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High-density lipoprotein benefits beyond the cardiovascular system: a potential key role for modulating acquired immunity through cholesterol efflux

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Commentary on 'Cholesterol accumulation in dendritic cells links the inflammasome to acquired immunity' by Westerterp *et al.*, Cell Metab 2017.

High-density lipoproteins (HDLs) are effective antioxidant, anti-inflammatory, anti-thrombotic, and cardioprotective particles capable of exerting vasculo- and cardioprotective effects.[1,2] Yet the most relevant function of HDL is to promote reverse cholesterol transport, a mechanism by which HDL particles mediate the movement of cholesterol of peripheral cells to the liver for excretion in the bile. It has been well established that HDL interacts with lipid rafts found in the surface of lipid-loaded macrophage and depletes cholesterol from these microdomains either in an active way through the adenosine triphosphate-binding cassette (ABC) transporters ABCA1 and ABCG1 or a passive/diffusional way (*Figure 1*). Through these mechanisms, HDL particles prevent foam cell formation, thereby protecting against atherosclerotic cardiovascular disease (CVD; *Figure 1*).[3] During the last years, however, it has become evident that the ability of HDL particles to act as cholesterol acceptors modulating lipid raft structure also affects the innate and adaptive immunity.[4] On the one hand, lipid rafts have been shown to function as platforms for the clustering and activation of toll-like receptors (TLRs), which are critically involved in the innate immune response by inducing cytokine and chemokine production, and on the other hand, lipid rafts contribute to the adaptive immune response, because they concentrate on major histocompatibility complex class II molecules in the cell surface of antigen-presenting cells.

Low HDL-cholesterol levels represent an important cardiovascular risk factor, and, accordingly, it was assumed that increasing HDL-cholesterol would exert beneficial effects in patients with symptomatic atherosclerotic heart disease.[5] However, observations from genetic studies and the disappointing outcomes from multiple clinical trials aimed to raising HDL with pharmacological therapies have evidenced that HDL-cholesterol levels do not necessarily reflect protection against CVD.[6] Moreover, human studies have evidenced that HDL function is better suited to predict CV events than HDL-cholesterol levels. For instance, a recent study in almost 3000 subjects free from CVD has linked impaired HDL-cholesterol efflux capacity with the prediction of atherosclerotic CV events.[7] Autoimmune disease such as systemic lupus erythematosus (SLE), rheumatoid arthritis, and Crohn's disease also feature low HDL-cholesterol levels suggesting a role for HDL particles in maintaining immune tolerance. As such, HDL particles have shown to suppress dendritic cell maturation and function thereby attenuating the adaptive immune response.[8] Interestingly, in concurrence with the CVD setting, HDL function is found to be altered in several autoimmune-related disorders. So far, few studies in animal models have suggested a link between defects on cholesterol efflux in macrophages and lymphocytes with the

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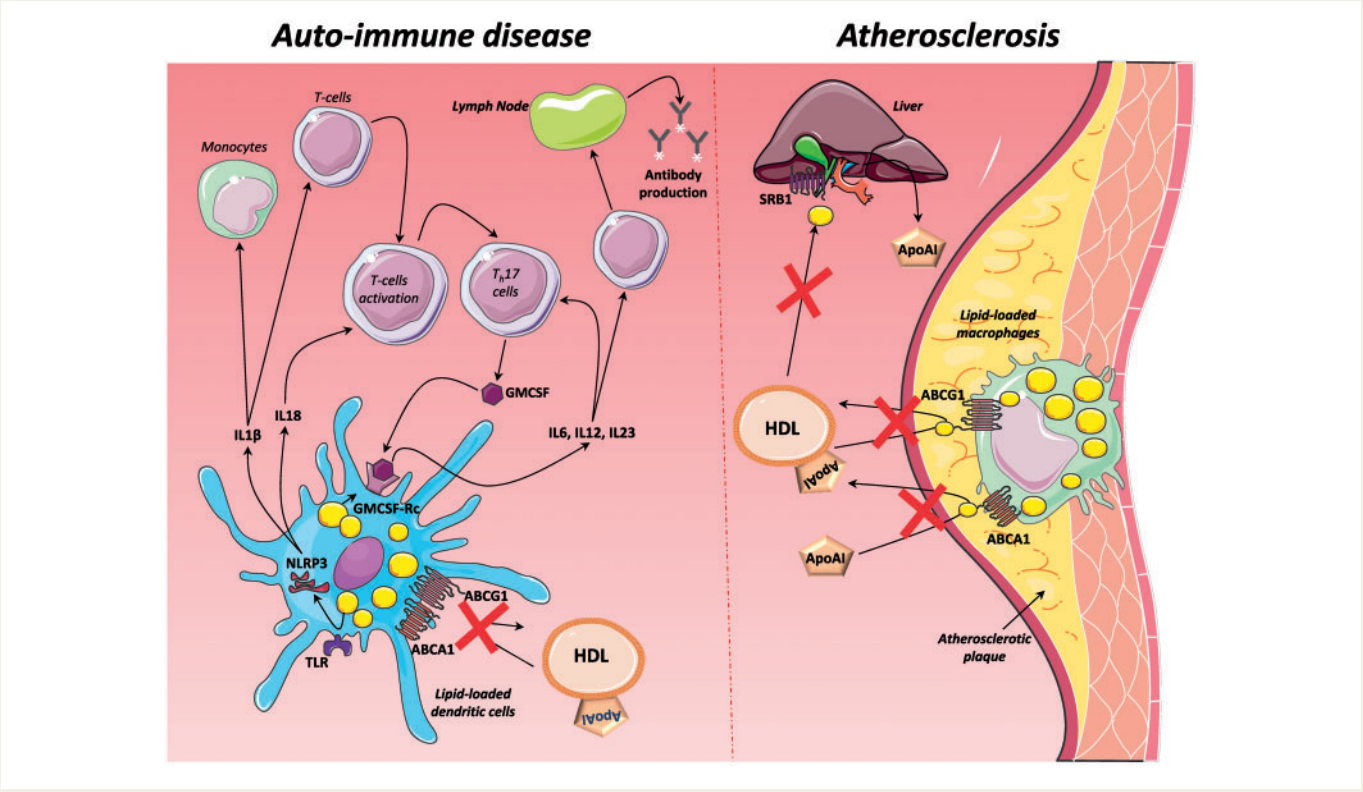

Figure 1 Role of HDL in atherosclerosis and auto-immune disease. The ATP-binding cassette transporters ABCA1 and ABCG1 prevent atherosclerosis progression by effluxing lipids from lipid-loaded macrophages to apolipoprotein (Apo)AI and HDL, respectively. Then, liver scavenger receptor class B type I (SR-B1) mediates uptake of HDL-derived cholesterol for further secretion in the bile. HDL also plays an important role in maintaining immune homeostasis. As such, appropriate HDL-related cholesterol efflux via ABCA1 and ABCG1 impedes intracellular cholesterol loading in dendritic cells preventing an aberrant inflammatory response. On the contrary, blockade or dysfunctional ABCA1 and ABCG1 receptors not only favor atherosclerosis progression but lead to the formation of lipid-loaded dendritic cells favoring the activation of the toll-like receptor (TLR)-related NLRP3 inflammasome pathway and an enhanced GMCSF-receptor surface expression overall inducing the production of interleukins known to promote auto-immune development.

development of certain autoimmune phenotypes. As such, cholesterol accumulation in ABCG1-deficient T-cells has been implicated in T-lymphocyte proliferative response by altering signalling from the plasma membrane,[9] and macrophage deficiency in liver- and/or retinoic X-receptor have shown to display an impaired macrophage uptake of apoptotic bodies (efferocytosis) leading to an aberrant inflammatory response and the consequent antibody development in a process dependent on ABCA1 transporter.[10,11] In this work, Westerterp *et al.*[12] demonstrate that impaired dendritic cells cholesterol efflux via ABCA1/G1 receptors induces the development of an autoimmune phenotype through an inflammatory-driven process independent of antigen presentation. Dendritic cells are determinant in the adaptive immune response and autoimmune diseases because of their efficient antigen-presenting role. Briefly, dendritic cells phagocyte antigens and/or pathogens, generate MHC-peptide complexes, migrate from the sites of antigen acquisition to secondary lymphoid organs and, finally, prime naive T-lymphocytes thereby driving the adaptive immune response. However, prolonged self-antigen presentation by dendritic cells to T-cells leads to the development of autoimmune disease.[13] In this work, the authors, by performing a series of sophisticated and elegant studies in transgenic mice, firstly, demonstrate that deletion of both cholesterol transporters (ABCA1 and ABCG1) in dendritic cells (i.e. DC-ABC^{DKO}) leads to an age-related development and adoption of an autoimmune phenotype that resembles that of SLE (*Figure 1*). Moreover, DC-ABC^{DKO} cells display a 'foam cell-like' phenotype supporting the need for proper dendritic cell-cholesterol efflux capacity in maintaining immune homeostasis. Interestingly, deletion of both transporters in macrophages or T-cells does not translate into the development of autoimmune disease or intracellular cholesterol loading, emphasizing the key role of dendritic cells in the development of this autoimmune disorder.

The authors also provide insights as to the mechanisms behind and demonstrate that, rather than being associated with antigen presentation, it is a consequence of an enhanced cholesterol-related activation of the *TLR*-related NLRP3 inflammasome pathway (*Figure 1*). Inflammasomes are intracellular innate immune systems that trigger the maturation of pro-inflammatory cytokines promoting the recruitment of innate immune defences. However, the fact that NLRP3 deficiency only partly reverses the autoimmune phenotype supports the contribution of the adaptive immune system in the overall inflammatory response. In this regard, the authors demonstrate higher expression of the granulocyte–macrophage colony-stimulating factor (GM–CSF) receptor in DC-ABC^{DKO} cells in concurrence with a higher release of GM–CSF from T-cells, which in turn have been previously activated by DC-ABC^{DKO} cells (*Figure 1*). Altogether, GM–CSF/GM–CSF receptor interaction leads to an enhanced dendritic cell secretion of interleukins (ILs) known to promote autoimmune development (IL-23, IL-12, and IL-6) as well as further induce T-cell

expansion, eventually closing the positive feedback loop that explains the detected SLE-like phenotype (*Figure 1*). Interestingly, treatment with reconstituted HDL reversed the inflammatory response by promoting passive cholesterol removal confirming the key contribution of intracellular lipid accumulation in inflammasome activation and overall IL release.

In contrast to immune-related disorders, the involvement of dendritic cells in the pathophysiology of atherosclerotic disease is less understood. Scarce literature indicates that they mainly contribute to present antigens (i.e. heat shock proteins and oxidized LDL) to specific T-cells activating the adaptive immune system and further recruiting leucocytes to the site of lesion with the consequent increase in atheromatous plaque vulnerability.[14] In fact, dendritic cells are usually detected in the shoulder regions of human unstable plaques and in close proximity to T-cells. However, whether dendritic cells may acquire a foam-like phenotype and exacerbate inflammasome activation and the release of inflammatory mediators need to be determined. In fact, macrophage NLRP3 inflammasome activation has been associated with cholesterol crystals accumulation and with the increasing severity of coronary artery disease.[15,16] Moreover, given that both innate and adaptive immune responses have key roles in atherosclerosis development and progression, a better understanding on the mechanistic insight into this remarkable ability of HDL to modulate the immune response through dendritic cells-related ABC transporters may represent a novel pathway to target atherosclerosis progression. Moreover, the fact that Apolipoprotein-AI (ApoAI) (the major protein constituent of HDL) not only is largely responsible for cholesterol unloading but also has shown to lessen T-cell response in atherosclerotic lesions[17] supports ApoAI mimetics administration as a potentially effective therapeutic approach. Collectively, however, all these observations strengthen the need to maintain HDL particles in a functional state to impede the development of chronic inflammatory disorders thereby reducing the burden of autoimmune- and atherosclerotic-diseases.

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