Commentary

MD-2 as a possible therapeutic target for atherosclerosis

Shuang Chen a,b, Timothy R. Crother a,b,*

a Department of Pediatrics, Division of Infectious Diseases and Immunology, Cedars-Sinai Medical Center, Los Angeles, CA 90048, USA
b Department of Biomedical Sciences, Infectious and Immunologic Disease Research Center, Cedars-Sinai Medical Center, Los Angeles, CA 90048, USA

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Atherosclerosis is the leading cause of cardiovascular disease (CVD). Immune cells and their mediators drive the chronic arterial inflammation that is a hallmark of atherosclerosis, with the addition of excessive lipid markers [1]. Despite inflammation being the major contributor to the development of atherosclerosis, the mainstay of treatments and prevention strategies focus predominantly on lowering blood pressure, cholesterol levels, and platelet counts. Recently, the importance of targeting inflammation was highlighted by the CANTOS trial, which demonstrated that targeting IL-1β proved therapeutically beneficial in reducing recurrence of cardiac events, independent of cholesterol levels [2]. Although these results helped strengthen the case for the inflammatory basis of human atherosclerosis, the exact mechanisms by which circulating IL-1β ablation benefited these patients are not well understood.

While Toll like Receptor 4 (TLR4) and Myeloid differentiation factor 88 (MyD88) signaling pathways are responsible in the production of IL-1β and IL-18, MyD88 signaling is equally involved in mediating cellular responses to IL-1β and IL-18. Endothelial cells and macrophages in human atherosclerotic plaque express TLR4 [3]. Studies have implicated MyD88-dependent arm of TLR signaling pathway to promote acceleration of atherosclerosis [4–6]. MD-2 is a 25-kD glycoprotein that binds to the extracellular domain of TLR4 and is therefore tethered on TLR4-expressing cells. It has been demonstrated that TLR4 does not induce a complete response in the absence of MD-2 [7]. Thus, while TLR4 has been clearly implicated in atherogenesis [6], the role of MD2 and its engagement with TLR4 in ox-LDL-induced inflammation and atherosclerosis has been overlooked.

In this issue of EBioMedicine, Liang and colleagues investigated the specific role of MD2 in atherosclerosis development [8]. They observed that MD2 protein amounts increased in aortas of Apoe−/− mice fed a high fat diet (HFD) compared with the normal/low-fat diet. Increased levels of soluble MD2 protein were also found in the blood of Apoe−/− mice fed a HFD and positively associated with levels of circulating tumor necrosis factor-α, an inflammatory cytokine. The levels of MD2 were consistently elevated in circulating mononuclear cells from patients with atherosclerotic disease. The authors demonstrated that MD2 protein mostly localises to CD68-positive macrophages. Macrophages, prototypical cells in the innate immune system, have been known to play a key role in lipid accumulation and inflammation during all stages of atherogenesis. Macrophages highly express TLR4 that can be activated by lipopolysaccharide, Hsps, and other microbial products to induce intracellular signaling through MyD88 and nuclear factor κB pathways, further promoting the inflammatory response and modulating subsequent adaptive responses. Liang et al reported that ox-LDL incubated for 24 h with mouse primary macrophages significantly increased MD2 protein production. In Apoe−/−/Md2−/− mice macrophages infiltrated less and inflammatory cytokine production was reduced compared with Apoe−/− mice fed HFD, despite no differences in Dil-ox-LDL uptake between wildtype and Md2−/− macrophages. Using a bone marrow transplantation model, they demonstrated that MD2 in bone marrow–derived hematopoietic cells is critical for atherosclerotic plaque formation and inflammatory signaling in lesions. Liang et al’s results shed new light on the role of MD2 in atherosclerosis and provide a mechanistic basis for ox-LDL-induced inflammatory responses. While this bone marrow chimera approach clearly shows the role of MD2 in hematopoietic cells, their specific role in macrophages in vivo still needs to be determined.

Importantly, the investigators showed that therapeutic inhibition of MD2 signaling by a small-molecule inhibitor, L6H9 may reduce atherosclerosis lesion in the Apoe−/− model. L6H9 binds directly to MD2 and prevents MD2-mediated TLR4 activation, and it was previously determined to have anti-inflammatory effects and cardiac protective activity [9]. In this study, L6H9 treatment prevented ox-LDL induced TNF-α and IL-6 production in macrophages, and reduced nuclear localization of NF-κB p65 subunit while increasing IkBα levels in primary macrophages. Finally, administration of L6H9 significantly improved atherosclerosis in HFD-fed Apoe−/− mice with a 40% reduction in plaques size. Thus, these data support the therapeutic potential of MD2 inhibitors in atherosclerosis-driven cardiovascular diseases (Fig. 1).

Because of the seemingly higher importance of TLR4, researchers paid more attention to TLR4 than MD2 in the past decades. However, blocking TLR4 can lead to severe side effects, and “inappropriate” immune responses, such as allergic Th2 responses or immunologic...
Therefore, targeting MD2 may be a viable option to curb atherosclerosis. So far, several MD2 inhibitors have been reported, binding directly to the MD2 pocket and blocking TLR4/MD2’s recognition of LPS, resulting in the prevention of proinflammatory signaling and septic shock. More studies may shed light on MD2 inhibitors to identify potential candidates in the treatment of atherosclerosis.

While in the CANTOS trial severe infections were an undesired side effect indicating the critical nature of IL-1β signaling in host defense, inhibition of MD2 may offer a more nuanced approach as this will only affect TLR4 signaling. Experimental atherosclerosis in animals provides an important research tool and proof-of-concept, but extrapolation to humans can be challenging and will require carefully controlled clinical trials. With the initial promise of the CANTOS trial beginning to open the floodgates of potential anti-inflammatory modalities in treating atherosclerosis, much work is still left to be done.

**Declaration of Competing Interest**

The authors declare no conflict of interests.

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