commentary

SAA, HDL biogenesis, and inflammation[1]

The understanding of Tangier disease has, over the last decade, focused attention on the role of the ABC transporter ABCA1 in the biogenesis of HDL (1). Both hepatic and extrahepatic ABCA1 expression are important in the regulation of plasma HDL levels (2). The level of HDL is thought to be critical in inhibiting lesion formation as well as in reducing the lipid load of preexisting atherosclerotic lesions. However, it is not only the level of HDL that is relevant for its atheroprotective properties; the quality of the HDL is also important (3). After infection and inflammation, HDL changes its composition (4, 5) and its anti-inflammatory properties (3). Among the changes in HDL composition associated with acute inflammation are a marked enrichment in serum amyloid A (SAA) and a reduction in paraoxonase (6).

The relationship of SAA with HDL and its atheroprotective function is a complex one. In this issue of the *Journal of Lipid Research*, Hu and colleagues (7) examine, in a series of elegant experiments, the interaction between SAA, HDL biogenesis, and the dependence of the formation of SAA-HDL on ABCA1. They studied ABCA1 knockout animals and the cells derived from them for further cell-based studies. Although these experiments furnish valuable new information about the complex relationship between SAA, ABCA1, HDL, and the properties of SAA, they also raise new questions that would be appropriate for further study. In brief, they demonstrate that, unlike the situation in normal mice, in which SAA is largely associated with HDL after the induction of SAA expression by injection of lipopolysaccharide (LPS), in the absence of ABCA1 there is of course little or no HDL and the small amount of SAA present in plasma is mostly found in the VLDL/LDL fraction. The livers of ABCA1 knockout mice have high levels of SAA mRNA, although not as high as in wild-type mice. SAA protein levels in the liver of LPS-treated mice are also similar in wild-type and knockout mice. In contrast, the amount of SAA secreted from primary hepatocytes derived from LPS-treated knockout animals is significantly lower than that secreted from wild-type mice, and all of the SAA secreted from the knockout hepatocytes was recovered as lipid-free protein. Thus, there was an apparent disproportionate reduction in the secretion of SAA by hepatocytes from the ABCA1-deficient mice. Is this a cell culture artifact? We think not, because hepatocytes from wild-type mice are able to secrete SAA in association with HDL-like particles, and comparable levels of apoA-I were secreted from the wild-type and knockout hepatocytes. The presumptive explanation for the small amount of SAA in the medium of the ABCA1-deficient hepatocytes is that there is intracellular or extracellular degradation of the SAA. A careful pulse-chase experiment with control and ABCA1 knockout hepatocytes, both infected with SAA-expressing adenovirus, could provide further insight into this apparent discrepancy. The use of LPS is avoided in this proposed experiment, as there are many changes that are induced in the liver in response to LPS stimulation that could confound the interpretation of the experiment.

These authors further showed that the incubation of cells in culture with exogenous SAA protein promoted phospholipid and cholesterol release from the cells as efficiently as apoA-I, but only in ABCA1-expressing cells. In addition, they observed that SAA was able to stabilize ABCA1 protein at least as effectively as apolipoprotein A-I (apoA-I). Both of these observations suggest that SAA resembles apoA-I in interacting directly with ABCA1. The promotion of the ABCA1-dependent release of lipids from cells by SAA is not unexpected. Apoprotein interaction with ABCA1 is not sequence-specific but appears to depend on the amphiphilicity of helices in apoproteins or synthetic peptides (8, 9), and SAA contains amphipathic helices. These apoproteins interact with ABCA1 only in their lipid-free/lipid-poor state, not when associated with HDL. However, HDL containing SAA may influence cholesterol trafficking into and out of cells, depending on the conditions (10, 11). In the context of tissue inflammation, the presence of SAA, either as the free protein or on HDL, may influence cellular cholesterol homeostasis.

Not only do apoproteins stabilize ABCA1; so does phospholipid transfer protein. The stabilization of ABCA1 by apoA-I is thought to involve signaling via a variety of cellular kinases that phosphorylate the transporter (1). This has not been studied in any detail for any of the other proteins that interact with ABCA1.

Recent experiments by Michael Phillips and his collaborators (12, 13) have dissected in detail the interaction of apoA-I with ABCA1. They have proposed a three-step model mechanism. A low-capacity apoprotein interaction with the transporter promotes cholesterol and phospholipid migration from the cytoplasmic leaflet of the plasma membrane that results in the enrichment of the

DOI 10.1194/jlr.E700012-JLR200

[1] See referenced article., *J. Lipid Res.* 2008, 49: 386-393.

exofacial leaflet with these lipids, which is resolved by exovesiculation to relieve the membrane stress. A high-capacity interaction between apoA-I and this region of the membrane then promotes the formation of nascent HDL. Apoprotein sequence requirements for the low- and high-capacity interactions are not the same. Although SAA contains amphipathic helices, they do not resemble the regularity of the amphipathic helices found in apoA-I. The studies reported by Hu and colleagues (7) lend themselves to further exploration of the precise mechanism by which SAA promotes lipid release and the determination of the sequences of SAA required for each of these interactions. The presumption is that the ABCA1-dependent lipid release by SAA generates a nascent discoidal HDL particle, but this requires further examination. The fate of these particles is also unknown. Cabana and colleagues (14) have reported that a proportion of the HDL containing SAA in the plasma exists, at least in mice, as an HDL particle devoid of other exchangeable apoprotein. This particle is spherical and thus contains core lipids such as cholesteryl ester and triglyceride. It is not known yet whether SAA is like apoA-I in possessing the ability to significantly activate LCAT, which would be required for the direct conversion of the nascent SAA discs into a spherical HDL containing only SAA.

The relative efficacy of apoA-I and SAA promotion of cellular lipid release will depend on their relative affinity for the transporter and for the lipid-enriched exovesicles. This has been studied in some detail for apoA-I (13), but not for SAA. Using the Phillips model as a guide, the advantage of elucidating the SAA sequence requirements for ABCA1 binding (low capacity) and lipid binding (high capacity) could be in the provision of probes for the study of the role of SAA in the context of the local vessel inflammation associated with atherosclerosis. Furthermore, there is the potential for SAA and apoA-I interacting with one another to remodel HDL and to generate free apoproteins that may function to promote both ABCA1-dependent lipid transport and the formation of discoid HDL. The careful studies of Hu and colleagues (7) have opened up these many relevant questions.

Godfrey S. Getz, *Associate Editor*
Catherine A. Reardon
The University of Chicago
g-getz@uchicago.edu

REFERENCES

1. Oram, J. F., and J. W. Heinecke. 2005. ATP-binding cassette transporter A1: a cell cholesterol exporter that protects against cardiovascular disease. *Physiol. Rev.* **85:** 1343–1372.
2. Singaraja, R. R., M. Van Eck, N. Bissada, F. Zimetti, H. L. Collins, R. B. Hildebrand, A. Hayden, L. R. Brunham, M. H. Kang, J. C. Fruchart, et al. 2006. Both hepatic and extrahepatic ABCA1 have discrete and essential functions in the maintenance of plasma high-density lipoprotein cholesterol levels in vivo. *Circulation.* **114:** 1301–1309.
3. Van Lenten, B. J., S. Y. Hama, F. C. de Beer, D. M. Stafforini, T. M. McIntyre, S. M. Prescott, B. N. La Du, A. M. Fogelman, and M. Navab. 1995. Anti-inflammatory HDL becomes pro-inflammatory during the acute phase response. Loss of protective effect of HDL against LDL oxidation in aortic wall cocultures. *J. Clin. Invest.* **96:** 2758–2767.
4. Cabana, V. G., J. R. Lukens, K. S. Rice, T. J. Hawkins, and G. S. Getz. 1996. HDL content and composition in acute phase response in three species: triglyceride enrichment of HDL a factor in its decrease. *J. Lipid Res.* **37:** 2662–2674.
5. Khovidhunkit, W., R. A. Memon, K. R. Feingold, and C. Grunfeld. 2000. Infection and inflammation-induced proatherogenic changes of lipoproteins. *J. Infect. Dis.* **181 (Suppl. 3):** 462–472.
6. Han, C. Y., T. Chiba, J. S. Campbell, N. Fausto, M. Chaisson, G. Orasanu, J. Plutzky, and A. Chait. 2006. Reciprocal and coordinate regulation of serum amyloid A versus apolipoprotein A-I and paraoxonase-1 by inflammation in murine hepatocytes. *Arterioscler. Thromb. Vasc. Biol.* **26:** 1806–1813.
7. Hu, W., S. Abe-Dohmae, M. Tsujita, N. Iwamoto, O. Ogikubo, T. Otsuka, Y. Kumon, and S. Yokoyama. 2008. Biogenesis of high density lipoprotein by serum amyloid A is dependent on ATP binding cassette transporter A-1 in the liver in vivo. *J. Lipid Res.* **49:** 386–393.
8. Stonik, J. A., A. T. Remaley, S. J. Demosky, E. B. Neufeld, A. Bocharov, and H. B. Brewer. 2004. Serum amyloid A promotes ABCA1-dependent and ABCA1-independent lipid efflux from cells. *Biochem. Biophys. Res. Commun.* **321:** 936–941.
9. Remaley, A. T., J. A. Stonik, S. J. Demosky, E. B. Neufeld, A. V. Bocharov, T. G. Vishnyakova, T. L. Eggerman, A. P. Patterson, N. J. Duverger, S. Santamarina-Fojo, et al. 2001. Apolipoprotein specificity for lipid efflux by the human ABCA1 transporter. *Biochem. Biophys. Res. Commun.* **280:** 818–823.
10. Tam, S. P., A. Flexman, J. Hulme, and R. Kisilevsky. 2002. Promoting export of macrophage cholesterol: the physiological role of a major acute-phase protein, serum amyloid A 2.1. *J. Lipid Res.* **43:** 1410–1420.
11. Artl, A., G. Marsche, S. Lestavel, W. Sattler, and E. Malle. 2000. Role of serum amyloid A during metabolism of acute-phase HDL by macrophages. *Arterioscler. Thromb. Vasc. Biol.* **20:** 763–772.
12. Vedhachalam, C., P. T. Duong, M. Nickel, D. Nguyen, P. Dhanasekaran, H. Saito, G. H. Rothblat, S. Lund-Katz, and M. C. Phillips. 2007. Mechanism of ATP-binding cassette transporter A1-mediated cellular lipid efflux to apolipoprotein A-I and formation of high density lipoprotein particles. *J. Biol. Chem.* **282:** 25123–25130.
13. Vedhachalam, C., A. B. Ghering, W. S. Davidson, S. Lund-Katz, G. H. Rothblat, and M. C. Phillips. 2007. ABCA1-induced cell surface binding sites for apoA-I. *Arterioscler. Thromb. Vasc. Biol.* **27:** 1603–1609.
14. Cabana, V. G., C. A. Reardon, B. Wei, J. R. Lukens, and G. S. Getz. 1999. SAA-only HDL formed during the acute phase response in apoA-I$^{+/+}$ and apoA-I$^{-/-}$ mice. *J. Lipid Res.* **40:** 1090–1103.