# Editorial

# What Fans the Fire

## Insights Into Mechanisms of Inflammation in Atherosclerosis and Diabetes Mellitus

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It is now widely accepted that inflammation and immunity play important roles in the pathogenesis of atherosclerosis and diabetes mellitus. Over the past 3 decades, it has been demonstrated that cells of both the innate and adaptive immune systems contribute to the progression of atherosclerosis and modulation of plaque stability.[1] Similarly, recent studies in experimental models of obesity and type II diabetes mellitus have shown that not only macrophages, but also T cells and B cells are involved in the modulation of adipose depots and the development of insulin resistance.[2–5] However, even with all that is known about the important cellular players in atherogenesis, a clear understanding of the mechanisms by which immune cells influence arterial plaque formation is lacking.

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Macrophages are considered the predominant cell involved in foam cell formation and atherosclerotic plaque development. Numerous studies have linked macrophage activation in atherosclerosis to Toll-like receptors (TLRs) and have demonstrated that "danger signals" such as oxidized low-density lipoprotein (LDL), heat shock proteins, and other products of oxidative stress induce adhesion molecule and chemokine production by cells of the vessel wall and by direct activation of macrophages in the lesion. For example, TLRs such as TLR-4, TLR-2, and TLR-6 (in concert with the scavenger receptor CD36 and TLR-4),[6–10] are activated by oxidized LDL and may exacerbate the inflammatory nature of the atherosclerotic plaque.

In addition to macrophages, other immune cells, including natural killer (NK) cells, T cells, and a unique group of cells, NK T (NKT) cells, have been identified in atherosclerotic plaques of both humans and mice, and depletion of these cells by either antibodies or genetic approaches has shown that these lymphocytes play important roles in lesion formation.[1] Specifically, NK and CD4$^+$ T helper 1 (Th1) cells, which produce interferon-γ, are known to promote atherosclerosis

and plaque instability,[11,12] whereas FoxP3$^+$ regulatory T cells, which produce interleukin [IL]-10, are protective.[13] Like their Th1 and NK cell counterparts, NKT cells are viewed as proatherogenic,[14] although recent studies have challenged this view.[15]

Unlike macrophages, which are activated by nonspecific signals associated with inflammation, injury, or infection, T cells require specific cues in the form of peptide antigens presented by antigen-presenting cells such as B cells, macrophages, and dendritic cells. Although T-cell activation promotes inflammation in the atherosclerotic lesion, the exact atherosclerosis-associated antigen recognized by T cells has not been identified. Molecules such as native LDL, oxidized LDL, and bacteria-associated antigens (in the case of infection) have been implicated as potential neoantigens.[16,17] Identification of the exact antigen might prove impossible, because it is likely that several are involved and vary among individuals. Therefore, targeting specific antigens as a means of therapy will likely prove impractical.

Conventional activation of T cells involves at least 2 signals. The first is initial recognition of antigen in the context of the appropriate major histocompatibility complex. The second signal is the ligation of a costimulatory receptor, which, depending on the molecule, can result in either enhanced immune responses, as is the case for CD28 and CD40L, or decreased immune responses, seen with ligation of CTLA-4, a mechanism thought to turn off T-cell responses once the inflammation or infection is resolved. Usually, there is a third signal for differentiation or proliferation in the form of a cytokine mediator. Unfortunately, although immune players in atherosclerosis have been well identified, the specific molecular mechanisms for immune-mediated progression of atherosclerosis remain somewhat elusive.

Because lymphocytes are found in abundance in the plaque, understanding their means of activation and relative contribution to intralesion inflammation is of high priority in the atherosclerotic field. In this issue of *Circulation,* Xia et al[18] introduce a new and likely important player in lymphocyte activation and atherogenesis. This group examined the role of a specific activating molecule, NK cell lectin-like receptor subfamily K (NKG2D), in atherosclerosis. NKG2D, also referred to as Klrk1, is a membrane-bound receptor expressed on NK cells, NKT cells, γδ T cells, and CD8$^+$ αβ T cells,[19] which, as discussed above, have all been implicated in atherosclerosis. NKG2D has several ligands, including retinoic acid early transcripts 1 (Rae-1), the minor histocompatibility antigen 60 (H60), and the mouse and human UL16-binding protein-like proteins (Mult1 and ULBP, respectively).[19] Similar to ligands for TLRs, the NKG2D

ligands can be considered danger signals because they are usually expressed at low levels under normal healthy conditions but are highly upregulated after injury or infection. Because of this, Xia et al set out to determine whether NKG2D and its ligands are upregulated in atherosclerotic plaques of mice and humans. Interestingly, 1 such NKG2D ligand, MIC, was detected at high levels in sera and isolated plaques from patients with type 2 diabetes mellitus. Likewise, using immunohistochemistry, the authors demonstrated that atherosclerotic plaques of apolipoprotein E–deficient (apoE$^{-/-}$) mice express high levels of 2 other NKG2D ligands, Rae-1 and H60, whereas nonatherosclerotic aortic regions do not. Analysis of specific cells with flow cytometry revealed that NKG2D ligands are upregulated on macrophages, endothelial cells, and hepatocytes of mice with experimental atherosclerosis. Although it is not clear what stimuli increase the expression of Rae-1 and H60 on cells of apoE$^{-/-}$ mice during atherogenesis, Xia et al nicely demonstrate that LDL, oxidized LDL, and advanced glycation end products upregulate Rae-1 expression on macrophages.[18] These data indicate that products associated with atherosclerosis and diabetes mellitus may be responsible for providing signals of cellular stress and damage.

To investigate the causal relationship between NKG2D and its ligands in atherosclerosis, Xia et al crossed NKG2D-deficient *Klrk1*$^{-/-}$ mice with apoE$^{-/-}$ mice and demonstrated that, compared with wild-type apoE$^{-/-}$ mice, the *Klrk*$^{-/-}$apoE$^{-/-}$ animals have a dramatic 80% reduction in aortic arch atherosclerosis.[18] The authors also studied the role of NKG2D in animals with a combination of type 1 diabetes mellitus and hyperlipidemia, both commonly associated with aggressive atherosclerosis. By treating mice with the β-cell toxin streptozotocin, Xia et al demonstrated that the absence of signaling via the NKG2D pathway results in an impressive decrease in atherosclerotic burden in the aortas of diabetic *Klrk*$^{-/-}$apoE$^{-/-}$ animals. Similar results were observed in streptozotocin-treated mice injected with a blocking antibody for the NKG2D receptor. The mechanism for this protection appeared to be related to overt decreases in inflammation as levels of serum cytokines such as IL-6, interferon-γ, and IL-12 were reduced. Surprisingly, levels of the antiinflammatory cytokine IL-10 were likewise decreased in all 3 tested conditions. Whether the reduction in IL-10 is a direct result of NKG2D blockade or the indirect result of an overall reduction in inflammation was not apparent. These findings by Xia et al have provided new insight into the inflammatory process in atherosclerosis (Figure). Their work has identified an alternative activation pathway in which injury or oxidative stress associated with atherogenesis might exacerbate detrimental immune responses in the atherosclerotic lesion, leading ultimately to plaque instability and rupture. In addition to showing that NKG2D participates in atherosclerosis, Xia et al demonstrate that this receptor/ligand axis might also contribute to inflammation associated with diabetes mellitus, which could accelerate atherosclerosis.[18] Because streptozotocin-induced diabetes mellitus leads to direct destruction of β cells by the drug and not by autoimmune T cells, future experiments to determine whether NKG2D blockage is a viable option for reducing autoimmune-associated islet destruction
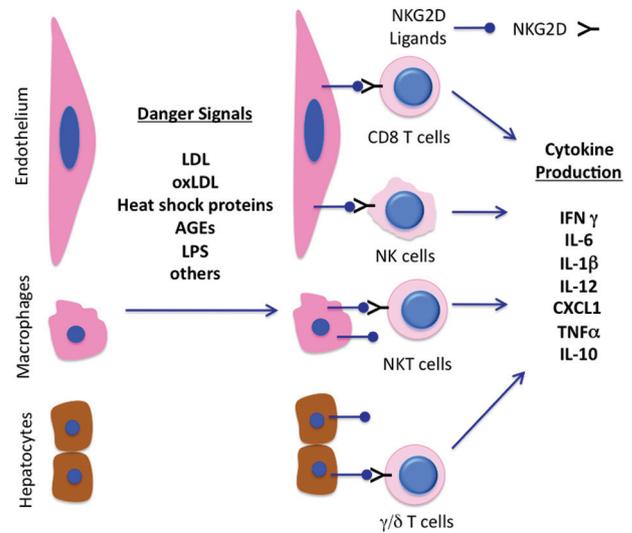


**Figure.** Schematic of NKG2D and ligands in promoting inflammation. Danger signals such as lipids (low-density lipoprotein [LDL], oxidized LDL [oxLDL]), heat shock proteins (HSPs), advanced glycosylation end products (AGEs), and components of infectious agents such as lipopolysaccharide (LPS) can upregulate specific ligands of NKG2D on endothelial cells, macrophages, and/or hepatocytes. This, in turn, leads to activation of subsets of T cells, natural killer T (NKT) cells, and natural killer (NK) cells with subsequent production of immunomodulatory cytokines. Activation of T cells and NKT cells requires ligation of T-cell receptors with specific antigens presented in major histocompatibility complexes or CD1d, respectively. It is also possible that cytokines such as interleukin (IL)-1β and IL-6 can be produced by endothelial cells, macrophages, and/or hepatocytes in direct response to danger signals or via interaction with activated lymphocytes. IFNγ indicates interferon-γ; TNFα, tumor necrosis factor-α; CXCL1, chemokine ligand 1.

or improvement of islet transplantation could prove interesting. It would be even more fascinating if inhibition of NKG2D signaling could prove beneficial in models of metabolic disease and associated exacerbated atherosclerosis.

One area left undetermined by this study is exactly which cells expressing the NKG2D receptor mediate the inflammation in concert with the ligand-expressing macrophages, endothelial cells, and hepatocytes. Although the authors clearly show that various NKG2D ligands are upregulated in atherosclerotic plaques of humans and apoE$^{-/-}$ mice, it is not as clear which NKG2D-expressing immune cells complete the signaling axis. Because NKG2D is expressed by NK cells, NKT cells, γδ T cells, and CD8$^+$ T cells, any of these could be involved, as illustrated in the Figure. However, of note, these cells make up a small fraction of the atherosclerotic plaque where, besides macrophages, CD4$^+$ T cells dominate. It is possible that activated NKG2D$^+$ CD4$^+$ T cells in the plaque or other positive lymphocytes in the adventitia or distal tissues such as the liver modulate the systemic inflammatory environment and that reduced activation of these cells, wherever they may reside, improves cardiovascular outcomes.

This study emphasizes the concept that atherosclerosis is much more than a disease of lipid dysregulation. Clinically, this is perhaps most evident in patients with autoimmunity who often have severely accelerated atherosclerosis and increased risk of myocardial infarction in the absence of

elevated LDL cholesterol.[20] With the exception of statins, which work by both modifying cholesterol and decreasing inflammation, treatments that specifically target immune activation in a controlled manner have not been identified. The work presented by Xia et al moves us 1 step closer to identifying therapeutic targets such as NKG2D that more precisely modulate immunity and inflammation to ultimately decrease atherosclerosis and other chronic inflammatory diseases.

## Disclosures

None.

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