commentary

SAA: a link between cholesterol efflux capacity and inflammation?[1]

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Serum amyloid A (SAA) concentration in plasma increases markedly following inflammation or infection, with the liver being the principal site of its synthesis. SAA was first reported to be associated with both human and animal HDLs in the late 1970s (1), but is also associated with other lipoprotein fractions (2, 3). Further studies showed that HDL particles isolated from endotoxin-treated mice contain up to two SAAs per apoA-I molecule (4). When SAA containing HDL was reinjected into mice it was cleared from the plasma more rapidly than apoA-I (5–8). However, isolation of SAA and its reconstitution with HDL to create SAA-enriched particles gives particles that on reinjection are more slowly cleared from plasma compared with native SAA containing HDL (5). HDL particles isolated from patients with myocardial infarction also contain SAA and are small (9), with a density similar to that of HDL_3 (10), but with a diameter resembling HDL_2. The lipid content of these particles is reduced and enriched in triglyceride compared with HDL from healthy people (10–12). During acute inflammatory stress the principal protein bound to HDL_3 is SAA (13), not apoA-I, showing a reversal in the protein composition from normal HDL. SAA increases the binding affinity of HDL to macrophages but reduces HDL binding to hepatocytes (14), suggesting that SAA directs HDL to preferentially remove cholesterol from sites of inflammation (15), possibly through the formation of preβ-HDL (16). HDL cholesteryl ester uptake from reconstituted HDL via SR-B1 is inhibited by the presence of SAA on the particles (17), but the efflux of free cholesterol is enhanced through both SR-B1 (18) and ABCA1 (18, 19) to SAA-containing HDL particles.

Early studies of the HDL particle focused mainly on changes in the lipid and protein content as well as the structure, size, and clearance of the particles from plasma. Extensive investigations into identifying HDL's protein cargo have suggested that the HDL proteome can be highly variable among individuals and altered in response to acute cardiac events (20). These studies suggest a need for accurate quantitative methods to carefully assess individual risk factors with regard to heart disease and HDL function. More recent work has begun to quantify the changes in the protein cargo of HDL under both normal conditions and inflammatory stress. Mass spectrometry is the principal tool used for these studies, employing one of two methods. The first method analyzes intact protein mixtures, called top-down proteomics (21–23), and often involves electron transfer dissociation or electron capture dissociation to obtain amino acid sequence information that helps confirm protein identification. In the second, more commonly used method called bottom-up proteomics (24–30), protein mixtures are digested with a single protease, usually trypsin. Protein identification is based on accurate mass analysis of peptides combined with MS/MS peptide sequencing. Quantitation of proteins, including those carried by HDL, depends on the method of analysis. Label-free, shotgun proteomics gives the greatest possibility of identifying all of the possible changes that are taking place with minimum manipulation of the samples. Label-free proteomics yields a more comprehensive picture of the cargo proteins carried by HDL, whereas methods that label peptides, like iTRAQ or stable-isotope labeling, can lead to a more quantitative assessment of individual protein concentration (20, 31). Label-free methods require careful attention to detail if accurate quantitation is desired and usually employs either spectral counting or measurements of ion abundance (32). When the content of specific target proteins carried by HDL is desired, multiple-reaction monitoring (33–35) with the addition of stable-isotope labeled peptides has worked well (36).

The largest number of HDL cargo proteins identified in a single study was 225 by Jorge et al. (20) who employed both iTRAQ and $^{16}O/^{18}O$ labeling. Comparison of HDL protein cargo between normolipidimic and CVD patients showed that there were differences in the levels of several cargo proteins carried by HDL_2 (25) and HDL_3 (37). Interestingly, an association between SAA and CVD was provided by Lepedda et al. (3), who extracted VLDL, LDL,

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and HDL from carotid endarterectomy samples and found that SAA levels were significantly increased in patients compared with controls. In another study, HDL isolated from CVD patient plasma identified 196 proteins associated with HDL particles, of which four showed increased expression in CVD patients, whereas a further seven proteins were decreased compared with normal controls (38). Using MS, and with verification by ELISA, SAA showed a highly significant increase in patient samples, whereas apoC-I levels showed a significant decrease compared with controls. Mass spectrometry combined with 2-D gel electrophoresis has also been used to precisely map the proteins that coelute with HDL and apoA-I (28).

In this issue of the *Journal of Lipid Research*, Vaisar et al. investigate how inflammation, an established risk factor for CVD, affects cholesterol efflux to HDL. The human participants were subjected to acute endotoxin-induced inflammation and changes in HDL cargo proteins were measured using mass spectrometric-based proteomic methods. As reported by others, Vaisar et al. found that inflammation did not change plasma HDL cholesterol levels, but that the amount of SAA1 and SAA2 carried by HDL was significantly increased. Unlike a previous study, they did not see any change in apoC-I levels (38) possibly due to differences in the stringency of the methods used to analyze the results. Notably, Vaisar et al. report a reduction in the cholesterol efflux to HDL isolated from endotoxin-treated subjects and demonstrate an inverse correlation between the concentration of HDL SAA 1 and 2 and cholesterol efflux capacity. These authors further recapitulated their findings using wild-type and SAA 1/2 deficient mice, in an experiment in which the SAA deficient mice were protected from a reduction in cholesterol efflux after inflammatory challenge. However, using the mouse model to provide a mechanism of how HDL SAA influences cholesterol efflux gave an array of results that raises the question, how much HDL SAA is required to disrupt cholesterol efflux capacity?

In summary, the report by Vaisar et al. demonstrates a link between HDL SAA and decreased cholesterol efflux from J774 macrophages. The SAA appears to displace other proteins from the plasma HDL particles, generating particles that are rich in SAA and defective in their efflux capacity. The next step may be to determine at what SAA plasma or particle concentration cholesterol efflux is affected, and whether SAA alters all HDL size classes equally, or if it is limited to the smaller, denser HDL_3 particles.

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