review

HDL serves as a S1P signaling platform mediating a multitude of cardiovascular effects

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Abstract The lysosphingolipid sphingosine 1-phosphate (S1P) is a component of HDL. Findings from a growing number of studies indicate that S1P is a mediator of many of the cardiovascular effects of HDL, including the ability to promote vasodilation, vasoconstriction, and angiogenesis, protect against ischemia/reperfusion injury, and inhibit/reverse atherosclerosis. These latter cardioprotective effects are being shown to involve the S1P-mediated suppression of inflammatory processes, including reduction of the endothelial expression of monocyte and lymphocyte adhesion molecules, decreased recruitment of polymorphonuclear cells to sites of infarction, and blocking of cardiomyocyte apoptosis after myocardial infarction. This review article summarizes the evidence that S1P as a component of HDL serves to regulate vascular cell and lymphocyte behaviors associated with cardiovascular (patho)physiology.— Argraves, K. M., and W. S. Argraves. **HDL serves as a S1P signaling platform mediating a multitude of cardiovascular effects. *J. Lipid Res.* 2007.** 48: **2325–2333.**

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In 1997, Yatomi et al. (1) were the first to show that the lysosphingolipid sphingosine 1-phosphate (S1P) was a component of human plasma, present at a concentration of ~200 nM. After these findings were published, Sachinidis et al. (2) reported that human LDL and HDL possess a signaling activity that promoted extracellular signal-regulated protein kinase phosphorylation in a pertussis toxin-sensitive manner. HPLC fractionation of plasma LDL and HDL showed that the lipoprotein-associated signaling activity had a chromatographic elution profile corresponding to that of purified S1P. Further evidence appeared demonstrating that ~65% of the S1P in blood is associated with the lipoproteins LDL, VLDL, and HDL, with the bulk of lipoprotein-associated S1P (~54%) bound to HDL (3). Measurements of the S1P content of human HDL and LDL particles show that these lipoproteins contain ~180 and 70 pmol S1P/mg protein, respectively (4). Together, these findings have set the stage for investigations into the mechanism of S1P incorporation into lipoproteins and the physiological significance of lipoproteins as carriers of S1P, and the findings of these investigations are summarized in this review.

THE SOURCE OF S1P IN BLOOD

Until recently, the source of S1P in the blood was uncertain. Sphingosine kinase, the enzyme that phosphorylates sphingosine to produce S1P, is expressed in platelets (1) as well as a variety of peripheral blood cells, including erythrocytes, neutrophils, and mononuclear cells (5). Even though platelets store and release large amounts of S1P upon stimulation by thrombin or calcium (1, 6, 7), platelet-deficient mice have normal plasma levels of S1P (8). Several recent studies have established that erythrocytes are the principal source of plasma S1P (8–10). Importantly, erythrocytes are unable to produce sphingosine and display relatively low levels of sphingosine kinase activity (10). Although the origin of sphingosine in vivo is uncertain, erythrocytes efficiently convert exogenous sphingosine to S1P. The fact that erythrocytes lack S1P-degrading enzymes (e.g., sphingosine lyase and S1P phosphatase) also permits them to accumulate large amounts of S1P (8, 10).

In the studies of Pappu et al. (8), plasma S1P levels were reduced by 90% in lethally irradiated wild-type mice engrafted with hematopoietic cells from bone marrow of sphingosine kinase-deficient mice. Although these findings support the conclusion that erythrocytes are the major source of plasma S1P, the origin of the remaining 10% of plasma S1P is not known. Extracellular generation of S1P, as opposed to cellular secretion of S1P, represents a possible source (11, 12). Sphingosine kinase has been shown to be secreted by endothelial cells, airway smooth muscle cells (SMCs), and monocytic cells (11, 13, 14). Work by Hla and colleagues (12) has shown that the sphingosine kinase 1a isoform is preferentially secreted by

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endothelial cells compared with the 1b and 1c isoforms. Sphingosine kinase is also present in human plasma, as shown by the finding that plasma depleted of both platelets and cells can mediate the formation of S1P when incubated with sphingosine (12). Furthermore, sphingomyelinase and ceramidase, the enzymes that act to sequentially produce sphingosine, are also secreted (15–17). The extent to which the extracellular biosynthesis of sphingosine and S1P contributes to levels of S1P in blood remains to be established.

ROLE OF ABC TRANSPORTERS IN S1P RELEASE

The mechanism by which S1P becomes associated with HDL or other plasma lipoprotein particles is not understood. The observation that isolated erythrocytes release S1P into plasma, but not into serum-free medium, indicates that a plasma factor, possibly HDL and/or albumin, is required to facilitate S1P secretion (9). Even though platelets do not appear to be the major contributor to plasma S1P levels, more is known about the mechanism by which S1P is released from platelets compared with erythrocytes. For example, studies by Aoki et al. (18) demonstrate that S1P released from stimulated human platelets becomes associated with both albumin and HDL in plasma. Similarly, Kobayashi et al. (19) showed that thrombin-induced release of S1P from platelets requires a lipid acceptor, such as albumin. Furthermore, they found that the process could be blocked by treatment with glyburide, an inhibitor of ABC transporters.

ABC transporters are known to mediate the efflux of cholesterol and phospholipids from plasma membranes to lipid acceptors, such as lipid-poor apolipoprotein A-I (apoA-I), HDL, and albumin (20). For example, ABCA1 mediates the apolipoprotein-dependent formation of HDL by facilitating lipid-poor apoA-I interaction with cells and acts as a translocator of cellular lipids across the plasma membrane to the lipid-poor apoA-I acceptor (21–24). The identity of the transporter responsible for S1P release in platelets is not yet known, but platelets express several ABC transporters, including ABCA1, ABCA3, ABCA7, ABCC1 [also known as multidrug resistance-associated protein 1 (MRP1)], and ABCC4 (MRP4) (25–28).

Recently, ABCC1 was shown to mediate the export of S1P from mast cells stimulated with an albumin-conjugated antigen (29). Although the S1P acceptor was not determined in that study, albumin is a likely candidate given the fact that ABCC1 has been shown to mediate the transport of phosphatidylcholine from the inner to the outer leaflet of the plasma membrane to albumin present in cell culture medium (30). Furthermore, ABCC1 also mediates the transport of glucosylceramide and sphingomyelin to albumin present in the medium of pig kidney-derived polarized cells (31). Although these studies establish that ABCC1 can transport S1P and other sphingolipids to albumin, this transporter has not been evaluated for the ability to mediate S1P efflux to HDL, the major carrier of S1P in plasma. Given the new evidence for the predominant role of erythrocytes in the regulation of plasma S1P levels, the involvement of erythrocyte ABC transporters in S1P release to HDL must now be examined. In this regard, it is important to note that erythrocytes mainly express ABCC1, ABCC4, and ABCC5 (MRP5) (28).

ROLE OF CFTR/ABCC7 IN S1P UPTAKE

Another member of the ABC transporter superfamily, cystic fibrosis transmembrane conductance regulator (CFTR), also known as ABCC7, has been shown to mediate the cellular uptake of S1P as well as the related phosphorylated lipids dihydrosphingosine 1-phosphate and lysophosphatidic acid (LPA) (32). Importantly, CFTR-mediated uptake of S1P negatively influences S1P bioavailability, apparently diverting it from interaction with S1P receptors. Such a mechanism could be important to the process by which S1P signaling is modulated in cells.

Whether CFTR binds directly to albumin-associated S1P and/or HDL-associated S1P and thereby mediates endocytosis or whether it regulates the uptake of S1P by some other receptor is not known. There is evidence for another ABC transporter, ABCA1, playing a general role in the endocytosis of ligands by other receptors (33, 34). For example, the endocytosis of LDL as well as transferrin is upregulated in fibroblasts having nonfunctional ABCA1 (33). Findings support a model in which ABCA1 is necessary for the maintenance of cross-leaflet phosphatidylserine distribution of the plasma membrane, thus influencing membrane curvature attributable to the alteration of relative surface area of the leaflets (35). Alterations in membrane curvature initiate vesiculation, leading to endocytosis (36). Although ABCA1 action seems to suppress endocytosis via effects on the dynamics of the plasma membrane, there is no evidence yet for CFTR/ABCC7 having a similar effect.

NITRIC OXIDE-MEDIATED VASODILATORY EFFECT OF HDL-ASSOCIATED S1P

Several recent studies illustrate the beneficial effects of the intravenous administration of HDL to endothelial function. For example, in hypercholesterolemic patients, impaired endothelial function (e.g., reduced vasodilator response to acetylcholine) can be normalized by intravenous administration of reconstituted HDL (37). Similarly, intravenous infusion of HDL reduces arterial blood pressure in rats whose mean blood pressure has been increased by endothelin treatment (38). Furthermore, in vitro treatment of precontracted rodent aortic segments with HDL, the lipid-containing fraction of HDL, or S1P leads to an ~50% increase in relaxation (38). In these studies, treatment with apoA-I, cholesterol, phosphatidylcholine, sphingomyelin, or the delipidated protein fraction of HDL did not cause vasodilation (38), thus implicating S1P as a mediator.

The vasodilatory effects of HDL in both humans (37) and rodents (38) have been shown to be dependent on

endothelial nitric oxide synthetase (eNOS) phosphorylation and the production of nitric oxide (NO) by the endothelium. The HDL vasodilatory effects are also partially dependent on phosphatidylinositol 3′-kinase (PI3K)/Akt pathway activation (38). These findings are perhaps consistent with evidence that HDL binding to scavenger receptor class B type I (SR-BI) leads to eNOS phosphorylation via the activation of both PI3K and mitogen-activated protein kinase (MAPK) pathways (39). Because the HDL component known to bind to SR-BI, apoA-I, produces no vasodilatory effect, HDL-associated entities such as S1P or other lysosphingolipids are candidate mediators. Although the vasodilatory effects of HDL in the rat studies of Nofer et al. (38) were not shown to be directly attributable to the S1P component of HDL, it was demonstrated that S1P as well as sphingosylphosphorylcholine (SPC) and lysosulfatide (LSF) could mediate the relaxation of aortic segments in vitro as well as vasorelaxation in vivo. Furthermore, studies using S1P3-deficient mice show that S1P3 mediates both the vasodilatory and eNOS activation effects of HDL as well as for three HDL-associated lysophospholipids, S1P, SPC, and LSF (38).

VASOCONSTRICTING EFFECTS OF S1P

In addition to the aforementioned dilatory effects on precontracted rat aortic segments in vitro and the vasorelaxation effects in vivo (38), S1P (associated with albumin) has also been shown to promote the vasoconstriction of rat cerebral arteries (40–42). Similarly, in dog hearts perfused with blood supplemented with S1P, a decrease in coronary blood flow was also observed compared with that in controls (43). S1P has also been shown to induce Rho kinase-dependent vasoconstriction of the spiral modiolar artery in the gerbil (44). Although S1P was found to promote a vasoconstriction response in rat cerebral arteries, peripheral rat arteries, including the carotid and femoral arteries, showed no constrictive response to S1P, and coronary arteries showed a weak constrictive response (41). The aorta also does not exhibit vasoconstriction in response to S1P (40). Thus, different blood vessels appear to respond differently to S1P with respect to vasoconstriction. Coussin et al. (40) attribute the disparate responses to S1P receptor expression levels, with S1P2 and S1P3 levels being considerably lower in the aorta compared with the cerebral artery.

There are conflicting reports regarding which S1P receptors are responsible for the vasoconstriction effects of S1P. Using S1P3 antisense treatments, the vasoconstrictor response of S1P on cerebral artery segments was reduced (41). By contrast, S1P2 antisense treatment of cerebral artery segments did not modify the S1P contractile response (41). However, Ohmori et al. (42) reported that a putative S1P2 antagonist, JTE-013, inhibited S1P-induced contraction of cultured coronary artery SMCs, whereas treatment with the S1P3 inhibitor suramin had no effect. Likewise, Nofer et al. (38) reported that the vasoconstrictive effect of S1P on nonprecontracted aorta segments was similar between wild-type and S1P3-deficient mice.

The dual effects of S1P on vascular tone (vasodilation vs. vasoconstriction) appear to be initiated via different pathways, one on endothelial cells, acting via S1P3 (38) to mediate vasodilation, and the second operable in vascular smooth muscle cells (VSMCs) to mediate vasoconstriction via as yet undefined S1P receptors. This latter pathway may come into play under pathological conditions in which endothelial barrier function has been compromised, allowing plasma HDL-associated S1P complexes to reach the VSMCs. Therefore, the development of S1P receptor-selective agonists may have therapeutic utility in the treatment of hypertension and hypotension, both risk factors for stroke.

HDL-ASSOCIATED S1P IN ATHEROSCLEROSIS

The ability of HDL to protect the arterial wall has been attributed to a number of its activities, including the ability to mediate reverse cholesterol transport and to inhibit monocyte adhesion, LDL oxidation, and the activation and aggregation of platelets (45). Several recent studies point to the ability of HDL to regulate S1P-dependent functions of endothelial cells, VSMCs, lymphocytes, and macrophages (**Table 1**). These findings also serve as an indication that the S1P cargo of HDL may account for many of the antiatherogenic activities of HDL. Indeed, the level of lipoprotein-associated S1P in blood has the greatest positive correlation with HDL cholesterol levels as opposed to LDL cholesterol levels (46). Zhang et al. (46) also report that S1P is associated with apoA-I- and apoA-I/apoA-II-containing HDL subclasses, but not with the apoE-containing subclasses of HDL. To date, studies have not compared the ability of different subclasses of HDL to mediate S1P signaling relative to the S1P concentration in each subclass. A number of studies have demonstrated that overexpression of the human apoA-I gene in transgenic mice confers protection against diet-induced atherosclerosis. In one such study, overexpression of human apoA-I conferred a change from a unimodal to a polydispersed HDL distribution, with an increase in preβ-migrating HDL species (47). It is possible that such an increase in preβ-HDL particle concentration translates to an increase in the level of plasma S1P available for signaling.

Even though epidemiological evidence indicates that high HDL is generally cardioprotective and that plasma S1P levels are highly correlated with HDL cholesterol (46), it cannot be concluded that the cardioprotective activities of HDL are S1P-mediated. One reason for this is the seemingly paradoxical evidence that the synthetic S1P analog, FTY720, may to some extent mediate cardioprotection by suppressing S1P signaling. However, it is possible that HDL-associated S1P (HDL-S1P) signaling can be cardioprotective via a mechanism separate from that by which FTY720 has been shown to act, such as suppression of endothelial cell activation (48).

TABLE 1. Cardiovascular related effects of HDL-S1P and HDL-FTY720

HDL-S1P-Mediated Effects	Ref.	FTY720-Mediated Effects[a]	Ref.
Endothelial cells			
Increased migration	72		
Endothelial tube formation	55, 57		
Increased proliferation	57, 58		
Decreased caspase 3 and 9 activation	59		
Activation of the phosphatidylinositol 3′-kinase/Akt pathway	57, 59		
Activation of Ras	56		
Activation of ERK	56, 57		
Decreased ERK activation in response to TNF-α	61		
Decreased vascular cell adhesion molecule-1 in response to TNF-α	48		
Decreased intercellular adhesion molecule-1 in response to TNF-α	48		
Decreased nuclear factor κB activation in response to TNF-α	61		
Decreased sphingosine kinase activity	61		
Increased NO production by endothelial NO synthetase	38		
Macrophages			
Decreased adhesion to endothelial cells	69		
		Decreased basal IL-6 secretion	63
		Decreased IL-6 secretion in response to LPS	63
		Decreased basal NO secretion	63
		Decreased NO secretion in response to LPS	63
		Increased basal secretion of IL-RA	63
		Increased secretion of IL-RA in response to IL-4	63
Vascular smooth muscle cells			
Reduced migration in response to platelet-derived growth factor and lysophosphatidic acid	50, 51		
T-cells			
		Inhibited T-cell egress/migration	73
Blood			
		Decreased numbers of circulating lymphocytes	63
		Decreased circulating levels of IL-6	63
		Decreased circulating levels of IL-12	63
		Decreased circulating levels of TNF-α	63
		Decreased circulating levels of soluble TNF-α receptor	63
		Decreased circulating levels of interferon-γ	63
Ischemia/reperfusion injury			
Decreased myocardial infarct size	69		
Decreased apoptotic cell death in cardiac infarct area	69		
Decreased recruitment of polymorphonuclear cells to the infarct site	69		
Atherosclerosis			
		Decreased atherosclerotic lesion size	63

ERK, extracellular signal-regulated protein kinase; IL-6, interleukin-6; LPS, lipopolysaccharide; NO, nitric oxide; RA, receptor antagonist; S1P, sphingosine 1-phosphate; TNF-α, tumor necrosis factor-α.

[a] These effects were in response to in vivo administration of the synthetic S1P analogue FTY720, which was shown to become phosphorylated and preferentially associated with HDL (63).

An additional dimension to the role of HDL in atherogenesis is that HDL can become transformed to a proinflammatory molecule under conditions of systemic inflammation (e.g., coronary heart disease, diabetes, metabolic syndrome, infection rheumatologic disease) (49). To date, HDL particles isolated from individuals in a proinflammatory state have not been assessed for alterations in the composition of S1P or other sphingolipids. Given evidence that at least one ABC transporter, ABCA1, is downregulated under conditions of systemic inflammation (50), it is possible that reduced ABC transporter-mediated S1P transport to HDL would result in the production of HDL with reduced S1P content. By contrast, there is evidence from in vitro studies that sphingosine kinase-1 is upregulated by the proinflammatory cytokine tumor necrosis factor-α (TNF-α), leading to increased intracellular levels of S1P. If this occurs in vivo under conditions of inflammation, then increased levels of HDL-S1P could be an expected result. A caveat to this supposition is that under conditions of acute or chronic inflammation, mice show no detectable increase in tissue S1P levels (49).

The remainder of this review will focus on the evidence for signaling mediated by HDL-S1P in the control of vascular cell and lymphocyte behaviors associated with atherosclerosis as well as ischemia/reperfusion injury.

HDL-S1P INHIBITS VSMC MIGRATION

Hallmarks of atherosclerotic pathogenesis are the migration and proliferation of VSMCs. Both HDL and S1P have been shown to inhibit the migration of VSMCs induced by platelet-derived growth factor (PDGF) (50, 51). The inhibitory effects of HDL and S1P on PDGF-induced VSMC migration can be suppressed by small interfering RNA to the S1P receptor, S1P2 (51). Conversely, overexpression of S1P2 enhances the antimigratory effects of HDL and S1P (50). By contrast to HDL, LDL has been found to stimulate the migration of VSMCs via the action

of LPA, which is found in higher levels in LDL compared with HDL (51). HDL or S1P can inhibit this response to LPA. Despite the fact that LDL contains S1P, LDL has no effect on VSMC migration induced by PDGF, unless the lipoprotein particle is treated with monoglyceride lipase to degrade the LPA component (51). Importantly, the S1P content of LDL is decreased, whereas the content of the LPA precursor, lysophosphatidylcholine, is increased during the copper oxidation of LDL (4). Together, these findings highlight the possibility that a balance may exist between S1P and LPA signaling that determines the direction of action of lipoproteins on VSMC migration, particularly in the context of oxidized LDL-induced neointima formation and the development of atherosclerosis.

Although VSMC proliferation has not been formally shown to be mediated by HDL, studies have shown that S1P (when tested as a complex with albumin) promotes VSMC growth via the MAPK/extracellular signal-regulated protein kinase-dependent activation of Elk-1 (52).

HDL-S1P PROMOTES ENDOTHELIAL CELL MIGRATION

In contrast to its effect on VSMCs, HDL promotes the migration of endothelial cells, and S1P has been shown to be the component responsible for this activity (53). This was established by showing that only the S1P-rich fraction of HDL stimulated human umbilical vein endothelial cell (HUVEC) migration (53). Furthermore, it was shown that antisense oligonucleotides to the S1P receptors, S1P1 and S1P3, blocked HDL-induced HUVEC migration (53). These findings are consistent with other studies showing that pertussis toxin, an antagonist of G-protein-coupled receptors, including S1P1 and S1P3, blocks the S1P-induced invasion of HUVECs into collagen and fibrin matrices (54).

HDL-S1P PROMOTES ENDOTHELIAL TUBE FORMATION

In addition to influencing endothelial cell migration, HDL-S1P has also been implicated in endothelial tube formation, the process by which newly formed networks of endothelial cells undergo lumenization. HDL-S1P particles reconstituted from purified apoA-I, S1P, and 1-palmitoyl-2-oleoyl phosphatidylcholine promoted human coronary artery endothelial cells (HCECs) to form capillary-like structures in vitro (55). By contrast, reconstituted HDL particles lacking S1P did not promote endothelial tube formation (55). The effects of HDL-S1P on HCEC endothelial tube formation could be inhibited by antisense oligonucleotides to the S1P receptors, S1P2 and S1P3 (55), and by pertussis toxin (56). Downstream signaling events in HDL- and S1P-mediated endothelial tube formation include activation of the MAPK, protein kinase C, and PI3K/Akt pathways (57).

HDL-S1P STIMULATES ENDOTHELIAL CELL GROWTH AND SURVIVAL

Several studies demonstrate that HDL has growth-promoting effects on endothelial cells. Early-passage endothelial cells exposed to HDL and transferrin grow as well as endothelial cells exposed to serum (58). Only recently have investigations focused on S1P as a potential mediator of the growth effects of HDL on endothelial cells. For example, treatment of HDL with alkaline phosphatase to degrade S1P has been shown to reduce its capacity to stimulate endothelial cell proliferation (57). Furthermore, pertussis toxin treatment of HCECs inhibits the activation of Ras and intermediates of the Ras pathway, a major proliferation-regulating cascade (56). Additionally, HDL treatment of endothelial cells leads to increased phosphorylation of p42/44 MAPK, and the MAPK inhibitor PD98059 and inhibits HDL-mediated proliferation and endothelial tube formation (56, 57). Furthermore, HDL-induced MAPK activation and endothelial tube formation are inhibited by the overexpression of dominant-negative Ras (56).

HDL has also been shown to promote endothelial cell survival in a lysosphingolipid-dependent manner. For example, HDL and S1P treatment of HUVECs activates the PI3K/Akt pathway, a major prosurvival/antiapoptotic signaling cascade (57). The PI3K/Akt pathway also mediates the endothelial survival-promoting effects of HDL-associated lysosphingolipids other than S1P (59). Evidence for this conclusion comes from the findings that suppression of Akt activity by wortmannin and LY-294002 or by a dominant negative Akt eliminates the antiapoptotic effects of SPC and LSF on endothelial cells (e.g., these treatments prevented the activation of caspase 9 and 3) (59). By contrast to HDL, VLDL isolated from mice on a regular chow diet provides little apoptosis protection to endothelial cells (60). However, VLDL isolated from mice fed a high-fat/high-cholesterol/cholate diet inhibits caspase 3/7 activation (60). This high-fat diet VLDL-related anti-caspase activity can be blocked by treatment with the S1P receptor antagonist VPC 23019 or by S1P3-specific small interfering RNA (60).

HDL-S1P SUPPRESSES ENDOTHELIAL CELL ACTIVATION

In response to a variety of stimuli, such as TNF-α, endothelial cells become activated and express the adhesion molecules vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1). HDL-S1P has been shown to inhibit endothelial cell expression of VCAM-1 and ICAM-1 in response to TNF-α (48). These effects are mediated through a dual mechanism involving HDL/apoA-I binding to SR-BI and the S1P receptor, S1P1 (48). These effects of HDL-S1P would be expected to reduce monocyte and lymphocyte interaction with endothelial cells and cell penetration into the subendothelial space or the intima of arterial walls, thereby reducing

the inflammatory response and foam cell formation in the intima.

HDL has also been shown to inhibit TNF-α-induced endothelial cell activation by inhibiting sphingosine kinase (61). Furthermore, HDL inhibits the TNF-α-induced activation of extracellular signal-regulated protein kinase and the transcription factor nuclear factor κB (61). Based on these observations, HDL-mediated inhibition of endogenous S1P production protects endothelial cells from the cytokine-induced upregulation of VCAM-1 and ICAM-1 via a mechanism that involves a signaling cascade sensitive to endogenously produced levels of S1P.

HDL-S1P AS AN IMMUNOMODULATOR

Recent studies demonstrate that plasma S1P is required for the process by which murine lymphocytes egress from the thymus to the blood (8). These findings are consistent with earlier observations that mice conditionally deficient in hematopoietic cell-S1P1 lack peripheral blood T-cells. Lymphopenia in these mice is attributed to the inability of the T-cells to egress from both the thymus and peripheral lymphoid organs (62). Lymphopenia is also observed in mice treated with the synthetic sphingosine analog FTY720 (63). The basis for this effect is that the phosphorylated synthetic analog promotes T-cell S1P1 endocytosis, leading to degradation of the receptor (64). This suppression of S1P1 expression inhibits the ability of T-cells to egress in response to a S1P signal and thus mimics the phenotype observed in mice genetically deficient in T-cell S1P1 expression. Although HDL has not yet been shown to facilitate the process of S1P-dependent lymphocyte egress, it has been implicated in the effects of FTY720, because the analog becomes preferentially incorporated into the HDL lipoprotein fraction of plasma after intraperitoneal injection (63). Based on these findings, HDL-FTY720 cannot be considered biochemically analogous to HDL-S1P with respect to T-cell migratory signaling.

Although FTY720 and S1P have opposite effects on T-cell migration, they appear to similarly downregulate cytokine levels. For example, FTY720 treatment of mice leads to reduction in plasma levels of the cytokines interleukin-6 (IL-6) and TNF-α, two markers of macrophage-dependent inflammatory processes (63). Indeed, peritoneal macrophages from FTY720-treated animals display both decreased proinflammatory cytokine secretion in response to lipopolysaccharide and increased anti-inflammatory mediators (e.g., IL-1 receptor antagonist) in response to IL-4 treatment, compared with non-FTY720-treated mice (63). Furthermore, administration of FTY720 to LDL receptor-deficient mice reduces atherosclerotic lesion size in the aortic root and brachiocephalic artery compared with control mice (63). Mechanistically, the antiatherogenic effects of FTY720 have been attributed in part to a reduction in the plasma levels of IL-6 and TNF-α. This response may be analogous to the suppression of T-cell secretion of proinflammatory cytokines (e.g., IL-4, IL-2, and interferon-γ) and the enhanced secretion of anti-inflammatory cytokines in response to S1P treatment (65, 66). Whether the capacity of both FTY720 and S1P to suppress cytokine levels mimics a normal function of HDL-associated S1P is not certain. However, in support of this possibility is the fact that HDL inhibits peripheral blood monocyte expression of IL-1β and TNF-α (67). Consistent with the concept that S1P has an anti-inflammatory role with respect to cytokine production is the finding that the production of proinflammatory cytokines (e.g., IL-1β) is reduced in response to silencing of the enzyme responsible for S1P degradation, SPP2 (68).

HDL-S1P AND ALBUMIN-S1P PROTECT AGAINST ISCHEMIA/REPERFUSION INJURY

Recent studies in the mouse have also demonstrated that administration of HDL-S1P before transient coronary ligation results in a 20% reduction in myocardial infarct size (69). Similar results were obtained when albumin-S1P was administered, although administration of LDL had no effect on infarct size. The effects of both HDL-S1P and albumin-S1P on myocardial infarct size were correlated with a decrease in the recruitment of polymorphonuclear cells to the infarct site, indicating that suppression of the inflammatory response is a potential factor in the cardioprotective effects. The ability of S1P to increase endothelial barrier activity/decrease vascular permeability (70) may be another factor underlying the capacity of HDL-S1P to decrease polymorphonuclear cell recruitment. Both HDL-S1P and albumin-S1P have been found to reduce macrophage adhesion to endothelial cells in vitro (69). Either HDL-S1P or albumin-S1P administration also reduces the level of apoptotic cell death in the myocardial infarction area.

The protective effects of HDL-S1P and albumin-S1P on cardiac ischemia/reperfusion injury, including suppressing leukocyte adhesion to an activated endothelium and blocking cardiomyocyte apoptosis, are negated in animals pretreated with an inhibitor of NO synthase, indicating that the effects are produced through a NO-dependent pathway (69). Similarly, HDL has been shown to enhance ischemia-induced angiogenesis in a NO-dependent manner (71). Finally, the cardioprotective effects of HDL-S1P and albumin-S1P appear to be mediated by S1P3 receptor signaling, because administration of either HDL-S1P or albumin-S1P conferred no protective effects against reperfusion injury in S1P3-deficient mice (69).

CONCLUSIONS

In <10 years since the discovery that S1P is a component of HDL, a spectrum of HDL-associated cardiovascular effects have been attributed to its S1P cargo. These findings raise numerous questions and highlight many directions for future investigations. Further experimentation is needed to directly compare the effects of HDL/HDL-S1P versus S1P/albumin-S1P on the various cardioprotec-

tive activities associated with HDL cholesterol. Also necessary is a greater understanding of the mechanisms underlying the biosynthesis of HDL-S1P and the potential involvement of ABC transporters and secreted sphingosine kinases and sphingosine phosphatases. Of particular interest is how HDL-S1P biosynthesis is affected by dyslipidemia and whether alterations in HDL-S1P biosynthesis/plasma levels of S1P underlie aspects of the pathology of atherosclerosis. In this regard, it will be important to establish whether S1P levels correlate with the risk of atherosclerosis. The application of technologies such as liquid chromatography-mass spectrometry now allow high-throughput quantification of plasma lipoprotein-associated S1P and other bioactive lysosphingolipids. Such analyses need to be performed in conjunction with studies that evaluate the relative abilities of the various subclasses of HDL to mediate S1P signaling.

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