The endothelium, once thought of as a cellophane-like inner lining for blood vessels, is now known as a selective barrier between blood and the surrounding tissue. 1,2 Endothelial cells (ECs) specialize their barrier function, as in brain ECs, where the blood–brain barrier protects neurons against toxins, or in liver ECs, where they are highly fenestrated for efficient filtering of the blood. 3 Angiogenesis also relies on regulation of EC junctions. 4 Finally, barrier function plays a role in diseases such as atherosclerosis, where poor barrier maintenance allows increased monocyte extravasation. 5 The increase in monocyte extravasation leads to macrophage colonization and the eventual buildup of plaques.

Vessels With Cingulin Are Leakproof
Chris Givens, Ellie Tzima

The endothelium, once thought of as a cellophane-like inner lining for blood vessels, is now known as a selective barrier between blood and the surrounding tissue. 1,2 Endothelial cells (ECs) specialize their barrier function, as in brain ECs, where the blood–brain barrier protects neurons against toxins, or in liver ECs, where they are highly fenestrated for efficient filtering of the blood. 3 Angiogenesis also relies on regulation of EC junctions. 4 Finally, barrier function plays a role in diseases such as atherosclerosis, where poor barrier maintenance allows increased monocyte extravasation. 5 The increase in monocyte extravasation leads to macrophage colonization and the eventual buildup of plaques.

See accompanying article on page 647

ECs make use of both adherens junctions and tight junctions (TJ) to regulate their barrier function. The better characterized adherens junctions form the first cell–cell contacts and may even be required for TJ formation. 2 Vascular endothelial cadherin makes up the transmembrane component of endothelial adherens junctions, whereas β-, γ-, and p120-catenin, among other proteins, form the cytoplasmic component of adherens junctions. TJs, however, consist of the transmembrane occludins and claudins, with an intracellular plaque that consists of zonula occludens proteins and cingulin. 2 Cingulin has been shown to recruit the RhoGEF guanine nucleotide exchange factor H1 (GEFH1) to TJ, thereby downregulating the activity of RhoA and downstream signaling. 6 Although expression of cingulin in ECs is low, the recently identified role of GEFH1 in permeability 7 raises the intriguing possibility that cingulin is also important in regulation of barrier function. In this issue of ATVB, a study by Schossleitner et al 8 provides novel insights into the function of cingulin in ECs. They interrogate expression levels of cingulin in different vascular beds and, using gain- and loss-of-function approaches, show that cingulin modulates endothelial barrier function.

Schossleitner et al 1 first characterized cingulin expression in the vasculature, demonstrating specific cingulin expression in ECs of large- and small-sized arteries and veins in human skin, lung, and brain. Interestingly, no expression was detected in muscle or heart. In ECs in vitro, cingulin formed a complex with zonula occludens-1 and GEFH1, whereas solubility experiments revealed that a portion of cingulin is bound to the cytoskeleton. Overexpression of tagged-cingulin constructs in ECs negatively regulated expression of claudin-5, the main endothelial claudin that participates in TJ formation. The authors then examined cingulin-dependent regulation of endothelial barrier function. Using transmission electron microscopy, the authors found that overexpression of cingulin lead to increased TJ formation in ECs. Cingulin overexpression also reduced endothelial proliferation, presumably a result of increased junctional integrity in cingulin-overexpressing cells. This is supported by barrier function studies, which revealed increased barrier function after cingulin overexpression. Transcellular resistance was increased ≤4-fold in ECs-overexpressing cingulin. Permeability to solutes was also reduced in cingulin-overexpressing cells, where diffusion of 70000 and 376 Da solutes was reduced. Taken together, these data suggest that cingulin is a major regulator of endothelial barrier function.

To characterize endothelial phenotypes in the absence of cingulin, Schossleitner et al 9 used a cingulin knockout mouse missing exon 1 of cingulin, which encodes the head domain of the protein. To assess in vivo barrier function in cingulin knockout mice, the authors injected biotin into the tail vein. Streptavidin staining revealed increased uptake in Purkinje cells and neurons of the area postrema, suggesting reduced blood–brain barrier function. In addition, ECs isolated from the cingulin knockout mice showed increased permeability to solutes, further suggesting that cingulin is required for endothelial barrier function. Further studies revealed major mislocalization of phosphorylated GEFH1 and p114RhoGEF in knockout mice. Both of these GEFs are important regulators of RhoA function. These data suggest that cingulin may regulate barrier function not only through TJ maintenance but also through regulation of RhoA-dependent cellular contractility.

This study places cingulin as an important regulator of endothelial barrier function. Although the molecular mechanisms responsible are unclear, loss of cingulin resulted in increased permeability in vitro and in vivo. Perhaps as a mark of its import to the vascular biology community, this study raises as many questions as it answers. What is the precise role of cingulin in TJ formation and endothelial barrier function? Cingulin might be critical for the structure of TJs, or its role could be more indirect, resulting from cingulin-dependent sequestration of RhoA GEFs. In addition, the demonstrated negative regulation of claudin-5 by cingulin raises interesting questions. The blood–brain barrier in claudin-5 knockout mice shows a selective increase in permeability to solutes under 600 Da. These results mirror the selective increase in permeability in the cingulin knockout mice, but the connection between the 2 results, if any, remains unclear. Is cingulin required for physiological vascular processes, such as vasculogenesis or angiogenesis? What about vascular pathologies, where barrier...
function is often aberrant? Cingulin may have undiscovered roles in atheroprone endothelium, where aberrant barrier function is at the root of plaque development. Further studies must be done on cingulin to fully understand its role in endothelial biology. We have come a long way because the cellophane days of the endothelium, and we still have much to learn. Hopefully, discovering more about endothelial junctions will help to reveal better therapeutics for a range of vascular maladies.

**Sources of Funding**
This study was supported by National Institutes of Health (NIH) HL117256 and NIH T32 HL069768 and a Wellcome Trust Senior Fellowship (to E. Tzima).

**Disclosures**
None.

**References**

**KEYWORDS:** Editorials ■ adherens junctions ■ blood–brain barrier ■ occludin ■ tight junctions
Vessels With Cingulin Are Leakproof
Chris Givens and Ellie Tzima

Arterioscler Thromb Vasc Biol. 2016;36:584-585
doi: 10.1161/ATVBAHA.116.307238
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2016 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/36/4/584

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/