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Liver-Artery Interactions via the Plasminogen-CD36 Axis in Macrophage Foam Cell Formation New Evidence for the Role of Remote Organ Crosstalk in Atherosclerosis

Hiroshi Iwata, MD, PhD; Masanori Aikawa, MD, PhD

therosclerosis is an inflammatory vascular disorder. APopulation studies linked hypercholesterolemia with coronary risk several decades ago. In the 1990s, preclinical and clinical evidence on lowering low-density lipoprotein (LDL) established that cholesterol not only promotes atherogenesis but also triggers the onset of acute thrombotic complications. Hypercholesterolemia promotes endothelial cell activation, recruiting circulating monocytes into the arterial wall, where they differentiate into macrophages. These proinflammatory phagocytes then become lipid-laden foam cells, a hallmark of atherosclerosis.¹⁻³ Although the direct role of loaded lipids remains obscure, these activated macrophage foam cells (as gauged by the expression of high levels of proinflammatory factors such as cytokines and chemokines) activate vascular smooth muscle cells (SMCs), endothelial cells, and neighboring macrophages and induce the infiltration of additional immune cells into the lesion, which amplifies the atherogenic milieu in arteries. Matrix-degrading enzymes released by foam cells, such as matrix metalloproteinases (MMPs), and thrombogenic factors (eg, tissue factor) may reduce the mechanical stability of atherosclerotic plaques and trigger acute thrombosis.4 A global proteomic analysis by Becker et al⁵ in control and cholesterol-loaded macrophages identified 46 proteins that respond to sterol loading of macrophages in vivo. These proteins participate in lipid binding, cytoskeletal regulation, and vesicle-mediated transport and are enriched in factors associated with causative roles in atherogenesis.5

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The underlying mechanisms for foam cell formation remained obscure until the 1980s.⁶ Joseph L. Goldstein and Michael S. Brown reported that although the incubation of macrophages in culture with native LDL did not substantially cause foam cell formation, the cells rapidly took up chemically modified LDL (acetyl LDL) and became foamy cells, which led to the discovery of the acetyl LDL receptor by the same group and the subsequent cloning of the scavenger receptor A-I by Monty Krieger and Tatsuhiko Kodama.

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Circulation is available at http://circ.ahajournals.org DOI: 10.1161/CIRCULATIONAHA.113.001461 In parallel, investigators including Daniel Steinberg and Guy M. Chisolm established that oxidative modification of LDL promotes foam cell formation and atherogenesis (the oxLDL hypothesis). Elevated production of reactive oxygen species by cells in atherosclerotic plaques, including macrophages, in individuals with hyperlipidemia and other risk factors (eg, smoking, diabetes mellitus) promotes oxidative modification of phospholipids on LDL particles, and oxidized LDL (oxLDL) indeed accumulates in arteries.⁷ But because the evidence did not support the acetyl LDL receptor mediating the binding of the majority of oxLDL to macrophages, the mystery of foam cell formation remained, and the hunt for other scavenger receptors continued. Subsequent in vitro and in vivo studies identified CD36 as a key receptor for oxLDL, contributing to foam cell formation and atherogenesis.⁸⁻¹⁰

Since its discovery as a receptor for thrombospondin, many studies have indicated that CD36 is a multiligand receptor expressed by various cell types, including platelets, myocytes, adipocytes, endothelial cells, epithelial cells, and phagocytes, and thus, it plays a wide variety of biological roles in multiple organs. In adipocytes, CD36 binds and transports long-chain fatty acids into cells, resulting in lipid storage. It participates importantly in fatty acid oxidation in cardiac and skeletal muscles. CD36 also contributes to the innate immune system as a pattern recognition receptor. Besides oxLDL, its ligands include advanced glycated endproducts, amyloid-β, microparticles, and surface components of apoptotic cells for efferocytosis, the clearance of apoptotic cells by phagocytes.¹¹ Efficient efferocytosis induces anti-inflammatory mediators, such as interleukin-10 and transforming growth factor- β . Impaired efferocytosis in advanced plaques leads to secondary necrosis, in which macrophages release toxic debris, oxidized lipids, and proinflammatory mediators. Clinical evidence links necrotic core size with the risk of plaque rupture and acute coronary events.

Interestingly, ligands increase CD36 expression. For instance, abundant oxLDL promotes macrophage production of CD36, which accelerates the uptake of oxidized lipids by the cells. Macrophages normally remove the excess cholesterol accumulated in the cells via the ATP-binding cassette transporters ABCA1 and ABCG1 ("cholesterol efflux") as an initial step of reverse cholesterol transport.¹² But overwhelming cholesterol influx, mediated by the amplifying interaction between oxLDL and CD36, outweighs the efflux process and loads large amounts of lipids into macrophages, forming foam cells. CD36 also serves as a signaling receptor and promotes diverse processes; it mediates oxLDL-induced activation of mitogen-activated protein kinases (JNK1 and JNK2) and nuclear factor–κB.^{13,14} CD36 signaling also modulates macrophage migration and thus promotes retention

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From the Center for Interdisciplinary Cardiovascular Sciences, Cardiovascular Division, Department of Medicine, Brigham Women's Hospital, Harvard Medical School, Boston, MA.

Correspondence to Masanori Aikawa, MD, PhD, Brigham Women's Hospital, Harvard Medical School, 3 Blackfan St, 17th Floor, Boston, MA 02115. E-mail maikawa@rics.bwh.harvard.edu

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of these proinflammatory cells in the lesion. CD36 binding to tissue factor containing microparticles and its role in platelet activation may increase thrombogenicity. In this issue of *Circulation*, Das et al¹⁵ demonstrate that plasminogen induces macrophage expression of CD36 via leukotriene B4 production and promotes lipid uptake, thus adding a new item to the list of CD36-mediated biology.

Plasminogen is a glycoprotein predominantly released by the liver into the blood circulation. Plasmin, a serine protease converted from plasminogen, can degrade many proteins in blood plasma. The best-known function of the plasminogen activation system is degradation of fibrin blood clots (fibrinolysis). Plasmin also participates in extracellular matrix degradation via activation of pro-MMP to their active form.¹⁶ Plasmin activity is tightly regulated by plasminogen activators (tissue plasminogen activator and urokinase plasminogen activator), as well as by plasminogen activator inhibitor-1. Extracellular matrix degradation by plasmin-MMP may contribute to plaque instability by thinning the fibrous cap. Activation of plasminogen in the presence of fibrin is also regulated by LDL and lipoprotein(a). Multiple plasmin receptors exist in many different cell types. Plasmin also promotes cell signaling. In the present study by Das et al,15 plasmin triggered macrophage production of leukotriene B4 (LTB4), which in turn activated gene transcription of the scavenger receptor CD36.

In addition to inducing fibrinolysis, the plasminogen activation system regulates vascular remodeling. Plasminogen deficiency enhances atherosclerosis formation in apolipoprotein E-deficient mice, which suggests a net antiatherogenic role.17 On the other hand, plasminogen deficiency diminishes the development of intimal hyperplasia after vascular injury in mice, probably because plasmin promotes SMC migration by activating MMP.¹⁸ Plasmin participates in the inflammatory process, such as in recruitment of monocytes/macrophages.¹⁹ Plasmin formation mediated by urokinase plasminogen activator receptor, but not by tissue plasminogen activator, appears to drive SMC migration.¹⁹ Macrophage-selective overexpression of urokinase plasminogen activator worsens atherosclerosis in apolipoprotein E-deficient mice. Further in vivo studies have provided inconsistent information on the role of plasminogen activator inhibitor-1 in atherogenesis in mice, and results from clinical studies on plasminogen activator inhibitor-1 inhibitors have been inconsistent. Collectively, the role of the plasminogen activation system in the size of vascular lesions remains incompletely elucidated. At least in the context of acute thrombotic complication, however, features other than plaque size (eg, macrophage foam cell numbers, necrotic core size, fibrous cap thinning) may play major roles. Das et al¹⁵ demonstrate that plasminogen promotes macrophage foam cell formation via induction of CD36. OxLDL uptake decreased in macrophages derived from plasminogen mutant mice compared with wild-type mice. Plasminogen deletion suppressed both binding and internalization of oxLDL. Foam cell-rich plaques often have large necrotic cores and thin fibrous caps and are prone to rupture. The study by Das et al¹⁵ therefore offers a new potential role of plasminogen in plaque stability.

Recent evidence suggests that the interplay between cell types in vascular lesions is more dynamic than previously imagined (Figure). In addition, interchangeability between cell types (eg, monocytes/macrophages versus SMCs) may exist.²⁰ Although most lesional macrophage foam cells originate from newly recruited monocytes, intimal SMCs can accumulate excess cholesterol esters and become SMC-derived foam cells. Interestingly, emerging evidence suggests that lipid loading in SMCs suppresses SMC markers and induces macrophage markers and phagocytic activity, which indicates the transdifferentiation of SMCs into macrophage foam cells.^{21,22} In contrast, a subpopulation of circulating monocytes may become SMC-like cells in vascular lesions, although the relative contribution depends on disease context (eg, chronic coronary atherosclerosis, in-stent restenosis, transplant arteriopathy).^{23–25} Iwata et al²³ demonstrated that some intimal cells express both macrophage markers (CD11b and F4/80) and smooth muscle- α actin. A subset of monocytes that express high levels of Ly6C (a marker of proinflammatory monocytes) appear to become SMC-like cells or smooth muscle progenitor cells in the intima of atherosclerotic plaques but may not fully differentiate into SMCs that express the smooth muscle myosin heavy-chain isoform SM1. Interestingly, this population expressed higher levels of MMPs than did SMCs of nonmonocyte origin, which suggests their specific role in plaque instability.23 These findings suggest dynamic and complex interactions between bone marrow-derived monocytes/ macrophages and SMCs. Spleen releases monocytes that contribute to inflammation in the peripheral organs in response to a cue from the site of an acute event that travels through the blood circulation.26 These findings suggest active crosstalk between arteries and remote organs such as bone marrow and spleen (Figure). Proatherogenic or antiatherogenic factors released from adipose tissue (eg, plasminogen activator inhibitor-1, adiponectin) also control vascular inflammation from remote organs. Chronic kidney disease causes high serum levels of phosphate, which promotes atherosclerosis and cardiovascular calcification and increases cardiovascular risk.

Das et al¹⁵ propose that plasminogen, a liver product, increases CD36 expression in macrophages and promotes lipid uptake and foam cell formation. Their findings suggest another interesting scenario for remote organ crosstalk in vascular disease, between the liver and arteries. Clinical evidence links blood levels of plasminogen with risk of coronary artery disease. Other liver-derived factors that are associated with cardiovascular risk include apolipoprotein B, apolipoprotein CIII, C-reactive protein, and fibrinogen. Proinflammatory cytokines (eg, interleukin-1β, interleukin-6) released from inflamed atherosclerotic plaques may travel to the liver and induce hepatocytes to produce C-reactive protein. Conversely, some evidence indicates that C-reactive protein not only reflects vascular inflammation as a marker but also exerts proinflammatory effects (eg. activation of monocytes/ macrophages and endothelial cells) or promotes thrombosis in the vasculature, which suggests more dynamic cross talk between the liver and the arteries via C-reactive protein as an atherogenic molecule.^{27,28} Another line of evidence, however, shows that C-reactive protein has antiatherogenic properties.²⁹

The potential interaction between plasminogen and CD36 opens a new window to the role of the liver in vascular diseases, and even the potential for 3- or 4-way cross talk between the liver, artery, bone marrow, and spleen.



Figure. Dynamic multiple organ crosstalk in the pathophysiology of atherosclerosis. Close and active interactions and possibly interchangeability between bone marrow–derived monocytes/macrophages and smooth muscle cells (SMC) may play an important role in development of atherosclerotic vascular lesions. The spleen may contribute to atherogenesis through release of inflammatory monocytes in response to a cue from the site of atherosclerosis that travels through the blood circulation. Adipose tissue remotely interacts with atherogenic arteries via various factors, such as adiponectin and plasminogen activator inhibitor-1. Moreover, chronic kidney disease promotes atherosclerosis and cardiovascular calcification with increasing cardiovascular risk. The crosstalk between atherogenic arteries and the liver has been proposed. Proinflammatory cytokines (eg, interleukin-1β and interleukin-6) released from inflamed atherosclerotic plaques may induce C-reactive protein production by hepatocytes in the liver. C-reactive protein produced by the liver conversely may promote vascular inflammation. In this context of liver-artery crosstalk, findings of the study by Das et al¹⁵ suggest a new paradigm of the role of the interaction of plasminogen and CD36 in macrophage foam cell formation. EC indicates endothelial cell.

How can we translate this exciting discovery on the plasminogen-CD36 axis into the clinic? Although strong clinical evidence suggests that LDL lowering by statins can prevent cardiovascular events, many patients still experience such events. Therefore, tremendous efforts in academia and industry have focused on the development of new antiatherosclerosis therapies beyond statins.³⁰ Recent insight into the role of proatherogenic cues, or cells released from remote organs, provides a new paradigm of cardiovascular therapeutics. Novel evidence for plasminogen-CD36 interaction may thus lead to a better understanding of atherogenesis and to new pharmaceutical interventions.

Despite its potential impact, this strategy faces several challenges. Plasminogen and CD36 are both multifunctional proteins. Various cell types express multiple plasmin receptors and CD36. The net effects of systemic suppression of plasminogen or CD36 or of plasmin binding to CD36 are difficult to predict. Complete systemic deletion of the gene in mice, which may induce compensatory changes in other genes, does not always reflect what will result from pharmacological modulations of the target molecule. The same global approach may not inform on the molecule's function in each cell type or organ when its expression is ubiquitous. Although timeconsuming and costly, cell-type-selective genetic manipulation remains a standard approach for addressing this issue. The emerging nanotechnology of the cell-type-targeted in vivo delivery system may enable the development and clinical use of safe and effective therapeutics (eg, small interfering RNA delivery in lipid nanoparticles). Targeting a specific function of pleiotropic molecules such as plasminogen and CD36 rather than their global suppression might enhance the efficacy of new therapies without promoting multiple major off-target effects. In addition, the net beneficial effects of suppressing the plasminogen-CD36 axis may depend on disease context (eg, the relative role of thrombosis, the dominancy of macrophages). Dealing with complex biology and crosstalk between several organs may require new approaches such as proteomics-based global network analysis.31

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Disclosures

None.

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