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Impact of genetic deletion of platform apolipoproteins on the size distribution of the murine lipoproteome

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ABSTRACT

Given their association with cardiovascular disease protection, there has been intense interest in understanding the biology of high density lipoproteins (HDL). HDL is actually a family of diverse particle types, each made up of discrete - but as yet undetermined – combinations of proteins drawn from up to 95 lipophilic plasma proteins. The abundant apolipoproteins (apo) of the A class (apoA-I, apoA-II and apoA-IV) have been proposed to act as organizing platforms for auxiliary proteins, but this concept has not been systematically evaluated. We assessed the impact of genetic knock down of each platform protein on the particle size distribution of auxiliary HDL proteins. Loss of apoA-I or apoA-II massively reduced HDL lipids and changed the plasma size pattern and/or abundance of several plasma proteins. Surprisingly though, many HDL proteins were not affected, suggesting they assemble on lipid particles in the absence of apoA-I or apoA-II. In contrast, apoA-IV ablation had minor effects on plasma lipids and proteins, suggesting that it forms particles that largely exclude other apolipoproteins. Overall, the data indicate that distinct HDL subpopulations exist that do not contain, nor depend on, apoA-I, apoA-II or apoA-IV and these contribute substantially to the proteomic diversity of HDL.

Biological significance: Plasma levels of high density lipoproteins (HDL) are inversely correlated with cardiovascular disease. These particles are becoming known as highly heterogeneous entities that have diverse compositions and functions that may impact disease. Unfortunately, we know little about the forces that maintain the composition of each particle in plasma. It has been suggested that certain 'scaffold' proteins, such as apolipoprotein (apo) A-I, apoA-II and apoA-IV, may act as organizing centers for the docking of myriad accessory proteins. To test this hypothesis, we took advantage of the genetic tractability of the mouse model and ablated these three proteins individually. We then tracked the abundance and size profile of the remaining HDL proteins by gel filtration chromatography combined with mass spectrometry. The results clearly show that certain cohorts of proteins depend on each scaffold molecule to assemble normal sized HDL particles under wild-type conditions. This work forms the basis for more detailed studies that will define the specific compositions of HDL subspecies with the possibility of connecting them to specific functions or roles in disease.

1. Introduction

The name high density lipoprotein (HDL) was given to those plasma lipoproteins that float within the density range of 1.063 to 1.210 g/mL during density gradient ultracentrifugation (UC) thus distinguishing them from low (1.019–1.063 g/mL, LDL) and very low density lipoproteins (0.95–1.006 g/mL, VLDL). In the laboratory, the HDL family is well known for its heterogeneity in terms of size, charge and composition - so much so that efforts have been made to standardize the nomenclature of HDL particles [1]. In the clinic, on the other hand, HDL has historically been treated as a single entity and tracked by its cholesterol content (HDL-C). Indeed, HDL-C levels in plasma are associated

Abbreviations: Apo, apolipoprotein; CVD, cardiovascular disease; CSH, calcium silica hydrate; HDL, high density lipoprotein; HDL-C, high density lipoprotein cholesterol; LDL, low density lipoprotein; MS, mass spectrometry; PL, phospholipid; STB, standard Tris buffer; UC, ultracentrifugation; VLDL, very low density lipoprotein; WT, wild-type.

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with protection from cardiovascular disease (CVD) and this clinical test has been used for many years as a tool to assess individual risk [2]. However, recent work has shown that across-the-board elevation of plasma HDL-C levels, either as a consequence of natural genetic mutation [3] or by pharmacologic manipulation [4,5], does not necessarily provide the cardio protective benefits predicted by the epidemiology. Nevertheless, an intact HDL pathway has recently been shown to be important in humans as non-functional SR-BI, the cell surface protein responsible for selective cholesteryl ester uptake into the liver and a critical component of reverse cholesterol transport, results in high HDL-C levels, but increased CVD risk [6].

Recent applications of modern mass spectrometry, tracked by the HDL Proteome Watch website [7], have shown that UC isolated HDL preparations contain about 95 consensus proteins [8–10]. These include the 'major' proteins like apolipoprotein (apo)A-I and apoA-II which together account for around 70% of total protein mass in UC-derived HDL. These are sometimes referred to as scaffold or platform proteins because of their key roles in maintaining HDL particle structure [11] and the possibility that they coordinate the binding of other proteins to the particles [12,13]. Numerous 'minor' HDL-associated proteins with known roles in lipid transport, innate immunity, coagulation, regulation of the complement system, metal ion transport and even glucose metabolism have also been identified (reviewed in [10,14]). As first argued in the classic paper of Kostner and Alaupovic [15], all of these auxiliary proteins cannot reside on the same particle due to the limited size of HDL. Work by our laboratory and others has shown that these proteins distribute throughout the HDL family in distinct patterns across particle density [16–18], size [19,20] and ionic character [21,22]. This strongly argues that HDL is actually a diverse collection of subspecies that vary widely in protein composition, and presumably function.

Unfortunately, little is known about the molecular basis underlying why proteins segregate among HDL subspecies. In the current work, we tested the hypothesis that the most abundant apolipoproteins in HDL act as organizing scaffolds that mediate the recruitment of specific subsets of other HDL proteins. We asked a simple question: if a given scaffold protein, say apoA-I, is responsible for mediating the assembly of other proteins into HDL, then the HDL particle size distribution of those proteins should be perturbed when apoA-I is genetically ablated. Conversely, proteins that do not depend on apoA-I should not be affected in its absence. Having recently demonstrated that the lipoproteome of both LDL and HDL-sized lipoproteins in the mouse exhibit a similar overall complexity to humans [23], we took advantage of the mouse model to test this hypothesis. We tracked the size distribution and relative protein levels of the plasma lipoprotein proteome in mice lacking each of the three most abundant HDL proteins, apoA-I, apoA-II and apoA-IV, and compared them to WT controls.

1.1. Experimental section

1.1.1. Animal models and plasma collection

All mouse strains were on the C57BL/6J background and were fed a standard chow diet at all times post weaning. Mice were maintained in American Association for Accreditation of Laboratory Animal Care-approved pathogen-free animal facilities, and the institutional laboratory animal medical services at University of Cincinnati approved all experimental protocols. ApoA-I knock out (KO) mice and age matched wild type (WT) controls were purchased from Jackson Laboratories. ApoA-II KO mice with age matched WT controls were provided by Dr. Catherine Reardon at the University of Chicago. The apoA-I KO and apoA-II KO cohorts were between 6 and 10 weeks of age at the time of blood sampling. The apoA-IV KO mice, derived from the original mouse line generated by the Breslow lab [24], were back-crossed for >10 generations on the C57BL6 background in the Tso lab [25]. They were 32 weeks old with an independent age-matched WT group. Blood was collected from ketamine anesthetized mice ($n = 3$ for each KO and control group) by cardiac puncture using citrate as the anticoagulant. Cellular components were pelleted by centrifugation at ~1590 $\times g$ for 15 min in a Horizon mini-E (Quest Diagnostics) at room temperature. Plasma was stored at 4 °C until gel filtration separation, usually within 16 h. The samples were never frozen.

1.1.2. Plasma separation by gel filtration chromatography

370 μL of plasma from each mouse was applied to three Superdex 200 gel filtration columns (10/300 GL; GE Healthcare) arranged in series on an ÄKTA™ FPLC system (GE Healthcare) [20]. The sample was separated at a flow rate of 0.3 mL/min in standard Tris buffer (STB) (10 mM Tris, 0.15 M NaCl, 1 mM EDTA, 0.2% NaN_3). Eluate was collected as 1.5 mL fractions on a Frac 900 fraction collector (GE Healthcare) maintained at 4 °C. Each fraction was assessed for protein by modified Lowry assay [26], and choline-containing phospholipid and total cholesterol by colorimetric kits from Wako (Richmond, VA).

1.1.3. Isolation of phospholipid-containing particles using calcium silica hydrate (CSH)

The fractions were then passed through a calcium silica hydrate (CSH) resin to bind components that contain phospholipid (PL), as previously described [20]. All PL-containing particles tightly associate with the CSH while non-lipid associated proteins are washed through. Briefly, in a 96 well filter plate (Millipore), 45 μg of CSH (from 100 mg/mL stock solution in 50 mM ammonium bicarbonate (AB)) per 1 μg of PL in 400 μL of fraction were mixed gently for 30 min at room temperature. The resin was eluted using a vacuum manifold and then washed with 50 mM AB buffer. Lipid-containing proteins that remained associated with the CSH were trypsinized with 1.5 μg of sequencing grade trypsin (Promega) overnight at 37 °C and the peptides were washed off the resin, reduced with dithiothreitol (200 mM; 30 min. at 37 °C) and carbamidomethylated with iodoacetamide (800 mM; 30 min. at room temperature). The peptides were then vacuum pelleted and stored at −20 °C until MS analysis.

1.1.4. Mass spectrometry analysis of fractions

Dried peptides were reconstituted in 15 μL of 0.1% formic acid in water. An Agilent 1100 series autosampler/HPLC was used to draw 0.5 μL of sample and inject it onto a C18 reverse phase column (GRACE; 150 × 0.500 mm) where an acetonitrile concentration gradient (5–30% in water with 0.1% formic acid) was used to elute peptides for on-line ESI-MS/MS by a QStar XL mass spectrometer (Applied Biosystems). Column cleaning was performed automatically with 2 cycles of a 5–85% acetonitrile gradient lasting 15 min each between runs.

1.1.5. MS data analysis

To identify the protein composition of lipid-containing particles in the gel filtration fractions, peak lists generated from an analysis of each fraction were scanned against the UniProtKB/Swiss-Prot Protein Knowledgebase (release 57.0, 03/2009) using the Mascot (version 2.1) search engine. Search criteria included: mouse taxonomy, variable modifications of Met oxidation and carbamidomethylation, both peptide tolerance and MS/MS tolerance were set to ±35 PPM, and up to 3 missed tryptic cleavage sites were allowed. Scaffold software (version Scaffold_2_04_00, Proteome Software) was used to validate MS/MS based peptide and protein identifications. Peptide identification required a value of 90% probability (using data from both Mascot and X!Tandem) using the Peptide Prophet algorithm [27]. Positive protein identification also required a value of 90% probability by the Protein Prophet algorithm [28]. Also, a minimum of 2 peptides were required unless the protein in question was found with single peptide hits in multiple consecutive fractions that were consistent across animal subjects. Since equal volumes of sample were applied to the MS analysis, the relative amount of a given protein present in a given fraction is proportional to the number of spectral counts (i.e. the number of MS/MS