Atherosclerosis is an inflammatory disease driven by hyperlipidemia. There is accumulating evidence that lipid metabolism and inflammation are closely linked. However, the cross talk between these processes in the development and progression of atherosclerosis are not fully defined. Macrophages mediate tissue innate immune response and lipid metabolism and, therefore, act as a key player at the crossroads of innate immunity and lipid homeostasis in atherosclerosis.

In lipid-loaded macrophages, cholesterol biosynthetic intermediates, such as desmosterol, and oysterolys derived from cholesterol, activate the transcription factor, liver X receptors. Liver X receptors form heterodimers with retinoid X receptors on the promoters of many genes involved in cholesterol metabolism, for example, ATP-binding cassette transporters A1 and G1 (ABCA1 and ABCG1), to upregulate their expression and enhance cholesterol efflux. When exceeding capacity, cholesterol begins to accumulate and macrophages become lipid-enriched. Cholesterol enrichment in the plasma membrane promotes the formation of toll-like receptor 4 (TLR4)–MD2 complexes, which enhance macrophage response to TLR4 ligands, such as lipopolysaccharide. Excessive free cholesterol can trigger crystal formation and inflammassome activation, which further exacerbates macrophage inflammation and cholesterol accumulation. Beyond promotion of cholesterol homeostasis, ligand-dependent conjugation of SUMO2/3 (small ubiquitin-like modifier) to liver X receptors targets them to receptor corepressor complexes and thus maintain suppression of target gene transcription. Conversely, through activation of interferon regulatory factor 3 (IRF3), TLR3 and TLR4 ligands inhibit the transcriptional activity of liver X receptor on its target genes, including ABCA1 and ABCG1, and disrupt lipid homeostasis. Thus, cross talk between inflammation and lipid metabolism in macrophages can synergistically exacerbate the pathological progress in atherosclerosis. Elucidation of the functional denominators linking macrophage activation and derailed cholesterol metabolism may facilitate the development of novel therapeutics in atherosclerosis (Figure 1).

In a recent issue of Circulation Research, Chen et al report that IRF 2–binding protein 2 (IRF2BP2) is a novel regulator of macrophage inflammation and lipid homeostasis that acts by maintaining anti-inflammatory transcription factor Krüppel-like factor 2 (KLF2) expression, therefore reducing susceptibility to atherosclerosis (Figure 2). IRF2BP1 and IRF2BP2 are transcriptional corepressors binding to the C-terminal repression domain of IRF2, a negative regulator of many interferon-responsive genes. IRF2BP2 also represses the nuclear factor of activated T cells (NFAT1)–dependent transactivation of NFAT-responsive genes, such as interleukin (IL)-2 and IL-4. Human genome-wide association studies (GWAs) identified genetic variants near IRF2BP2 associated with elevated plasma total cholesterol and low-density lipoprotein (LDL) cholesterol, although no association with coronary heart diseases (CHD) has been revealed in current GWASs. Besides, in a classical in vitro model of macrophage polarization using human monocyte-derived macrophage, IRF2BP2 mRNA was massively suppressed by M1 polarization (lipopolysaccharide and interferon-γ) and induced by M2 polarization (IL-4). These studies suggested the potential role of IRF2BP2 in macrophage polarization and lipid metabolism, but the mechanisms and impact on disease remained undetermined, prompting Chen et al to pursue their novel studies.

Chen et al present a combination of cell and mouse studies, mechanistic molecular experiments, and human genetics that advance our understanding of IRF2BP2 in regulating inflammation and lipid metabolism in macrophages and modulating atherosclerosis in rodent models and humans. First, the authors described that IRF2BP2 regulates macrophage inflammation and lipid homeostasis in vitro. Bone marrow–derived macrophages (BMDM) derived from myeloid-specific Irf2bp2 KO mice (Ly5M−/−/Ir2bp2−/−, abbreviated as KO) showed higher inflammatory M1 markers (IL1b, Tnf, Ccl2, and Nos2) under basal condition and during stimulation, but reduced IL-4–induced M2 markers (Arginase1, Retnlab, Mgl1, and Mrc1). In wild-type (Irf2bp2+/+) BMDM, modified LDL loading upregulated Abca1 and Aebcl expression. The effects were markedly impaired in Irf2bp2 KO BMDM. This was accompanied by increased [3H]-cholesterol–labeled acetylated LDL uptake and reduced [3H]-cholesterol efflux to lipoprotein acceptors, as well as increased apoptosis. As a result of increased
Luciferase reporter assay indeed suggested that the deletion mutation leads to reduced luciferase translation. Importantly, carriers for homozygous deletion polymorphism showed reduced IRF2BP2 expression in peripheral blood mononuclear cells.

The authors performed mRNA microarray to determine differentially expressed genes between wild-type and KO BMDM. One of the top differentially expressed genes suppressed in KO BMDM is Klf2, an anti-inflammatory transcription factor. KLF2 inhibits the transcriptional activity of both NF-kB and activator protein 1, in part by means of recruitment of transcriptional coactivator p300/CREB binding protein–associated factor. Klf2 protein levels were barely detectable in atherosclerosis lesion of Irf2bp2 KO Ldlr−/− mice. To establish the functional requirement of KLF2 in IRF2BP2-mediated effects, lentivirus-mediated Klf2 overexpression in Irf2bp2 KO BMDM attenuated inflammation and improved cholesterol handling. Critically, the authors showed that IRF2BP2 is required for myocyte enhancer factor 2–mediated KLF2 transcriptional activation, and that homozygous IRF2BP2 mutation carriers also showed lower KLF2 protein levels in peripheral blood mononuclear cells.

These mechanistic and translational studies define a novel molecular pathway controlled by IRF2BP2 in atherosclerosis. Yet, as with any new molecular targets with translational and therapeutic potential, many questions remain to be answered. Perhaps of greatest clinical importance is the need to clarify the human genetics at the IRF2BP2 locus. The initial GWAS discoveries were for total and LDL cholesterol, yet large CHD data sets (eg, CARDIoGRAM [Coronary ARtery DIsease Genome wide Replication and Meta-analysis]) have not identified a convincing CHD signal for these same variants. This might be a matter of sample size/power for CHD or more likely lack of coverage of the 3′-UTR deletion polymorphism on the GWAS SNP (single nucleotide polymorphism) array. The authors do provide evidence for an association of the 3′-UTR deletion with CHD, but this is a relatively small study without replication and, in this data set, there was no association with plasma lipids. Thus, it is important that large-scale studies replicate the CHD association and, if confirmed, define whether this is likely to be mediated through plasma lipids. In clinical context, it is noteworthy that all in vitro macrophage work was in mouse BMDM, and human studies were limited to peripheral blood mononuclear cells. Whether monocyte-derived macrophages of mutation carriers show reduced IRF2BP2 (eg, via expression quantitative trait loci or allele-specific expression) and enhanced inflammatory response or deficiency in cholesterol metabolism remain an open question. Because of functional and transcriptional differences between mouse and human macrophages, particularly in innate immune and lipid responses, functional genomic studies in human macrophages, either primary monocyte-derived macrophages or human-induced pluripotent stem cell–derived macrophages (which permit gene editing), are required to confirm the clinical relevance of the Irf2bp2 rodent macrophage phenotype. These studies are required to provide convincing evidence for the role and likely intermediate human mechanism in CHD, as well as provide a compelling rationale for investment in translational therapeutics.

The role of IRF2BP2 is not simply to increase KLF2 expression. BMDM isolated from LysM-Cre–mediated
myeloid-specific Klf2 knockout mice showed enhanced adhesion to endothelial cells, but were similar to wild-type macrophages in response to polarization and lipid accumulation. In this context, although Klf2 overexpression rescued the phenotype of Irf2bp2 KO in BMDM, it is unlikely that direct activation of macrophage Klf2 expression will reproduce all Irf2bp2 effects. Macrophage might not be the only effector cell of IRF2BP2 effects. In the current study, cell-specific Irf2bp2 KO mice were generated by LysM-Cre/loxP–mediated recombination, which will delete the target gene in macrophages, and granulocytes. The broader relevance of IRF2BP2 in other myeloid cells, for example, neutrophils, with inflammatory action in atherosclerosis should be considered. In addition, LysM-Cre transgene is expressed in Kupffer cells. As the author demonstrated, the LysM-Cre Irf2bp2 KO mice fed a high-fat diet showed elevated serum total cholesterol and lipoprotein profile compared with wild-type mice, possibly because of the ablated Irf2bp2 in liver Kupffer cells. Because no difference in lipid profiles was seen between Irf2bp2 KO and HET mice, the broader relevance of IRF2BP2 in Kupffer cells and in hepatic lipoprotein metabolism should not be overlooked.

Chen et al have advanced the field by identifying the impact and the mechanisms of the anti-inflammatory actions of IRF2BP2 in atherosclerosis. The most important advance is the insight into new mechanisms of IRF2BP2 function at the crossroads of innate immunity and lipid homeostasis. This may provide opportunities for the development of novel anti-inflammatory therapeutics, but much remains to be determined. Although ablation of Irf2bp2 worsens atherosclerosis, whether overexpression of Irf2bp2 is beneficial remains unclear. Because overexpression of Klf2 induces marked hepatic triglycerides accumulation in mice, it is uncertain what the adverse effects of IRF2BP2 activation could be in humans. Indeed, the actions of IRF2BP2 across multiple cells and tissues raise concerns for specificity of targeting in chronic disease, as well as for limiting adverse effects. If successful, however, restoring IRF2BP2 expression and signaling in human macrophages could become a promising strategy to limit inflammatory response and lipid dysregulation in cardiometabolic diseases, but extent of IRF2BP2 activation may be critical.

In summary, this intriguing target that functions at the junction of lipid metabolism and inflammation in atherosclerosis underscores the importance of cross talk between lipid metabolism and immunity in cardiovascular pathology. Undoubtedly, this knowledge will advance our understanding of macrophages in complex disease and provide greater opportunity to target macrophage phenotypes and functions in atherosclerosis and cardiometabolic disorders.

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**Disclosures**

None.

**References**


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