High-density lipoprotein (HDL) cholesterol levels are inversely proportional to the risk of atherosclerosis. However, the evidence that raising HDL per se will reduce atherosclerosis and thereby cardiovascular events remains controversial, and mechanisms of putative HDL-mediated atheroprotection remain unclear. In addition to canonical role of HDL in mobilizing excess cholesterol from arterial wall macrophages (the first step in reverse cholesterol transport), there is compelling evidence to support the anti-inflammatory effects of HDL, both dependent and independent of its cholesterol transport capacities.1

Macrophages serve as important orchestrators of inflammation at the intersection of immunity, metabolism, and cardiovascular diseases. Yet anti-inflammatory effects of HDL in macrophages are less well understood. In a recent issue of Nature Immunology, De Nardo et al2 report that anti-inflammatory effects of HDL in macrophages are mediated through the induction of activating transcription factor 3 (ATF3), an ancient transcriptional modulator that provides negative feedback on toll-like receptor (TLR) innate immune signaling.

The authors first describe that pretreatment with both native HDL and reconstituted HDL (apoA-I and phospholipids) inhibited cytokine production by multiple distinct TLR ligands in murine bone marrow–derived macrophages (BMDM), human peripheral blood mononuclear cells, and TLR ligand–treated mice, an in vivo model of acute inflammation and liver injury. Although HDL is known to interact and neutralize the TLR4 ligand lipopolysaccharide directly, the authors showed that HDL did not interact with TLR1/2 ligands (Pam3CSK4) and the TLR9 ligand (CpG DNA) and did not directly modulate TLR1/2/9 activation, signaling transduction (eg, CpG activation of p38 mitogen-activated protein kinase and Jnk kinase) or nuclear factor-κB translocation (Figure). This suggested a mechanism involving de novo transcription of an inflammatory repressor in the absence of effects on early TLR signaling.

To identify candidate transcription factors, the authors performed microarray comparing mRNA expression in resting BMDMs with that in HDL-pretreated BMDMs subsequently stimulated with CpG. The authors identified Atf3 from the wealth of regulated genes, in part, because predicted binding sites for ATF3 were present in 28 of the 33 genes most significantly repressed by HDL. ATF3 is a key transcriptional regulator of innate immune response genes that is induced by TLR and other innate immune ligands. It acts as a negative feedback on TLR signaling by inactivating target genes through reduction of histone acetylation. HDL increased Atf3 mRNA and protein expression in BMDM, with further potentiation by stimulation of TLR ligands. ATF3 chromatin immunoprecipitation (ChIP)-seq in wild-type and Atf3-deficient BMDM revealed that ATF3 is specifically enriched at the promoters of cytokines found to be regulated by HDL both in vitro and in vivo. HDL injection in Apoe-deficient mice fed a high-fat diet induced Atf3 mRNA expression in liver Kupffer cells, but not in hepatocytes. And HDL-mediated protection against carotid artery injury and TLR ligand–induced acute inflammation was lost in Atf3-deficient mice. Ultimately, in macrophages, HDL is responsible for broad attenuation of inflammatory responses via the activation of ATF3.

This work defines a novel and potentially fundamental molecular pathway targeted by HDL in macrophages. This may be a key mechanism of anti-inflammatory and antiatherogenic signaling of HDL. However, many questions remain unanswered. The ChIP-seq experiments suggest that CpG treatment alone reduced ATF3 binding to macrophage target genes, but with HDL-pretreatment CpG actually enhanced ATF3 binding to targets. This cannot be explained on the basis of HDL-induction of Atf3 mRNA and protein but suggests additional HDL-remodeling of chromatin that limits ATF3 removal from targets during CpG treatment. The underlying mechanism(s) have to be addressed. The broader relevance of HDL modulation of ATF3 in other cell systems known to be targets of HDL anti-inflammatory actions is unknown and, as acknowledged by the authors, many of these effects may be independent of ATF3.

The importance of this work to atherosclerosis, the primary disease target for HDL-therapeutics, is an open question. HDL modulation of macrophage ATF3 in vivo was demonstrated in rodent Kupffer cells but not in macrophages in atherosclerotic lesions, and in vitro human studies were limited to peripheral blood mononuclear cells but did not examine macrophages. Importantly, Apoe–/– mice deficient in Atf3 have accelerated atherosclerosis,3 but it is unclear whether this is driven by upregulation of macrophage cholesterol metabolism genes, innate immune genes, or both. More fundamentally, the constituents of HDL responsible for induction and modulation...
of ATF3 are not yet known. In this study, reconstituted HDL containing only apoA-I and phospholipids, as well as native HDL,2 with its rich cargo of >100 proteins and many lipid species, were both equally active in inducing ATF3. Which macrophage receptors (eg, ATP-binding cassette transporters A1 and G1, scavenger receptor BI, or lysosphingolipid receptors) and signaling pathways mediate HDL modulation of ATF3 are unclear. This knowledge is key because the HDL effect is not simply a matter of increased ATF3 expression but also seems to involve additional chromatin remodeling. In this context, it is unlikely that direct activation of macrophage ATF3 expression (as occurs with CpG treatment) will mimic HDL-like effects and will produce the desired therapeutic effect in atherosclerosis. Thus, targeting ATF3 in a HDL-mimetic manner should be the goal.

To date, there have been no reports of ATF3 genetic associations with human atherosclerotic cardiovascular disease. This can be viewed in several ways. First, more studies are required and links may emerge with time. Second, HDL may modulate ATF3 in humans but this genetic pathway may lack atheroprotective potential. Third, ATF3 functions could be atheroprotective but genetic variation in this pathway may have diverse effects that confer both harm and benefit in humans. Additional genetic and pharmacological studies of ATF3 in atherosclerosis models and across diverse pathophysiological scenarios are required specifically to understand the cellular and physiological consequences of targeting this pathway in vivo. Modulation of this ancient and fundamental innate immune regulatory pathway raises the spectre of on-target host toxicity that may limit therapeutic possibilities.

In summary, De Nardo et al2 have advanced the field by identifying a specific molecular pathway for anti-inflammatory actions of HDL in macrophages opening up new opportunities for understanding HDL salutary actions and exploiting therapeutic potential. Perhaps the most important advance is the insight into new mechanisms of HDL function and the possibility of more refined metrics of HDL functions, based on a better understanding of its anti-inflammatory actions in macrophages. Undoubtedly, this will expand the foundation for the development of HDL-related therapeutics in atherosclerosis and beyond.

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References

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