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High-density lipoprotein heterogeneity and function in reverse cholesterol transport

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Abstract

Purpose of review—HDL is a cardioprotective lipoprotein, at least in part, because of its ability to mediate reverse cholesterol transport (RCT). It is becoming increasingly clear that the antiatherogenic effects of HDL are not only dependent on its concentration in circulating blood but also on its biological 'quality'. This review summarizes our current understanding of how the biological activities of individual subclasses of HDL particles contribute to overall HDL performance in RCT.

Recent findings—Recent work indicates that apolipoprotein A-I-containing nascent HDL particles are heterogeneous and that such particles exert different effects on the RCT pathway. RCT from macrophages has been examined in detail in mice and the roles of plasma factors (lecithin-cholesterol acyltransferase, cholesterol ester transfer protein, phospholipid transfer protein) and cell factors (ATP-binding cassette transporter A1, ATP-binding cassette transporter G1, scavenger receptor class B type 1) have been evaluated. Manipulation of such factors has consistent effects on RCT and atherosclerosis, but the level of plasma HDL does not reliably predict the degree of RCT. Furthermore, HDL cholesterol or apolipoprotein A-I levels do not necessarily correlate with the magnitude of cholesterol efflux from macrophages; more understanding of the contributions of specific HDL subspecies is required.

Summary—The antiatherogenic quality of HDL is defined by the functionality of HDL subspecies. In the case of RCT, the rate of cholesterol movement through the pathway is critical and the contributions of particular types of HDL particles to this process are becoming better defined.

Keywords

apolipoprotein A-I; atherosclerosis; cholesterol efflux; HDL; reverse cholesterol transport

Introduction

Epidemiological evidence indicates that HDL particles serve an antiatherogenic function in that high levels of HDL cholesterol (HDL-C) are associated with a decreased risk of cardiovascular disease [1]. Consistent with this antiatherogenic function, acute elevation of HDL-C by infusion of synthetic HDL particles can induce regression of atherosclerotic plaque in patients [2]. An important way that HDL achieves these effects is by promoting the reverse cholesterol transport (RCT) pathway, which involves the transport of excess cholesterol from peripheral tissues (including macrophages) back to the liver for eventual

excretion from the body [3–5]. The ability of HDL to remove cholesterol and oxysterols from cells such as macrophages in the arterial wall is linked to the anti-inflammatory and immunosuppressive functions of this lipoprotein [6,7**]. These capabilities coupled with the antioxidant, antithrombotic and vasodilatory capabilities of HDL [8*] give rise to the overall cardioprotective behavior of HDL. It is becoming increasingly apparent that these antiatherogenic effects of HDL are not only dependent on its concentration in circulating blood but also on its biological quality [2,4,9]. This review considers the recent progress that is being made in understanding the biological activities of different HDL subspecies and how these activities contribute to the antiatherogenic performance of the mixture of heterogeneous HDL particles that comprise plasma HDL-C and mediate RCT.

HDL heterogeneity

Traditionally, HDL isolated by ultracentrifugation is defined as the lipoprotein with density in the range 1.063–1.21 g/ml. However, HDL constitutes a heterogeneous group of particles differing in density, size, electrophoretic mobility, lipid composition and apolipoprotein content [10] (Fig. 1). Therefore, HDL can be fractionated into discrete subclasses by different techniques according to their physicochemical properties. Human HDL can be separated by ultracentrifugation into two main subfractions on the basis of density, HDL₂ (1.063–1.125 g/ml) and HDL₃ (1.125–1.21 g/ml), which is relatively protein-rich [11]. HDL₂ and HDL₃ can be further divided on gradient gel electrophoresis into HDL_{2b} (10.6 nm), HDL_{2a} (9.2 nm), HDL_{3a} (8.4 nm), HDL_{3b} (8.0 nm) and HDL_{3c} (7.6 nm) in decreasing order of particle diameter [12]. In addition, HDL can be separated into two main subpopulations on the basis of electrophoretic mobility: the major subfraction has a relatively high negative surface charge density and is called α -HDL, whereas the other fraction is called pre β -HDL [13,14]. Most of the HDL particles in plasma are α -HDL [15] and pre β -HDL represents only approximately 5% of total apolipoprotein A-I (apoA-I) [3]. Choline-containing phospholipids (phosphatidylcholine, lysolecithin, sphingomyelin) represent 90–95% of the phospholipids in HDL [16]. The calculated lipid composition is 137 phospholipids, 50 free (unesterified) cholesterol, 90 cholesterol ester and 19 triacylglycerol molecules in an HDL₂ particle, and 51 phospholipids, 13 free cholesterol, 32 cholesterol ester and nine triacylglycerol molecules in an HDL₃ particle [17]. Pre β -HDL contains mainly apoA-I and phospholipids with small amounts of cholesterol [3,18]. Pre β -HDL has been resolved into pre β ₁, pre β ₂ and pre β ₃ HDL particles according to increasing size by two-dimensional gel electrophoresis [14]. HDL can also be separated on the basis of apolipoprotein composition [19] into several subpopulations using immunoaffinity methods [20]: two major apoA-I-containing HDL particles exist, that is, LpA-I+A-II, which includes both apoA-I and apoA-II (molar ratio ~2/1), and LpA-I, which contains only apoA-I. About 65% of the total recovered weight of apoA-I in human plasma is found in LpA-I+A-II and approximately 25% is found in LpA-I. The majority of LpA-I has the same density and size as HDL₂, whereas the majority of LpA-I+A-II floats with HDL₃ [20]. Table 1 summarizes the main characteristics of the major human HDL subclasses, including pre β -HDL, HDL₂ and HDL₃.

Remodeling by plasma factors is a critical part of HDL metabolism and underlies the dynamic nature of HDL particles. HDL is a substrate for the plasma factors listed in Table 2 and the lipolytic and lipid transfer activities of these factors cause the various subpopulations of HDL particles that exist in human plasma [10,21] to continually interconvert. As shown in Fig. 2 in section ‘reverse cholesterol transport pathway’ below, the changes in HDL particle shape and size caused by these plasma factors are also central to the participation of HDL in the RCT pathway. The key function of lecithin-cholesterol acyltransferase (LCAT) is to form cholesterol ester and, at a constant apoA-I concentration, larger discoidal HDL particles are the preferred substrate [22]. The remaining enzymes

listed in Table 2, hepatic lipase and endothelial lipase, are involved in releasing fatty acids from phospholipids and triacylglycerol. The lipid transfer proteins, cholesterol ester transfer protein (CETP) and phospholipid transfer protein (PLTP) are involved in moving cholesterol ester, triacylglycerol and phospholipid molecules among HDL and other lipoprotein particles [23*,24*]. As shown in Table 2, the interconversion of HDL particles is frequently accompanied by the release of apoA-I molecules into the aqueous phase; rearrangements of HDL particles that are accompanied by a decrease in net surface area lead to desorption of apoA-I molecules and vice-versa. Such cycling of apoA-I molecules between HDL particles and the aqueous phase is critical for HDL metabolism [18,25].

The pool of recycling lipid-free (poor) apoA-I, together with newly synthesized apoA-I secreted by hepatocytes and enterocytes, is involved in the biogenesis of nascent HDL particles by interaction with ATP-binding cassette transporter A1 (ABCA1) [26,27]. Studies over the last several years have shown that multiple sizes of nascent HDL particles (diameters in the range 7–20 nm) are created concurrently when apoA-I is incubated with ABCA1-expressing cells [28–31]. Work in our laboratory has revealed that the predominant HDL species formed are approximately 9 and 12 nm discoidal particles that contain either two or three apoA-I molecules [30]. These particles are also heterogeneous with respect to phospholipid composition and cholesterol content. ApoA-I is the only protein constituent of these nascent HDL particles but, interestingly, if apoM is also expressed by the cells, the particle size increases. ApoM may function intracellularly to transfer lipid onto the HDL particles during or after their formation by ABCA1 [32*]. Depending upon cell type, α -HDL and/or pre β -HDL particles can be formed by the apoA-I/ABCA1 reaction [29]. Besides discoidal particles, pre β 1-HDL (or lipid-poor apoA-I) containing a single apoA-I molecule, three to four phospholipid molecules and one to two cholesterol molecules can be formed [33]. Pre β 1-HDL is both a product and a substrate in ABCA1-mediated efflux of cellular phospholipids and cholesterol to apoA-I. Lipid-poor apoA-I participates similarly to lipid-free apoA-I in the ABCA1 reaction, but increasing lipidation reduces the ability of apoA-I to react with ABCA1 [31]. Overall, studies of HDL biogenesis indicate that a heterogeneous population of particles is created; subsequent remodeling by plasma factors refines this mixture (which also includes surface remnants created by the action of lipoprotein lipase on triacylglycerol-rich lipoproteins [34]) while preserving the heterogeneity.

The functionality of HDL is impaired in humans with chronic inflammatory diseases, including atherosclerosis [35], and these deleterious effects can involve oxidative modification of apoA-I [36*]. The HDL dysfunction involves reductions in both anti-inflammatory and cholesterol transport properties. Small dense HDL₃ particles seem to be particularly sensitive to such oxidative damage and loss of antiatherogenic function [37]. The presence of oxidatively modified HDL particles increases the overall heterogeneity of the HDL pool.

Reverse cholesterol transport pathway

It is clear from the RCT pathway summarized in Fig. 2 that apoA-I is involved in all stages, including the formation of nascent HDL particles, HDL remodeling by LCAT and delivery of HDL cholesterol to the liver via scavenger receptor class B type 1 (SR-BI). ApoA-I exists in several physical states in that it is associated with different HDL subspecies. These various HDL particles interact differently with key proteins in the pathway; these proteins include the cell surface receptors ABCA1, ABCG1 and SR-BI that mediate cholesterol transport between HDL and the cell, as well as LCAT in the plasma that esterifies HDL-associated cholesterol.

The first step in the macrophage RCT process involves the efflux of cholesterol to HDL. The four pathways contributing to this effect are summarized in Table 3 (for reviews, see [3,38–40]). The aqueous diffusion pathway is largely responsible for cholesterol efflux to human serum from mouse peritoneal macrophages containing normal levels of cholesterol; in addition, there are minor contributions from ABCA1 and SR-BI [41]. Cholesterol loading of such cells approximately doubles the fractional cholesterol efflux due to a large increase in the ABCA1 contribution and a smaller increase in the ABCG1 contribution. The situation is different with cholesterol-loaded human macrophages in that ABCG1 activity does not contribute to cholesterol efflux to HDL; as with mouse macrophages, ABCA1 plays a major role in mediating cholesterol efflux [42**].

Much has been learned about the molecular mechanisms underlying the four cellular cholesterol efflux pathways listed in Table 3. The aqueous diffusion pathway mediates the bidirectional flux of cholesterol between the cell plasma membrane and HDL in the extracellular medium; the direction of net cholesterol mass transport is determined by the cholesterol concentration gradient as reflected by the free cholesterol/phospholipids ratios in the donor and acceptor particles [38]. The rate-limiting step in efflux from cells is normally the desorption of cholesterol molecules from the cell plasma membrane. The rate of this step is influenced by interactions of free cholesterol molecules with neighboring phospholipid molecules [38,43]; the rate is increased by higher phospholipid unsaturation and decreased by a higher membrane sphingomyelin content [44,45]. The various HDL subclasses are equally effective acceptors in this pathway because the efflux is not affected significantly by alterations in HDL particle size [46]. The diffusion of cholesterol molecules between the cell plasma membrane and HDL particles can be facilitated by SR-BI. Thus, binding of HDL to SR-BI promotes cholesterol efflux [47], apparently by forming a complex [48,49] that contains a hydrophobic channel along which cholesterol molecules can diffuse [50]. Because larger HDL particles bind better to SR-BI [51], such particles are more effective in supporting cholesterol efflux via this receptor [49]. ABCG1 is a member of the ABC family of membrane transporters and is involved in controlling intracellular cholesterol homeostasis [52*]. Expression of ABCG1 enhances efflux of cholesterol to HDL [53] by increasing the pool of plasma membrane free cholesterol and reorganizing it so that it can desorb more readily into the extracellular medium [54*]. Larger HDL₂ and smaller HDL₃ particles are equally effective acceptors, as expected for the aqueous diffusion pathway. In contrast, the acceptor for cellular cholesterol efflux via the ABCA1 pathway is pre β 1-HDL [lipid-free (poor) apoA-I] (Table 3). In this case, there is simultaneous efflux of free cholesterol and phospholipids [55] due to microsolubilization of plasma membrane lipids [56–57] and creation of discoidal nascent HDL particles (c.f. section ‘HDL heterogeneity’). Pools of intracellular cholesterol are also mobilized by the activity of ABCA1 [58,59]. The concentration of active ABCA1 in the cell plasma membrane determines the rate of lipid efflux and nascent HDL particle formation. Strikingly, increased membrane phospholipid unsaturation inhibits ABCA1-mediated cholesterol efflux [60], which, as mentioned above, is opposite to the effects of increased phospholipid unsaturation on efflux via the aqueous diffusion mechanism. Binding of apoA-I to ABCA1 prevents its intracellular degradation and increases the level of transporter in the plasma membrane [61]. Importantly, it has been shown recently that ABCA1 is palmitoylated and that this post-translational modification localizes it to the plasma membrane and regulates its lipid efflux ability [62**]. In addition to the nascent HDL particles, such cholesterol efflux can involve the release of microparticles [30,63**], a fact that is often overlooked in analyzing and interpreting the results of apoA-I-mediated efflux via ABCA1. The discoidal nascent HDL particles created by the apoA-I/ABCA1 reaction are effective acceptors of cellular cholesterol effluxed by the ABCG1 pathway [64,65].

The cholesterol incorporated into nascent HDL particles is transported through the plasma compartment by the various HDL remodeling steps summarized in section 'HDL heterogeneity' and Fig. 2. Eventually, the free cholesterol and cholesterol ester in HDL are delivered to the liver. Hepatocytes express SR-BI, which mediates the selective uptake of the HDL cholesterol [21]. This two-step process involves binding of HDL particle to the receptor, followed by diffusion of cholesterol ester and free cholesterol molecules into the cell plasma membrane. Large diameter (10 nm) HDL particles bind better to SR-BI and deliver more cholesterol ester than small (8 nm) HDL particles [49,51]. The presence of apoA-II in an HDL particle modulates both binding to SR-BI and selective uptake of cholesterol [66]. SR-BI protein is situated in both the basolateral and canalicular membranes of hepatocytes [67*]; in the former location, it promotes cholesterol influx and in the latter, it promotes cholesterol efflux. The rate of biliary cholesterol secretion, a key step in the RCT pathway (Fig. 2), is related to the hepatic expression level of SR-BI [68*].

In-vivo measurement of reverse cholesterol transport

There is great interest in understanding the ability of HDL to mediate RCT from macrophages because this ability underlies the antiatherogenic functions of the lipoprotein. One of the first attempts to examine RCT in intact animals was published in 1999 by Stein *et al.* [69] who introduced cationized LDL containing labeled cholesterol into the thigh muscle of mice and rats and then followed the disappearance of the labeled cholesterol from this depot. In these studies, the cholesterol was not initially present in macrophages and no attempt was made to examine the individual components of the RCT pathway. In an effort to study RCT as an integrated system, an experimental protocol that measures the flux of labeled cholesterol from macrophages in the peritoneal cavity of mice to the plasma, liver and feces has been developed [70]. In this system murine macrophages, either established cell lines or primary peritoneal or bone marrow, are enriched with radiolabeled cholesterol and injected into recipient mice. At timed intervals, plasma is taken to measure the appearance of labeled cholesterol and at the termination of the study, generally 48 h following injection of the macrophages, the mice are sacrificed and the amount of macrophage-derived labeled cholesterol is measured in the plasma, liver and feces. This protocol, which has been used in a number of studies, has several advantages: it measures the movement of cholesterol out of the macrophages, which is the first step in RCT (Fig. 2), and traces its fate to the last step in RCT, the excretion into the feces; the recipient mice can be genetically manipulated so that the individual steps in RCT can be either enhanced or eliminated so that the contribution of each step can be individually assessed; and the recipient mice can be treated with drugs or diets that may impact on RCT. The basic protocol of introducing macrophages containing labeled cholesterol into the peritoneal cavity of mice has been widely adapted by numerous investigators. A modification of the original protocol has been introduced [71]; this protocol introduces the macrophages subcutaneously into mice and uses two labeled sterols (cholesterol and sitostanol) to permit correction for animal-to-animal variation in biliary cholesterol absorption in the intestine.

Among the numerous studies using the integrated mouse RCT protocol are those that examined the contribution of individual steps in RCT (Fig. 2). The earliest experiment using the macrophage to feces protocol investigated RCT in mice overexpressing human apoA-I [70]. This study validated the experimental protocol and demonstrated greater movement of labeled cholesterol from macrophages to a number of tissues in the mice overexpressing apoA-I. A comparison of RCT in mice expressing either wild-type human apoA-I or apoA-I_{Milano} showed no difference, suggesting that the beneficial effects of apoA-I_{Milano} apparently are not related to more efficient RCT [72*]. Human apoA-II can also maintain effective RCT from macrophages to feces [73].

The role of LCAT in RCT was studied using human apoA-I transgenic mice overexpressing human LCAT [74**]. As both overexpression and reduction in LCAT had only a modest effect on RCT, the results suggest that LCAT may not have a major impact on total macrophage efflux, and RCT may not be as dependent on LCAT as previously thought. This conclusion is consistent with studies demonstrating that in patients with partial or full genetic deficiencies in LCAT, there is no enhanced preclinical atherosclerosis [75]. The studies of LCAT and RCT provide an example of how different HDL particles can differentially mediate both in-vitro cell cholesterol efflux and in-vivo RCT [74**]. Overexpression of LCAT increased HDL-C, but this elevation in HDL-C did not increase RCT from macrophages to feces. Interestingly, the plasma from these mice had a reduced ability to promote cell cholesterol efflux *ex vivo* via ABCA1. An explanation for this observation is that increased esterification by LCAT resulted in an increase in the production of mature HDL particles and a reduction in lipid-poor pre β -HDL. When homozygous or heterozygous LCAT-null mice were used as macrophage recipients, there was a 45% reduction in labeled cholesterol in the feces of homozygous mice and no difference in RCT between wild-type and heterozygous mice. When the sera from these mice were tested for cholesterol efflux efficiency using J774 cells, total cell efflux was similar to that obtained with wild-type sera, but these sera were more efficient in promoting cellular efflux via the ABCA1 pathway [74**]. This result can be attributed to the greater level of pre β -HDL present in the sera from LCAT-deficient mice. Thus, the level of LCAT activity determines the array of HDL particles present in the plasma, and this in turn influences the efficiency of the various efflux pathways in the removal of cell cholesterol (c.f. Table 3).

The in-vivo mouse RCT protocol has been used in a limited number of studies designed to probe the role played by two important plasma transfer proteins (c.f. Table 2), CETP [76] and PLTP [77] on RCT. The macrophage to feces pathway was quantitated by injection of cholesterol-labeled and cholesterol-enriched J774 cells into mice stably expressing human CETP in their liver. From this study, which used a number of different genetically manipulated mouse models, it was concluded that liver expression of CETP promotes macrophage RCT and that this effect is dependent on the LDL receptor [76]. Recent studies focusing on the role of PLTP in RCT illustrate how the mouse RCT assay can be used to study the effect of systemic or macrophage-specific expression of a protein [77]. Thus, hepatic overexpression of PLTP in either wild-type or apoA-I transgenic mice impaired RCT, but when wild-type mice were injected with macrophages obtained from PLTP transgenic mice, there was no impact on RCT.

In many of the studies described above, the primary manipulation of the RCT pathway was in components of the pathway that were present in the plasma of the recipient mice. Thus, levels of apoA-I, LCAT, CETP and PLTP varied and the effects of the changes in these proteins on RCT were quantitated. In addition to this approach, the macrophage to feces RCT protocol provides a system that can determine the contribution of various cell-related factors to RCT. One of the best examples of this is a study in which macrophages harvested from mice deficient in either ABCA1, SR-BI or ABCG1 were injected into recipient mice [78]. Using this approach, the relative contributions of the various efflux pathways could be determined, and it was found that elimination of either ABCA1 or ABCG1 reduced RCT and knockdown of both of these proteins produced an additive reduction in RCT. In contrast, RCT in mice receiving macrophages lacking SR-BI was similar to that observed with mice receiving wild-type macrophages [78]. The importance of ABCA1 in promoting RCT has been documented in other investigations using the in-vivo mouse RCT protocol [73,79,80]. Thus, mouse RCT results illustrated the importance of both ABCA1 and ABCG1 in RCT and the lack of a contribution of SR-BI. The relative importance of these proteins in mediating efflux of cholesterol from mouse macrophages *in vitro* (section 'reverse

cholesterol transport pathway') is consistent with their contributions to RCT, as estimated from the in-vivo mouse RCT system [78].

In addition to the studies described above, determination of the rate of macrophage RCT in mice has been used to quantitate the effects on RCT of inflammation [81^{*}], dietary manipulations [82] and pharmacologic interventions [83,84]. The numerous genetic interventions that have been investigated using the mouse RCT protocol have resulted in effects on RCT that are largely consistent with the effects of the same interventions on atherosclerosis (Table 4) [85^{*}]. The results in Table 4 show that macrophage RCT mediated by HDL has important implications for the development of atherosclerosis, but the HDL contribution does not correlate well with the HDL-C levels. For instance, the increase in HDL-C due to elimination of SR-BI is associated with decreased RCT and increased atherosclerosis. These results indicate that the rate of macrophage RCT and the quality rather than the quantity of HDL are important in determining the antiatherogenic effects of HDL. It should be noted that though it is generally agreed that ABCA1 plays an important role in pre β 1-HDL-mediated RCT from macrophages, this transporter is not involved in the centripetal movement of cholesterol from peripheral parenchymal cells to the liver and intestine, at least not in mice on a low-cholesterol diet [86^{**}].

Serum efflux potential

The first step in RCT is cholesterol efflux from macrophages (Fig. 2), and to aid in understanding how these HDL parameters modulate RCT, attention has focused on defining how HDL composition and subclass distribution affect this efflux process. When investigating a limited number of HDL particles that differ in composition and size, comparisons of cellular cholesterol efflux efficiency and efflux pathways can be made by directly isolating and adding the purified particles to the culture media. However, as understanding of the complexity of HDL subfractions has developed, it is very difficult to isolate enough of all of the different particles that are in serum, because of the large number and low concentrations of many of the HDL subfractions. As a consequence of this limitation, determining the efflux efficiency of HDL subfractions has been investigated by exposing cells having different efflux pathways to the total HDL fraction from a number of serum specimens and then establishing the relative efflux efficiencies of each type of HDL particle by determining the correlations between the HDL subfractions and efflux via the different efflux pathways [87]. Using this approach, two HDL subspecies correlated significantly with ABCA1-mediated efflux from macrophages to human serum, namely, small, lipid-poor pre β 1-HDL particles and intermediate-sized α 2-HDL particles. In contrast to the limited number of HDL particles that were associated with ABCA1 efflux, SR-BI efflux correlated with a large number of HDL subfractions, all of which were particles that contained significant levels of phospholipids [87]. Efflux of cell cholesterol via the ABCG1 pathway exhibited an HDL subfraction efflux pattern generally similar to that of SR-BI [54^{*}].

The efficiency of any serum specimen or HDL preparation in removing cholesterol from cells depends on both the HDL subfraction distribution and the level of expression of the different efflux pathways (Table 3) operational in the specific cell being studied. The selection of cholesterol donor cell is important and macrophages, either established cell lines or primary cells, are particularly useful. Macrophages express, to varying degrees, all of the cholesterol efflux pathways (section 'reverse cholesterol transport pathway'), but there is great heterogeneity in the macrophage populations in tissues [88], and different pathways are expressed depending upon the origin of the cells [89^{**}] and how the cells were derived [90]. Because of the differences in contribution of different pathways, a prediction of the efflux efficiency of serum or isolated HDL cannot be made based simply on the level of

HDL-C or apoA-I. This is exemplified by a recent study of de La Liera-Moya *et al.* [91**] who measured efflux from cAMP-treated J774 macrophages to the total HDL fraction isolated from a number of normolipemic human serum specimens. In this study, there were a number of serum samples that contained similar concentrations of either HDL-C or apoA-I but promoted significantly different levels of cell cholesterol efflux. Further analysis demonstrated that the differences in efflux efficiency observed with sera having similar HDL-C or apoA-I levels were a reflection of elevated efflux via the ABCA1 pathway, which in turn was associated with differences in the level of pre β 1-HDL in the serum. Thus, in this experimental system, total apoA-I was similar, but sera having a greater percentage of the apoA-I as pre β 1-HDL enhanced efflux from cells expressing high levels of ABCA1 [91**]. These findings provide a striking demonstration of how HDL quality rather than quantity governs the functionality.

Conclusion

The antiatherogenic quality of HDL is not simply defined by the plasma HDL-C level but rather by the functionality of HDL subspecies. In the case of RCT, the rate of cholesterol movement through the pathway is critical [85*] and the contributions of particular types of HDL particles to this are becoming better defined, especially in terms of interactions with cell-surface cholesterol transporters. The ability of HDL to transport cholesterol provides additional indirect cardioprotective benefits; for instance, ABCA1-mediated efflux of cholesterol from arterial macrophages to apoA-I suppresses inflammation [92*,93*]. The fact that intimal arterial smooth muscle foam cells have impaired ABCA1 activity [94**] further underscores the critical role played by this transporter in preventing atherosclerosis. The tissue-specific contributions of ABCA1 to atherosclerosis susceptibility remain controversial; hepatic ABCA1 clearly plays an antiatherogenic role but, depending upon the particular animal model, expression of ABCA1 in macrophages may or may not be antiatherogenic [95**]. The successful development of HDL-based therapies will benefit from the increasing knowledge of the functionalities of HDL subspecies, as well as a means for reliably measuring RCT in humans [96*].

Acknowledgments

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Papers of particular interest, published within the annual period of review, have been highlighted as:

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Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 271–272).

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Figure 1. Factors contributing to the structural heterogeneity of HDL particles

Different subclasses of the depicted spherical HDL particle can be isolated on the basis of differences in particle diameter and density (lipid/protein ratio). Variations in the surface charge lead to subclasses that can be separated due to differences in electrophoretic mobility. Differences in apolipoprotein composition permit isolation of HDL particles containing either apoA-I alone (LpA-I) or apoA-I along with apoA-II (LpA-I + A-II) by immunoaffinity methods. HDL particles can also be distinguished by their shape: spherical plasma HDL particles contain a neutral lipid core, whereas the discoidal HDL particles that occur in lymph do not. See text for further details. CE, cholesterol ester; TG, triacylglycerol.

Figure 2. Schematic overview of the major pathways involved in HDL-mediated macrophage cholesterol efflux and reverse cholesterol transport to the liver

ApoA-I is produced by the liver and acquires free cholesterol and phospholipid from liver and peripheral cells (including macrophages) via the ATP-binding cassette transporter A1 (ABCA1) to form nascent (discoidal) HDL particles. Nonlipidated apoA-I is cleared by the kidney. Free cholesterol efflux from macrophages to HDL particles is also promoted by the ABCG1 transporter and scavenger receptor class B type 1 (SR-BI). As discussed in section ‘HDL heterogeneity’, the free cholesterol in discoidal HDL particles is converted to cholesteryl ester by lecithin-cholesterol acyltransferase (LCAT) activity leading to the formation of mature, spherical HDL particles. Phospholipid transfer protein (PLTP) mediates transfer of phospholipids from VLDL into HDL, thereby providing phospholipids for the LCAT reaction. Mature HDL particles can be remodeled to smaller particles with the release of apoA-I by the actions of hepatic lipase and endothelial lipase, which hydrolyze HDL triglycerides and phospholipids, respectively (Table 2). In humans, but not rodents, HDL cholesterol ester can be transferred to the VLDL/LDL pool by cholesteryl ester transfer protein (CETP) and taken up by endocytosis into hepatocytes via interaction with the LDL receptor (LDLR). HDL cholesterol ester and free cholesterol are also transferred directly to hepatocytes via SR-BI-mediated selective uptake. Cholesterol taken up by the liver can be recycled back into the ABCA1 pathway, secreted into bile as either free cholesterol or bile acids, or assembled into lipoprotein particles that are secreted back into the circulation (not shown). CE, cholesterol ester; FC, free cholesterol; TG, triacylglycerol.

Table 1

Characteristics of human HDL subclasses

Property	Pre β -HDL	HDL ₂	HDL ₃
Molecular weight	Pre β 1: 71 000 Pre β 2: 325 000	360 000	175 000
Electrophoretic mobility	Pre β	α	α
Density (g/ml)	1.210	1.063–1.125	1.125–1.210
Subpopulations	Pre β 1, Pre β 2, Pre β 3	HDL _{2b} , HDL _{2a}	HDL _{3a} , HDL _{3b} , HDL _{3c}
Diameter (nm)		8.8–12.9	7.2–8.8
	Pre β 1: 5.4–7	HDL _{2b} : 9.7–12.9	HDL _{3a} : 8.2–8.8
	Pre β 2: 12–14	HDL _{2a} : 8.8–9.7	HDL _{3b} : 7.8–8.2
			HDL _{3c} : 7.2–7.8

Table 2

Plasma factors involved in remodeling of HDL

HDL conversion	Plasma factors
Disc → sphere	LCAT
Large sphere → small sphere + free apoA-I	CETP and hepatic lipase
Large sphere → small sphere	Endothelial lipase
Sphere → larger and smaller spheres + free apoA-I	PLTP

CETP, cholesteryl ester transfer protein; LCAT, lecithin-cholesterol acyltransferase; PLTP, phospholipid transfer protein.

Table 3

Pathways for cholesterol efflux from cells to plasma

Efflux pathway	Energetics	Preferred HDL acceptor	Acceptor binding
Aqueous diffusion	Passive	HDL ₂ ~ HDL ₃ (LpA-I ~ LpA-I + A-II)	No
SR-BI	Passive	HDL ₂ > HDL ₃	Yes
ABCG1	Active	HDL ₃ = HDL ₃	No
ABCA1	Active	Pre β 1-HDL [lipid-free (poor) apoA-I]	Yes

ABCA1, ATP-binding cassette transporter A1; SR-BI, scavenger receptor class B type 1.

Table 4

Relationship between plasma HDL-cholesterol, macrophage reverse cholesterol transport and atherosclerosis in response to genetic manipulation in mice

Genetic manipulation	Tissue	Effect on plasma HDL-C	Effect on macrophage RCT	Effect on atherosclerosis
ApoA-I overexpression	Liver	Increase	Increase	Decrease
ApoA-I knockout	Whole body	Decrease	Decrease	Increase
SR-BI overexpression	Liver	Decrease	Increase	Decrease
SR-BI knockout	Whole body	Increase	Decrease	Increase
CETP expression	Liver	Decrease	Increase	Decrease
LCAT overexpression	Liver	Increase	Decrease	Increase
LCAT + CETP expression	Liver	Variable	Neutral	Neutral
LCAT knockout	Whole body	Decrease	Neutral	Variable
ABCA1 knockout	Macrophage	No change	Decrease	Increase
ABCG1 knockout	Macrophage	No change	Decrease	Decrease
ABCA1 + ABCG1 knockout	Macrophage	No change	Decrease	Increase
SR-BI knockout	Macrophage	No change	Neutral	Variable

ABCA1, ATP-binding cassette transporter A1; CETP, cholesteryl ester transfer protein; HDL-C, HDL-cholesterol; LCAT, lecithin-cholesterol acyltransferase; RCT, reverse cholesterol transport; SR-BI, scavenger receptor class B type 1. Table adapted from [85[•]].