Chronic inflammation contributes critically to the pathogenesis of cardiometabolic disorders. Accumulation of inflammatory phagocytes and lipid-laden foam cells in aortic lesions are particular hallmarks of atherosclerosis. Over a century ago, scientists began to associate lipids with atherosclerosis, which was later supported by clinical evidence that linked elevated low-density lipoprotein (LDL) levels and coronary risk. However, the addition of native LDL to cultured macrophages did not induce foam cell formation in the research laboratories. This mystery remained unsolved until the 1980s when Goldstein and Brown first demonstrated in vitro that macrophages take up acetyl LDL and become foamy cells. Steinberg and Witztum, and others established that oxidized LDL (oxLDL) promotes foam cell formation and atherogenesis. Another breakthrough came in the early 1990s: discovery of a series of macrophage scavenger receptors that recognize modified LDL. Kodama and Freeman from the Krieger laboratory discovered scavenger receptor types AI and AII, and Endeman et al identified CD36, a scavenger receptor in the class B family. CD36 and type A scavenger receptors play principle roles in lipid accumulation in macrophages. Macrophages also express other scavenger receptors, including scavenger receptor B I and II, and lectin-like oxLDL receptor-1 (LOX-1).

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The Src-family kinases, the largest family of non-receptor tyrosine kinases, consist of 9 family members: Src, Fyn, Yes, Frk, Btk, Fgr, Hck, Lck, and Lyn. Src-family kinases comprise 3 main domains: Src homology 3 (SH3), SH2 and the kinase catalytic domain (SH1). Src kinase activity is regulated by conformational changes modulated by autophosphorylation, phosphorylation by other tyrosine kinases (eg, C-terminal Src kinase, Csk) and dephosphorylation by numerous protein tyrosine phosphatases. In contrast to the ubiquitous expression of Src, Fyn, Yes, and Frk, the other 5 kinases are expressed in hematopoietic cells, including monocytes/macrophages. Src-family kinases not only play prominent roles in cancer biology, but also regulate macrophage functions, including foam cell formation and proinflammatory cytokine expression mediated by CD36, effecotyosis, and lesion development and instability in a mouse model of atherosclerosis.

CD36, a multi-ligand receptor expressed on monocyte/macrophages and various other type cells, plays a wide variety of biological roles, including promoting foam cell formation and atherogenesis. CD36 contains 1 glycosylated extracellular domain, 2 transmembrane domains and 2 short cytoplasmic domains. The carboxy terminal intracellular domain can transduce signals through formation of molecular complexes with coreceptors (eg, Toll-like receptor 4 (TLR4) and TLR6) and Src-family kinases (eg, Lyn), in response to exogenous and endogenous ligands (eg, oxLDL) (Figure). Ligation of CD36 by either copper-oxLDL or specific NOxLDL (oxidized by the myeloperoxidase/nitrite system) induces the phosphorylation of Lyn and JNK1/2 (mitogen-activated kinases Jun-kinase) in murine peritoneal macrophages. Further, Vav family Rho guanine nucleotide exchange factors (Vav1, Vav2 and Vav3) contribute to CD36-dependent oxLDL uptake in vitro and lesion progression in experimental atherosclerosis. Most recently, iron transporter Na+/K+-ATPase was reported as a new modulator in the CD36-Lyn signaling complex. The interaction of CD36 with Lyn kinase also induces the activation of TLR4-TLR6 heterodimer and the downstream NF-kB signaling (Figure).

TLRs specifically recognize pathogen-associated molecular patterns and endogenous ligands, and play fundamental roles in innate immunity and infectious diseases as well as atherosclerosis. At least 5 TLRs (TLR1, TLR2, TLR4, TLR5 and TLR6) exist on the surface of monocyte/macrophages. Using deficient mice, earlier in vivo studies demonstrated that TLR2 and TLR4 promote atherogenesis in either apolipoprotein E- or LDL receptor-null mice fed a high-fat diet, whereas the deletion of either TLR1 or TLR6 produced no effects on atherogenesis in mice. Multiple studies demonstrated that TLR4 signaling critically contributes to foam cell formation and inflammatory cytokine expression in macrophages exposed to minimally-modified LDL (mmLDL) or extensively oxLDL.

The molecular mechanisms for lipoprotein uptake triggered by mmLDL have been extensively investigated. Because of insufficient modification to be phagocytized by macrophages through CD36 and other scavenger receptors, mmLDL and...
induces the interaction of TLR4 with Src, but not with Fyn or Lyn in the Src kinases family, as demonstrated by immunoprecipitation analysis. These different responses may result from the different degrees of oxidation and distinct mixtures of bioactive components in mmLDL and oxLDL particles. However, it remains obscure whether TLR4-Src signaling also activates downstream JNK1/2 and Vav1 in oxLDL-stimulated macrophages, which deserves further investigation (Figure).

Interestingly, Syk and Src-family kinases are both engaged by CD36 to trigger the internalization of CD36 and oxLDL. Because both oxLDL and mmLDL might be present in the subendothelial space of atherosclerotic lesions, simultaneous exposure to different types of modified LDL and activation of different signaling pathways (Syk and Src kinase) mediated by TLR4 on macrophages may contribute to macrophage heterogeneity, lipid accumulation and foam cell formation.

The evidence previously suggested that Syk kinase interacts with TLR4 through its SH2 domain. Because Src kinase also contains SH2 and SH3 domains, it might interact directly with cholesteryl ester hydroperoxides (active components produced by 12/15 lipoygenase in LDL) instead require TLR4 and spleen tyrosine kinase (Syk) to initiate the signaling to enhance oxLDL uptake by macrophages in vitro and by circulating monocytes in vivo. In resting macrophages, Syk constitutively binds to the intracellular TIR domain of TLR4, and mmLDL stimulation induces the phosphorylation of both TLR4 and Syk, which results in the activation of Vav1-Ras-Raf-MEK1/2-Raf signaling cascades and stimulates the inflammatory response and foam cell formation. OxLDL also activates Src kinase in a TLR4-dependent manner and induces CD36 expression, lipid uptake and foam cell formation, which may involve the activation of JNK1/2, Vav1 and PPAR-γ. Both inflammatory responses and foam cell formation significantly contribute to atherosclerosis. LDL, low-density lipoprotein; mmLDL, minimally oxidized LDL; oxLDL, extensively oxidized LDL; TLR, Toll-like receptor; NF-κB, nuclear factor κB; PPAR-γ, peroxisome proliferator-activated receptor γ.
the tyrosine residues of TLR4. TLR4 signaling is transduced through the interaction of intracellular TIR domain with at least 5 different adaptor molecules, including MyD88 and TRIF-related adaptor molecule (TRAM). TLR4-mediated effects of mmLDL on macrophages are mainly MyD88-independent. Consistently, silencing of either MyD88 or TRAM has no significant effect on the TLR4-Src interaction. Earlier studies have shown, however, that Src kinase also associates with other receptors, including CD14 and TLR2, and CD14 can form complexes with TLR2 or TLR4 in lipid rafts. It is plausible that the TLR4-Src interaction may associate with other adaptor proteins or coreceptors (eg, TLR2, CD14 or CD36) to form a multi-component complex.

Yang et al report another novel finding: silencing either TLR4 or Src suppresses the induction of CD36 by oxLDL, implicating involvement of CD36 in the late phase of oxLDL uptake and foam cell formation modulated by the TLR-Src signaling axis. Via a positive feedback mechanism, oxLDL uptake mediated by CD36 or TLR4 further increases the expression of CD36 and TLR4. Specific oxidized lipids generated from internalized oxLDL can serve as ligands to activate nuclear hormone receptor PPAR-γ and increase CD36 transcription, which promotes further uptake of oxLDL. In addition, reactive oxygen species released by the activated macrophages in atherosclerotic plaques fuel the oxidative modification of phospholipids on LDL particles and expand the local pool of oxLDL. Understanding the interplay between CD36 and TLR4 mediated by Lyn and Src kinases may shed more light on the complex biology of foam cell formation stimulated by oxLDL. PPAR-γ may be one of the transcription factors activated by TLR4/Src signaling, which remains undetermined (Figure).

Furthermore, immunostaining of atherosclerotic lesions from femoral arteries by Yang et al suggests enrichment of macrophages expressing both TLR4 and Src kinase, consistent with a previous finding in human coronary arteries. These results indicate the pathological relevance of TLR4-Src signaling in atherosclerotic disease. A previous report and the present study by Yang et al demonstrate that pan-Src family kinase inhibitor (PP2) significantly attenuates the lipid accumulation in macrophages induced by oxLDL. The latest study by Medina et al reported that deficiency of 2 myeloid-specific Src-family kinases (Hck and Fgr) enhances plaque instability, which raises a caution about potential therapeutic strategies designed for simultaneous targeting of several src family kinases in plaque inflammation. Recently, a specific Src kinase inhibitor (KX2-391) was successfully developed and assessed in clinical trials of patients with advanced stages of cancer. Whether the TLR4-Src signaling pathway plays an important role in foam cell formation in vivo warrants further investigation using either a specific Src kinase inhibitor or myeloid lineage-selective deficiency of the src gene in well-defined mouse models of atherosclerosis.

References