Endothelial Protection by High-Density Lipoproteins
From Bench to Bedside
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Abstract—There are several potential mechanisms by which HDLs protect against the development of vascular disease. One relates to the unique ability of these lipoproteins to remove cholesterol from the arterial wall. Another is the ability of HDL to prevent and eventually correct endothelial dysfunction, a key variable in the pathogenesis of atherosclerosis and its complications. HDLs help maintain endothelial integrity, facilitate vascular relaxation, inhibit blood cell adhesion to vascular endothelium, reduce platelet aggregability and coagulation, and may favor fibrinolysis. These functions of HDLs complement their activity in arterial cholesterol removal by providing an excellent rationale for favorably influencing pathological processes underlying a variety of clinical conditions, such as accelerated atherosclerosis, acute coronary syndromes, and restenosis after coronary angioplasty, through a chronic or acute elevation of plasma HDL concentration. (Arterioscler Thromb Vasc Biol. 2003;23:●●●-●●●.)

Key Words: high-density lipoproteins ■ endothelium ■ endothelial dysfunction ■ atherosclerosis

Human HDLs are a heterogeneous class of lipoproteins of high density (1.063 to 1.21 g/mL) and small diameter (5 to 17 nm). Most HDL particles contain apolipoprotein A-I (apoA-I) as the major protein component. Several other proteins, including apolipoprotein (apo) A-II, apoCs, apoE, minor apolipoproteins, lecithin:cholesterol acyltransferase (LCAT), paraoxonase (PON1), and platelet-activating factor acetylhydrolase (PAF-AH), are associated with HDL and impart significant physiological functions. The plasma concentration of HDL is routinely quantified as HDL cholesterol (HDL-C). However, differences in lipid and protein composition characterize several major and minor HDL particle subpopulations, which differ in density, size, shape, and surface charge.1 Although the physiological significance of these different particles is mostly undefined, some of them display peculiar functional properties, at least in vitro.2-4

Several prospective epidemiological studies provided overwhelming evidence that a low plasma HDL-C is a major, independent risk factor for the development of an acute coronary event.5 Studies in patients with rare disorders of HDL metabolism and in genetically modified animal models support a causal relationship between low HDL and development of atherosclerotic vascular disease. The atheroprotective activity of HDL is often explained by the unique ability of these lipoproteins to remove cholesterol from peripheral tissues, including the arterial wall, and transport it to the liver for excretion in the bile. The role of HDL and of their various subpopulations in this process, termed reverse cholesterol transport,6 has been recently reviewed in detail in this journal.7 The present review will focus on the effects of HDL on vascular endothelium and will discuss how the in vitro evidence from cell-culture studies translates into in vivo HDL-mediated endothelial protection, which may be relevant in the development and prevention of vascular disease.

Endothelial Dysfunction and Cardiovascular Disease
Traditionally, the endothelium has been considered an inert component of the vessel wall. During the past 2 decades it has become evident that the vascular endothelium plays an important role in the maintenance of vascular homeostasis through the production of a variety of substances that modulate vascular tone, inflammation, and hemostasis. Given its unique anatomical position, the endothelium serves as a primary target for mechanical and biochemical injuries caused by traditional risk factors, such as hypertension, hyperlipidemia, diabetes mellitus, and cigarette smoking. Injury to the endothelium results into deleterious alterations of endothelial physiology, also referred to as endothelial dysfunction, which represent a key early step in the development of an atherosclerotic lesion and are implicated in plaque progression and destabilization, thrombus generation, myocardial ischemia-reperfusion injury, and pathological arterial wall remodeling after coronary procedures.

Endothelial dysfunction has been reported in patients with primary hypoalphalipoproteinemia, a genetic lipoprotein disorder characterized by low plasma HDL-C and apoA-I levels and high coronary heart disease (CHD) risk.8,9 Moreover, a low plasma HDL concentration is an independent predictor of endothelial dysfunction in healthy individuals and hyperlip-
HDL and Vascular Tone

Nitric Oxide

NO is an endothelium-derived signaling molecule that activates guanylate cyclase in vascular smooth muscle cells (SMCs) to induce relaxation. A decrease in NO bioavailability is a prominent feature of endothelial dysfunction. In the endothelial cell, NO is generated by a constitutive endothelial NO synthase (eNOS), which is primarily localized to caveolae, cholesterol-rich microdomains of the plasma membrane that contain a variety of signal transduction molecules. eNOS becomes activated in response to multiple stimuli, including hemodynamic shear stress and agonists of diverse G protein-coupled cell-surface receptors.

Incubation of cultured endothelial cells with HDL activates eNOS in a process that involves the binding of apoA-I to the scavenger receptor-BI (SR-BI). However, eNOS is not activated by lipid-free apoA-I. Exactly how HDL activate eNOS is not clear. The enzymatic activity of eNOS is regulated by a variety of mechanisms, including membrane localization, intracellular calcium and ceramide, and phosphorylation. HDL interaction with SR-BI modifies membrane cholesterol distribution and morphology, thus potentially influencing eNOS activity. This same interaction leads to an intricate activation of kinase (Akt and mitogen-activated protein kinase [MAPK]) cascades, which may involve an increase of intracellular ceramide, ultimately resulting in eNOS phosphorylation and activation. HDL also enhance eNOS expression in cultured human endothelial cells.

In vivo studies provide additional support to the concept that HDLs prevent endothelial dysfunction by promoting endothelial NO production. Early investigations by quantitative coronary angiography and intravascular ultrasound analysis showed a positive correlation between plasma HDL concentration and NO-dependent coronary vasodilation. More recently, the plasma HDL-C concentration has been found to be an independent predictor of NO-dependent peripheral vasodilation in healthy individuals, hyperlipidemic and diabetic patients, and patients with CHD. A short-term treatment with niacin in patients with low HDL causes an elevation of plasma HDL with a parallel increase of NO-mediated vasodilation. Even more striking, the intravenous infusion of sHDL in hypercholesterolemic subjects rapidly restores the altered endothelium-dependent vasodilation by increasing NO bioavailability.

Prostacyclin

Prostacyclin (PGI2) is a potent endothelium-derived vasodilator that binds IP receptors on vascular SMC and acts synergistically with NO to induce smooth muscle relaxation. PGI2 is synthesized from arachidonate derived from phospho-
lipsidic cellular membranes or from exogenous sources, eg, phospholipids and cholesteryl esters of circulating lipoproteins. The enzyme responsible for PGI₂ production is cyclooxygenase (Cox), which exists as 2 different isoforms, a constitutive Cox-1 and an inducible Cox-2.

Incubation of cultured endothelial cells with HDL causes a dose-dependent increase of PGI₂ release, which is prevented by a Cox inhibitor, implying an effect on PGI₂ synthesis. The 2 major HDL subfractions, HDL₂ and HDL₃, are equally effective. Delipidated HDL apolipoproteins also enhance PGI₂ production, but to a lower extent than intact HDL. This finding suggests that activation of different mechanisms by HDL lipids and apolipoproteins may ultimately enhance the production of PGI₂. HDL can provide endothelial cells with arachidonate, which then acts as substrate for Cox-mediated PGI₂ synthesis. Because HDL cholesteryl esters are the most efficient donors of arachidonate for PGI₂ production, it is tempting to speculate that the HDL-induced PGI₂ synthesis may depend on SR-BI-mediated selective uptake and hydrolysis of HDL cholesteryl esters. SR-BI would thus appear as a major player in HDL-induced vasodilation, mediating the production of 2 potent vasodilators, NO and PGI₂. Finally, HDLs increase basal and cytokine-induced expression of the Cox-2 enzyme. Because cytokine-induced expression of Cox-2 is mediated through the transcription factor nuclear factor (NF)-κB, it was hypothesized that HDLs enhance Cox-2 expression through NF-κB activation. However, HDLs have no effect or even inhibit cytokine-induced nuclear translocation and DNA binding of NF-κB, indicating that the upregulation of Cox-2 by HDL occurs through an NF-κB-independent pathway. Because MAPK is also involved in cytokine-induced endothelial Cox-2 expression and HDLs trigger MAPK signaling in endothelial cells through SR-BI binding, it is tempting to speculate that HDLs may induce Cox-2 and eNOS (see above) expression in endothelial cells through a common SR-BI-dependent and MAPK-dependent signaling pathway. This is consistent with data on other cell types, eg, SMCs, showing that HDL enhance Cox-2 expression through MAPK activation.

An increased PGI₂ release is also observed in isolated rabbit hearts infused with homologous or heterologous HDL. HDLs additionally enhance the postischemic release of PGI₂ in isolated hearts, indicating that the HDL-induced PGI₂ production may participate in the heart’s own effort to upregulate its defense against the deleterious effects of ischemia-reperfusion.

There is limited information on the effects of HDL on PGI₂ synthesis/release in vivo in humans, but the plasma HDL-C level has been found to correlate with the plasma concentration of the stable PGI₂ metabolite 6-keto PGF₁α.

Endothelin

Endothelin is a potent vasoconstrictor peptide that binds to specific G protein-coupled receptors on SMCs to reverse the response to NO. Endothelial cells are the major source of endothelin-1 (ET-1), the most important isoform in the vascular system.

Early studies with bovine aortic endothelial cells showed a stimulatory effect of HDL on ET-1 production and secretion. These findings are in sharp contrast with those of a recent study, where human endothelial cells were cultured on a 2-chamber model system, which reproduces the physiological state where ET-1 is released toward the underlying intimal smooth muscle in a polar fashion. HDLs inhibited the secretion of ET-1 on the opposite side of the culture on which they were applied, suggesting that HDL may indeed prevent the vasoconstrictor effects of ET-1. Additional investigations are needed to clarify the effects of HDL on ET-1 secretion and to understand their relevance for endothelial function in vivo.

HDL and Inflammation

Cell Adhesion

Injury to vascular endothelium induces the expression of cell adhesion molecules (CAMs), such as vascular cell adhesion molecule-1, intercellular adhesion molecule-1, E-selectin, and P-selectin, which attract monocytes and other leukocytes to adhere to the endothelial surface and subsequently transmigrate into the intimal tissue. CAMs are synthesized in the endothelial cells by a variety of stimuli, including shear stress and proinflammatory cytokines, as tumor necrosis factor-α (TNF-α).

HDLs downregulate TNF-α–induced CAM expression in cultured human umbilical vein endothelial cells. The HDL₃ subfraction is more effective than HDL₂. shDL containing apoA-I and phospholipids, but neither phospholipid vesicles nor lipid-free apoA-I, prevent TNF-α–induced CAM upregulation; the shape and apolipoprotein/phospholipid composition of shDL remarkably influence their ability to affect CAM expression, with spherical particles made with apoA-I and linoleate-containing phosphatidylcholine being the most effective. The inhibition of CAM expression by HDL results in a significant reduction of leukocyte adhesion to cultured endothelial cells.

The molecular mechanisms by which HDLs downregulate TNF-α–induced CAM expression are poorly understood. Whereas HDLs have the potential to bind and inactivate TNF-α, an interference of HDL with the binding of TNF-α to its receptor is excluded by the observation that the inhibitory activity remains when HDLs are removed before cell activation. Therefore, the effect of HDL is likely secondary to a perturbation of signaling pathways at postreceptor sites. Because the transcription factor NF-κB is essential for TNF-α–induced CAM expression, HDL may act by inhibiting NF-κB activation. Indeed, HDLs block the TNF-α–induced nuclear translocation and DNA binding of NF-κB by interrupting a sphingosine kinase signaling pathway upstream of NF-κB activation and inhibiting NF-κB transactivation by the AP-1 transcription factor. It is noteworthy that the opposite mechanism, eg, NF-κB activation, has been invoked to explain Cox-2 induction by HDL (see above). This apparent discrepancy is concealed by the demonstration that HDLs indeed differentially modulate cyto-
kine-induced Cox-2 and E-selectin expression in cultured endothelial cells.30

Although studies are all consistent in showing the ability of HDL to modulate CAM expression in umbilical vein endothelial cells, conflicting results have been obtained with cells that more directly model blood vessels affected by atherosclerosis, ie, aortic and coronary endothelial cells. HDL and sHDL failed to downregulate TNF-α–induced CAM expression in human aortic and coronary endothelial cells,42,43 but sHDL inhibited the cytokine-induced expression of E-selectin in porcine aortic endothelial cells.44 The reasons for these discrepant findings are not immediately apparent.

Several studies in experimental animals provide evidence for HDL-mediated downregulation of CAM expression in vivo. Elevation of plasma HDL concentration by overexpression of apoA-I in apoE-deficient mice, an animal model of human atherosclerosis, significantly inhibits CAM expression on vascular endothelium and monocyte recruitment into the arterial wall.35 The infusion of sHDL reduces endothelial vascular cell adhesion molecule-1 expression and monocyte recruitment into the arterial wall of apoE-deficient mice,46,47 inhibits interleukin-1β–induced E-selectin expression in a porcine model of acute inflammation,48 and decreases intercellular adhesion molecule-1 and P-selectin expression in a rat model of hemorrhagic shock.41 In humans, a low plasma HDL concentration is associated with enhanced plasma levels of soluble CAMs,8 a surrogate marker of endothelial CAM expression.

Platelet-Activating Factor
PAF is a highly bioactive phospholipid that exerts a broad range of biological effects, such as stimulation of cell adhesion, vascular permeability, platelet aggregation, and smooth muscle contraction. PAF is generally released from the producing cells into the extracellular space and acts on target cells by binding to a specific G protein–coupled receptor. However, the PAF synthesized by activated endothelial cells remains on the cell membrane, where it mediates juxtacrine activation and subsequent adhesion of blood mononuclear cells.

HDLs inhibit, in a dose-dependent manner, agonist-induced production of PAF by cultured human endothelial cells; both lipid and apolipoprotein components are required for full inhibition.48 The biological activity of PAF is abolished by hydrolysis of the acetyl residue through the action of intracellular and extracellular PAF-AHs. Human plasma PAF-AH is in part associated with HDLs,49 which also bind and transport other PAF-degrading enzymes, eg, LCAT and paraoxonase.50,51 Therefore, by limiting PAF production by endothelial cells and enhancing its degradation by circulating enzymes, HDL may prevent PAF–induced adhesion of leukocytes to the activated endothelium, which may well contribute to the in vivo antiadhesive effects of HDL described above.

Inflammation Affects HDL Structure and Function
HDLs have been described as chameleon-like lipoproteins, because they are anti-inflammatory in the basal state but could loose their anti-inflammatory properties during an acute-phase response and chronic inflammatory states.52 In these conditions, HDLs undergo remarkable structural alterations through the acquisition of acute-phase reactants, such as serum amyloid A and ceruloplasmin, and by losing part of apoA-I, apoA-II, paraoxonase, and PAF-AH.53 Such modified HDLs are less effective than native HDL in protecting LDL against oxidative modifications and in activating LCAT.52 Whether inflammation also affects the anti-inflammatory actions of HDL is not known, but the enrichment of HDLs with serum amyloid A does not impair their capacity to inhibit CAM expression in cultured endothelial cells.39

HDL and Hemostasis

Platelet Adhesion and Reactivity
Besides being crucial mediators in the regulation of vascular tone, endothelium-derived NO and PGI2 have a powerful antithrombotic effect, inhibiting platelet aggregation by increasing cGMP and cAMP contents, respectively. PAF, instead, stimulates platelet aggregation. As described above, HDLs enhance NO and PGI2 production and limit PAF activity. Von Willebrand factor (vWF) is another protein expressed by endothelial cells that plays an essential role in platelet adhesion and aggregation; the circulating vWF levels are inversely correlated with plasma HDL,53 suggesting that HDL may inhibit vWF production. Therefore, by modulating the production/activity of a variety of endothelium-derived factors, such as NO, PGI2, PAF, and vWF, HDL may affect both vascular tone and thrombogenicity. It is noteworthy that a high plasma HDL-C level in humans is associated with a reduced ex vivo thrombogenic potential54 and that the injection of sHDL in an animal model of arterial thrombosis significantly reduces thrombus formation on the injured endothelial surface.55

Coagulation
Tissue factor (TF) is a membrane-bound protein that initiates the extrinsic coagulation pathway by mediating the activation of factors IX and X by factor VII. HDLs do not affect TF production by endothelial cells,56 but may suppress TF activity through a specific interaction between HDL apolipoproteins and TF.57,58 TF induction of the extrinsic coagulation pathway is counterbalanced by a serine-protease inhibitor known as TF pathway inhibitor (TFPI). Circulating TFPI consists of 2 forms, a free and a lipoprotein-bound TFPI. The latter associates preferentially with small, dense LDL and HDL subpopulations,59 and a significant correlation exists between TFPI activity and the plasma concentration of HDL,60 especially of dense HDL30 particles.61 HDLs were also reported to enhance the anticoagulant activity of activated protein C and protein S.62 The observation that the infusion of sHDL into human volunteers limits the procoagulant state associated with endotoxemia63 supports the concept that HDLs may exert a significant anticoagulant effect in vivo in humans.

Fibrinolysis
The endothelium participates in the regulation of fibrinolysis by producing and releasing plasminogen activators, such as tissue plasminogen activator, and inhibitors, such as plasminogen activator inhibitor. An early report indicated that HDLs reduce tissue plasminogen activator secretion and mRNA
levels in unstimulated and stimulated human endothelial cells, but this observation has not been confirmed by others. Both studies are consistent in showing no or weak effect of HDL on plasminogen activator inhibitor production. HDL apolipoproteins directly enhance urokinase-induced plasminogen activation in a purified in vitro system through an unknown mechanism. This activity may lead to the formation of unstable thrombi with an increased tendency to spontaneously dissolve, as observed in rats injected with sHDL.

### HDL and Endothelium Integrity

A continuous and intact monolayer of endothelial cells is required for the maintenance of normal vessel wall properties. The integrity of endothelium may be transiently or chronically disrupted by endothelial cell turnover, traumatic injury, or pathological damages. Under these circumstances, a rapid regeneration of the endothelium through the migration and proliferation of endothelial cells is a critical process in the response to injury. Incubation of cultured bovine aortic endothelial cells with HDL enhances endothelial cell migration; this effect is specific and comparable to that of basic fibroblast growth factor, the prototypical agonist of endothelial cell movement. The molecular mechanisms underlying the promigratory activity of HDL are not known, but the signaling pathway seems to be different from that of fibroblast growth factor. HDLs also stimulate the proliferation of bovine and human endothelial cells. Initially, it was proposed that the HDL-induced proliferation occurs through a protein kinase C–mediated pathway; HDL apolipoproteins were required for this effect. More recent data suggest the mitogenic effect of HDL to be mediated by a rise in intracellular pH and calcium, initiated by phospholipase C activation. The lipid fraction of HDL is responsible for the rise of intracellular calcium, suggesting that activation of 2 different signaling pathways by HDL apolipoproteins and lipids may ultimately enhance proliferation of endothelial cells.

Endothelial cell apoptosis may lead, through loss of cell number, to increased permeability of vascular endothelium, blood cell adhesion to the vessel wall, SMC proliferation, and enhanced blood coagulation. Inflammatory cytokines, products of lipid peroxidation, and growth factor deprivation are potent apoptotic stimuli for endothelial cells, activating a tightly regulated series of intracellular biochemical reactions, which ultimately lead to protein and nucleic acid degradation.

HDL and sHDL protect cultured human endothelial cells from TNF-α–induced apoptosis; the effect is dose-dependent, occurs at physiological HDL concentrations, and is mediated through inhibition of caspase 3 activity. The apolipoprotein composition of sHDL affects their antiapoptotic activity, with apoA-I–containing particles being the most effective. HDLs also suppress the mitochondrial pathway of apoptosis, which is induced by growth factor deprivation. This occurs through activation of Akt, an antiapoptotic protein kinase that maintains mitochondrial integrity, thus inhibiting cytochrome c release, and activation of caspases 3 and 9. Neither HDL apolipoproteins nor purified apoA-I or apoA-II inhibit this pathway of apoptosis; in contrast, the HDL lipid fraction and, in particular, HDL-carried lysosphingolipids exert a potent antiapoptotic effect. It is noteworthy that Akt activation after HDL binding to SR-BI has been implicated in HDL-induced eNOS activation and NO production (see above).

The in vivo relevance of these findings for the recovery of the endothelium during normal or pathological arterial wall remodeling remains to be determined. Nevertheless, the elevation of plasma HDL by sHDL infusion in experimental animals remarkably reduces neointima formation after balloon injury, and a high plasma HDL level is independently and strongly related to the risk of restenosis and to the time of...
restenosis in patients with CHD who underwent a successful coronary angioplasty.\textsuperscript{73}

Conclusions and Perspectives

The capacity of HDL to prevent and correct endothelial dysfunction complements the well-known role of these lipoproteins in arterial cholesterol removal by providing an excellent rationale for favorably influencing pathological processes underlying a variety of clinical conditions, such as accelerated atherosclerosis, acute coronary syndromes, and restenosis after coronary angioplasty (Figure 2) through the enhancement of the concentration or biological function of HDL. Three classes of drugs presently used for the treatment of dyslipidemias can actually increase plasma HDL, fibrates, niacin, and statins. These drugs were not originally developed with the objective of raising plasma HDL, and indeed their effect on HDL is just part of a more extended spectrum of activity on lipid and lipoprotein metabolism. New, HDL-targeted drugs are in preclinical or clinical development.\textsuperscript{74,75}

An alternative to the long-term increase of plasma HDL could soon become available.\textsuperscript{78–79}

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