

High density lipoprotein: it's not just about lipid transport anymore

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Plasma levels of high density lipoprotein cholesterol (HDL-C) have long been associated with protection against cardiovascular disease (CVD) in large populations. However, HDL-C has been significantly less useful for predicting CVD risk in individual patients. This has ignited a new debate on the merits of measuring HDL quantity versus quality in terms of protective potential. In addition, numerous recent studies have begun to uncover HDL functions that vary surprisingly from traditional lipid transport roles. In this paper, we review recent findings that point to important functions for HDL that go well beyond lipid transport. These discoveries suggest that HDL might be a platform that mediates protection from a host of disease states ranging from CVD to diabetes to infectious disease.

The traditional view

High density lipoprotein (HDL) is a circulating, non-covalent assembly of amphipathic proteins (~50% by mass) that stabilize lipid emulsions composed of a phospholipid monolayer (PL) (~25%) embedded with free cholesterol (~4%), with a core of triglycerides (TG) (~3%) and cholesteryl esters (CE) (~12%). Plasma levels of HDL cholesterol (HDL-C) are a well known negative risk factor for the development of cardiovascular disease (CVD). A widely accepted basis for the inverse relationship between human plasma HDL-C and CVD is the ability of HDL, and its major protein constituent apolipoprotein (apo)A-I, to mediate reverse cholesterol transport (RCT). In this process, excess cholesterol and other lipids (originally delivered from the liver via low density lipoproteins) are returned for catabolism. The primary targets are lipid-laden macrophages in the vessel wall, harbingers of the fatty streaks and atherosclerosis that can ultimately progress to myocardial infarction or stroke [1].

A large body of evidence supports the notion that HDL-mediated RCT is essential for human cardiovascular health. In addition to numerous *in vitro* studies that demonstrate cellular cholesterol efflux to HDL, there are well established biochemical pathways that process HDL lipid via numerous circulating enzymes and transfer proteins, and via receptor-mediated uptake into the liver. The *in vivo* RCT assay developed by Rader and colleagues clearly shows that apoA-I overexpression in mice promotes release of macrophage cholesterol first into the plasma compartment and

then eventually to the feces, concomitant with a clear decrease in atherosclerosis susceptibility in this model [2]. However, recent studies have provided tantalizing clues that HDL might be more than it appears. The application of mass spectrometry (MS) based proteomic approaches has revealed unexpected diversity in the HDL proteome (Box 1). Interestingly, only about one-third of HDL proteins are known to mediate lipid transport. The rest play roles in such areas as protease inhibition, complement regulation and acute phase response. This suggests that HDL has broader functions (Figure 1). On an evolutionary scale, atherosclerosis is a relatively recent affliction that strikes well after reproductive age, and would not be expected to be a major driving force for genetic evolution of either apoA-I or HDL. It follows that HDL probably evolved under selection pressure to support more basic survival functions.

Anti-inflammatory functions

Aside from RCT, the next best recognized HDL function is its role as an anti-inflammatory regulator. It accomplishes this through interactions with both the vascular endothelium and circulating inflammatory cells. For example, HDL limits the extent to which endothelial cells can become activated by proinflammatory cytokines, resulting in reduced expression of adhesion molecules [3]. This was elegantly demonstrated *in vivo*, in a study in which infusion of rabbits with reconstituted (r)HDL reduced vascular inflammation by inhibiting endothelial cell adhesion molecule expression [4]. The effect was diminished when the rHDL particle was generated with non-enzymatically glycated apoA-I [5] such as might occur in type II diabetes (T2D).

HDL can also inhibit production of chemoattractant molecules such as monocyte chemoattractant protein (MCP)-1 [6], a chemokine responsible for the recruitment of monocytes, dendritic cells and T lymphocytes to sites of injury and inflammation. Additionally, HDL can modulate vascular tone by affecting the production of nitric oxide (NO), a key mediator of vascular smooth muscle cell contraction. It accomplishes this by stimulating the activity of endothelial nitric oxide synthase (eNOS) to boost NO production, triggering vasorelaxation [7]. Earlier *in vitro* work demonstrated that apoA-I can increase eNOS activity via AMP-activated protein kinase (AMPK) activation [8], and via the phosphoinositide 3-kinase (PI3K)/AKT and mitogen activated protein kinase signaling pathways [9]. *In vivo* studies confirmed increased AKT and extracellular

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Box 1. HDL proteome complexity

Since the first isolation of HDL by ultracentrifugation over 60 years ago, hundreds of studies have aimed to characterize its protein complement. Until recently, HDL was believed to contain predominantly apoA-I, apoA-II and a limited number of less abundant proteins associated with (i) lipid transport or lipoprotein integrity, i.e., the apolipoproteins, (ii) lipid metabolism or transfer, and (iii) acute phase response. The known functions of these proteins fit well into the general dogma of a primary role for HDL in RCT. However, recent applications of MS based proteomics have provided a new appreciation for the complexity of the HDL proteome by detecting more than 50 distinct HDL associated proteins. Many of these have known functions that do not easily fit into the RCT paradigm. For example, several complement proteins, such as C3, C1 inhibitor and complement factor H were discovered. Additionally, protease inhibitors, including several members of the serine protease inhibitor (SERPIN) family were also found. These findings clearly link HDL to innate immunity and proteolytic pathways involved in inflammation and coagulation. The importance of these discoveries has been emphasized by the demonstration that the proteomic profiles of HDL are altered in patients with CVD, and can even be partially normalized by lipid modification therapies. These techniques have also been used to demonstrate that distinct clusters of proteins can segregate to specific HDL subfractions, including a very recent study using gel filtration as the mode of separation [67–69]. Such comparative proteomic studies offer the hope that new biomarkers can be identified to predict not only CVD, but possibly other disease states linked to the novel HDL functions reviewed in this paper.

signal related kinase (ERK)1/2 phosphorylation in apoA-I transgenic animals, and decreased AKT and ERK1/2 phosphorylation in the aortas of apoA-I deficient mice [10].

Two cell surface proteins have also been implicated in HDL-mediated modulation of vascular tone, the scavenger receptor class B member (SR-BI) and the transporter ABCG1 (ATP-binding cassette, sub-family G, member 1). SR-BI colocalizes with eNOS in the caveolae of vascular endothelial cells, and interaction with HDL directly activates eNOS activity [11]. ABCG1 is strongly expressed in endothelial cells, and is known to promote the efflux of cholesterol and oxysterols to HDL, and to mediate various important intracellular processes [12]. It has been demonstrated that formation of eNOS dimers, which is necessary for proper enzyme function, is inhibited in the aortas of ABCG1 deficient (ABCG1^{-/-}) mice fed high cholesterol or western diets [13]. The oxysterol 7-ketocholesterol tended to accumulate in the aortic endothelial cells, and the femoral arteries from these mice displayed impaired vasorelaxation in response to the agonist acetylcholine. Treatment with HDL prevented 7-ketocholesterol-induced disruption of eNOS dimer formation and restored eNOS activity in wild type aortic endothelial cells, but not in ABCG1^{-/-} cells. Furthermore, ABCG1^{-/-} mouse endothelial cells displayed increased surface expression of inflammatory markers such

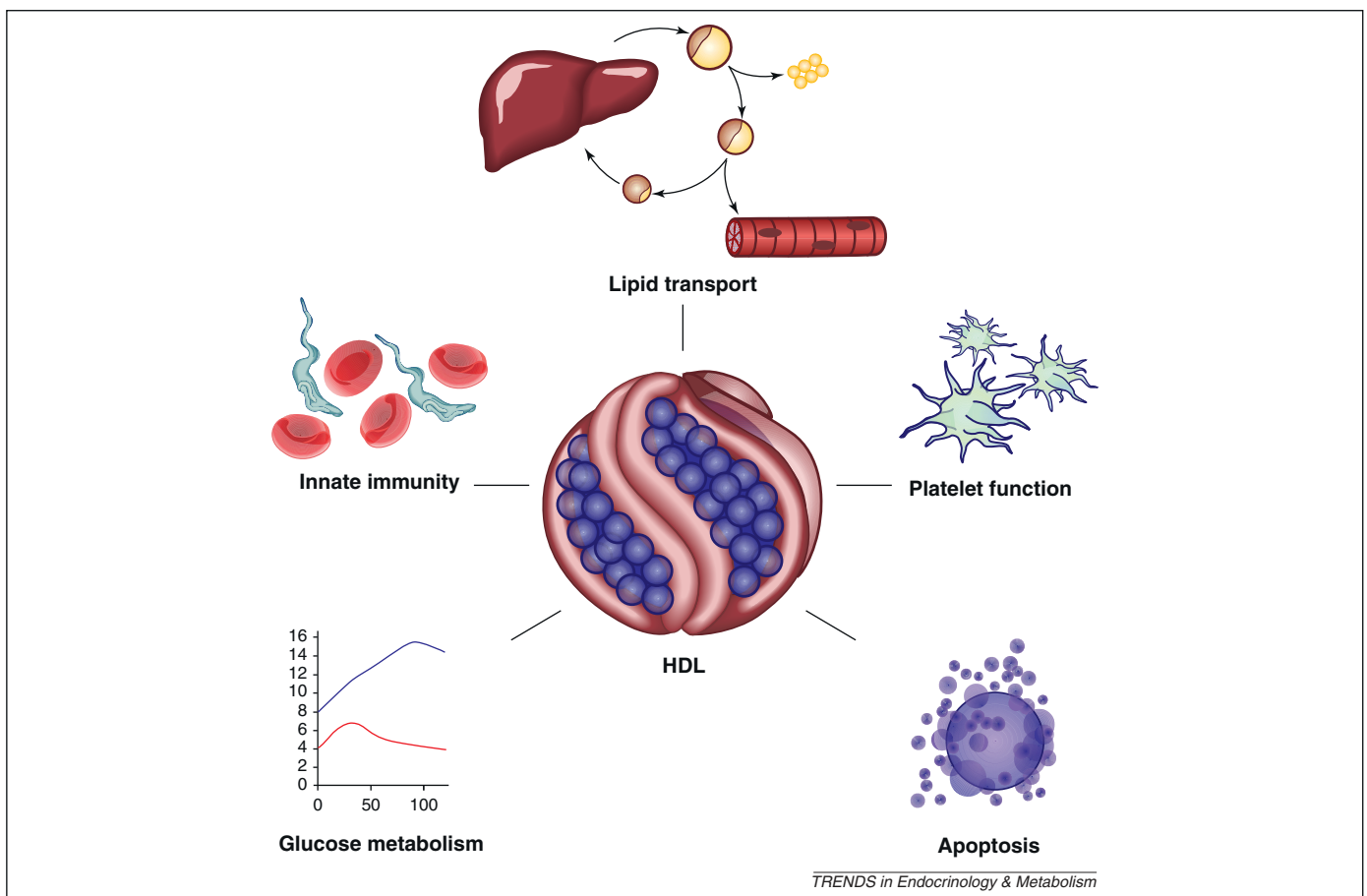


Figure 1. The increasing functional heterogeneity of high density lipoprotein. Numerous recent studies have begun to uncover HDL functions that vary surprisingly from traditionally recognized lipid transport roles. Further exploration of these functions will be useful for understanding the potential of HDL as a treatment option for non-cardiovascular pathologies.

as E-selectin and intercellular adhesion molecule-1 and increased secretion of interleukin-6 and MCP-1 [14]. These findings correlated with increased endothelial cell activation in the ABCG1^{-/-} mouse, suggesting that HDL-mediated lipid removal is an important mechanism in reducing vascular inflammation.

HDL also interacts directly with circulating leukocytes to limit inflammation. For example, apoA-I on HDL can inhibit activation of the monocyte cell surface protein CD11b, an integrin involved in vascular adhesion [15]. Interestingly, this process was dependent on another ATP binding cassette transporter, ABCA1 (member of subfamily A). In this gene lie the molecular defects responsible for Tangier's disease, in which patients exhibit markedly depressed HDL levels. The transporter plays a crucial role in HDL formation and cholesterol efflux via an interaction with lipid-free forms of apoA-I, which are either secreted after synthesis in the liver and intestine, or displaced from intact lipoproteins. The involvement of two ABC transporters with established cholesterol efflux capacity is strongly suggestive of a mechanistic link between the cholesterol efflux and anti-inflammatory properties of HDL. This connection has recently been elegantly demonstrated at the molecular level (Box 2).

Finally, the binding of HDL to macrophage-derived cytokines and growth factors might limit the proinflammatory activity of these proteins. For example, progranulin is a proinflammatory protein secreted by macrophages, which has been shown to bind to plasma apoA-I. Incubation with apoA-I or HDL suppresses progranulin induced expression of inflammatory markers in HEK293 cells [16].

Innate immune functions

The innate immune system represents the first line of defense against invading microorganisms. Accumulating evidence supports the idea that HDL is an integral component of innate immunity, mediating diverse functions that defend against viral, bacterial and parasitic infections. The finding that HDL is host to several complement factors

reinforces this view and suggests that HDL acts as a platform for the assembly of potent immunomodulatory complexes that regulate antimicrobial activity [17,18]. In addition, because infection and inflammation are tightly linked processes, the ability of HDL to regulate the amplitude of the inflammatory response might work in conjunction with these direct antimicrobial effects to influence the outcome of the infection.

Bacterial infection

HDLs are conserved in lower vertebrates such as fish, where they are expressed at high levels in plasma, as well as in tissues that constitute the primary defense barriers to bacterial infection [19]. Recent studies have demonstrated that apoA-I has potent bactericidal and bacteriostatic effects against both Gram-positive and Gram-negative bacteria, and vaccination with bacterial preparations increased apoA-I levels and enhanced the antibacterial activity of serum [20]. This antimicrobial effect is also effective against pathogens that infect humans, suggesting that HDLs might have originally evolved as components of primitive innate immune systems [19,21]. In addition to their direct antibacterial effects, mammalian HDLs have also been shown to protect against the adverse consequences of a bacterial infection by limiting the toxicity of bacterial components, many of which are responsible for life-threatening pathophysiological changes in the host. This toxin-neutralizing activity is largely attributed to apoA-I, and has been shown to be effective against enterohemolysin [22], lipopolysaccharide (LPS), and lipoteichoic acid [23–27].

Viral infection

The antiviral effects of human serum have been known for some time, and HDLs are known to account for a significant proportion of this activity, neutralizing both DNA and RNA viruses, and both enveloped and non-enveloped viruses [28]. The precise mechanism for HDL-mediated antiviral activity is incompletely understood, but current evidence suggests that it can involve direct viral inactivation, interference with viral entry into the cell, or inhibition of virus-induced cell fusion [28,29].

Parasitic infestation

The most well understood mechanism for the antimicrobial effects of HDL involves *Trypanosoma brucei*, a eukaryotic parasite that is the causative agent of African sleeping sickness [30]. Humans are naturally resistant to infection by the subspecies *T. brucei brucei* because the parasite is highly susceptible to lysis by trypanosome lytic factor (TLF), present in human serum [31].

TLF is composed of at least two independent circulating complexes: TLF1 and TLF2 [32]. TLF1 is bound to a subfraction of HDL and contains the primate-specific proteins apoL-I and haptoglobin-related protein (Hpr) [31,33]. TLF2 contains the same two proteins, but is part of a lipid-poor complex comprising IgM and apoA-I [34,35]. Within the circulation, the Hpr component of TLF1 associates with hemoglobin (Hb), generating a Hb-charged TLF1 particle that is efficiently taken up by the parasite via a receptor-mediated endocytic process that trypanosomes use to ac-

Box 2. An anti-inflammatory receptor

The ABCA1 transporter is an important regulator of cholesterol efflux from lipid laden cells in the periphery to lipid-free apoA-I, a crucial step in reverse cholesterol transport and HDL production. Interestingly, Oram and colleagues have published a series of papers demonstrating that the apoA-I/ABCA1 interaction not only results in a transfer of cellular lipid to form HDL, but also mediates outside-in signaling events. One consequence is a triggering of enhanced apoA-I binding to ABCA1 mediated by Janus kinase (JAK)2 to reinforce lipid transfer [72]. More recently, a second arm of this pathway has been identified in which JAK2 activation by apoA-I binding to ABCA1 promotes the association of the STAT3 (signal transducer and activator of transcription 3) transcription factor with ABCA1 [73]. Once bound, STAT3 is phosphorylated to its activated state and translocates to the cell nucleus, where it activates anti-inflammatory gene expression programs. An important result of this is a reduced inflammatory response to bacterial LPS. This discovery has solidified the relationship between the lipid transport functions of apoA-I and the inflammatory response pathways in macrophages. It also raises the intriguing possibility that HDL or its components might be beneficial in other chronic inflammatory conditions such as rheumatoid arthritis.

Box 3. Anti-HDL warfare

If there is any doubt that HDL plays an important role in host defense, it might be of interest to consider some of the adaptations that invading pathogens have evolved to specifically target HDL or its components. For example, *Streptococcus pyogenes*, a group A streptococcal bacterium responsible for tonsillitis, pharyngitis, and toxic shock syndrome, secretes a protein called serum opacity factor (SOF). Originally described for its ability to opacify the normally translucent plasma of infected individuals, SOF has recently been found to specifically target HDL particles by direct binding to apoA-I and apoA-II [70]. This causes a dramatic redistribution of the HDL neutral lipid cargo into large, protein-poor microemulsions. Although the exact effect of this reaction on the competitive fitness of the bacterium has not yet been identified, it is likely that SOF evolved as a virulence factor designed to subvert the antibacterial properties of intact HDL particles. Interestingly, parasites have also developed innovative strategies to frustrate HDL-based defenses. In contrast to *T. b. brucei*, humans can acquire African sleeping sickness by infection with the related subspecies *Trypanosoma brucei rhodesiense*. The ability of *T. b. rhodesiense* to infect humans has been attributed to its secretion of serum resistance-associated protein (SRA), a virulence factor that directly interacts with the C-terminal helix of HDL-associated apoL-I and inhibits its antitrypanosomal activity [33]. However, variants of apoL-I have recently been identified in the African population that do not bind SRA and thus retain lytic activity against *T. b. rhodesiense* [71]. These apoL-I variants are associated with high rates of renal disease in African Americans, suggesting that the selection pressure to acquire an SRA-resistant variant of apoL-I in Africa might have contributed to the development of a risk factor for kidney disease.

quire heme from the host bloodstream [36]. Once inside the parasite, TLF1 is delivered to the lysosome by the endocytic pathway, where progressive acidification dissociates apoL-I from the complex, allowing it to insert into the lysosomal membrane. The bcl2-related pore-forming domain in the apoL-I protein triggers an influx of chloride ions into the lysosome, followed by water, resulting in swelling and trypanosome lysis. The pathway by which TLF2 enters the parasite is less clear, but the lysis mechanism appears to be the same. Interestingly, parasites that reside within the phagolysosomes of macrophages, such as *Leishmania sp.*, are not protected from TLF because the infected macrophages deliver plasma TLF directly into the vacuole in which the *Leishmania* organisms reside [37]. HDL is a sufficiently important threat that invading pathogens have evolved defense mechanisms or infection strategies that directly target HDL (Box 3).

Modulation of glucose metabolism

T2D is characterized by a lack of glucose control due to the development of insulin resistance. Patients with T2D also display dyslipidemia with low HDL-C concentrations [38]. It has been shown by cell culture, animal and human studies that apoA-I gene expression is decreased by elevated glucose levels and increased by insulin [39], but there is emerging evidence that HDL, and apoA-I in particular, might also modulate glucose metabolism directly.

In *ex vivo* experiments, it was found that recombinant apoA-I improved glucose uptake associated with increased AMP-activated protein kinase (AMPK) activity in mouse skeletal muscle. It was also demonstrated that the absence of apoA-I in mice resulted in higher fasting blood glucose levels associated with reduced AMPK activity, and increased hepatic gluconeogenesis determined by increased

phosphoenolpyruvate carboxykinase and glucose-6-phosphatase expression levels [40]. Furthermore, apoA-I was internalized through a clathrin-dependent endocytotic process before activating AMPK and acetyl-coenzyme A carboxylase in cell culture experiments. Recent observations indicate that infusions of rHDL particles reduced plasma glucose, increased insulin secretion and promoted glucose uptake in skeletal muscle of patients with T2D [41]. Based on cell culture experiments, those authors suggested that the AMPK signaling pathway might be involved in the rHDL-mediated increase in glucose uptake. Regarding the observed increased insulin levels in the patients with T2D, the report provided the first evidence, using a murine β cell line, that HDL directly stimulates insulin secretion from pancreatic β cells. In a follow-up study using transformed β cell lines and primary islets under basal and high-glucose conditions, it was determined that insulin secretion can be mediated by apoA-I and apoA-II, in their lipid-free forms, as constituents of rHDL or of HDL isolated from human plasma [42]. Furthermore these effects were calcium dependent and involved expression of ABCA1, ABCG1, and SR-BI. The importance of ABCA1 in modulating insulin secretion has been emphasized by the observation that mice with specific inactivation of ABCA1 in β cells had markedly impaired glucose tolerance and defective insulin secretion, but normal insulin sensitivity [43].

Besides its direct effect on glucose homeostasis, there is recent evidence that HDL might improve insulin resistance and obesity through its anti-inflammatory actions. The apoA-I mimetic peptide L-4F, known to have antioxidant properties, was investigated to determine if it would ameliorate insulin resistance and diabetes in genetically obese mice. It was found that L-4F treatment reduced adiposity and inflammation, and improved glucose tolerance in genetically obese *ob/ob* mice [44]. The observed reduction in glucose levels and prevention of fat mass accumulation was later attributed to upregulation of heme oxygenase expression and downregulation of endocannabinoid receptor-1 expression, resulting in adipose tissue remodeling [45]. In addition, the authors of that report detected increased AKT and AMPK phosphorylation in the aortas of L-4F treated mice, which could be prevented by inhibitors of PI3K activity. However, pharmacological inhibition only partially prevented the glucose lowering effect of L-4F in *ob/ob* mice, indicating the possible existence of alternative mechanisms. Another recent study in mice showed that apoA-I and its mimetic D-4F, an absorbable L-4F stereoisomer, increased energy expenditure by upregulating expression of UCP-1 in brown adipose tissue, thus adding an ulterior antiobesity function to HDL [46].

Antiapoptotic functions

HDL has been demonstrated to inhibit apoptotic activity in at least six different cell types including vascular endothelial and smooth muscle cells, some leukocytes, pancreatic β cells, cardiomyocytes and even bone-forming osteoblasts. Several different modes of action have been identified, involving both the protein and lipid components of HDL, which can act directly to influence cellular signaling or by various indirect mechanisms to prevent apoptosis. The protein component of HDL, consisting primarily of

apoA-I, has been shown to be responsible for about 70% of HDL mediated inhibition of oxidized LDL-induced apoptosis in human microvascular endothelial cell line-1 [47].

There is evidence that some of the less abundant HDL proteins might also be involved. One minor HDL protein called paraoxonase (PON) 1 has been found to be important for HDL binding and protection of macrophages from apoptotic events. This effect is the result of increased expression of SR-BI via PON 1 mediated activation of ERK1/2 and PI3K signaling pathways [48]. Another HDL associated protein, alpha-1-antitrypsin, can prevent apoptosis in a more indirect manner. This protein inhibits elastase-induced degradation of the extracellular matrix, thus preventing detachment and subsequent apoptosis of vascular smooth muscle cells [49].

The lipid composition of HDL can also play roles in antiapoptotic signaling. Recently, reports have indicated important roles for sphingosine 1 phosphate (S1P), a common component of HDL, in the protection from apoptosis of cardiomyocytes, pancreatic β cells and endothelial cells [50–53]. In addition to SR-BI, these protein and lipid components of HDL are likely to interact with other cell surface proteins to produce these effects. For example, the ATP binding cassette transporters ABCA1 and ABCG1 have both been implicated in the antiapoptotic effects of HDL on macrophages [54,55]. Furthermore, a known receptor for apoA-I and HDL, called cell surface F1-ATPase (an enzyme related to mitochondrial F1-ATPase), can inhibit apoptosis independent of ABC transporters and SR-BI. Interaction of F1-ATPase with HDL not only protects against apoptotic signaling but also stimulates endothelial cell proliferation [56]. Different density fractions of HDL can have varying antiapoptotic capacities, with small dense HDL_{3c} having the most potent effects, shown on endothelial cells and osteoblasts [47,57]. Interestingly, this activity is impaired in patients with metabolic syndrome, possibly due to the changes in HDL particle protein and lipid composition seen in these patients [58].

Influence on stem cells and embryogenesis

Another functional property of HDL currently under investigation is a role in the maturation of stem cells. Bone marrow cells (BMCs) are a key source of vascular progenitor cells that contribute to vessel repair upon endothelial denudation. BMCs are thought to be constantly shuffling back and forth between the bone marrow and the circulation, allowing them to migrate to sites of injury in response to proinflammatory cytokines. Once there, they can differentiate into the cell types that are needed to effect endothelial repair.

It was observed that treatment of lineage-negative BMCs with lipid-free apoA-I induced a change in their morphology and promoted expression of CD31, an adhesion molecule present in endothelial cells. This increased their ability to bind to both fibronectin and cultured endothelial cells [59]. A mutant of apoA-I lacking a key lipid binding site failed to promote these transformations, suggesting that apoA-I might mediate these effects via lipid efflux. The authors of that report suggested that apoA-I stimulation of BMC differentiation might be a mechanism by which HDL mediates vessel repair. The ability of apoA-I

to mobilize cellular lipids also appears to play a role in the proliferation of hematopoietic stem and progenitor cells (HSPCs). For example, it was found that ABCA1 and ABCG1 deficient (ABCA1^{-/-}ABCG1^{-/-}) mice, which have extremely low levels of circulating HDL, displayed a five-fold increase in HSPCs compared with controls [60]. When ABCA1^{-/-} ABCG1^{-/-} bone marrow was transplanted into apoA-I transgenic mice, which display elevated levels of HDL, these levels decreased significantly. These results suggest that HDL might inhibit leukocytosis (elevated levels of circulating leukocytes) and reduce monocyte infiltration into vascular lesions.

Additionally, HDL is the predominant lipoprotein in follicular fluid, and might play a role in oocyte development and embryogenesis [61]. For example, a negative correlation has been found between human follicular HDL-C levels and embryo fragmentation during *in vitro* fertilization [62]. HDL metabolism might also affect embryo viability *in vivo*. Liver specific SR-BI knockout female mice with significantly decreased HDL uptake by the liver and large dysfunctional circulating HDL were infertile. However, reconstitution of SR-BI expression in the knockout animals by adenovirus mediated gene delivery restored both HDL morphology and fertility [63], consistent with a role for HDL in female reproductive physiology.

Effects on platelet function

HDL has been known to affect platelet function for many years, although the effects have been complex, and varied between experimental systems [64]. Recent evidence has revealed that the cholesterol homeostatic functions of HDL and apoA-I might significantly affect platelet function. Mice that lack SR-BI are known to be thrombocytopenic (low platelet count). Their platelets exhibit reduced ability to aggregate and this has been correlated to abnormally high levels of free cholesterol in the cells [65]. This suggests that platelet SR-BI interactions with HDL can modulate cellular cholesterol levels for optimal platelet function.

This assertion was supported by a more recent study in which patients with T2D were infused with reconstituted HDL preparations [66]. The infused patients exhibited a 50% decrease in platelet aggregation response versus controls. The effect was attributed to the phospholipid fraction of the particles with apoA-I having no role, consistent with the idea that SR-BI-mediated cholesterol efflux can beneficially modulate platelet function.

The discovery of several HDL proteins with known roles in platelet function such as the complement family and platelet activating factor acylhydrolase (Box 1), strongly suggest that more ties between HDL and platelet function exist, which go beyond cholesterol efflux. More work in this area is clearly needed.

Conclusions and future perspectives

Taking into account the diverse set of functions described above, and adding in the increasing appreciation of the compositional and structural heterogeneity of HDL, it is difficult to imagine that all these functions are mediated by the relatively limited number of HDL subspecies that are currently characterized. It is becoming clear that the term 'HDL' refers to an ensemble of discrete particles, each with

their own complement of proteins and lipids that endow the host particle with distinct and varied functionalities. Indeed, many of these particles might play important physiological roles that have little to do with RCT or protection from heart disease. Some of these functions are closely related to the ability of HDL to modify the behavior of a target cell or organism by removing lipids. However, an increasing number appear to be mediated via interactions with cell surface proteins to trigger distinct signaling pathways to alter cell function. Although many of these studies are still in the initial stages, it would seem that it is an exciting time for the study of HDL metabolism.

In our view, two key challenges lie before the field of HDL research with respect to these alternative functions. First, we need a better understanding of the subparticle makeup of the fractions classically referred to as 'HDL'. New technologies for alternative particle isolation and analysis and clever strategies for identifying distinct particle functions on the background of staggering compositional complexity will have to be developed to meet this important challenge. Once these subspecies are identified and characterized, it will be easier to correlate specific functions for these particles. Second, research needs to be directed at identifying additional roles for HDL outside of the classic purview of cardiovascular disease. The strong ties between inflammation and innate immunity make HDL a promising target for treatment or prevention of diseases such as those caused by opportunistic pathogens or chronic inflammatory states. This knowledge will not only be useful for understanding the potential of HDL as a treatment option for non-cardiovascular pathologies, but it could also be crucial for understanding the consequences of pharmacological manipulation of HDL-C levels. Indeed, current pharmacological therapies such as niacin or those in development including cholesteryl ester transfer protein inhibition and apoA-I transcription stimulation aim to raise plasma HDL cholesterol in the generic sense without direct knowledge of the functionality (or lack thereof) of the elevated species. It will be important to ascertain the effect of these manipulations, not only on those subspecies linked to CVD protection, but also on these peripheral functions, which might be just as important.

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