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Plugging Vascular Leak by Sphingosine Kinase From Bone Marrow Progenitor Cells

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The bone marrow is a rich reservoir of cells that are mobilized in response to physiological stress signals (hypoxia, inflammation) or pathological states (cancer, chronic inflammation).[1] Although mobilization of myeloid cells that participate in innate immune responses and inflammation has been appreciated for some time, recent studies have focused on so-called bone marrow–derived progenitor cells (BMPCs).[1] It is now appreciated that distinct progenitor population, which can differentiate into cells with both myeloid and vascular markers, exit the bone marrow and enter into tissues in response to chemokine cues. Indeed, BMPCs may circulate widely in the body; recent work has shown that sphingosine 1-phosphate (S1P) signaling is used as a egress signal for these cells to reenter the lymphatic system to eventually return to the bone marrow.[2] Once in the tissue, BMPCs respond to extracellular signals to differentiate into cells that are closely associated with the vascular system. Although opinions are divided whether BMPCs are incorporated into the vascular tree or are perivascular, it is clear that their presence is functionally important for inflammation, neoplasia and angiogenesis.[3] Indeed, several clinical trials are currently testing the efficacy of bone marrow cell therapy in ischemic tissues.[4,5]

Such bone marrow–derived cells modulate vascular and tissue responses by elaborating cytokines, chemokines and lipid mediators.[1,3] Interestingly, the lipid mediator S1P has been the subject of recent interest as a regulator of vascular and immune systems.[6] It is produced by the metabolism of sphingomyelin, an abundant phospholipid essential for the formation of membrane domains such as rafts and caveolae.[7] Sphingomyelinase hydrolyzes sphingomyelin to form ceramide, which is further degraded by ceramidase to form sphingosine. Sphingosine levels are kept low because sphingosine kinase (Sphk) catalyzes the phosphorylation into S1P. Subsequently, S1P lyase catalyzes its irreversible degradation of S1P into hexadecenal and phosphoethanolamine, intermediates in the biosynthesis of phospholipids. Many of the steps in the S1P metabolic pathway are reversible. For example, S1P is converted by S1P phosphatase enzymes into sphingosine, which itself is converted back into ceramide by ceramide synthases and eventually into sphingomyelin by sphingomyelin synthases. S1P is released into the extracellular environment by various cells, including those of the hematopoietic system.[8] It is highly enriched in the circulatory and lymphatic systems but is lower in interstitial fluids of tissues, thus creating a gradient. This gradient is essential as a chemotactic cue in the traverse of various hematopoietic cells into lymphatic and vascular channels.[9]

S1P exerts powerful effects on the vascular endothelium.[6] The prototypical S1P receptor ($S1P_1$) was originally cloned as an abundant and inducible mRNA from human endothelial cells.[10] Activation of S1P receptors in endothelial cells result in the redistribution of adherens junction proteins into areas of cell–cell contact and the their assembly. For example, treatment of human endothelial cells in vitro with S1P results in the rapid translocation of VE-cadherin, β-catenin, and α-catenin to areas of cell–cell contact.[11] Indeed Triton X-100 solubility of adherens junction proteins decreases with S1P treatment, suggesting that signaling from S1P receptors induce the assembly of adherens junction in endothelial cells. This signaling event requires the heterotrimeric G_i protein and small GTPases Rac and Cdc42.[11,12] This effect of S1P (via the $S1P_1$ receptor) results in tightening of junctions and increase in transmonolayer electric resistance in vitro.[13] Furthermore, acute agonism of $S1P_1$ with the pharmacological agent FTY720 results in profound suppression of vascular endothelial growth factor–induced vascular permeability in vivo.[14] In addition, S1P treatment suppressed lipopolysaccharide-induced pulmonary vascular permeability in canine and murine models.[15] Indeed, inhibition of $S1P_1$ signaling with a specific pharmacological antagonist resulted in the increased vascular permeability in the lung and the skin tissues.[16] Moreover, a recent study using conditional Sphk-knockout mice showed that reduction in plasma S1P resulted in increased basal vascular permeability in the lung and decreased survival during platelet activating factor-induced anaphylactic shock.[17] These data form the basis for the emerging concept that tonic signaling of the endothelial cell $S1P_1$ receptor is needed for maintenance of the homeostatic barrier property of the vascular system. In addition, during infection and inflammation, $S1P_1$ receptor system is required for the restoration of normal vascular barrier function. This is clinically important because increased fluid retention in the lung caused by abnormal vascular permeability is a significant clinical problem in infectious diseases.

Given the importance of S1P signaling in pulmonary edema, in this issue of *Circulation Research*, Zhao et al report the role of Sphk enzyme in the BMPCs in the restoration of vascular permeability in a murine model of lipopolysaccha-

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ride-induced lung injury.[18] They show that intravenous therapy with BMPCs improved pulmonary edema and lethality from lipopolysaccharide. Interestingly, BMPCs from *Sphk1*$^{-/-}$ mice did not provide similar protection, suggesting that secretion of S1P from the BMPCs in the local environment and activation of $S1P_1$ receptors on the pulmonary vasculature protected the lung tissue from excessive vascular leak. In support of this, the authors show that BMCP from wild-type mice secreted S1P and induced transendothelial monolayer resistance in a $S1P_1$-dependent manner. Indeed, the ability of BMPCs to induce adherens junctions in endothelial cell monolayers required signaling by small GTPases Rac and Cdc42, which is very similar to signal transduction pathways induced by S1P-induced activation of its receptor on endothelial cells.[12] These data are of importance in the understanding of pathogenesis of pulmonary edema induced during infections. In addition, from a therapeutic standpoint, activation of $S1P_1$ receptors by agonists should aid in the alleviation of pulmonary vascular leak. Indeed, S1P receptor modulators have been tested for potential clinical application in the control of autoimmune reactions in multiple sclerosis.[19] However, the prototypical S1P receptor modulator, FTY720 has a complex mode of action: although it is a potent agonist on $S1P_1$, it acts as a functional antagonist because it downregulates $S1P_1$ receptors and induces ubiquitinylation-dependent proteosomal degradation of the receptor.[6] Thus, an agonist of $S1P_1$ that does not induce receptor downregulation or degradation is needed to inhibit pathological vascular permeability in the lung. In the absence of such a pharmacological tool, BMPC therapy appears promising.

Despite these promising findings, data in the report suggest that more complex mechanisms may be at play. Characterization of BMPCs from wild-type and *Sphk1*$^{-/-}$ mice indicated significant differences in cell surface marker expression. Interestingly, progenitor/stem cell markers were reduced and differentiated vascular and hematopoietic markers were elevated in *Sphk1*$^{-/-}$ BMPCs, suggesting that lack of Sphk1 enzyme facilitates cellular differentiation. Indeed, work from the laboratory of Gamble has shown that Sphk1 regulates the rate of endothelial progenitor cell differentiation.[20] The mechanism by which Sphk1 regulates renewal and/or differentiation of BM stem cells and progenitors is not known, but G protein–coupled receptor–independent mechanisms may be involved. Indeed, because Sphk1 occupies a central position in the sphingolipid metabolic pathway, lack of this enzyme may alter cell fate in rapidly proliferating progenitor populations that require tight control of membrane turnover. Alternatively, intracellular signaling function of sphingolipid metabolites, such as sphingosine, ceramide or S1P itself may be involved. Thus, functional differences in BMPCs from wild-type versus *Sphk1*$^{-/-}$ mice may be involved in the differential protective functions of these two populations. Second, Zhao et al noted that pulmonary retention of wild-type and *Sphk1*$^{-/-}$ BMPCs are very different. Although it is not known why injected *Sphk1*$^{-/-}$ BMPCs are not retained in the lungs, differential cellular adhesion or survival may be responsible. Thus, in addition to released S1P, phenotypic alterations between BMPCs from wild-type and *Sphk1*$^{-/-}$ mice may explain the differences in pulmonary protection.

Non-standard Abbreviations and Acronyms	
BMPC	bone marrow–derived progenitor cell
S1P	sphingosine 1-phosphate
Sphk	sphingosine kinase

Several critical questions are highlighted by the interesting results of Zhao et al. For example, how is Sphk1 activated in BMPCs? What is the functional role of Sphk1 in BMPCs? What cytokines are elaborated by *Sphk1*$^{-/-}$ BMPCs and does S1P signaling play a role in migration and/or egress of BMPCs in the lung? Ultimately, one would like to know if BMPCs are recruited physiologically during infection to help restore pulmonary microvascular function. Nevertheless, these results also suggest exciting opportunities for BMPC-based therapies to control infectious diseases. In addition, Sphk1 and $S1P_1$ function were shown to be critical for human ES cell proliferation.[21] Therefore, this system may be a fundamental signaling pathway in stem/progenitor cell biology.

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Disclosures

None.

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