The Enigma of Sphingosine 1-Phosphate Synthesis
A Novel Role for Endothelial Sphingosine Kinases

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Sphingosine 1-phosphate (S1P) is well known as a key sphingolipid messenger molecule in the cardiovascular system, yet many fundamental features of its synthesis, transport, and action remain enigmatic. Indeed, the very name “sphingolipid” was coined to reflect the mysterious characteristics of this class of lipids. It is now well established (through the work of Timothy Hla and of many others subsequently) that S1P binds to and activates a family of G protein–coupled S1P receptors located in vascular endothelial cells, cardiac myocytes, blood platelets, and vascular smooth muscle cells (among other cell types) and elicits a broad range of physiological responses (reviewed elsewhere). In vascular endothelial cells, S1P elicits such diverse responses as cell survival, proliferation, angiogenesis, cell migration, permeability, and endothelial NO synthase activation. The intracellular signaling pathways stimulated by S1P have been extensively characterized, yet the cellular origins of S1P and the pathways for its transport and action in cardiovascular tissues remain incompletely understood. The concentration of S1P in normal human plasma is within the range of several hundred nanomolar, and this amphipathic lipid is highly protein bound, mostly to HDL and albumin. Importantly, S1P receptors bind their ligand with an affinity in the nanomolar range; therefore, many important questions remain. What controls S1P binding to plasma proteins? Where does all this plasma S1P come from? Important clues to the latter question are provided in a recent report from the laboratory of Timothy Hla, published in this issue of Circulation Research and reporting the novel and important discovery: the vascular endothelium is an important source of plasma S1P through the actions of sphingosine kinases (SphKs) in endothelial cells. For many years, conventional wisdom held that blood platelets represent the principal source for S1P in the plasma. Elegant studies by Igarashi (no relation to J.I.) showed that blood platelets contain abundant quantities of sphingosine kinase, the key enzyme involved in synthesizing S1P from sphingosine, and many laboratories have shown that blood platelets can secrete S1P following platelet activation by thrombin and other ligands. Moreover, platelets are characterized by having a low activity of S1P lyase, a key enzyme involved in S1P degradation. Thus, blood platelets contain abundant S1P, and can secrete the lipid in response to agonist stimulation. So convincing were these observations that S1P was dubbed by many investigators, including ourselves, as a “platelet-derived lipid mediator.” There is no doubt that platelets do contain and secrete S1P, but are they the sole, or even the principal, source of S1P in plasma? Recent research suggests that the answer is no. Indeed, a recent article reported that erythrocytes are a major source of plasma S1P. Venkataraman et al. used multiple complementary experimental approaches to test their hypothesis that endothelial cells are a key source of plasma S1P. They first explored the half-life of plasma S1P using a new 3H-labeled synthetic reagent C17-S1P. This lipid differs slightly from endogenous S1P (C18-S1P), and these 2 lipid species are readily distinguishable in high-performance liquid chromatographic analysis, permitting the authors to determine the abundance of the exogenous 3H-labeled C17-S1P following injection of the compound into the mouse tail vein. The authors estimated the t1/2 of plasma C17-S1P to be on the order of ~15 minutes, suggesting the presence of highly active S1P-producing and -degrading pathways within the body. The authors next analyzed plasma S1P levels in mice that had targeted deletions of the critical S1P synthetic enzyme sphingosine kinase, of which there are 2 mammalian isoforms (SphK1 and SphK2). To test the contribution of hematopoietic cells to plasma S1P, the authors performed bone marrow transplant experiments in which marrow from wild-type mice was transfused into gene-targeted SphK1/SphK2-deficient mice (these mice were engineered to have a homozygous deletion of SphK1 but were heterozygous for SphK2 deletion because complete deletion of all 4 SphK alleles is embryonic lethal). The authors subjected either wild-type or SphK-deficient mice to whole body irradiation to eliminate hematopoietic cells, then transfused bone marrow derived from mice having the other genotype into the irradiated animals. At baseline, the mice that were genetically deficient in SphK had lower plasma S1P levels, and transfusion of wild-type cells to these mice was able to rescue plasma S1P levels, as expected. However, to the surprise of the authors, the plasma S1P concentration of wild-type mice did not decrease after total body irradiation and remained at normal levels following transfusion of bone marrow from the SphK-deficient animals. The authors also found that pharmacological treatments that cause anemia or thrombocytopenia similarly failed to attenuate plasma S1P levels. So, where is the S1P coming from?

These observations raised the possibility that some other nonhematopoietic cell type must produce sufficient S1P such

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that plasma S1P levels are maintained in the face of anemia and thrombocytopenia, or even in the presence of transplanted blood components lacking SphK. Suspicions immediately fell on vascular endothelial cells as an alternative source of S1P. The authors and others have previously shown that cultured vascular endothelial cells are able to express SphK1 and can release S1P into the culture media.\(^1\) Infection of Sphk1-null mice with SphK1-expressing adenovirus led to expression of Sphk1 protein in vascular endothelial cells, as well as hepatocytes, and to the restoration of plasma S1P levels. The authors favor the hypothesis that it is the endothelial cells, rather than the hepatocytes, that are making S1P because cultured hepatocytes do not synthesize or release much S1P, but cultured endothelial cells do. This plausible hypothesis gains even greater credence from the experiments of the authors showing that laminar shear stress leads to an increase in S1P release from cultured endothelial cells, associated with downregulation in the abundance of the S1P-degrading enzymes S1P lyase and S1P phosphatase.

Taken together, the results in the report by Venkataraman et al\(^3\) quite convincingly establishes that vascular endothelial cells are an important source of plasma S1P. However, several limitations in this study should be considered. The specific quantitative contribution of endothelium-derived S1P may have been more rigorously established by studying mice in which endothelial S1P synthesis was completely abrogated by endothelial cell-specific targeting of the SphK1 and SphK2 genes. A cell type–specific knockout would be essential for these studies because the homozygous SphK1-null/SphK2-null mice are not viable.\(^6\) Another concern relates to the use of the S1P analog C17-S1P to determine the half-life of plasma S1P in vivo because it is not clear that this analog and endogenous S1P behave equivalently as the substrates of S1P-metabolizing enzymes. Also confusing the analyses of C17-S1P half-life that were performed by the authors is the use in these studies of albumin as the S1P carrier: in normal plasma, S1P is found principally in HDL, with a smaller fraction of the lipid found in albumin or LDL.\(^8\) Indeed, major questions remaining in our understanding of S1P biology relate to the pathways that modulate S1P binding to plasma proteins. For example, there may be an important relationship between HDL-associated S1P and modulation of endothelial nitric oxide synthase activation.\(^9\) It also seems likely that there are important local pathways for the modulation of S1P metabolism at specific sites in the vasculature, which may be variously regulated by local hemodynamic forces, by the state of platelet aggregation, and by the influence of paracrine factors that modulate S1P responses in the vascular wall (Figure).

These studies also indicate that we need to seek a deeper understanding of the subcellular localization of SphK and other enzymes involved in S1P synthesis, as well as the targeting of some of these S1P-related enzymes is subjected to regulation by hemodynamic shear stress forces on these cells. Endothelial cells thus represent a major source of the plasma S1P pool, in addition to the S1P released from blood platelets or RBCs; these cell types had been previously thought to represent the principal sources of plasma S1P. As also shown in this figure, most of the S1P in plasma is protein-bound, complexed either with HDL or with albumin, as depicted at top right. Because the total plasma S1P concentration exceeds the binding constant of the ligand for S1P receptors, the release of S1P from its carrier proteins may represent a key point of control of S1P actions in vasculature. The factors that modulate S1P plasma protein dynamics are less well characterized, and elucidating the relevant regulatory pathways is essential to more fully understanding the vascular actions of S1P. Note also that within vascular endothelial cells, sphingolipids (including S1P) are mostly targeted to plasmalemmal caveolae, which are specialized flask-shaped signaling microdomains in which these lipids function both as substrates and products of the S1P-metabolizing enzymes. The S1P-metabolizing enzymes at least transiently need to associate with caveolae; however, the subcellular localization of these enzymes remains largely unknown.

Figure. S1P metabolism in the vascular wall and in blood. Shown is a schematic presentation of how vascular endothelial cells and blood cells may metabolize S1P, a key sphingolipid mediator of many fundamental vascular functions. The figure depicts how resulting S1P residing in the plasma may modulate S1P receptors at the endothelial cell surface and also shows S1P reversibly binding to albumin and HDL in the plasma. Shown at the bottom of the figure is a schematic of a vascular endothelial cell at its membrane interface with the vessel lumen. Endothelial cells contain enzyme activities that metabolize S1P and related lipid species. SphKs catalyze the phosphorylation of sphingosine (Sph) to produce S1P. S1P lyase and S1P phosphatase (P'ase) are enzymes that degrade S1P. The report by Venkataraman et al in this issue of Circulation Research shows that vascular endothelial cells produce S1P and contribute to a significant proportion of the plasma S1P pool; other sources of plasma S1P include blood platelets and red blood cells.\(^3\) Venkataraman et al also found that the abundance of at least...
S1P metabolites and S1P receptors and of S1P itself. In endothelial cells, the S1P1 receptor is dynamically targeted to sphingolipid-rich signal-transducing microdomains termed plasmalemmal caveolae (see Figure). Caveolae-targeted sphingolipids function both as substrates and products of the S1P-metabolizing enzymes, which at least transiently would need to associate with this subcellular organelle.5,6 S1P1 receptors are reversibly targeted to caveolae,7 where the caveolae resident protein caveolin-1 modulates S1P-dependent signaling,7 as well as other critical molecular constituents that influence cardiovascular signal transduction pathways.8,9 The report by Venkataraman et al provides key additional evidence for caveolae-targeted mechanochemical and receptor-modulated signaling pathways in the regulation of S1P responses in the vasculature. In uncovering a central role for the vascular endothelium as an important source for plasma S1P, this work stimulates new and provocative questions concerning the role and regulation of this enigmatic lipid mediator, sphingosine 1-phosphate.

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