A key question in unlocking the mystery of accelerated atherosclerosis that often accompanies type 1 and 2 diabetes and leads to increased incidence and severity of heart attacks and strokes is, what are the precise biochemical and molecular signaling pathways that perturb the diabetic blood vessel leading to acceleration of vascular inflammation and atherosclerosis? Extensive evidence implicates elevated levels of glucose as a critical culprit in these phenomena. Beyond a critical threshold, it is apparent that increased glucose levels significantly exceed homeostatic requirements, and divert fundamental pathways leading to untoward nonphysiological effects in the cells.

Glucose may be dangerous, not solely because of its direct effects, but because of its long-term consequences. The studies of the Diabetes Control Clinical Trials (DCCT) and the Epidemiology of Diabetes Interventions and Complications Research (EDIC) Groups showed definitively that earlier rigorous glycemic control, despite eventual normalization of glucose levels compared with conventionally-treated type 1 diabetic subjects, exerted long-term protection against surrogate (carotid intima-media thickness) and frank end points (cardiovascular events and death) of cardiovascular disease.1–2 Why is the diabetic vessel so vulnerable to increased glucose? A plausible hypothesis is that it’s not just glucose; rather, excessive glucose is accompanied by clever accomplices in the pathways leading to irreversible vascular injury, some of which may be directly influenced by adverse signals emitted by hyperglycemia. For example, the nonenzymatic glycation and oxidation of proteins and lipids results in the generation of Advanced Glycation Endproducts (AGEs), key signaling species that perturb endothelium, smooth muscle cells and monocytes to ignite vascular inflammation and contribute to accelerated atherogenesis and the development and progression of vulnerable atherosclerotic plaques.3 AGEs, by virtue of their interaction with Receptor for AGE (RAGE), trigger generation of oxidative species and activation of downstream proinflammatory and prothrombotic pathways. Importantly, in addition to their ability to activate signaling pathways, such oxidative species may also serve as co-factors for the further generation of AGEs.4–5

Along the way, natural decoys emerge in an attempt to thwart the adverse effects of glucose and its consequences in the vessel wall. Although en balance, glucose and its consequences seem to win the war, efforts to uncover innate defenses are critical. Seizing on such basic science observations may lead to identification of novel agents to protect the diabetic vasculature and, thereby, mount a better defense against glucose and its deadly accomplices. In this issue of Circulation Research, Whetzel and colleagues demonstrate that physiologically-relevant concentrations of sphingosine-1-phosphate (SIP) prevent the interaction of monocytes with type 1 diabetic endothelium, largely via the sphingosine-1-phosphate receptor S1P1.6 Experimental evidence suggested that the antiinflammatory signals elicited by SIP were, in part, via inhibition of NF-κB translocation to the nucleus.

Monocyte-endothelial interaction represents a key step in atherogenesis; one which is enhanced in diabetes. SIP is generated primarily from the degradation of ceramide to sphingosine; subsequent phosphorylation generates SIP. SIP resides normally on lipoproteins, particularly HDL, and albumin in the circulation. SIP is normally generated in the vasculature and released by cells such as platelets, leukocytes and endothelial cells in concentrations similar to that studied by Whetzel and colleagues. How diabetes may impact on the generation or release of SIP is not well-understood. Further, if SIP is released in concentrations relevant to those studied by these authors, why, then, is it apparently ineffective in the war against glucose and its consequences? Is it possible that SIP is vulnerable to modifications that reduce its potential effectiveness as an antiinflammatory signal? Alternatively, or in addition, is it possible that in the diabetic state, SIP is subjected to more rapid degradation and inactivation via sphingosine-1 phosphate phosphatase (SPP1) by cleavage into species that do not display antiinflammatory potential7? Answers to these questions may be useful in harnessing the anti-inflammatory properties of SIP.

Whetzel and colleagues demonstrate that although SIP prevents endothelial induction of Vascular Cell Adhesion Molecule-1 (VCAM-1) in diabetic mice, its vascular protective effects may not fully depend on the generation of this molecule. Rather, time course studies indicated that the impact of SIP was exerted within minutes; even at 30 minutes, SIP reduced monocyte-endothelial interactions in type 1 diabetic endothelial cells. Two questions arise from this observation; first is it possible that the effects of SIP at the earliest time points were because of mobilization of calcium stores? In addition to activation of downstream signal transduction pathways such as NF-κB, it has been shown that SIP results in mobilization of calcium in human endothelial cells.8 Certainly, mobilization of calcium stores
rapidly modulates cellular properties, including changes in actin-cytoskeleton which may substantially alter cellular interactions. Second, as suggested by the authors, their studies did not interrogate the potential impact of S1P on monocytes in their reduced adhesion to diabetic endothelial cells. It is plausible that rapid changes in monocyte behavior, perhaps via calcium mobilization and S1P receptors, potently impacted on S1P-mediated reduction in monocyte adhesion to diabetic endothelial cells. Future studies might assess the time course of biochemical and molecular effects of S1P on monocyte properties in the hyperglycemic milieu, particularly, the impact on cellular migration.

Whetzel and colleagues used biochemical agonists and antagonists, and receptor-devoid mutant endothelial cells, where available, to dissect the potential contributions of distinct S1P receptors. The balance of their evidence supports that S1P1 is the major receptor mediating the effects of S1P in diabetic endothelial cells. In the absence of viable S1P1 global or tissue-targeted endothelial S1P1 null mice, the conclusions regarding the specific role of S1P1 reached by the authors rely on biochemical agonists or antagonists of these pathways. Future studies might be directed at small interfering RNA (siRNA) knockdown exclusively of S1P1 or other endothelial-expressed S1P receptors in cultured endothelial cells to firmly establish the precise role of S1P1 in this process. These concepts are relevant, as it remains to be determined if administration of S1P, for example, reflects a feasible and safe therapeutic strategy for the diabetic blood vessel. Alternatively, is it possible that targeting the receptors of S1P, such as S1P1, might enhance the risk to benefit ratio of targeting this axis in vivo? Indeed, in this context, other studies have supported the endothelial-protective impact of S1P-S1P1 interaction. Lee and colleagues showed that via S1P1 (as elucidated by siRNA knockdown studies), S1P induced endothelial tight junctions and, thereby, enhanced endothelial barrier integrity.9

However, distinct reports suggest that S1P in small arteries may cause pathophysiological vasoconstriction. Scherer and colleagues studied the gerbil spiral modiolar artery and demonstrated that S1P induced a concentration-dependent constriction of the spiral modiolar artery, with an EC50 of 115 nmol/L, similar to the concentrations used by Whetzel and colleagues. S1P1 is the major receptor mediating the effects of S1P in diabetic endothelial cells. In the absence of viable S1P1 global or tissue-targeted endothelial S1P1 null mice, the conclusions regarding the specific role of S1P1 reached by the authors rely on biochemical agonists or antagonists of these pathways. Future studies might be directed at small interfering RNA (siRNA) knockdown exclusively of S1P1 or other endothelial-expressed S1P receptors in cultured endothelial cells to firmly establish the precise role of S1P1 in this process. These concepts are relevant, as it remains to be determined if administration of S1P, for example, reflects a feasible and safe therapeutic strategy for the diabetic blood vessel. Alternatively, is it possible that targeting the receptors of S1P, such as S1P1, might enhance the risk to benefit ratio of targeting this axis in vivo? Indeed, in this context, other studies have supported the endothelial-protective impact of S1P-S1P1 interaction. Lee and colleagues showed that via S1P1 (as elucidated by siRNA knockdown studies), S1P induced endothelial tight junctions and, thereby, enhanced endothelial barrier integrity.9

This is especially relevant because the properties of S1P in tumors and tumor vasculature may favor conditions in which growth and metastasis is encouraged. S1P may contribute favorably to processes linked to angiogenesis, such as stimulation of DNA synthesis, chemotactic motility, and capillary tube formation of endothelial cells, in part via regulation of VEGF and PDGF.12 Based on basic science observations such as these, Visentin and colleagues reported on the characterization of an anti-S1P antibody tested as a potential therapeutic agent in reducing tumor growth, invasion and angiogenesis.13

Thus, taken together, the biology and biological context of S1P may vary depending on the cell types, the type of macroenvironment and the specific microenvironment, and the distinct S1P receptors in individual tissues. Indeed, this situation is not much different than that faced in tackling the complex problems of diabetes and its complications. For example, if S1P were targeted as a therapeutic agent in diabetes, it would be important to delineate how such proangiogenic effects of S1P might modulate properties of retinal vascular endothelial cells. Among the most fascinating aspects of diabetes complications is the differential impact of hyperglycemia on distinct vascular beds, as in the retina, chronic injury and presumably ischemic foci stimulate untoward angiogenesis and processes that vitally impact vision. In striking contrast, in the peripheral vasculature consequent to ischemia, for example, markedly impaired capillary responses may leave the affected limb vulnerable to amputation. In the latter case, S1P might be expected to exert substantial benefit. Its potential impact in retinopathy would need to be rigorously addressed, however, in preclinical investigation.

The intriguing studies of Whetzel and colleagues should stimulate further experimentation on S1P as a potential therapeutic agent in diabetes with increased emphasis placed on creating a more human-like microenvironment in rigorous in vivo testing. Specifically, it is important to consider in the context of atherosclerosis per se, that type 1 diabetic nonobese-diabetic mice studied by Whetzel and colleagues do not spontaneously develop this disease. Their studies need to be taken to the next step, with the role of S1P probed in a lipid-enriched environment. How might the availability or potential modification of S1P be impacted in an hyperlipidemic and hyperglycemic environment? This remains to be elucidated experimentally.

Finally, in waging the war on diabetes and vascular injury, it is important to consider that the diabetic macrovascular wall must truly reflect a markedly different environment than that observed in euglycemic disease. Although distinct groups of researchers focus on lipids, AGEs, oxidative stress, S1P and the like, what seems to be underappreciated is that these species are not generated and modified in a vacuum. Rather, it is quite likely that their interplay in the hyperglycemic milieu continually creates new biochemical species which, even if short-lived, may have long-term impact on the vasculature and its properties. How do changes in fatty acid homeostasis influence ceramide and sphingosine homeostasis in Type 1 and Type 2 diabetes? How do such modified glucose-, lipid-, ceramide-, and reactive oxidative species
moieties, for example, interact with each other, and how do these new super-species interface with the innate and adaptive immune mechanisms?

In conclusion, Whetzel and colleagues have set the stage for stimulation of new debates and hypotheses in diabetic vascular complications. Is the diabetic vessel wall simply just “more of the same” injuries that plague the euglycemic vessel wall? We do not think so. We do believe, though, that the intriguing and, yes, pleiotropic biology of S1P should serve as springboard to reignite the debates on causes of vascular injury in diabetes. Only by uncovering and dissecting each aspect, both the beneficial and maladaptive properties, of present (and future) therapeutic targets in active preclinical or clinical investigation, may we win the war on diabetes and its assault on the vasculature.

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