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EDITORIAL COMMENT

# Vascular Inflammation and Hyperlipidemia

## The Neutrophil Within*

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In this issue of *JACC: Basic to Translational Science*, Osaka et al. (1) reported that neutrophils contributed to vascular inflammation and identified a mechanism that involved induction of the neutrophil chemoattractant CXCL1 by lipids and subsequent neutrophil adhesion to the vessel wall. The investigators further demonstrated that citrullination, the conversion of arginine to citrulline, of neutrophil histones was central to hyperlipidemia-induced neutrophil adhesion to the vascular wall. They used atherosclerosis-prone, low-density lipoprotein receptor null ($LDLR^{-/-}$) mice, fed with a high-fat diet, and a combination of pharmacologic approaches that included lipid-lowering drugs and inhibitors of citrullination, as well as in vivo and in vitro neutrophil adhesion experiments, to support this conclusion. Because histone citrullination is involved in neutrophil extracellular trap (NET) formation, the investigators suggested NET formation as a plausible mechanism that also contributed to the hyperlipidemia-induced vascular inflammation preceding atherosclerosis.

Hyperlipidemia results in cholesterol and triglyceride deposits in the blood vessel wall, which often leads to atherogenesis and atherosclerosis, an arterial disease process characterized by the subendothelial accumulation of lipoproteins, immune and vascular wall cells, as well as the extracellular matrix (2). Atherogenesis can lead to blood flow restriction, atherothrombosis, and an increased risk for heart attack and stroke. Hyperlipidemia and vascular inflammation are not only independently associated with atherosclerosis but are also interconnected processes. For instance, lipoproteins function as damage-associated molecular patterns that trigger an early innate immune response, which, if unresolved, transitions into chronic nonresolving inflammation that often leads to arterial damage and thrombosis-induced organ infarction. Until recently, the innate immune response in atherosclerosis was believed to be predominantly mediated by monocytes and macrophages through increased hematopoiesis, enhanced recruitment into the vessel wall, and activation partly mediated by interactions of the macrophage scavenger receptors and toll-like receptors with oxidized LDL and apolipoprotein CIII, respectively.

Clinical data also support that inflammation, determined with the biomarker C-reactive protein (CRP), largely parallels LDL cholesterol in patients, and that statins and other lipid-lowering drugs reduce both CRP and LDL cholesterol (3), supporting hyperlipidemia and inflammation as 2 intimately related, and possibly, interconnected processes. The contribution of neutrophils, the earliest innate cell responders in the inflammatory response, to vascular inflammation and atherogenesis, has been less mechanistically explored. Osaka et al. (4) built on their previous work that demonstrated that neutrophils activated through the complement system adhered to the vascular wall in wild-type mice fed with a high-fat diet. They used $LDLR^{-/-}$ mice in their current work, which unlike wild-type mice, are prone to develop atherosclerosis in hyperlipidemic conditions, and suggested that these mechanistic findings had potential implications in atherosclerosis. Because the studies were terminated after only 4 weeks of a

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high-fat diet, whether hyperlipidemia induction of neutrophil adhesion to the vascular wall had consequences in atherosclerosis plaque formation remains to be investigated.

Neutrophils exert physiological functions through multiple mechanisms that include phagocytosis, degranulation, release of reactive oxygen species, and NET formation, which were described approximately 2 decades ago as a defense mechanism in response to infection. NETs are neutrophil-released fragments of extracellular DNA that contain histones and granular proteins with antimicrobial and pro-inflammatory properties. Since their discovery, NETs have been found in a large number of pathological inflammatory conditions, ranging from diabetes to cancer, autoimmunity, and recently, in COVID-19 (5,6). In these settings of persistent "sterile" inflammatory conditions, NETs are believed to be drivers of pathological inflammation, in contrast to their beneficial role of "trapping" pathogens to rapidly clear infection.

Osaka et al. (1) uncovered a potential novel role for NETs in the pathophysiology of vascular inflammation induced by the high-fat diet in the atheroprone LDLR$^{-/-}$ mouse preclinical model. The investigators demonstrated that CXCL1, which was increased in plasma of high-fat diet fed LDLR$^{-/-}$ mice, activated the enzyme peptidyl arginine deiminase 4 (PAD4), which mediates the conversion of arginine to citrulline, and induces histone citrullination. Histone hypercitrullination results in chromatin decondensation and is involved in NET formation (5). Although the investigators clearly demonstrated activation of enzymes and pathways that are involved in NET-release, as well as increased neutrophil adhesion to endothelial cells in vitro and in vivo, the presence of NETs in this context was not evaluated. The questions that remain unanswered are whether NETs are involved in neutrophil adhesion to the vascular endothelium, and how NETs themselves may participate directly or indirectly in adhesion. Two intriguing possibilities are that the granule content of NETs activate endothelial adhesion molecules that serve as receptors for neutrophil adhesion ligands or that NETs directly adhere to the endothelium. Whether NET-releasing neutrophils are the same ones that adhere to the endothelium was also not reported. Some reports indicated that histone citrullination by PAD4 was not sufficient to induce chromatin decondensation, opening the possibility that neutrophil adhesion in this setting was induced in a NET formation independent manner. Nevertheless, this work provides insights into the role that neutrophils play in vascular inflammation and suggests novel potential mechanisms that connect hyperlipidemia with early systemic inflammation and focal adhesion of neutrophils to the vessel wall that may precede atherosclerosis.

The investigators used intravital microscopy in the femoral artery and convincingly demonstrated increased adhesion of neutrophils using the LysM-Cre reporter mice in the LDLR$^{-/-}$ background. Because of the sparsity of circulating neutrophils in mouse blood, the use of chlodronate liposomes helps eliminate the monocyte and/macrophage Green Fluorescent Protein (GFP) adhesion background to visualize neutrophil adhesion more clearly. However, there is the possibility that chlodronate did not deplete the totality of the macrophages; the efficiency of the clodronate treatment used was not reported. The investigators also convincingly demonstrated increased plasma levels of CXCL1 and triglycerides, as well as induction of histone 3 citrullination in bone marrow neutrophils isolated from LDLR$^{-/-}$ mice fed a high-fat diet or in control neutrophils treated with such plasma. Moreover, antagonism of CXCR2, the receptor of CXCL1, or pharmacological inhibition of peroxisome proliferator-activated receptor α to achieve lipid lowering with permafibrate both prevented neutrophil adhesion to the femoral artery and citrullination. CXCL1 alone and plasma from LDLR$^{-/-}$ mice fed a high-fat diet both induced citrullination of neutrophils ex vivo, which supported that the neutrophil chemoattractant CXCL1 was the predominant signal responsible in this response. However, it is also possible that triglycerides and/or LDL cholesterol contributed to this phenomenon in addition to CXCL1, because both permafibrate and the PAD4 inhibitor Thr-Asp-F-amidine (TDFA) showed similar effects in the neutrophil function in this setting. These studies were all performed in bone marrow–derived neutrophils, and adhesion was monitored in the femoral artery at a time (4 weeks of a high-fat diet) in which plaque formation did not develop in LDLR$^{-/-}$ mice. Whether these mechanisms reflected recruitment to the atherosclerotic plaque or could precede plaque formation later on was something not investigated in this work and will require further investigation. Once these questions are answered, we could conceive the consequences of this mechanism for atherogenesis.

Neutrophils have previously been reported to be involved in experimental mouse models of lipid-driven chronic inflammation, and NETs have been detected in human atherosclerotic plaques, particularly in superficial erosions, rather than lipid-rich plaques (4,5). Perhaps the most novel aspect of the study by Osaka et al. (1) is the identification of CXCL1 as the specific driver of neutrophil activation and

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endothelial adhesion in response to a high-fat diet, uncovering an early response mechanism that may lead to atherosclerosis. Identification of the CXCL1-induced histone citrullination pathway associated with neutrophil endothelial adhesion may open new avenues for therapeutic approaches. CXCR2 antagonists or monoclonal antibodies to CXCL1 have shown efficacy in reducing inflammation in rheumatoid arthritis and perhaps could be used for early intervention to prevent or slow down vascular inflammation and subsequent plaque progression and atherosclerosis. In contrast, permafibrate, which is efficient at lowering lipids, may also have anti-inflammatory effects by reducing lipid induction of CXCL1 and consequent citrullination of neutrophils and vascular inflammation. Permafibrate has been shown to decrease hepatic inflammation in patients, and it could be speculated that it is through mechanisms similar to this one (7). Recently, NETs have been reported in the plasma and lungs of patients with severe COVID-19 (6). Because obesity and metabolic syndrome are associated with poor prognosis in patients with severe COVID-19, is it possible that the mechanisms reported may be responsible for severe COVID-19? Further research is needed to selectively inhibit neutrophil pathological functions without compromising immune protection.

Osaka et al. (1) remind us that neutrophils are important early players in high-fat, diet–induced vascular inflammation, provide insights into new pathways that can be targeted with available drugs, and their work contributes to support the hypothesis that the beneficial effects of lipid-lowering inhibitors may be also through dampening inflammation by altering neutrophil functions.

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