S1P and the birth of platelets

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Recent work has highlighted the multitude of biological functions of sphingosine 1-phosphate (S1P), which include roles in hematopoietic cell trafficking, organization of immune organs, vascular development, and neuroinflammation. Indeed, a functional antagonist of $S1P_1$ receptor, FTY720/Gilenya, has entered the clinic as a novel therapeutic for multiple sclerosis. In this issue of the *JEM*, Zhang et al. highlight yet another function of this lipid mediator: thrombopoiesis. The $S1P_1$ receptor is required for the growth of proplatelet strings in the bloodstream and the shedding of platelets into the circulation. Notably, the sharp gradient of S1P between blood and the interstitial fluids seems to be essential to ensure the production of platelets, and S1P appears to cooperate with the CXCL12–CXCR4 axis. Pharmacologic modulation of the $S1P_1$ receptor altered circulating platelet numbers acutely, suggesting a potential therapeutic strategy for controlling thrombocytopenic states. However, the $S1P_4$ receptor may also regulate thrombopoiesis during stress-induced accelerated platelet production. This work reveals a novel physiological action of the S1P/$S1P_1$ duet that could potentially be harnessed for clinical translation.

S1P and immune/hematopoietic cell trafficking

The lipid mediator S1P has received significant attention as an extracellular factor that signals via G protein–coupled receptors to regulate lymphocyte trafficking and vascular function (Blaho and Hla, 2011; Cyster and Schwab, 2012; Obinata and Hla, 2012). It is now established that an S1P gradient exists between vascular and nonvascular compartments (Schwab et al., 2005; Hla et al., 2008). High levels of S1P are found in blood and lymph, whereas the actions of degradative enzymes in tissue compartments such as secondary lymphoid organs keep its concentration low. This S1P gradient is necessary for the directional egress of lymphocytes from secondary lymphoid organs and the thymus into the circulatory system. Interference with the gradient by pharmacologic or genetic manipulation of S1P lyase (Schwab et al., 2005), genetic knockout of sphingosine kinases (Pham et al., 2010), S1P transporter (Spns2; Fukuhara et al., 2012), or the lipid phosphate phosphatase-3 (LPP3; Bréart et al., 2011) results in the attenuation of lymphocyte egress, and lymphopenia. Similar mechanisms may also be operative in the trafficking of dendritic cells, NKT cells, and hematopoietic progenitor cells (Massberg et al., 2007; Cyster and Schwab, 2012). These findings attest to the generality of the S1P gradient-dependent trafficking paradigm for lymphocytes and other hematopoietic cells.

Detailed investigations of S1P receptors have also shown that $S1P_1$ receptor on immune cells is required for ligand-dependent egress (Grigorova et al., 2009; Allende et al., 2010). The rate of egress is determined by the net effect between retention signals and egress signals. In the case of lymph node–resident T cells, a key retention signal is determined by the chemokine CCL21 signaling via CCR7 (Pham et al., 2008). Activation of $S1P_1$ by S1P appears to be the only egress signal identified to date. Intravital two-photon fluorescence microscopy studies have shown that $S1P_1$ signaling on immune cells allows probing of the endothelial lining of cortical sinuses with cellular processes that ultimately allows productive egress. The endothelial $S1P_1$ receptor appears to be dispensable for egress, whereas plasma membrane residence of $S1P_1$ on lymphocytes is one of the key factors that determine egress rates (Thangada et al., 2010). It is likely that such mechanisms are applicable to many situations in which hematopoietic cells traffic into the S1P-rich environments via trans-endothelial egress.

S1P and platelets

Early work identified that S1P is released by activated platelets stimulated with thrombin or ADP (Yatomi et al.1995, 1997). Platelets carry endothelial cell-protective cargo (trophogens) such as platelet-derived growth factor (PDGF), vascular endothelial growth factor, CXCL12, fibroblast growth factor (FGF), and stem cell factor, among others, and thrombocytopenic states lead to vascular endothelial dysfunction and breach of vascular barrier (Nachman and Rafii, 2008). S1P seems to "nourish" the endothelium, supporting the integrity of the vascular bed by activating endothelial $S1P_1$ receptors. In addition, platelets also express S1P receptors; however, their role in platelet biology has remained elusive.

Zhang et al. (2012) report that S1P signaling via its multifunctional receptor $S1P_1$ is important in platelet production from megakaryocytes. Even though multiple S1P receptors are expressed in megakaryocytes (i.e., $S1P_{1,2,4}$), $S1P_1$ is unique in that it is required for two specific events important in platelet formation and release.

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The positioning of megakaryocytes to the endothelial lining of the bone marrow sinusoids via the VCAM1/VLA4 adhesion pair is known to be critical for thrombopoiesis (Hamada et al., 1998; Majka et al., 2000; Avecilla et al., 2004; Schulze et al., 2006). In $S1P_1$ knockout megakaryocytes, the positioning itself was not altered; however, the directional migration of proplatelet-containing cytoplasmic extensions into the circulatory compartment was inhibited. These data, coupled with in vitro studies using S1P gradients, suggest that compartmentalized $S1P_1$ signaling is important for directional growth of proplatelet-containing megakaryocyte processes. $S1P_1$ signaling is also required for the shedding of proplatelets in a Rac-dependent manner. Because it is well established that $S1P_1$ couples to the G_i-dependent Rac activation (Lee et al., 2001), the findings suggest that active signaling by this S1P receptor is required to complete the final stages of thrombopoiesis (Fig. 1). The importance of this pathway was demonstrated in the hematopoietic-specific *S1pr1* knockout mice, which showed severe thrombocytopenia.

These findings also highlight the cooperative action of different GPCRs in megakaryocytes in ensuring optimal thrombopoiesis. Previous studies have determined that endothelial cell expression of CXCL12 and its action on megakaryocytes via CXCR4 GPCR is important for the interaction and positioning of the mature megakaryocytes in their proper vascular niche (Avecilla et al., 2004). Indeed, provision of CXCL12 and FGF-4 (another endothelial-active cytokine; Konishi et al., 1996) was able to support platelet formation even in thrombopoietin knockout mice. Thus, CXCR4 supports megakaryocyte interaction and positioning at the vascular niche, whereas $S1P_1$ supports polarized proplatelet process formation and release into the circulation.

The intracellular signaling mechanisms used by CXCR4 in megakaryocytes to allow interaction with endothelial cells are not well understood. The CXCR4 receptor can activate multiple G proteins, such as G_i, G_q, and $G_{12/13}$ (Alkhatib, 2009). How such pathways lead to endothelial–megakaryocyte interactions is not well understood. However, $S1P_1$ is known to activate the Gi pathway exclusively (Windh et al., 1999). This results in activation of Rac-dependent cortical actin assembly (Lee et al., 2001). In addition to inducing actin cytoskeleton rearrangement, the $S1P_1$–Rac pathway also potently induces microtubule dynamics (Paik et al., 2004; Obinata and Hla, 2012). Therefore, $S1P_1$-dependent Rac activation is critical for process extension and the release of proplatelets. Indeed, a small molecule inhibitor of Rac potently blocked platelet release.

A previous study examined $S1P_4$, another megakaryocyte-expressed S1P receptor that possesses different signaling properties (Golfier et al., 2010). This receptor is strongly induced in megakaryocyte differentiation, but upon gene deletion, platelet numbers were not altered. However, stress-induced thrombopoiesis was slightly delayed in *S1pr4* KO mice in this study, suggesting a possible function under accelerated platelet generation. Further, a significant number of *S1pr4* KO megakaryocytes exhibited abnormal cellular morphology characterized by cytoplasmic vacuolation and nuclear ploidy changes. In contrast, *S1pr4* KO megakaryocytes did not exhibit alterations in proplatelet generation in vitro. Thus, $S1P_4$ may also have a role in thrombopoiesis, even though its exact significance in physiological and stress-induced thrombopoiesis needs further elucidation.

Recent studies also show that $S1P_1$ is intimately involved in flow-dependent signal transduction in the endothelium (Jung et al., 2012). In vascular endothelial cells, $S1P_1$ is necessary for shear stress–induced signaling events, which culminate in the stabilization of newly formed vascular networks (Gaengel et al., 2012; Jung et al., 2012). Notably, $S1P_1$ GPCR

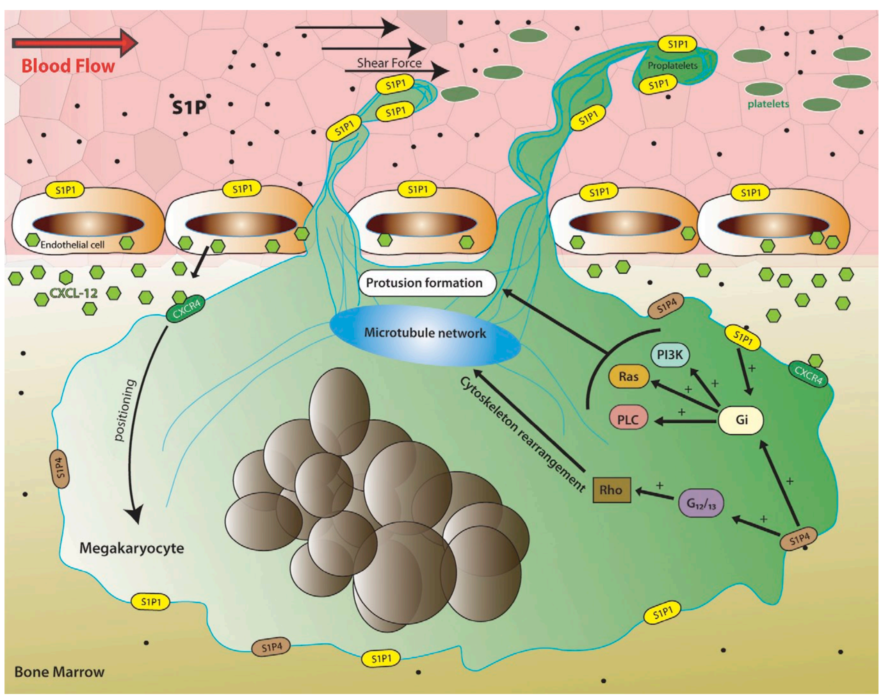

Figure 1. $S1P_1$ receptor on megakaryocytes is required for thrombopoiesis. The $S1P_1$ receptor, which activates the G_i protein, Ras GTPase, PI-3-kinase (PI3K), and phospholipase C (PLC) pathways, regulates the formation of proplatelet-containing cytoplasmic protrusions and release of platelet fragments. CXCR4 expression is required to position mature megakaryocytes in the appropriate vascular niche for platelet formation, and $S1P_1$ receptor is essential for process formation and proplatelet release. Both actin-based and microtubule cytoskeleton changes may be required for such events, which are likely to require both plasma-derived S1P and shear forces exerted by blood flow. The $S1P_4$ receptor may also regulate thrombopoiesis because it is also highly expressed in megakaryocytes. However, endothelial $S1P_1$ is essential for vascular stability and homeostasis.

can signal in response to laminar shear stress in a ligand-independent manner. Either the $S1P_1$ GPCR itself contains a mechanosensitive domain or is capable of associating with a mechanosensor, thus promoting signal transduction in a ligand-independent manner (Jung et al., 2012). Therefore, it is possible that proplatelet release from the transendothelial processes into the circulation requires S1P-dependent and flow-dependent mechanisms.

S1P therapeutics and potential modulation of platelet biology

The ability of the S1P–$S1P_1$ axis to regulate immune cell trafficking was harnessed in the novel treatment paradigm for multiple sclerosis (MS), in which autoreactive immune cells migrate into the central nervous system (CNS) and destroy myelin-containing axons, leading to astrogliosis and neuronal deficit. Treatment of MS patients with the S1P1 receptor antagonist Fingolimod (a.k.a., FTY720/Gilenya) resulted in the disruption of normal trafficking patterns as indicated by the reduced numbers of circulating central memory type T cells that are IL-17^+ (LaMontagne et al., 2006; Brinkmann et al., 2010; Cohen et al., 2010). In mouse models of experimental autoimmune encephalomyelitis, $S1P_1$ receptor inhibitors induced profound lymphopenia and reduced penetration of inflammatory cells into the CNS (Chun and Hartung, 2010). Fingolimod and related $S1P_1$ receptor-targeting drugs are functional antagonists; even though they act as agonists upon initial binding to $S1P_1$, they induce irreversible receptor internalization, resulting in a reduced plasma membrane residence of $S1P_1$ (Oo et al., 2011). Zhang et al. (2012) show that FTY720 administration causes a rapid increase in platelet numbers in mice, suggesting that acute agonistic action of FTY720 on the megakaryocyte $S1P_1$ receptors induced platelet release. Thus, it may be possible to therapeutically regulate platelet deficiencies by targeting $S1P_1$. However, because this pathway influences immune cell trafficking and vascular endothelial cell function, mechanism-based potential adverse events should be considered for translational approaches.

Several clinical conditions are associated with thrombocytopenia. In various infectious conditions, such as sepsis and Dengue hemorrhagic fever, platelet counts are markedly reduced and pose a significant risk for hemorrhage. Thus, in many hematological malignancies, as well as after administration of bone marrow cytotoxic therapies, the ability to increase platelet counts acutely may be useful. Thus, activation of this pathway with a long-lasting agonist of $S1P_1$ may be beneficial not only in increasing platelet counts but also in preserving endothelial function. It is important to note that current $S1P_1$ receptor modulators act as functional antagonists because of their ability to induce irreversible receptor internalization (Oo et al., 2011). In this scenario, such compounds are unlikely to be effective inducers of platelet formation. Thus, a new generation of $S1P_1$ agonists will need to be developed to therapeutically harness this system. Because the S1P pathway is involved in the terminal steps of thrombopoiesis, it is likely that $S1P_1$ agonists will have to be used in conjunction with other inducers of thrombopoiesis, for example, thrombopoietin, a key megakaryocyte differentiation factor.

These recent findings of Zhang et al. (2012) have highlighted a novel function of the lipid mediator S1P that signals through its multifunctional receptor $S1P_1$. This pathway may be potentially useful in therapeutic modulation of thrombocytopenia. However, because of the multitude of biological systems that $S1P_1$ regulates, any therapeutic strategy will need to consider possible adverse events. Novel agents that selectively target $S1P_1$ receptors in a cell- or tissue-specific manner will likely be needed to fully harness this potential translational opportunity.

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