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EDITORIAL

Further evidence that high-density lipoprotein is a chameleon-like lipoprotein

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This editorial refers to 'Serum amyloid A: high-density lipoproteins interaction and cardiovascular risk'†, by S. Zewinger *et al.*, on page 3007.

Zewinger *et al.*[1] studied 3310 patients undergoing coronary angiography in the Ludwigshafen Risk and Cardiovascular Health (LURIC) Study and determined that serum amyloid A (SAA) concentrations predicted all-cause and cardiovascular mortality. Patients with low SAA levels and higher high-density lipoprotein (HDL) cholesterol levels had lower all-cause and cardiovascular mortality. In contrast, patients with high SAA levels and higher HDL cholesterol levels had increased all-cause and cardiovascular mortality. The authors derived a formula to calculate the levels of biologically 'effective' HDL cholesterol based on SAA and HDL cholesterol data from the LURIC Study. They validated this approach using two other populations, one with high SAA levels and very high risk for cardiovascular events: 1255 participants with type 2 diabetes on haemodialysis in the German Diabetes and Dialysis Study (4D) and a population-based survey of inhabitants of Augsburg, Germany (KORA S4 Study). In the KORA S4 Study, 4261 participants were recruited; subjects with previous myocardial infarction ($n = 77$), stroke ($n = 48$) or with missing values for HDL cholesterol or SAA were excluded ($n = 102$) as well as those lost to follow-up ($n = 7$). In these populations, HDL cholesterol levels did not predict outcomes, but higher levels of the calculated biologically 'effective' HDL cholesterol were associated with reduced risk for all-cause mortality as well as reduced cardiovascular endpoints. *In vitro* studies showed that SAA-supplemented HDL reduced endothelial nitric oxide (NO) production and increased endothelial production of reactive oxygen species, leading to the loss of the ability of the HDL to decrease adhesion of mononuclear cells to TNF-α-treated endothelial cells. The authors concluded that 'SAA turned HDL into a pro-inflammatory particle'.

In 1994, Liao *et al.*[2] demonstrated that mildly oxidized low-density lipoprotein (LDL), which has been implicated in the development of atherosclerosis dramatically induced hepatic mRNA levels for inflammatory isotypes of SAA in mice genetically susceptible to develop atherosclerosis, but not in mice genetically resistant to atherosclerosis.

In 1995, Van Lenten *et al.*[3] reported that anti-inflammatory HDL became pro-inflammatory during the acute phase response in both rabbits and humans. The acute phase response in rabbits was accompanied by high levels of SAA that displaced the main protein in HDL apolipoprotein A-I (apoA-I). In contrast to normal HDL, which was anti-inflammatory based on *in vitro* assays, the SAA-enriched HDL was pro-inflammatory. Moreover, SAA levels significantly correlated with the extent of aortic atherosclerosis and with the inflammatory properties of HDL, but not with HDL cholesterol levels.

The main protein in LDL is apoB, a protein that does not exchange between particles. The main protein in HDL is apoA-I, which readily moves between particles. Indeed, all proteins associated with HDL are continuously moving on and off the HDL particles. It was hypothesized that HDL is a shuttle whose size is estimated by measurement of HDL cholesterol, but the contents of the shuttle are not described by this measurement.[4,5] It was also hypothesized that HDL evolved as part of the innate immune system and is a chameleon-like lipoprotein.[4,5] In the absence of an acute phase response or systemic inflammation, the proteins associated with HDL are anti-inflammatory. However, in the presence of an acute phase response, or in the presence of systemic inflammation, the proteins associated with HDL are pro-inflammatory.[4,5] Thus HDL can be thought of as an amplification system, enhancing inflammation in the presence of an acute phase response or in the presence of systemic inflammation, but which promotes the maintenance of an anti-inflammatory state in the absence of an acute phase response or systemic inflammation.[4,5]

Consistent with this hypothesis, Ansell *et al.*[6] reported that subjects with high levels of HDL cholesterol with coronary heart disease had pro-inflammatory HDL, while normal subjects had anti-inflammatory HDL. Besler *et al.*[7] demonstrated that HDL

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from patients with coronary artery disease lacked endothelial anti-inflammatory effects and did not stimulate endothelial repair because it failed to induce endothelial nitric oxide (eNOS) production. This was found to be due to the activation of endothelial lectin-like oxidized LDL receptor 1, which triggered endothelial protein kinase C βII activation, which in turn inhibited eNOS-activating pathways and eNOS-dependent NO production.[7]

While the liver is thought to be the major source for SAA during an acute phase response, it is known that peripheral tissues can also significantly contribute SAA by local synthesis.[8] Song *et al.*[9] reported that SAA stimulates cytokine production in monocyte-macrophages *in vitro*. They also reported that subjects with coronary atherosclerosis showed a significant release of SAA into the coronary circulation that was not seen in subjects without coronary atherosclerosis.[9] Evidence that atherosclerosis is not the only inflammatory disease with abnormal HDL comes from the work of Watanabe *et al.*[10] They demonstrated the association of acute phase proteins, including SAA, and complement factors with pro-inflammatory HDL in rheumatoid arthritis. HDL taken from subjects with rheumatoid arthritis had impaired ability to stimulate cholesterol efflux.[11] Charles-Schoenman *et al.*[12] studied 36 patients with rheumatoid arthritis that were treated with tofacitinib (an oral Janus kinase inhibitor) for 6 weeks and found a trend toward lower SAA levels (51.93 ± 95.61 vs. 24.97 ± 48.96 mg/L; $P = 0.0588$) and HDL-associated SAA levels (34.76 ± 62.83 vs. 17.79 ± 34.45 mg/L; $P = 0.0647$), suggesting that resolution of inflammation decreases both plasma levels of SAA and levels of HDL-associated SAA.

Vaisar *et al.*[13,14] challenged humans with a low dose of endotoxin and found that only two proteins, SAA1 and SAA2, were differentially abundant in inflammatory HDL. The ability of HDL from the endotoxin-treated subjects to accept cholesterol from macrophages was inversely correlated with the content of SAA1/2 in the HDL.[13,14] While the composition and function of HDL from the endotoxin-treated subjects was significantly changed, the lipid composition of HDL and the plasma levels of HDL cholesterol, LDL cholesterol and triglycerides did not change.[13,14] In mouse studies, in the absence of inflammation, the protein composition of HDL from wild-type mice was virtually identical to that of HDL from mice null for *Saa1/2* and hence lacking SAA1 and SAA2. Treatment of wild-type mice with silver nitrate induced an acute phase response and markedly remodelled the HDL proteome of the wild-type mice, but not that of the mice null for SAA1 and SAA2.[13,14] HDL obtained from the wild-type mice after silver nitrate treatment showed a marked decrease in its ability to promote cholesterol efflux from macrophages that was inversely correlated with the SAA content of the HDL.[13,14] In contrast, HDL obtained after silver nitrate treatment from the mice null for *Saa1/2* retained its ability to promote cholesterol efflux.[13,14] Study of the proteome of HDL taken from both mice and humans after induction of an inflammatory state also indicated that SAA alters HDL's biological function in part by replacing cardioprotective proteins that are present in HDL in the non-inflamed state.

The work of Castrillo *et al.*[15] demonstrated that a basic response of macrophages to either bacterial or viral infection is to significantly reduce cholesterol efflux. The changes in the HDL proteome induced by an acute phase response or systemic inflammation likely evolved to enhance this component of the innate immune system.

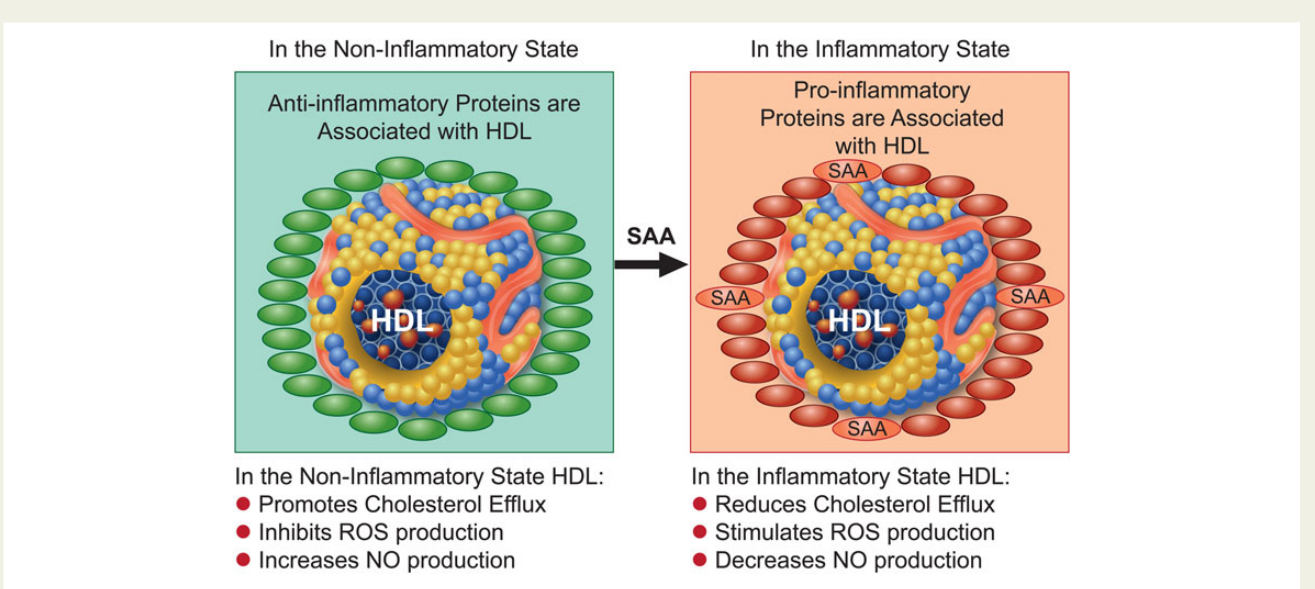

Figure 1 A schematic representation of the chameleon-like nature of HDL. In the non-inflammatory state, HDL contains little serum amyloid A (SAA) and promotes cholesterol efflux from macrophages, inhibits the production of reactive oxygen species (ROS) and increases nitric oxide (NO) production from endothelial cells. In the presence of an acute phase response or systemic inflammation the positive acute phase reactant SAA is produced by the liver and other tissues and associates with HDL. In the process of SAA associating with HDL, the HDL proteome is remodelled by reducing cardioprotective proteins associated with HDL. As a result, HDL-mediated cholesterol efflux is reduced, ROS production is stimulated and NO production is decreased, which leads to an enhancement of the inflammatory state.

The studies by Zewinger *et al.*[1] as reported in this issue of the *Journal* provide further evidence of the chameleon-like nature of HDL. In the absence of an acute phase response or systemic inflammation, the HDL proteome constitutes anti-inflammatory particles, but in the presence of an acute phase response or systemic inflammation the HDL proteome is remodelled to constitute particles that enhance the inflammatory response (*Figure 1*). This system likely evolved to provide protection against viral and bacterial infections at a time when humans did not live long enough to suffer from chronic inflammatory diseases such as atherosclerosis or rheumatoid arthritis.[4,5] The work of Zewinger *et al.*[1] suggests that therapies that modulate this aspect of the innate immune system may have the potential to improve the outcomes of such chronic inflammatory diseases.

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Disclosures

A.M.F. is a principal and officer in Bruin Pharma.

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