Editorial

High-density lipoprotein lifts the "dark web" cast by neutrophils

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In a recent publication, in *Circulation*, Westerterp and co-workers (1) reveal novel mechanisms for how NLRP3 inflammasome activation enhances atherosclerosis and further show that high-density lipoprotein (HDL) counteracts this activation. They report that ATP Binding Cassette A1 and G1 (ABCA1/ABCG1) deficiency in myeloid cells increases plasma interleukin-18 (IL-18) levels in *Ldlr*$^{-/-}$ mice on a western type diet by a NLRP3 inflammasome-dependent process. The knock-out of *Nlrp3* or *Caspase1/11* obliterated the increase in IL-18, confirming a central role of the NLRP3 inflammasome in inflammation induced by myeloid ABCA1/G1 deficiency. This may turn out to be a key finding in unraveling the complicated relationship between HDL, inflammation and atherosclerosis.

The inverse association between HDL-cholesterol and cardiovascular disease has been long known (2,3); however, recent attempts to increase HDL cholesterol by novel drugs have failed in their goal to reduce cardiovascular events (4,5). This paradox could be related to the surprising complexity of the protein and lipid composition of HDL, which can alter HDL function. Recent studies have demonstrated that among the many purported HDL anti-atherogenic functions, cholesterol efflux is closely inversely associated to cardiovascular risk and in fact appears to better correlate with the anti-atherogenic property of HDL than HDL-cholesterol (6,7). HDL actively accepts free cholesterol from the membrane of peripheral cells through the specific transporters ABCA1 and ABCG1, as well as by a passive concentration gradient dependent process (8).

The infiltration of inflammatory cells in atherosclerotic lesions and increased levels of circulating cytokines in patients with cardiovascular disease have also revealed the importance of inflammation in the pathogenesis of atherosclerosis (9). Most notably, interleukin 1β (IL-1β), a pro-inflammatory cytokine, has been shown to induce the expression of adhesion molecules in endothelial cells, stimulate the proliferation of smooth muscle cells in lesions and notably, activates cells of innate immunity, especially macrophages (10). In the CANTOS trial, canakinumab, a neutralizing monoclonal antibody against IL-1β, was shown to reduce the occurrence of cardiovascular events in secondary prevention, independent of any changes in plasma lipids (11). IL-1β is first produced as pro-IL-1β and proteolytically activated by Caspase 1, which in turn is activated by the inflammasome, a large multi-component molecular complex found in the cytoplasm of cells involved in innate immunity (12). Another pro-inflammatory cytokine activated by Caspase 1 is IL-18, which is also associated with atherosclerosis (13).

The inflammasome initiates the innate inflammatory response by the recognition of "danger signals". In particular, the NLRP3 inflammasome appears to be critical for atherogenesis; abrogation of NLRP3 function in bone marrow transplantation studies blocks atherosclerosis development in animal models (14). NLRP3 expression levels are also increased in the aorta of patients with coronary atherosclerosis (15). Neutrophils constitute the most abundant leukocytes in blood and are important effectors of innate immunity, particularly inflammasome

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activation, but their role in atherosclerosis has been difficult to establish (16). Even less understood is how HDL may modulate this process.

In their publication, Westerterp *et al.* found that, at early stages of atherosclerosis, myeloid deficiency in ABCA1/G1 caused neutrophilia, increased neutrophil infiltration in atherosclerotic plaques and a greater extent of neutrophil extracellular traps (NETs), which are extracellular nets made of DNA and granular proteins that can trap pathogens and are released by neutrophils when they are activated (1). Furthermore, they showed that the absence of inflammasome machinery, namely NLRP3 and Caspase1/11, blocked all these steps. Previously, transplantation of *Abca1/Abcg1* deficient bone marrow into *Ldlr*$^{-/-}$ mice showed that neutrophilia and monocytosis increased atherosclerotic lesions (17). Thus, ABCA1/G1 deficiency in progenitor cells in bone marrow would cause systemic myeloid cellular cholesterol accumulation, inflammasome activation and neutrophil proliferation in bone marrow, ending with neutrophil recruitment in atherosclerotic plaque. Remarkably, neutrophil granule proteins can also attract monocytes to the atherosclerotic lesion site (16).

Although there are some conflicting studies on this point, phagocytosis of cholesterol crystals by macrophages appears to result in lysosomal damage, which then triggers NLRP3 inflammasome activation (14,18). In their publication, Westerterp *et al.* also observed that increased esterified and free cholesterol in splenic neutrophils was associated with inflammasome activation (1), which would provide a mechanism whereby early cholesterol deposition in atherosclerotic plaque could trigger inflammation.

In a previous study, we showed that incubation of THP-1 cells and human primary macrophages with HDL decreased the expression of inflammasome components, such as NLRP3 and IL-1β, and also reduced the activation of Caspase1 (19). Similarly, Westerterp and co-workers found that the injection of reconstituted HDL (rHDL) into myeloid ABCA1/G1 deficient *Ldlr*$^{-/-}$ mice reversed the increase in IL-18 (1), also suggesting that HDL can counteract inflammasome activation. In this model, the HDL effect could only be exerted by enhancing passive cholesterol efflux from cells, since myeloid cells lacked ABCA1/G1 transporters. This suggests that cholesterol enrichment in cell membranes, in the form of cholesterol crystals or not, can potentiate inflammasome activation.

As mentioned above, NLRP3 inflammasome induces Caspase1 activation, leading to the secretion of IL-1β and IL-18 (12). However, under some circumstances a non-canonical pathway can be involved, mediated by murine Caspase11, or the human counterparts Caspase4/5, activated by lipopolysaccharide (LPS) (20). Westerterp *et al.* found elevated Caspase11 activation in neutrophils and macrophages. Moreover, they performed LPS mortality experiments on myeloid ABCA1/G1 deficient *Ldlr*$^{-/-}$ mice, with and without concomitant knock-out of *Nlrp3* or *Caspase1/11*. Interestingly, *Caspase1/11* knock-out mice were resistant to LPS-induced death, regardless if they were myeloid ABCA1/G1 deficient or not; whereas myeloid ABCA1/G1 deficient animals, were the most susceptible to LPS-induced death, even when they lack NLRP3 expression in their myeloid cells (1). These data suggest greater inflammasome priming in myeloid ABCA1/G1 deficiency, and that inflammasome activation in ABCA1/G1 deficiency is upstream of NLRP3. In this scenario, cholesterol accumulation in myeloid cell membranes would favor inflammasome activation by the non-canonical pathway.

Tangier Disease is a rare autosomal recessive disease due to ABCA1 deficiency that leads to a marked reduction in HDL cholesterol and the accumulation of cholesterol in peripheral tissues, particularly macrophages. Westerterp and co-workers found increased levels of IL-1β and IL-18 in Tangier Disease patients in comparison to controls, suggesting NLRP3 inflammasome activation (1). Besides Tangier Disease, other more common conditions characterized by increased "C" reactive protein (CRP), a marker for low-grade inflammation, also have been shown to have reduced levels of ABCA1 in myeloid cells. Type 2 diabetic patients, for example, have decreased expression of *Abca1* in blood leukocytes (21). Similar results have been observed in monocytes from obese/overweight patients (22) and in chronic kidney disease (CKD) patients (23). Furthermore, in type 2 diabetes and in CKD there is increased IL-18 and IL-1β in circulating monocytes (24,25), suggesting a possible pro-atherogenic role of the inflammasome in these common disorders.

In conclusion, this interesting study shows how the deficiency of ABCA1 and ABCG1 in myeloid cells induces cholesterol accumulation in cell membranes, which then favors NLRP3 inflammasome activation or Caspase11 mediated non-canonical inflammasome activation, even in the absence of cholesterol crystals. It also provides a novel mechanism for linking the cholesterol efflux function of HDL with inflammation.

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Finally, these results reveal that NLRP3 or the non-canonical pathway for inflammasome activation could be potential targets for the development of new drugs for the prevention of cardiovascular disease.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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