

## Review Article

# Sphingosine-1-phosphate: A bioactive lipid that confers high-density lipoprotein with vasculoprotection mediated by nitric oxide and prostacyclin

Cristina Rodríguez\*; María González-Díez\*; Lina Badimon; José Martínez-González

Centro de Investigación Cardiovascular (CSIC-ICCC), Hospital de la Santa Creu i Sant Pau, Barcelona, Spain

### Summary

Sphingosine-1-phosphate (S1P) is a bioactive lipid generated in the intracellular membranes from the metabolism of sphingomyelin. Once secreted/exported by cells of haematopoietic origin and vascular cells S1P interacts with plasma proteins and accumulates in high-density lipoprotein (HDL). Growing evidence indicates that HDL-associated S1P is responsible for the beneficial effects of these lipoproteins on vasorelaxation, cell survival, cell adhesiveness, angiogenesis and synthesis of two powerful endogenous anti-atherogenic and anti-thrombotic molecules such as nitric oxide (NO) and prostacyclin (PGI<sub>2</sub>). It is likely that

vascular effects of HDL-S1P are regulated by the local expression of S1P receptors. Five G protein-coupled receptors (S1P<sub>1</sub> to S1P<sub>5</sub>), with differential expression patterns and dissimilar coupling mechanism to G protein subunits, have been identified in the vasculature. This review is focused on the central role of S1P as a bioactive component that confers vasculoprotective properties to HDL by eliciting a wide range of biological responses on endothelial and smooth muscle cells largely dependent on the up-regulation of NO and prostacyclin.

### Keywords

High-density lipoproteins, sphingosine-1-phosphate, prostacyclin, nitric oxide, vascular function

**Thromb Haemost 2009; 101: 665–673**

## Introduction

Sphingosine-1-phosphate (S1P) is a bioactive lysophospholipid present in plasma that is stored in and released from circulating cells and that can be locally produced by vascular cells (1–3). S1P is generated intracellularly from sphingomyelin, a eukaryotic specific phospholipid essential for the formation of membrane rafts and caveolae (1). S1P is flipped or transported to the extracellular environment, process that involves specific ABC-type transporters (4), and binds to albumin and plasma lipoproteins in particular to high-density lipoproteins (HDL) (5).

The cardioprotective effects of HDL have been extensively demonstrated through experimental and epidemiological studies (6–9). They potentially rely on the ability of HDL to promote cholesterol efflux but also on its anti-oxidant, anti-inflammatory and anti-thrombotic actions (10–12). S1P is the most active lipid component of HDL and plays a key role in vascular biology as an extracellular mediator of a wide spectrum of biological responses (13). S1P is responsible, at least in part, for many of the

cardiovascular effects of HDL including the ability to modulate vascular tone (14), promote angiogenesis (15, 16), inhibit/reverse atherosclerosis (17) and protect against ischaemia/reperfusion injury (18). S1P has emerged as a pleiotropic lipid mediator that seems to play a critical role in pathophysiological conditions such as atherosclerosis, hypertension and diabetes (19). Therefore, it is likely that regulation of S1P biological activity could be a therapeutic target to control vascular disorders (20). In this context, a synthetic S1P analogue (FTY720) efficiently prevented atherosclerosis development (17, 21). Moreover, HMG-CoA reductase inhibitors (statins), lipid-lowering drugs whose beneficial effect on cardiovascular morbidity and mortality have been widely demonstrated and that have direct vascular effects (22), are able to modulate the expression S1P receptors in vascular cells and potentiate the response to HDL-associated sphingolipids (23, 24). This review is focussed on the role of S1P and its receptors in the vascular effects elicited by HDL, in particular those related to the modulation of nitric oxide (NO) and prostacyclin production.

Correspondence to:

Dr. José Martínez-González

Centro de Investigación Cardiovascular (CSIC-ICCC)

Hospital de la Santa Creu i Sant Pau

Antoni M<sup>o</sup> Claret 167, 08025 Barcelona, Spain

Tel.: +34 93 5565896, Fax: +34 93 5565559

E-mail: jmartinez@csic-iccc.org

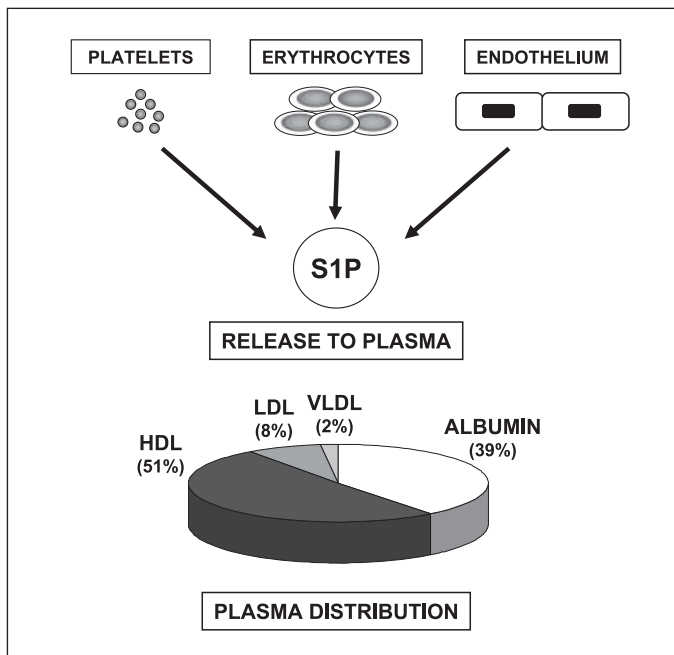
\*These authors contributed equally.

Received: October 17, 2008

Accepted after minor revision: January 21, 2009

Prepublished online: March 11, 2009

doi:10.1160/TH08-10-0675



**Figure 1: Plasma sphingosine-1-phosphate (S1P) source and transport by lipoproteins.** S1P is released into plasma from activated platelets, erythrocytes and endothelial cells, although their relative contribution is unknown. Lipoproteins and albumin act as acceptors of S1P being HDL the main carrier of plasma S1P.

## Plasma S1P levels and sources of S1P

In mammals S1P is found mainly in the blood and lymph. Platelets have been traditionally considered the main source of blood S1P because they store considerable amounts of S1P (1). However, S1P levels are normal in platelet-deficient animals and platelet depletion did not decrease plasma S1P levels (3, 25). Recent studies have revealed erythrocytes as the main blood cell incorporating, storing and releasing S1P (2); in fact, strong positive correlations between plasma S1P concentration and red blood cell-related parameters have been found (26). Vascular en-

**Table 1: S1P receptors in human vascular cells and monocytes/macrophages.**

Cell type	S1P receptor relative expression	Reference
Endothelial cells	S1P <sub>1</sub> and S1P <sub>3</sub> ; S1P <sub>2</sub> ND	29
	S1P <sub>1</sub> > S1P <sub>3</sub> > S1P <sub>2</sub>	30
	S1P <sub>1</sub> ; S1P <sub>3</sub> ND	31
VSMC	S1P <sub>2</sub> >S1P <sub>3</sub> >S1P <sub>1</sub>	30
	S1P <sub>3</sub> ; S1P <sub>2</sub> in aorta but not coronary; S1P <sub>1</sub> ND	31
	S1P <sub>2</sub> > S1P <sub>3</sub> ; S1P <sub>1</sub> , S1P <sub>4</sub> and S1P <sub>5</sub> ND	32
	S1P <sub>2</sub> > S1P <sub>1</sub> > S1P <sub>3</sub> > S1P <sub>5</sub> ; S1P <sub>4</sub> ND	33
	S1P <sub>2</sub> > S1P <sub>3</sub> > S1P <sub>1</sub> > S1P <sub>5</sub> ; S1P <sub>4</sub> ND	24
Monocyte/macrophage	S1P <sub>3</sub> >S1P <sub>4</sub> >S1P <sub>1</sub> >S1P <sub>2</sub> >S1P <sub>5</sub>	91

ND, not detected; S1P, sphingosine-1-phosphate; VSMC, vascular smooth muscle cells.

dothelium has also emerged as a major contributor of plasma S1P (3). S1P is synthesised intracellularly by phosphorylation of sphingosine by sphingosine kinase (1) and exported to the extracellular environment by transporters of the ABC family (4). Lipoproteins and albumin act as S1P acceptors. Plasma S1P is mainly found in a lipoprotein-bound form (~60%) HDL being the major carrier (~85%) (Fig. 1) (5). By binding to lipoproteins S1P is protected from degradation and hence extend its half-life; however, the association to lipoproteins could also reduce S1P bioactivity. Indeed, human serum S1P concentrations (300 nM to 1 μM) largely exceed the K<sub>d</sub> values of S1P receptors and the bioactive S1P fraction in plasma has been estimated in approximately 10 nM (27). The interaction of S1P with lipoproteins would avoid the full activation of S1P receptors contributing to temper acute reactions and to maintain tonic, more sustained long-term responses. Therefore, plasma S1P is dynamically controlled by various factors including release from cells, distribution among plasma proteins and degradation by ectophosphatase.

## S1P receptors in vascular cells

S1P was initially considered to be a second messenger. However, the discovery of G protein-coupled receptors (GPCR) for S1P significantly improved the understanding of the cellular actions of S1P and emphasised its role as an extracellular lipid mediator. In the vasculature, S1P induces dramatic responses in endothelial and vascular smooth muscle cells (VSMC) acting as ligand for high-affinity cell surface receptors. Five subtypes of GPCR termed S1P<sub>1</sub>–S1P<sub>5</sub> (formerly *Endothelial differentiation genes*, EDGs) have been identified so far: S1P<sub>1</sub> (*EDG-1*), S1P<sub>2</sub> (*EDG-5*), S1P<sub>3</sub> (*EDG-3*), S1P<sub>4</sub> (*EDG-6*) and S1P<sub>5</sub> (*EDG-8*) (28). These receptors bind S1P with a K<sub>d</sub> of 8–20 nM and exhibit a low affinity for other related lipids such as lysophosphatidic acid (LPA) or sphingosylphosphorylcholine (SPC). They are widely expressed but exhibit differential expression patterns depending on the cell type thereby specifying cellular responses. In the vasculature, the precise distribution of S1P receptors has not been clearly established, although S1P<sub>1</sub>, S1P<sub>2</sub> and S1P<sub>3</sub> seem to be the preponderant receptors expressed in vascular cells (Table 1) (29–34). This expression pattern seems to conform to that reported for other species, although contrasting results have been published depending on the vascular beds analysed (34).

The molecular details on the binding and the mechanism of S1P receptor activation are not well understood. S1P receptors exhibit dissimilar coupling mechanism to G-protein subunits. S1P<sub>1</sub> is coupled to G<sub>i/o</sub> proteins, preferentially G<sub>iα1</sub> and G<sub>iα3</sub>, S1P<sub>2</sub> is associated to G<sub>i/o</sub>, G<sub>12/13</sub> and G<sub>q</sub>; S1P<sub>3</sub> activates either G<sub>i/o</sub>, G<sub>q</sub> or G<sub>12/13</sub> proteins, while S1P<sub>4</sub> and S1P<sub>5</sub> signal through G<sub>i/o</sub> or G<sub>12/13</sub> and G<sub>i/o</sub> or G<sub>12</sub> subunits, respectively (13, 28). The complexity of S1P coupling to G proteins drives the regulation of multiple downstream signalling pathways, eliciting an intricate pattern of cellular responses depending on the relative expression levels of each S1P receptor (Table 2). Despite this apparent redundancy in intracellular signalling pathways linked to S1P receptors, Sensken et al. (35) have recently shown that certain S1P receptor agonists are able to selectively activate particular signalling pathway coupled to these receptors.

**Table 2: Effects elicited by SIP or its synthetic analogue FTY720 on vascular cells and monocytes/macrophages.**

Cell type	Cellular process	Effect	Reference
Endothelial cells	Nitric oxide (NO) synthesis	↑	14, 39, 67–69
	Cell adhesion molecules	↓↑	53–55, 57, 58
	Apoptosis	↓	62–65
	Migration	↑	67–69, 71, 72
	Proliferation	↑	15, 16, 67–69, 73
	Cytoskeletal rearrangement	↑	67–69
	Vessel formation	↑	15, 16, 73
	Endothelial barrier integrity	↑	74–77
	EPC number and bioactivity	↑	82
VSMC	Migration/Proliferation	↓↑	30, 32, 83–86
	Vasodilation/Vasoconstriction	↓↑	39, 41, 50, 51
	Prostacyclin synthesis	↑	24, 33
	MCP-1 synthesis	↓	21, 89
Monocytes/macrophages	Phagocytic activity	↑	91
	Inflammatory response (TLR2 signalling)	↓	93
	Anti-inflammatory phenotype	↑	94
	Endothelial interaction	↓	59–61
	Pro-inflammatory cytokines	↓	17

EPC: Endothelial progenitor cell; MCP-1: monocyte chemoattractant protein-1; TLR2, Toll-like receptor 2; VSMC, vascular smooth muscle cells.

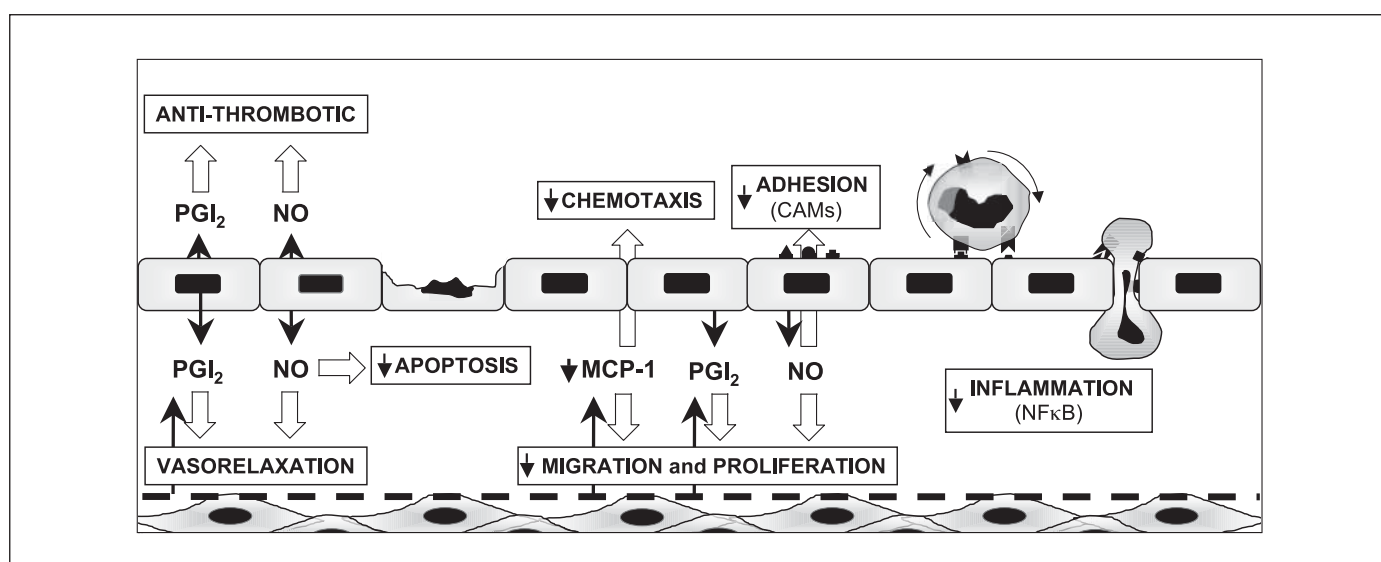
### Vascular effects of HDL mediated by SIP

Reverse cholesterol transport is the most widely accepted mechanism for the protection exerted by HDL. Additionally, HDL activates S1P receptors and triggers a number of effects on vascular cells among them up-regulation of NO and prostacyclin, two molecules that mediate a wide spectrum of vasoprotective functions. In fact, prostacyclin acts synergistically with NO to induce VSMC relaxation, inhibit platelet activation and prevent VSMC migration and proliferation (Fig. 2). Therefore, it is likely that the widely demonstrated effects of HDL on vascular reactivity, and

its anti-atherogenic and anti-thrombotic actions could be partially attributed to the modulation of these molecules by SIP contained in HDL.

### SIP in HDL-induced synthesis of vasorelaxant/antithrombotic molecules

Among the atheroprotective properties of HDL, improvement of endothelial function stands out as one of the most significant. Clinical studies have demonstrated a direct association between HDL levels and endothelium-dependent flow-mediated dilation (FMD); as such, in hypercholesterolemic individuals endothelial

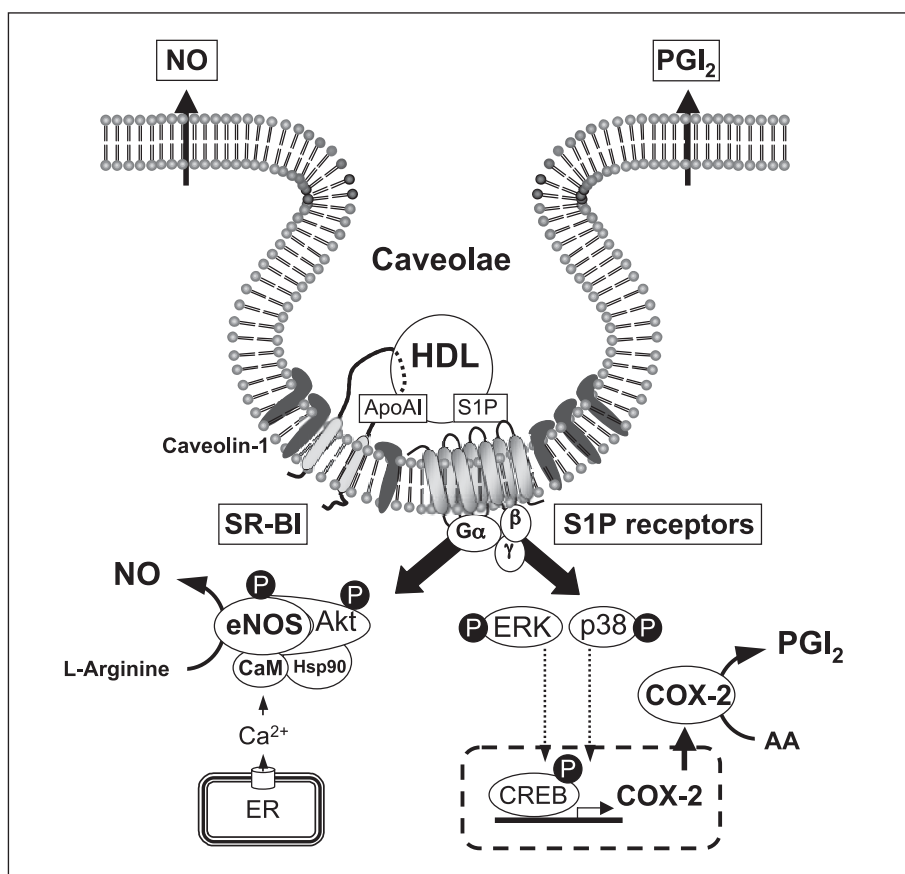


**Figure 2: Essential role of nitric oxide (NO) and prostacyclin (PGI<sub>2</sub>) in vascular responses mediated by high-density lipoprotein (HDL)-associated sphingosine-1-phosphate (SIP).** CAMs, cell adhesion molecules; MCP-1, monocyte chemoattractant protein-1; NFκB, nuclear factor-κB.

function is restored by acute administration of reconstituted HDL (11). One of the vasoprotective effects of HDL rely on the stimulation of endothelial NO release. HDL binds to scavenger receptor-BI (SR-BI) and through a phosphatidylinositol-3 kinase (PI3K)/Akt-dependent mechanism induces the phosphorylation of eNOS increasing its catalytic activity (36, 37). Furthermore, HDL extends the half-life of eNOS contributing to enhance NO production (38). The nature of the lipoprotein component responsible for HDL-vasodilatory properties remained undefined until Nofer et al. (14) uncovered that lysophospholipids carried by HDL, such as S1P, SPC and lysosulfatide (LSF) fully emulated the stimulatory effect of HDL on Akt activation, eNOS phosphorylation, NO production and NO-dependent vasorelaxation. These authors showed that the S1P<sub>3</sub> receptor is critical for these HDL-induced effects, which were absent in aortas or endothelial cells from S1P<sub>3</sub>-deficient mice (14). Accordingly, FTY720 (a structural S1P analogue) elicited an endothelium-dependent vasodilatory response in phenylephrine pre-contracted aortic rings from wild-type animals but not from either S1P<sub>3</sub>- or eNOS-deficient mice (39). Although S1P<sub>3</sub> receptor seems to play a key role in HDL-dependent eNOS regulation the contribution of other S1P receptors should not be excluded. In fact, statins up-regulate the endothelial expression of S1P<sub>1</sub> (the major receptor in endothelial cells) thereby enhancing NO production in response to HDL (23). Based on these observations, a cooperative mechanism has been suggested by which HDL binding to SR-BI brings S1P into proximity of S1P<sub>1</sub> and S1P<sub>3</sub> receptors providing a suitable context for a proper signal transduction. This

occurs mainly in caveolae, membrane microdomains that are specifically enriched in sphingolipids and contain a wide array of receptors including SR-BI and S1P receptors (Fig. 3) (36, 40). The intracellular sphingolipid pathway, and more specifically shingosine kinase activity, seems also to contribute to the vasorelaxant response and eNOS induction elicited by S1P in pre-contracted rat aorta (41). However, accumulating evidence suggest that endothelial dependent vasorelaxation is mainly regulated by S1P receptor-mediated mechanisms.

Besides NO, HDL regulates vascular reactivity through the synthesis of prostacyclin (PGI<sub>2</sub>), the major eicosanoid synthesised in the mammalian vasculature (42–44). The rate-limiting enzyme in the biosynthesis of PGI<sub>2</sub> is prostaglandin endoperoxide H synthase (cyclooxygenase, COX) which catalyses the conversion of arachidonic acid to PGH<sub>2</sub>, the first committed step in the biosynthesis of a wide range of eicosanoids. We and others have shown that HDL increases PGI<sub>2</sub> synthesis in both VSMC and endothelial cells through a mechanism depending on the up-regulation at transcriptional level of the inducible COX isoform (COX-2) (42–45), and that COX-2 inhibitors prevent the induction of PGI<sub>2</sub> production caused by HDL in vascular cells (46). In the light of growing evidence, not the least the clinical failure of chronic coxibs use, it begins to be clear that COX-2-derived prostanoids are key protective molecules for the cardiovascular system (47). Indeed, steady laminar shear stress selectively and sustainedly up-regulates COX-2 expression and PGI<sub>2</sub> synthesis in vascular endothelium while turbulent shear stress (typical of vascular areas prone to develop atherosclerosis) does not (48).



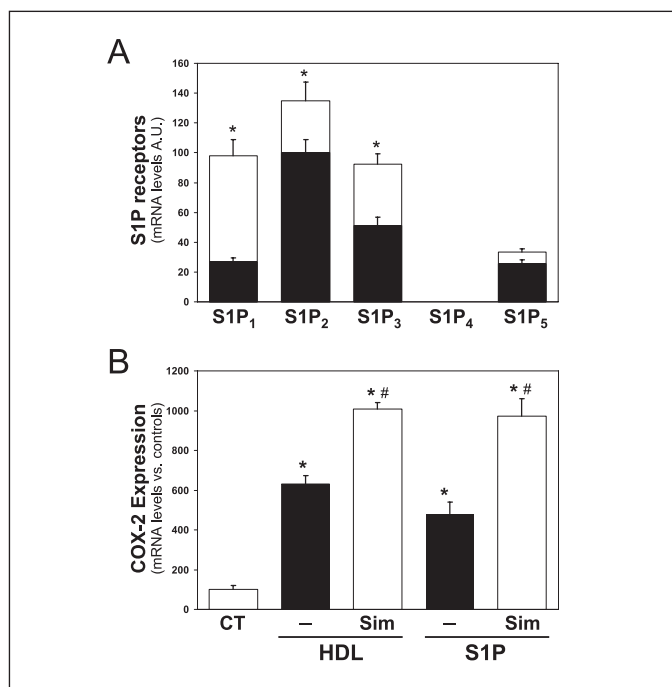
**Figure 3: Sphingosine-1-phosphate receptors mediate the activation of endothelial nitric oxide synthase (eNOS) and the up-regulation of cyclooxygenase-2 (COX-2) induced by HDL.** HDL increases Akt-dependent phosphorylation of eNOS through a cooperative mechanism that involves binding to scavenger receptor B-I (SR-BI) and the S1P receptor S1P<sub>3</sub>. Activated eNOS leads to an increase in NO biosynthesis through a catalytic process that requires the release of eNOS from membrane-bound eNOS/caveolin-1 complexes and the association with calcium-activated calmodulin (CaM) and heat-shock protein 90 (Hsp90). S1P receptors are also involved in the up-regulation of COX-2 and the increase in prostacyclin (PGI<sub>2</sub>) release induced by HDL in vascular cells. HDL (and free S1P) activates S1P receptors and downstream signalling pathways (p38 mitogen-activated protein kinase (p38) and extracellular signal regulated kinase-1/2 [ERK]) leading to activation of cAMP response element binding protein (CREB) that up-regulates COX-2 expression. AA, arachidonic acid.

The ability of HDL to induce COX-2/PGE<sub>2</sub> is abolished by HDL delipidation and it is affected by the lipid composition of the diet suggesting the involvement of a lipid component of HDL (46). Indeed, subsequent research revealed that free S1P mimics the induction of COX-2 expression/PGE<sub>2</sub> production caused by HDL in VSMC (24, 34). The effect was completely inhibited by pertussis toxin, an inhibitor of G(i/o) proteins. Moreover, using specific S1P receptor antagonists it has been shown that S1P receptors, in particular the two main subtypes expressed by VSMC (S1P<sub>2</sub> and S1P<sub>3</sub>), participate in COX-2-mediated PGE<sub>2</sub> release induced by both free S1P (24, 34) and HDL (34). Signalling downstream S1P receptors involves mitogen-activated protein kinases (both ERK1/2 and p38 MAPK) that lead to cAMP response element binding protein (CREB) phosphorylation and up-regulation of COX-2 expression (24). Interestingly, pre-treatment of human VSMC with simvastatin increases HDL-induced PGE<sub>2</sub> release (49), effect that has been recently associated to the up-regulation of S1P receptors and the consequent enhancement of COX-2 expression produced by HDL (Fig. 4) (24). Therefore, the up-regulation of S1P receptors by statins, reported in both endothelial cells and VSMC (23, 24), could contribute to the pleiotropic effects of these drugs, linked in this case to the vasoprotective effect of HDL.

Nevertheless contrasting results have been published concerning the vasomotor activity of free S1P. Indeed, while vasodilatory effects have been reported in pre-contracted arteries *ex vivo*, S1P has shown to induce vasoconstriction in native, non-contracted arteries (50, 51). The differential expression pattern of S1P receptors exhibited by endothelial cells and VSMC as well as the dissimilar concentration of S1P used in the different studies might help to explain these confusing results. Indeed, endothelial cells express mainly S1P<sub>1</sub> whose activation increases intracellular calcium that triggers induction of NO synthesis and vasorelaxation (19). By contrast, the activation of S1P<sub>2</sub>, the main S1P receptor present in VSMC, also causes a rise in intracellular calcium levels but activates phospholipase C-beta (PLC-β) and Rho kinase mediating cell contraction (19).

### S1P in the modulation of endothelial cell adhesiveness by HDL

Monocyte recruitment and adhesion to vascular endothelium is a key event in the pathogenesis of atherosclerosis. Dysfunctional endothelium expresses cell adhesion molecules (CAMs) in response to atherosclerotic risk factors and inflammatory mediators, thereby promoting mononuclear cell trafficking across the endothelial monolayer (Fig. 2). Cockerill et al. (52) early reported that HDL inhibit cytokine-induced expression of CAMs, including vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and E-selectin. This effect was associated with the up-regulation of COX-2 and the concomitant increases of prostacyclin synthesis (52). More recently, it has been shown that HDL-associated sphingolipids (SPC or LSF) attenuate the expression of CAMs induced by tumour necrosis factor (TNF)α via Akt-nuclear factor-kappaB (NFκB) signalling pathways (53). S1P contained in HDL is required for this effect that involves both SR-BI and S1P receptors (especially S1P<sub>1</sub>) and the activation of eNOS (54). The involvement of eNOS/NO is in agreement with the widely re-



**Figure 4: Simvastatin induces the expression of sphingosine-1-phosphate (S1P) receptors and increases cellular response to high-density lipoproteins (HDL).** A) Relative mRNA levels of S1P receptors in human vascular smooth muscle cells (VSMC) in the absence (black bars) or presence of simvastatin (white bars).  $P < 0.005$ :\* vs. controls. B) Pre-treatment of VSMC with simvastatin (sim) potentiates the induction of COX-2 mRNA levels produced by either HDL or S1P. [ $P < 0.005$ :\* vs. controls; # vs. cells treated with HDL or S1P]. A.U. arbitrary units. (modified from ref. 24).

ported ability of NO to modulate the expression of endothelial CAMs both *in vitro* and *in vivo*.

Concerning the effect of free S1P on CAM expression opposite effects have been reported (55–61). It should be taken into account, however, that in most studies showing stimulatory effects of S1P on VCAM-1 and ICAM-1 expression high S1P concentrations were used; thus, the physiological relevance of these studies is dubious. Endogenous S1P synthesised by the sphingosine kinase pathway also mediates the induction of endothelial CAM expression stimulated by TNFα or globular adiponectin through a NFκB-dependent mechanism (56, 57). Accordingly, the inhibition of cytokine-mediated CAM induction by HDL has been related to a disturbance of the sphingosine kinase pathway and a consequent reduction of intracellular S1P synthesis (56). This dual and apparently paradoxical effect of S1P could be explained by the ability of S1P receptors to mediate stimulatory or inhibitory signalling for expression of CAMs. Stimulation seems to occur mainly through S1P<sub>3</sub> and involves NFκB activation, while inhibition is mediated by S1P<sub>1</sub>, the main receptor expressed by endothelial cells, and involves PI3K/Akt/eNOS pathway (54, 58). Interestingly, functional analyses *in vitro* and *in vivo* have consistently shown a suppressive action of S1P on monocyte-endothelial interactions, effect mediated by S1P receptors (59–61) (in particular by S1P<sub>1</sub>) and associated to the inhibition of RhoA activity (60) and to the rearrangement of integrins α5β1 and αvβ3 (61).

### S1P in the modulation of endothelial cell survival/apoptosis by HDL

The endothelium is a selective permeability barrier to blood molecules and plays a critical role modulating VSMC function and maintaining vascular haemostasis. However, the exposure to risk factors or harmful agents can induce endothelial cell apoptosis disrupting endothelial monolayer integrity and contributing to cardiovascular diseases. HDL prevents apoptosis and preserves endothelial cell survival. Indeed, HDL suppresses the mitochondrial pathway of apoptosis inhibiting caspase-9 and -3 activities, reactive oxygen species (ROS) generation, dissipation of mitochondrial potential and cytochrome c release (62). Moreover, HDL activates Akt and causes phosphorylation of the Akt target BAD, which favours BAD dissociation from BCL-X<sub>L</sub> that is then free to inhibit mitochondria-mediated apoptosis (62). Interestingly, these effects are mimicked by HDL lysosphingolipids such as SPC, LSF and S1P (62, 63). The role of S1P in the cytoprotection elicited by HDL is further supported by the fact that S1P desensitisation or HDL-charcoal treatment abolishes such effects (64). An increase in NO production, that requires the activation of the receptor S1P<sub>1</sub>, underlies the anti-apoptotic action of S1P (63). Furthermore, the powerful atheroprotective features of small dense HDL3 particles could be related not only with their exacerbated cellular cholesterol efflux potential, but also with the stronger anti-apoptotic and anti-oxidative properties conferred by their enrichment in S1P (65). Conversely, oxidised low-density lipoproteins (oxLDL) are cytotoxic due to harmful substances such as lysophosphatidylcholine but also to the oxidation-mediated decrease of S1P content (64). Finally, the ability of VLDL from certain apolipoprotein E mouse genotypes to antagonise the anti-caspase activity of HDL is counteracted by diets that enable VLDL with an anti-apoptotic activity related to its increased capacity to activate S1P<sub>3</sub> (66).

### S1P in the pro-angiogenic effects of HDL

HDL exhibits pro-angiogenic properties that could be associated, at least in part, to its S1P content. S1P itself behaves as an angiogenic mediator able to increase NO production, to stimulate endothelial cell migration and proliferation and to promote cytoskeletal rearrangement (67–69). Furthermore, angiogenic factors such as vascular endothelial growth factor (VEGF) up-regulate S1P<sub>1</sub> expression sensitizing vascular endothelium (70). The S1P fraction of HDL seems also to account for the induction of endothelial cell proliferation and the formation of new vessels induced by this lipoprotein (15, 16). In fact, activation of S1P receptors (S1P<sub>1</sub> and S1P<sub>3</sub>) is required for HDL-induced cell migration via PI3K/Akt, p38 MAPK, Rho/Rho kinase and Ras-Raf1-dependent signalling pathways (71, 72). Accordingly, combined suppression of these receptors with specific anti-sense oligonucleotides dramatically reduced endothelial cell motility in response to HDL and S1P (71, 72). Moreover, recently Matsuo et al. (73) reported that reconstituted HDL (rHDL) particles containing S1P induced endothelial cell proliferation and tube formation by the activation of S1P<sub>2</sub> and S1P<sub>3</sub> receptors and the Akt/ERK/eNOS pathways.

S1P contained in HDL could also favour endothelial barrier integrity, a critical aspect in the final stages of angiogenesis to assure new vessel stabilisation. In fact, several studies revealed

that S1P enhances endothelial integrity through a complex mechanism that involves the modulation of cytoskeleton, adherent junction and focal adhesion (74). S1P increases trans-endothelial electrical resistance (TEER), an index of endothelial integrity, and enhances endothelial barrier function (75, 76). Accordingly, Argraves et al. (77) demonstrated that the content of S1P is critical for the regulation of endothelial barrier by HDL via a S1P<sub>1</sub>/Akt pathway. These authors also observed that the enhanced migratory activity exhibited by endothelial cells in the presence of either free S1P or HDL correlates with TEER.

Finally, an additional mechanism underlying HDL pro-angiogenic effects could be the improvement of endothelial progenitor cell (EPC) number and bioactivity observed in clinical and experimental studies (78–80). This HDL effect is associated to the up-regulation of eNOS and the prevention of EPC apoptosis (78). In this regard, it has been reported that FTY720 increases CXCR4 function *in vitro* and *in vivo* supporting homing and proliferation in haematopoietic progenitor cells (81). Moreover, both S1P and FTY720 improved blood flow recovery in ischaemic hind limbs in mice, effect that was dramatically reduced in S1P<sub>3</sub> deficient mice (82). Therefore, it is tempting to speculate that S1P/S1P receptors could contribute to such beneficial effects of HDL.

### S1P in the modulation of VSMC migration and proliferation by HDL

VSMC migration and proliferation are hallmarks of the pathogenesis of atherosclerosis and restenosis post-angioplasty. LDL and HDL regulate these processes in an opposite manner. LDL induces VSMC migration while HDL is able to counteract the migratory activity induced by chemoattractant agents such as LPA or platelet-derived growth factor (PDGF) (83, 84). Both HDL (83, 84) and S1P (30, 32, 83, 84) have shown anti-migratory effects on VSMC mediated by S1P receptors. In fact, S1P receptor desensitisation (incubating VSMC with an excess of S1P) reverses the inhibitory effects of HDL and S1P on PDGF-induced VSMC spreading and migration (83). Moreover, these inhibitory actions of HDL and S1P were suppressed by knock-down of the S1P<sub>2</sub> receptor (84). Interestingly, LDL depletion of LPA allows LDL to inhibit VSMC migration, effect that was also related with S1P<sub>2</sub> receptor activation (80). Thus, it seems that the ratio LPA/S1P determines the ability of lipoproteins to modulate VSMC migration in a positive or negative manner.

As has been described for other S1P effects the action of free S1P on VSMC migratory and proliferative responses seems to be bimodal, and contrasting results showing either stimulatory (85, 86) or inhibitory effects (30, 32) have been published. These opposite responses seem to be mediated by different S1P receptors. S1P<sub>1</sub> acts as a typical chemotactic receptor and its over-expression potentiates VSMC migratory and proliferative responses (87). By contrast, S1P<sub>2</sub> uniquely acts as a chemorepellent receptor and inhibits VSMC migration (30, 32, 88). Therefore, in normal quiescent human VSMC a low S1P<sub>1</sub>/S1P<sub>2</sub> ratio results in inhibition of migration.

HDL also inhibits the synthesis and secretion of monocyte chemoattractant protein-1 (MCP-1), a pro-inflammatory chemokine that modulates VSMC migration and proliferation and monocyte infiltration. This HDL effect is mediated by inhibition

of NAD(P)H oxidase-dependent ROS generation and is mimicked by the HDL-associated sphingolipids (S1P and SPC) and by the phosphorylated form of the S1P analogue FTY720 (89). Accordingly, FTY720 inhibits thrombin-induced release of MCP-1 in isolated aortic segments from wild-type but not from S1P<sub>3</sub><sup>-/-</sup> mice (21). Furthermore, it has been shown that the ablation of either S1P<sub>3</sub> or SR-BI receptors abolishes the modulation of MCP-1 production by either HDL or S1P suggesting the need of cooperative mechanisms between both receptors (89).

Interestingly, HDL suppresses VSMC cell cycle progression by stimulating prostacyclin synthesis leading to inhibition of G1 phase progression through an IP-dependent mechanism (90). Finally, in addition to the inhibitory effects of S1P and HDL directly acting on VSMC, the up-regulation of endothelial NO and prostacyclin, two molecules with well-recognised anti-migratory and anti-proliferative effects on VSMC may further contribute to prevent atherosclerosis and restenosis.

### S1P in the modulation of monocyte/macrophage function by HDL

Mononuclear cells play a key role in the atherosclerotic process, and the effectiveness of the S1P analogue FTY720 reducing atherosclerosis in animal models has been mainly attributed to the ability of this drug to modulate the function of monocytes/macrophages and suppress the machinery involved in the migration of these cells into the vascular wall (17, 21). Monocytes/macrophages express S1P receptors (Table 1) (91), but scarce and somewhat contradictory information is available about direct regulatory functions of S1P on these cells (Table 2). It has been reported that S1P alter macrophage gene expression pattern, including up-regulation of CD32, and promoting macrophage phagocytic activity (91). Conversely, in animal models of inflammation S1P and FTY720 exerted protective properties (92). Anti-inflammatory effects of S1P carried by HDL on macrophage biological activity, has also been recently reported by Dueñas et al. (93) that described a selective attenuation of the Toll-like receptor 2 (TLR2) signalling and the consequent blockade of NFκB cascade. In agreement with this, S1P modulated the response of macrophages favouring the switch from pro-inflammatory M1 to anti-inflammatory M2 phenotype as Hugues et al. recently shown (94). Finally, consistent with an anti-inflammatory and atheroprotective role for S1P in a recent study performed in LDL receptor-deficient mice the amelioration of atherosclerotic lesions by FTY720 was associated to a decrease in plasma levels of pro-inflammatory cytokines secreted by activated

macrophages and to the inhibition of macrophage activation markers (17).

## Conclusions and perspectives

The atheroprotective properties of HDL raise the interest of therapeutic strategies to enhance plasma HDL levels. Although the un-expected failure of ILLUMINATE phase III trial due to unwanted side effects (95) has not hampered the development of new drugs for increasing HDL levels, alternative approaches aimed to improve HDL-mediated vascular responses should be also considered. In this context, the ability of a simple lipid carried by HDL to resume most of vascular effects of these lipoproteins as well as the atheroprotective properties exhibited by the S1P analogue FTY720 in animal models support the interest of S1P and S1P receptors as possible pharmacological targets to increase vascular protection. Considering the variety of S1P effects and the existence of multiple receptor subtypes, it provides a range of potential therapeutic targets currently investigated. This task may be hampered, however, by the broad tissue/cell distribution of S1P receptors and by the potential functional antagonism among S1P receptors. Interestingly, despite this apparent complexity it has been shown that certain S1P receptor agonists are able to selectively activate particular signalling pathways coupled to S1P receptors. Deciphering the complex interplay between S1P signalling as well as the molecular mechanism of S1P-receptor activation will provide clues for the better understanding of the functional relationship among S1P receptors and for approaching a rational design of selective agonists of S1P receptors (or S1P receptor-mediated signalling pathways). Therefore, despite the growing progress in the knowledge of vascular S1P receptor-mediated responses further research will be required to elucidate their roles under physiological and pathophysiological conditions and to develop more selective molecules aimed to treat specific disorders.

## Acknowledgements

We apologise to colleagues whose work has not been directly cited due to space limitations. Original work from our group mentioned in this paper was supported by grants from Sociedad Española de Cardiología (Beca Pfizer de investigación 2008), from the Ministerio de Sanidad y Consumo-ISCIII (grant PI061480 and Red Temática de Investigación Cardiovascular RECAVA [RD06/0014/0027]) and from the Ministerio de Educación y Ciencia (SAF-2006-07378 and SAF-2006-10009).

## References

1. Yatomi Y, Ozaki Y, Ohmori T, et al. Sphingosine 1-phosphate: synthesis and release. *Prostaglandins Other Lipid Mediat* 2001; 64: 107–122.
2. Hänel P, Andréani P, Gräler MH. Erythrocytes store and release sphingosine 1-phosphate in blood. *FASEB J* 2007; 21: 1202–1209.
3. Venkataraman K, Lee YM, Michaud J, et al. Vascular endothelium as a contributor of plasma sphingosine 1-phosphate. *Circ Res* 2008; 102: 669–676.
4. Mitra P, Oskeritzian CA, Payne SG, et al. Role of ABC1 in export of sphingosine-1-phosphate from mast cells. *Proc Natl Acad Sci USA* 2006; 103: 16394–16399.
5. Okajima F. Plasma lipoproteins behave as carriers of extracellular sphingosine 1-phosphate: is this an atherogenic mediator or an anti-atherogenic mediator? *Biochim Biophys Acta* 2002; 1582: 132–137.
6. Rhoads GG, Gulbrandsen CL, Kagan A. Serum lipoproteins and coronary heart disease in a population study of Hawaii Japanese men. *N Engl J Med* 1976; 294: 293–298.
7. Badimon JJ, Badimon L, Galvez A, et al. High density lipoprotein plasma fractions inhibit aortic fatty streaks in cholesterol-fed rabbits. *Lab Invest* 1989; 60: 455–461.
8. Badimon JJ, Badimon L, Fuster V. Regression of atherosclerotic lesions by high density lipoprotein plasma fraction in the cholesterol-fed rabbit. *J Clin Invest* 1990; 85: 1234–1241.
9. Ibanez B, Vilahur G, Cimmino G, et al. Rapid change in plaque size, composition, and molecular footprint after recombinant apolipoprotein A-I Milano (ETC-216) administration: magnetic resonance imaging study in an experimental model of atherosclerosis. *J Am Coll Cardiol* 2008; 51: 1104–1109.
10. Assmann G, Nofer JR. Atheroprotective effects of high-density lipoproteins. *Annu Rev Med* 2003; 54: 321–341.

11. Mineo C, Deguchi H, Griffin JH, Shaul PW. Endothelial and antithrombotic actions of HDL. *Circ Res* 2006; 98: 1352–1364.
12. Barter PJ, Puranik R, Rye KA. New insights into the role of HDL as an anti-inflammatory agent in the prevention of cardiovascular disease. *Curr Cardiol Rep* 2007; 9: 493–498.
13. Spiegel S, Milstien S. Sphingosine-1-phosphate: an enigmatic signalling lipid. *Nat Rev Mol Cell Biol* 2003; 4: 397–407.
14. Nofer JR, van der Giet M, Tölle M, et al. HDL induces NO-dependent vasorelaxation via the lysophospholipid receptor S1P3. *J Clin Invest* 2004; 113: 569–581.
15. Miura S, Fujino M, Matsuo Y, et al. High density lipoprotein-induced angiogenesis requires the activation of Ras/MAP kinase in human coronary artery endothelial cells. *Arterioscler Thromb Vasc Biol* 2003; 23: 802–808.
16. von Otte S, Paletta JR, Becker S, et al. Follicular fluid high density lipoprotein-associated sphingosine 1-phosphate is a novel mediator of ovarian angiogenesis. *J Biol Chem* 2006; 281: 5398–5405.
17. Nofer JR, Bot M, Brodde M, et al. FTY720, a synthetic sphingosine 1 phosphate analogue, inhibits development of atherosclerosis in low-density lipoprotein receptor-deficient mice. *Circulation* 2007; 115: 501–508.
18. Theilmeier G, Schmidt C, Herrmann J, et al. High-density lipoproteins and their constituent, sphingosine-1-phosphate, directly protect the heart against ischemia/reperfusion injury in vivo via the S1P3 lysophospholipid receptor. *Circulation* 2006; 114: 1403–1409.
19. Alewijnse AE, Peters SL. Sphingolipid signalling in the cardiovascular system: good, bad or both? *Eur J Pharmacol* 2008; 585: 292–302.
20. Yatomi Y. Sphingosine 1-phosphate in vascular biology: possible therapeutic strategies to control vascular diseases. *Curr Pharm Des* 2006; 12: 575–587.
21. Keul P, Tölle M, Lucke S, et al. The sphingosine-1-phosphate analogue FTY720 reduces atherosclerosis in apolipoprotein E-deficient mice. *Arterioscler Thromb Vasc Biol* 2007; 27: 607–613.
22. Martínez-González J, Badimon L. Influence of statin use on endothelial function: from bench to clinics. *Curr Pharm Des* 2007; 13: 1771–1786.
23. Igarashi J, Miyoshi M, Hashimoto T, et al. Statins induce S1P1 receptors and enhance endothelial nitric oxide production in response to high-density lipoproteins. *Br J Pharmacol* 2007; 150: 470–479.
24. González-Díez M, Rodríguez C, Badimon L, Martínez-González J. Prostacyclin induction by high-density lipoprotein (HDL) in vascular smooth muscle cells depends on sphingosine 1-phosphate receptors: effect of simvastatin. *Thromb Haemost* 2008; 100: 119–126.
25. Pappu R, Schwab SR, Cornelissen I, et al. Promotion of lymphocyte egress into blood and lymph by distinct sources of sphingosine-1-phosphate. *Science* 2007; 316: 295–298.
26. Ohkawa R, Nakamura K, Okubo S, et al. Plasma sphingosine-1-phosphate measurement in healthy subjects: close correlation with red blood cell parameters. *Ann Clin Biochem* 2008; 45: 356–363.
27. Hla T, Venkataraman K, Michaud J. The vascular S1P gradient-Cellular sources and biological significance. *Biochim Biophys Acta* 2008; 1781: 477–482.
28. Waeber C, Blondeau N, Salomone S. Vascular sphingosine-1-phosphate S1P1 and S1P3 receptors. *Drug News Perspect* 2004; 17: 365–382.
29. Kimura T, Watanabe T, Sato K, et al. Sphingosine 1-phosphate stimulates proliferation and migration of human endothelial cells possibly through the lipid receptors, Edg-1 and Edg-3. *Biochem J* 2000; 348: 71–76.
30. Tamama K, Kon J, Sato K, et al. Extracellular mechanism through the Edg family of receptors might be responsible for sphingosine-1-phosphate-induced regulation of DNA synthesis and migration of rat aortic smooth-muscle cells. *Biochem J* 2001; 353: 139–146.
31. Mazurais D, Robert P, Gout B, et al. Cell type-specific localization of human cardiac S1P receptors. *J Histochem Cytochem* 2002; 50: 661–670.
32. Ryu Y, Takuwa N, Sugimoto N, et al. Sphingosine-1-phosphate, a platelet-derived lysophospholipid mediator, negatively regulates cellular Rac activity and cell migration in vascular smooth muscle cells. *Circ Res* 2002; 90: 325–332.
33. Damirin A, Tomura H, Komachi M, et al. Sphingosine 1-phosphate receptors mediate the lipid-induced cAMP accumulation through cyclooxygenase-2/prostaglandin I2 pathway in human coronary artery smooth muscle cells. *Mol Pharmacol* 2005; 67: 1177–1185.
34. Michel MC, Mulders AC, Jongasma M, et al. Vascular effects of sphingolipids. *Acta Paediatr Suppl* 2007; 96: 44–48.
35. Sensken SC, Stäubert C, Keul P, et al. Selective activation of G $\alpha$  i mediated signalling of S1P3 by FTY720-phosphate. *Cell Signal* 2008; 20: 1125–1133.
36. Yuhanna IS, Zhu Y, Cox BE, et al. High-density lipoprotein binding to scavenger receptor-BI activates endothelial nitric oxide synthase. *Nat Med* 2001; 7: 853–857.
37. Mineo C, Yuhanna IS, Quon MJ, Shaul PW. High density lipoprotein-induced endothelial nitric-oxide synthase activation is mediated by Akt and MAP kinases. *J Biol Chem* 2003; 278: 9142–9149.
38. Rämetsä ME, Rämetsä M, Lu Q, et al. High-density lipoprotein increases the abundance of eNOS protein in human vascular endothelial cells by increasing its half-life. *J Am Coll Cardiol* 2003; 41: 2288–2297.
39. Tölle M, Levkau B, Keul P, et al. Immunomodulator FTY720 Induces eNOS-dependent arterial vasodilatation via the lysophospholipid receptor S1P3. *Circ Res* 2005; 96: 913–920.
40. Igarashi J, Michel T. Agonist-modulated targeting of the EDG-1 receptor to plasmalemmal caveolae. eNOS activation by sphingosine 1-phosphate and the role of caveolin-1 in sphingolipid signal transduction. *J Biol Chem* 2000; 275: 32363–32370.
41. Roviezzo F, Bucci M, Delisle C, et al. Essential requirement for sphingosine kinase activity in eNOS-dependent NO release and vasorelaxation. *FASEB J* 2006; 20: 340–342.
42. Viñals M, Martínez-González J, Badimon JJ, et al. HDL-induced prostacyclin release in smooth muscle cells is dependent on cyclooxygenase-2 (Cox-2). *Arterioscler Thromb Vasc Biol* 1997; 17: 3481–3488.
43. Viñals M, Martínez-González J, Badimon L. Regulatory effects of HDL on smooth muscle cell prostacyclin release. *Arterioscler Thromb Vasc Biol* 1999; 19: 2405–2411.
44. Cockerill GW, Saklatvala J, Ridley SH, et al. High-density lipoproteins differentially modulate cytokine-induced expression of E-selectin and cyclooxygenase-2. *Arterioscler Thromb Vasc Biol* 1999; 19: 910–917.
45. Norata GD, Callegari E, Inoue H, Catapano AL. HDL3 induces cyclooxygenase-2 expression and prostacyclin release in human endothelial cells via a p38 MAPK/CRE-dependent pathway: effects on COX-2/PGL-synthase coupling. *Arterioscler Thromb Vasc Biol* 2004; 24: 871–877.
46. Escudero I, Martínez-González J, Alonso R, et al. Experimental and interventional dietary study in humans on the role of HDL fatty acid composition in PGI2 release and Cox-2 expression by VSMC. *Eur J Clin Invest* 2003; 33: 779–786.
47. Martínez-González J, Badimon L. Mechanisms underlying the cardiovascular effects of COX-inhibition: benefits and risks. *Curr Pharm Des* 2007; 13: 2215–2227.
48. Topper JN, Cai J, Falb D, Gimbrone MA Jr. Identification of vascular endothelial genes differentially responsive to fluid mechanical stimuli. *Proc Natl Acad Sci USA* 1996; 93: 10417–10422.
49. Martínez-González J, Escudero I, Badimon L. Simvastatin potentiates PGI<sub>2</sub> release induced by HDL in human VSMC: effect on Cox-2 up-regulation and MAPK signalling pathways activated by HDL. *Atherosclerosis* 2004; 174: 305–313.
50. Ohmori T, Yatomi Y, Osada M, et al. Sphingosine 1-phosphate induces contraction of coronary artery smooth muscle cells via S1P2. *Cardiovasc Res* 2003; 58: 170–177.
51. Salomone S, Potts EM, Tyndall S, et al. Analysis of sphingosine 1-phosphate receptors involved in constriction of isolated cerebral arteries with receptor null mice and pharmacological tools. *Br J Pharmacol* 2008; 153: 140–147.
52. Cockerill GW, Rye KA, Gamble JR, et al. High-density lipoproteins inhibit cytokine-induced expression of endothelial cell adhesion molecules. *Arterioscler Thromb Vasc Biol* 1995; 15: 1987–1994.
53. Schmidt A, Geigenmüller S, Völker W, Buddecke E. The antiatherogenic and antiinflammatory effect of HDL-associated lysosphingolipids operates via Akt  $\rightarrow$  NF- $\kappa$ B signalling pathways in human vascular endothelial cells. *Basic Res Cardiol* 2006; 101: 109–116.
54. Kimura T, Tomura H, Mogi C, et al. Role of scavenger receptor class B type I and sphingosine 1-phosphate receptors in high density lipoprotein-induced inhibition of adhesion molecule expression in endothelial cells. *J Biol Chem* 2006; 281: 37457–37467.
55. Lee H, Lin CI, Liao JJ, et al. Lysosphingolipids increase ICAM-1 expression in HUVEC through a Gi and NF- $\kappa$ B-dependent mechanism. *Am J Physiol Cell Physiol* 2004; 287: C1657–1666.
56. Xia P, Vadas MA, Rye KA, et al. High density lipoproteins (HDL) interrupt the sphingosine kinase signalling pathway. A possible mechanism for protection against atherosclerosis by HDL. *J Biol Chem* 1999; 274: 33143–33147.
57. Kase H, Hattori Y, Jojima T, et al. Globular adiponectin induces adhesion molecule expression through the sphingosine kinase pathway in vascular endothelial cells. *Life Sci* 2007; 81: 939–943.
58. Kimura T, Tomura H, Mogi C, et al. Sphingosine 1-phosphate receptors mediate stimulatory and inhibitory signalings for expression of adhesion molecules in endothelial cells. *Cell Signal* 2006; 18: 841–850.
59. Whetzel AM, Bolick DT, Srinivasan S, et al. Sphingosine-1 phosphate prevents monocyte/endothelial interactions in type 1 diabetic NOD mice through activation of the S1P1 receptor. *Circ Res* 2006; 99: 731–739.
60. Tani M, Kawakami A, Nagai M, et al. Sphingosine 1-phosphate (S1P) inhibits monocyte-endothelial cell interaction by regulating of RhoA activity. *FEBS Lett* 2007; 581: 4621–4626.
61. Aoki S, Yatomi Y, Shimosawa T, et al. The suppressive effect of sphingosine 1-phosphate on monocyte-endothelium adhesion may be mediated by the rearrangement of the endothelial integrins  $\alpha$ (5) $\beta$ (1) and  $\alpha$ (v) $\beta$ (3). *J Thromb Haemost* 2007; 5: 1292–1301.
62. Nofer JR, Levkau B, Wolinska I, et al. Suppression of endothelial cell apoptosis by high density lipoproteins (HDL) and HDL-associated lysosphingolipids. *J Biol Chem* 2001; 276: 34480–34485.

63. Kwon YG, Min JK, Kim KM, et al. Sphingosine 1-phosphate protects human umbilical vein endothelial cells from serum-deprived apoptosis by nitric oxide production. *J Biol Chem* 2001; 276: 10627–10633.
64. Kimura T, Sato K, Kuwabara A, et al. Sphingosine 1-phosphate may be a major component of plasma lipoproteins responsible for the cytoprotective actions in human umbilical vein endothelial cells. *J Biol Chem* 2001; 276: 31780–31785.
65. Kontush A, Therond P, Zerrad A, et al. Preferential sphingosine-1-phosphate enrichment and sphingomyelin depletion are key features of small dense HDL3 particles: relevance to antiapoptotic and antioxidative activities. *Arterioscler Thromb Vasc Biol* 2007; 27: 1843–1849.
66. Mihovilovic M, Robinette JB, DeKroon RM, et al. High-fat/high-cholesterol diet promotes a S1P receptor-mediated antiapoptotic activity for VLDL. *J Lipid Res* 2007; 48: 806–815.
67. Lee OH, Kim YM, Lee YM, et al. Sphingosine 1-phosphate induces angiogenesis: its angiogenic action and signalling mechanism in human umbilical vein endothelial cells. *Biochem Biophys Res Commun* 1999; 264: 743–750.
68. English D, Welch Z, Kovala AT, et al. Sphingosine 1-phosphate released from platelets during clotting accounts for the potent endothelial cell chemotactic activity of blood serum and provides a novel link between hemostasis and angiogenesis. *FASEB J* 2000; 14: 2255–2265.
69. Rikitake Y, Hirata K, Kawashima S, et al. Involvement of endothelial nitric oxide in sphingosine-1-phosphate-induced angiogenesis. *Arterioscler Thromb Vasc Biol* 2002; 22: 108–114.
70. Igarashi J, Erwin PA, Dantas AP, et al. VEGF induces S1P1 receptors in endothelial cells: Implications for cross-talk between sphingolipid and growth factor receptors. *Proc Natl Acad Sci USA* 2003; 100: 10664–10669.
71. Kimura T, Sato K, Malchinkhuu E, et al. High-density lipoprotein stimulates endothelial cell migration and survival through sphingosine 1-phosphate and its receptors. *Arterioscler Thromb Vasc Biol* 2003; 23: 1283–1288.
72. Miura S, Tanigawa H, Matsuo Y, et al. Ras/Raf1-dependent signal in sphingosine-1-phosphate-induced tube formation in human coronary artery endothelial cells. *Biochem Biophys Res Commun* 2003; 306: 924–929.
73. Matsuo Y, Miura S, Kawamura A, et al. Newly developed reconstituted high-density lipoprotein containing sphingosine-1-phosphate induces endothelial tube formation. *Atherosclerosis* 2007; 194: 159–168.
74. McVerry BJ, Garcia JG. In vitro and in vivo modulation of vascular barrier integrity by sphingosine 1-phosphate: mechanistic insights. *Cell Signal* 2005; 17: 131–139.
75. Lee JF, Zeng Q, Ozaki H, et al. Dual roles of tight junction-associated protein, zonula occludens-1, in sphingosine 1-phosphate-mediated endothelial chemotaxis and barrier integrity. *J Biol Chem* 2006; 281: 29190–29200.
76. Xu M, Waters CL, Hu C, et al. Sphingosine 1-phosphate rapidly increases endothelial barrier function independently of VE-cadherin but requires cell spreading and Rho kinase. *Am J Physiol Cell Physiol* 2007; 293: C1309–1318.
77. Argraves KM, Gazzolo PJ, Groh EM, et al. HDL-associated sphingosine-1-phosphate promotes endothelial barrier function. *J Biol Chem* 2008; 283: 25074–25081.
78. Noor R, Shuaib U, Wang CX, et al. High-density lipoprotein cholesterol regulates endothelial progenitor cells by increasing eNOS and preventing apoptosis. *Atherosclerosis* 2007; 192: 92–99.
79. Tso C, Martinic G, Fan WH, et al. High-density lipoproteins enhance progenitor-mediated endothelium repair in mice. *Arterioscler Thromb Vasc Biol* 2006; 26: 1144–1149.
80. Petoumenos V, Nickenig G, Werner N. High density lipoprotein exerts vasculoprotection via endothelial progenitor cells. *J Cell Mol Med* 2008 Aug 14; epub ahead of print.
81. Kimura T, Boehmler AM, Seitz G, et al. The sphingosine 1-phosphate receptor agonist FTY720 supports CXCR4-dependent migration and bone marrow homing of human CD34+ progenitor cells. *Blood* 2004; 103: 4478–4486.
82. Walter DH, Rochwalsky U, Reinhold J, et al. Sphingosine-1-phosphate stimulates the functional capacity of progenitor cells by activation of the CXCR4-dependent signaling pathway via the S1P3 receptor. *Arterioscler Thromb Vasc Biol* 2007; 27: 275–282.
83. Tamama K, Tomura H, Sato K, et al. High-density lipoprotein inhibits migration of vascular smooth muscle cells through its sphingosine 1-phosphate component. *Atherosclerosis* 2005; 178: 19–23.
84. Damirin A, Tomura H, Komachi M, et al. Role of lipoprotein-associated lysophospholipids in migratory activity of coronary artery smooth muscle cells. *Am J Physiol Heart Circ Physiol* 2007; 292: H2513–2522.
85. Hobson JP, Rosenfeldt HM, Barak LS, et al. Role of the sphingosine-1-phosphate receptor EDG-1 in PDGF-induced cell motility. *Science* 2001; 291: 1800–1803.
86. Lockman K, Hinson JS, Medlin MD, et al. Sphingosine 1-phosphate stimulates smooth muscle cell differentiation and proliferation by activating separate serum response factor co-factors. *J Biol Chem* 2004; 279: 42422–42430.
87. Kluk MJ, Hla T. Role of the sphingosine 1-phosphate receptor EDG-1 in vascular smooth muscle cell proliferation and migration. *Circ Res* 2001; 89: 496–502.
88. Takuwa Y. Subtype-specific differential regulation of Rho family G proteins and cell migration by the Edg family sphingosine-1-phosphate receptors. *Biochim Biophys Acta* 2002; 1582: 112–120.
89. Tölle M, Pawlak A, Schuchardt M, et al. HDL-associated lysosphingolipids inhibit NAD(P)H oxidase-dependent monocyte chemoattractant protein-1 production. *Arterioscler Thromb Vasc Biol* 2008; 28: 1542–1548.
90. Kothapalli D, Fuki I, Ali K, et al. Antimitogenic effects of HDL and APOE mediated by Cox-2-dependent IP activation. *J Clin Invest* 2004; 113: 609–618.
91. Duong CQ, Bared SM, Abu-Khader A, et al. Expression of the lysophospholipid receptor family and investigation of lysophospholipid-mediated responses in human macrophages. *Biochim Biophys Acta* 2004; 1682: 112–119.
92. Peng X, Hassoun PM, Sammani S, et al. Protective effects of sphingosine 1-phosphate in murine endotoxin-induced inflammatory lung injury. *Am J Respir Crit Care Med* 2004; 169: 1245–1251.
93. Dueñas AI, Aceves M, Fernández-Pisonero I, et al. Selective attenuation of Toll-like receptor 2 signalling may explain the atheroprotective effect of sphingosine 1-phosphate. *Cardiovasc Res* 2008; 79: 537–544.
94. Hughes JE, Srinivasan S, Lynch KR, et al. Sphingosine-1-phosphate induces an antiinflammatory phenotype in macrophages. *Circ Res* 2008; 102: 950–958.
95. Nissen SE, Tardif JC, Nicholls SJ, et al. Effect of torcetrapib on the progression of coronary atherosclerosis. *N Engl J Med* 2007; 356: 1304–1316.