumma L et al (2016d) Mouse macrophage polarity and ROCK1 activity depend on RhoA and non-apoptotic Caspase 3. Exp Cell Res 341:225–236

Liu Y, Chen W, Wu C et al (2017) Macrophage/monocyte-specific deletion of RhoA down-regulates fractalkine receptor and inhibits chronic rejection of mouse cardiac allografts. J Heart Lung Transplant 36:340–354

Mahajan-Thakur S, Bien-Möller S, Marx S et al (2017) Sphingosine 1-phosphate (S1P) signaling in glioblastoma multiforme—a systematic review. Int J Mol Sci 18:E2448

Ohki S, Iizuka K, Ishikawa S et al (2001) A highly selective inhibitor of Rho-associated coiled-coil forming protein kinase, Y-27632, prolongs cardiac allograft survival of the BALB/c-to-C3H/He mouse model. J Heart Lung Transplant 20:956–963

Pan S, Gray NS, Gao W et al (2013) Discovery of BAF312 (Siponimod), a potent and selective S1P receptor modulator. ACS Med Chem Lett 4:333–337

Parri M, Chiarugi P (2010) Rac and Rho GTPases in cancer cell motility control. Cell Commun Signal 8:23

Poniatowski LA, Wojdasiewicz P, Krawczyk M et al (2017) Analysis of the role of CX3CL1 (Fractalkine) and its receptor CX3CR1 in traumatic brain and spinal cord injury: insight into recent advances in actions of neurochemokine agents. Mol Neurobiol 54:2167–2188

Quarta S, Camprubí-Robles M, Schweigreiter R et al (2017) Sphingosine-1-phosphate and the S1P3 receptor initiate neuronal retraction via RhoA/ROCK associated with CRMP2 phosphorylation. Front Mol Neurosci 10:317

Sun XJ, Chen M, Zhao MH (2018) Rho GTPases are involved in S1P-enhanced glomerular endothelial cells activation with anti-myeloperoxidase antibody positive IgG. J Cell Mol Med 22:4550–4554

Wojdasiewicz P, Poniatowski LA, Kotela A et al (2014) The chemokine CX3CL1 (fractalkine) and its receptor CX3CR1: occurrence and potential role in osteoarthritis. Arch Immunol Ther Exp 62:395–403

Zhang L, Kloc M, Tejpal N et al (2012) ROCK1 inhibitor abrogates chronic rejection in rat cardiac model system. Open J Organ Transp Surg 2:46–51

Zhou C, Ling MT, Kin-Wah Lee T et al (2006) FTY720, a fungus metabolite, inhibits invasion ability of androgen-independent prostate cancer cells through inactivation of RhoA-GTPase. Cancer Lett 233:36–47