

# Sphingosine 1-phosphate

Subjects: Biochemistry & Molecular Biology

Contributor: Markus Gräler

The breakdown of the endothelial cell (EC) barrier contributes significantly to sepsis mortality. Sphingosine 1-phosphate (S1P) is one of the most effective EC barrier-stabilizing signaling molecules. Stabilization is mainly transduced via the S1P receptor type 1 (S1PR1). Here, we demonstrate that S1P was autonomously produced by ECs. S1P secretion was significantly higher in primary human umbilical vein endothelial cells (HUVEC) compared to the endothelial cell line EA.hy926. Constitutive barrier stability of HUVEC, but not EA.hy926, was significantly compromised by the S1PR1 antagonist W146 and by the anti-S1P antibody Sphingomab. HUVEC and EA.hy926 differed in the expression of the S1P-transporter Spns2, which allowed HUVEC, but not EA.hy926, to secrete S1P into the extracellular space. Spns2 deficient mice showed increased serum albumin leakage in bronchoalveolar lavage fluid (BALF). Lung ECs isolated from Spns2 deficient mice revealed increased leakage of fluorescein isothiocyanate (FITC) labeled dextran and decreased resistance in electric cell-substrate impedance sensing (ECIS) measurements. Spns2 was down-regulated in HUVEC after stimulation with pro-inflammatory cytokines and lipopolysaccharides (LPS), which contributed to destabilization of the EC barrier. Our work suggests a new mechanism for barrier integrity maintenance. Secretion of S1P by EC via Spns2 contributed to constitutive EC barrier maintenance, which was disrupted under inflammatory conditions via the down-regulation of the S1P-transporter Spns2.

Keywords: S1P receptor ; inflammation ; S1P transporter ; spinster homolog 2 ; barrier dysfunction

---

## 1. Introduction

Endothelial cell (EC) barriers are important intercellular structures that regulate the movement of fluids and dissolved substances into tissues. Maintaining barrier function is particularly important at sites where fluids need to be efficiently separated from tissues such as the vasculature, lymph vessels, gut, brain, and lung <sup>[1]</sup>. Several different junctional complexes are involved in barrier formation, including tight and adherens junctions, gap junctions, and desmosomes <sup>[2]</sup>. Adherens junctions are formed by cadherins and nectins and provide a mechanical linker similar to zippers, while tight junctions are formed by claudins, occludin, and junctional adhesion molecules in the transmembrane regions and perform most of the sealing functions to prevent passage of fluids and molecules <sup>[3][4]</sup>. The bioactive sphingolipid signaling molecule sphingosine 1-phosphate (S1P) and its G protein-coupled receptor S1PR1 are critical mediators of adherens junction assembly <sup>[5]</sup>. The deletion of S1PR1 in ECs or the deletion of the two known S1P-producing sphingosine kinases (SphK1 and SphK2) in hematopoietic cells and ECs of adult mice result in severe disruption of the EC barrier <sup>[6][7][8]</sup>. Despite this apparent phenotype, the exact mechanism of barrier maintenance by S1P is still unknown. One of the most puzzling questions is of how high S1P concentrations in circulation that are sufficient to induce activation-induced internalization and desensitization of S1PR1 are able to constitutively maintain the vascular EC barrier. Two models were proposed as potential explanations <sup>[9]</sup>: (1) the static model postulating that there is constantly sufficient S1PR1 expression on the luminal cell surface of ECs even at high S1P concentrations, due to efficient receptor recycling and (2) the dynamic model suggesting that S1PR1 is only expressed on the tissue-facing side of vascular ECs which are activated by S1P leaking through the EC barrier and subsequently induce adherens junction assembly and EC barrier stabilization. In either case, reduced S1P leakage and decreased barrier stability occur until the amount of S1P leaking through the EC barrier increases again and starts a new cycle of EC barrier formation. The validity of either of these models has not yet been verified.

The collapse of the EC barrier is a life-threatening condition and a major severity factor in sepsis <sup>[9]</sup>. The S1P concentration in circulation decreases significantly during systemic inflammation <sup>[10][11][12][13]</sup>. Whether or not this observed decrease of S1P has something to do with the vascular EC barrier collapse is not known. Previous data indicate that even low amounts of S1P in plasma are sufficient to maintain S1P and S1PR1 mediated lymphocyte circulation <sup>[14]</sup>.

## 2. Specifics

Here, we show that, in vitro, ECs can autonomously produce and secrete S1P, rendering their ability to maintain EC barrier formation largely independent from exogenously added S1P. The S1P transporter Spinster homolog 2 (Spns2) plays a crucial role in the proper release of S1P into the extracellular space. Our work has uncovered an important function of Spns2 in ECs to regulate barrier stability. Spns2 deficient mice demonstrated significantly reduced EC barrier formation presumably due to the lack of S1P exportation from ECs. Furthermore Spns2, but not S1PR1, was down-regulated in ECs stimulated with lipopolysaccharides (LPS) and pro-inflammatory cytokines. Inflammation-induced EC barrier breakdown due to down-regulation of Spns2 resulted in decreased S1P release. Thus, decreased exportation of S1P from ECs due to reduced expression of Spns2 may contribute to EC barrier dysfunction during inflammation. This mechanism may be particularly important in sepsis, where inflammation-induced collapse of the EC barrier significantly contributes to increased morbidity and mortality. The observed stable expression of S1PR1 and the most likely local autocrine and paracrine activity of Spns2-released S1P points to local approaches for S1P supplementation in tissues rather than systemic alteration of S1P in circulating plasma as a potential medical intervention.

---

## References

1. Stevens, T.; Garcia, J.G.; Shasby, D.M.; Bhattacharya, J.; Malik, A.B. Mechanisms regulating endothelial cell barrier function. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 2000, 279, L419–L422. [Google Scholar] [CrossRef] [PubMed]
  2. Vasileva, E.; Citi, S. The role of microtubules in the regulation of epithelial junctions. *Tissue Barriers* 2018, 6, 1539596. [Google Scholar] [CrossRef] [PubMed]
  3. Campbell, H.K.; Maier, J.L.; DeMali, K.A. Interplay between tight junctions & adherens junctions. *Exp. Cell Res.* 2017, 358, 39–44. [Google Scholar] [PubMed]
  4. Hartsock, A.; Nelson, W.J. Adherens and tight junctions: Structure, function and connections to the actin cytoskeleton. *Biochim. Biophys. Acta* 2008, 1778, 660–669. [Google Scholar] [CrossRef]
  5. McVerry, B.J.; Garcia, J.G. Endothelial cell barrier regulation by sphingosine 1-phosphate. *J. Cell. Biochem.* 2004, 92, 1075–1085. [Google Scholar] [CrossRef]
  6. Allende, M.L.; Yamashita, T.; Proia, R.L. G-protein-coupled receptor s1p1 acts within endothelial cells to regulate vascular maturation. *Blood* 2003, 102, 3665–3667. [Google Scholar] [CrossRef]
  7. Burg, N.; Swendeman, S.; Worgall, S.; Hla, T.; Salmon, J.E. Sphingosine 1-phosphate receptor 1 signaling maintains endothelial cell barrier function and protects against immune complex-induced vascular injury. *Arthritis Rheumatol.* 2018, 70, 1879–1889. [Google Scholar] [CrossRef]
  8. Camerer, E.; Regard, J.B.; Cornelissen, I.; Srinivasan, Y.; Duong, D.N.; Palmer, D.; Pham, T.H.; Wong, J.S.; Pappu, R.; Coughlin, S.R. Sphingosine-1-phosphate in the plasma compartment regulates basal and inflammation-induced vascular leak in mice. *J. Clin. Invest.* 2009, 119, 1871–1879. [Google Scholar] [CrossRef]
  9. Schnoor, M.; Garcia Ponce, A.; Vadillo, E.; Pelayo, R.; Rossaint, J.; Zarbock, A. Actin dynamics in the regulation of endothelial barrier functions and neutrophil recruitment during endotoxemia and sepsis. *Cell. Mol. Life Sci.* 2017, 74, 1985–1997. [Google Scholar] [CrossRef]
  10. Coldewey, S.M.; Benetti, E.; Collino, M.; Pfeilschifter, J.; Sponholz, C.; Bauer, M.; Huwiler, A.; Thiemermann, C. Elevation of serum sphingosine-1-phosphate attenuates impaired cardiac function in experimental sepsis. *Sci. Rep.* 2016, 6, 27594. [Google Scholar] [CrossRef]
  11. Frej, C.; Linder, A.; Happonen, K.E.; Taylor, F.B.; Lupu, F.; Dahlback, B. Sphingosine 1-phosphate and its carrier apolipoprotein m in human sepsis and in Escherichia coli sepsis in baboons. *J. Cell. Mol. Med.* 2016, 20, 1170–1181. [Google Scholar] [CrossRef]
  12. Gomes, L.; Fernando, S.; Fernando, R.H.; Wickramasinghe, N.; Shyamali, N.L.; Ogg, G.S.; Malavige, G.N. Sphingosine 1-phosphate in acute dengue infection. *PLoS ONE* 2014, 9, e113394. [Google Scholar] [CrossRef] [PubMed]
  13. Winkler, M.S.; Nierhaus, A.; Holzmann, M.; Mudersbach, E.; Bauer, A.; Robbe, L.; Zahrt, C.; Geffken, M.; Peine, S.; Schwedhelm, E.; et al. Decreased serum concentrations of sphingosine-1-phosphate in sepsis. *Crit. Care* 2015, 19, 372. [Google Scholar] [CrossRef] [PubMed]
  14. Pappu, R.; Schwab, S.R.; Cornelissen, I.; Pereira, J.P.; Regard, J.B.; Xu, Y.; Camerer, E.; Zheng, Y.W.; Huang, Y.; Cyster, J.G.; et al. Promotion of lymphocyte egress into blood and lymph by distinct sources of sphingosine-1-phosphate. *Science* 2007, 316, 295–298.
-

