Atheroprone flow promotes inflammation in endothelial cells, and this process is critical for pathogenesis of many chronic inflammatory conditions such as coronary and carotid artery atherosclerosis, as well as abdominal aortic aneurysm. Signal mediators activated by atheroprone (disturbed) flow that have been described include nuclear factor κB and protein kinase ζ, which is very different from atheroprotective (steady laminar) flow.¹ In this issue of Circulation, an article from Xiao et al² shows the critical role of sterol regulatory element binding protein 2 (SREBP2) on atheroprone flow–mediated Nod-like receptor protein 3 (NLRP3) inflammasome activation. In particular, they showed that atheroprone flow induced both mature form of SREBP2 (SREBP2-N) and SREBP2 mRNA induction, which transcriptionally increase NADPH oxidase 2 (Nox2) and NLRP3 expression, thereby leading to interleukin 1β (IL-1β) expression and endothelial inflammation (Figure 1). In this editorial, we briefly review the NLRP3 inflammasome and SREBP activation system, which play a key role in modulating atheroprone flow–mediated endothelial cell inflammation. We also discuss the following important considerations for the future: the role of local NLRP3 and IL-1β expression, mechanisms for two different types of flow (atheroprone flow versus atheroprotective flow) on SREBP2 activation, and other NLRP3 activators including thioredoxin-interacting protein (TXNIP).

The inflammasome is a protein complex that serves as a platform for the maturation of caspase-1 subsequent activation, leading to proteolytic maturation and secretion of IL-1β and IL-18 (Figure 8 in Xiao et al²). Three essential components of inflammasomes are a sensor protein, the adapter protein ASC, and the inflammatory protease caspase-1. As a sensor protein, Nod-like receptor (NLR) family (NLRP1, NLRP3, and NLRP4) and AIM2 function as pathogen sensors and form an inflammasome complex. NLRP3 is unique in that it responds to numerous diverse physical and chemical triggers, unlike other NLRP members. NLRP3 also integrates concomitant stresses of cell damage present during inflammation, such as extracellular adenosine triphosphate (ATP), reactive oxygen species (ROS), uric acid crystals, cholesterol crystals, and IAPP (islet amyloid polypeptide).³ ⁴ In the current study, Xiao et al have shown that atheroprone flow (oscillatory flow) increased NLRP3 expression and subsequent cleaved caspase-1 and IL-1β expression compared with pulsatile shear flow in vitro. The authors also found that the levels of the cleaved caspase-1, IL-1β, and NLRP3 were higher in the aortic arch (predominantly exposed to atheroprone flow) than thoracic aorta in vivo, supporting the crucial role of atheroprone flow–induced NLRP3 inflammasome activation on the development of atherosclerosis. The induction of NLRP3 and Nox2 by SREBP2 transactivation is proposed as a novel mechanism of atheroprone flow effects.

However, the evidence for the role of NLRP3 activation during the process of atherosclerosis is controversial⁵–⁷ (Figure 1). Cholesterol crystal in the plaque itself can activate NLRP3 inflammasome and release IL-1β from mouse and human macrophages.⁶ Cholesterol crystals are recognized as a hallmark of atherosclerotic lesions. Although crystals are prevalent in advanced atherosclerotic lesions, Duewell et al⁶ have reported that cholesterol crystals were not only detected in necrotic cores but also in subendothelial areas that are rich in immune cells, even in an early stage of atherosclerosis. Depletion of NLRP3 or ACS in macrophages inhibits cholesterol crystal–mediated caspase-1 cleavage and IL-1β release and subsequent atherosclerosis formation in the background of low-density lipoprotein receptor knockout mice (LDLR−/−). These data support the crucial role of NLRP3 inflammasome complex in cholesterol crystals–mediated inflammation and atherosclerosis.⁶ However, these results have been challenged by the recent publication from Mena et al⁷ which showed no differences of atherosclerosis between double knock mice of Nlrp3−/− crossed with ApoE−/− mice and control ApoE−/− mice.⁷ Two notable differences of those studies are (1) LDLR−/− versus ApoE−/− mice, and (2) knock out from bone marrow–derived cells versus whole body. In this current issue, Xiao et al nicely showed that endothelial-specific SREBP2 deficiency inhibited NLRP3 induction and atherosclerosis in the ApoE−/− background.⁵ But because the function of NLRP3 inflammasome in vessel wall, including endothelial cells, especially in ApoE−/− background, remains unclear, it will be crucial to determine the role of endothelial NLRP3 inflammasome in atherosclerosis.

The importance of NLRP3 inflammasome in the process of atherosclerosis has been supported by several studies that

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Atheroprone Flow Activation of the Sterol Regulatory Element Binding Protein 2 and Nod-Like Receptor Protein 3 Inflammasome Mediates Focal Atherosclerosis

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focus on IL-1β. In IL-1β−deficient mice crossed with atherogenic apolipoprotein E knockout mice (ApoE−/−), a significant decrease in atherosclerosis with stabilization of the atherosclerosis plaque was exhibited. Importantly, treatment of ApoE−/− mice with IL-1 receptor antagonists also significantly inhibits atherosclerosis. To evaluate the clinical efficacy of this concept, the CANTOS (Canakinumab Anti-inflammatory Thrombosis Outcomes Study) has been ongoing. The purpose of this study is to evaluate the effect of IL-1β inhibition by canakinumab (a human monoclonal antibody that selectively neutralizes IL-1β) as compared with placebo on recurrent myocardial infarction, stroke, and cardiovascular death among stable patients with coronary artery disease who show persistent elevation of high sensitivity C-reactive protein despite contemporary prevention strategies.

SREBPs, including SREBP-1a, -1c, and 2, regulate the transcription of a number of genes involved in the cellular sterol and lipid homeostasis. In particular, SREBP2 is ubiquitously expressed, and relatively selective activator of cholesterol synthesis, as opposed to fatty acid synthesis, and regulates HMG-CoA reductase and low-density lipoprotein receptor genes. Because low levels of cholesterol stimulate SREBPs, the crucial role of SREBPs on liver steatosis and hyperlipidemia has been reported. However, the other roles of SREBPs in innate immunity and autophagy, which are not directly related with lipid homeostasis, have also been reported. The functions of SREBP in endothelial cells unrelated to lipid homeostasis remain unclear. To our knowledge, this is the first report to show SREBP2 regulates endothelial inflammation via increasing NLRP3 and Nox2 expression, which is not directly related to the regulation of lipid homeostasis.

Importance of shear stress in vascular biology and pathophysiology is highlighted by the fact that steady laminar flow is protective against atherosclerosis, whereas disturbed flow is a strong risk factor of the disease. Previously, the authors have reported that atheroprone flow can cause sustained SREBP1 activation with nuclear localization. In contrast, atheroprotective flow also temporarily increased nuclear transport, but could not be sustained. SREBPs are bHLH-LZ family transcription factors, and inactive precursors (pre-SREBPs) of newly synthesized SREBPs are cleaved subsequently by proteases, then release the SREBP's NH2-terminal portion of SREBP-N (SREBP-N; Figure 2). This mature form of SREBP-N enters the nucleus and binds the sterol regulatory element 1 (SRE-1), and increases target genes expression (Figure 2). Therefore, there are 2 possible steps by which flow can regulate SREBP activation. The first is the process of pre-SREBPs cleavage in

![Figure 1](http://circ.ahajournals.org/)

**Figure 1.** Scheme for sterol regulatory element binding protein 2 (SREBP2)-mediated Nod-like receptor protein 3 (NLRP3) inflammasome activation. IL indicates interleukin; Nox, NADPH oxidase; ROS, reactive oxygen species; and TXNIP, thioredoxin-interacting protein.

![Figure 2](http://circ.ahajournals.org/)

**Figure 2.** Scheme for sterol regulatory element binding protein (SREBP) activation regulated by 2 different types of flow (atheroprone flow vs atheroprotective flow). Akt indicates v-akt thymoma viral oncogene homolog; AMPK, AMP-activated protein kinase; bHLH, basic helix-loop-helix; GSK, glycogen synthase kinase; mTORC, mammalian target of rapamycin complex; NLRP, Nod-like receptor protein; Nox, NADPH oxidase; SIRT, Sirtulin; and SRE, sterol regulatory element.
the extranucleus, and the second is nuclear SREBP-N transcriptional activity regulation. In the current issue, Xiao et al showed the acceleration of the process of pre-SREBPs cleavage, which is the first step of SREBP activation, by showing the increased level of SREBP-N induced by atheroprone flow. In the Discussion, the possible involvement of Akt induced by atheroprone flow was suggested by the authors, but because Akt directly regulates nuclear SREBP-N, which is the second step of SREBPs activation as we will explain below (Figure 2), further evaluation will be necessary.

Recently, the role of posttranslational modification on the regulation of SREBP activation has been discussed. Insulin increases SREBP-N transcriptional activity via activating phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin complex 1 (mTORC1) kinase pathway. Furthermore, Akt activation inhibits GSK3 kinase activation, which leads to ubiquitination and proteosomal degradation of the active transcription factor of SREBP-N by direct phosphorylation. In contrast to Akt, the inhibitory effect of AMP-activated protein kinase (AMPK) and Sirtulin1 (SIRT1) on SREBP activation has been reported. SREBP can be directly phosphorylated by AMPK, leading to suppress protein processing and nuclear translocation, and repress target gene expression, and SIRT1 directly deacetylates SREBP-1c and increases its degradation. Of note, Dr Shyy’s group has previously reported the specific activation of AMPK and induction of SIRT1 by atheroprone flow (Figure 2). However, it is not clear whether atheroprone flow–mediated Akt activation can explain sustained activation and nuclear localization of SREBP, and atheroprotective flow–induced AMPK and SIRT1 induction inhibit these events. These issues, including the specificity of each flow’s pattern differences on SREBP activation, need to be clarified in future studies. As mentioned above, because cholesterol crystal formation was observed in subendothelial areas in an early stage of atherosclerosis, atheroprone flow–induced SREBP2 activation may accelerate cholesterol crystal formation, which may indirectly activate NLRP3 inflammasome.

Our group has reported that atheroprone flow increased TXNIP expression. TXNIP can promote atherosclerosis by multiple mechanisms. These data are relevant because it has been reported that ROS induced TXNIP-NLRP3 association, and TXNIP deficiency inhibited ROS-mediated NLRP3 inflammasome activation. Therefore, it is possible that atheroprone flow–induced TXNIP expression may be involved to this NLRP3 inflammasome activation. However, because other reports were unable to reproduce some of TXNIP function on NLRP3, further investigation is necessary (Figure 1). Furthermore, although the crucial role of ROS on NLRP3 inflammasome activation has been well established, the source and type of inflammasome-activating ROS is under controversy (Figure 1). It is very intriguing that Xiao et al showed the crucial role NADPH oxidase 2 (Nox2) on caspase-1 activation and IL-1β expression in endothelial cells in the current article. However, the idea of the involvement of Nox on NLRP3 inflammasome activation was not supported in macrophages. The deletion of Nox1, Nox2, and Nox4 in macrophages did not show any defect in inflammasome activation. The macrophage-deficient Nox2 showed robust decrease of ROS production, but only a minimum reduction in inflammasome activation. Instead, ROS generated by mitochondria having reduced mitochondria membrane potential leads to NLRP3 inflammasome activation. Of note, mitochondria is also very sensitive to ROS, which disrupts the electron respiratory chain, and further increases ROS production. The contribution of Nox family and mitochondrial ROS production on endothelial NLRP3 inflammasome activation needs further investigation.

In conclusion, Xiao et al have shown that atheroprone flow–mediated SREBP2 activation increases NLRP3 inflammasome activation via increasing NLRP3 and Nox2 expression, leading to endothelial inflammation and atherosclerosis formation. Previously, the authors’ group showed the unique sustained SREBP nuclear localization induced by atheroprone flow but not by atheroprotective flow, supporting the critical role of SREBP2 activation under atheroprone flow, but the mechanisms by which the atheroprone flow but not atheroprotective flow activates SREBP activation need further clarification. In addition, the contribution of endothelial NLRP3 inflammasome on atherosclerosis, and source of ROS, which activates endothelial NLRP3, will be important to understand. It is clear that atheroprone flow–mediated endothelial inflammation is a highly regulated process that is increasingly as attractive a target for therapeutic intervention as steady laminar flow–mediated atheroprotective pathways.

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References

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