Lipopolysaccharide, Toll-Like Receptors, and the Immune Contribute to Atherosclerosis

To the Editor:

Stoll and colleagues present an intriguing and timely review on the potential contribution of lipopolysaccharide (LPS) to development of atherosclerotic plaque.1 Recent studies have now provided important new insights into how LPS and other pathogen-associated molecular patterns (PAMPs) might directly contribute to atherosclerosis and suggest that the contribution of immune mechanisms to atherogenesis may be more important that presently realized. Walton et al recently showed that modified but not native low-density lipoproteins (LDL) upregulate TLR4 in endothelial cells, are recognized by TLR4 in a CD14-independent fashion, and cause increased endothelial cell expression of IL-8.2 Studies from Witztum’s laboratory indicate that modified LDL signals mediated by TLR4 cause actin polymerization and spreading of macrophages that result in decreased phagocytic activity of apoptotic cells and enhanced uptake of modified LDL.3 Recent in vivo studies provide more direct insights in the role of TLR signaling events in atherosclerosis. Bjorkbacka et al reported that genetic deficiency of CD14 in apolipoprotein E (apoE)-null mice had no effect on early lesion development.4 Because CD14 seems to be important for LPS-induced TLR4 signaling, this might at first imply that TLR4 signaling is not involved in atherosclerosis. However, results from our laboratory showed that TLR4−/−/apoE−/− mice demonstrated reduced atherosclerosis.5 These findings collectively are most consistent with the interpretation that TLR4 signaling contributes to atherosclerosis, and either LPS is not the ligand, CD14 is not essential to transduce LPS signals through TLR4, or some other ligand is interacting with TLR4 in a CD14-independent manner to influence plaque development. Further direct support for the conclusion that atherosclerosis is affected by TLR4 signaling arises from reports that apoE−/− mice that harbor a genetic deficiency in MyD88 (a common adaptor on which transduction of signals through TLR4 and most other TLRs heavily depends) show a marked decrease in atherosclerotic plaque size and evidence of substantially blunted vascular inflammation.6,7 These studies and others provide convincing evidence for a prominent role for TLR signaling in atherosclerosis.8 But more importantly, they implicate innate immunity in the pathogenesis of the disease, because TLRs represent the major proximal sensory apparatus by which the host detects the presence of foreign pathogens.6 It has been clear for some time that atherosclerosis involves inflammation, but it may not be adequately appreciated that inflammation is usually a manifestation of an immune response, which in turn suggests intimate and critical involvement of host defense mechanisms in the pathogenesis of the disease. Host defense depends on three fundamental mechanisms to eliminate pathogens: phagocytic engulfment, inflammation that is directly or indirectly toxic, and direct attack by cytotoxic cells such as natural killer cells and natural killer T cells. All three are involved in atherosclerosis. Immune cells such as dendritic cells, mononuclear phagocytes, and T cells are present at the earliest stages of atherosclerosis, are key participants in all phases of development and destabilization of plaque, and may even precede lesion formation.8 Phagocytosis of cellular debris, lipids, and pathogens in plaque has been widely documented. Cytotoxic immune cells such as natural killer T cells are present in atheroma, appear to accelerate plaque development, and may contribute to necrosis and apoptosis.9 Understanding how host defense influences atherogenesis may have profoundly important therapeutic implications. Work from our laboratory and others has been directed toward favorably influencing the immune system in atherosclerosis by developing a vaccine that could forestall, reverse, or protect against plaque development.10 These efforts proceed shoulder to shoulder with exciting progress toward creating vaccines to protect against diseases such as cancer.11 In our view, if we can apply the seminal advances made in immunology over the last decade12 to vascular biology, we will be richly rewarded with a quantum leap in both our understanding of the mechanisms involved in atherosclerosis and our ability to prevent and treat its manifestations.

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In Response:

We thank Doherty et al for their eloquent comments and for expanding the scope of discussion regarding the potential role of endotoxin as an inflammatory mediator of atherosclerosis. The authors correctly point out the emerging evidence that alternative ligands such as oxidized lipid mediators may activate the TLR4 signaling pathway in atherosclerosis. These studies support the premise that innate immunity, particularly that of TLR4-mediated signaling, is directly implicated in the pathogenesis of atherosclerosis.
Identification of the ligand(s) for TLR4 that promote atherosclerosis in vivo is a challenging task and may be difficult to elucidate from studies in mice. Doherty et al suggest that because deletion of TLR4, but not CD14, ameliorated atherosclerosis in apolipoprotein E (apoE)-deficient mice,1,2 an alternative ligand (rather than endotoxin) may activate TLR4 signaling to promote atherosclerosis. We would like to point out some features of those studies that would tend to limit the contribution of endotoxin to innate immune activation. First, the mice were fed a high cholesterol diet, resulting in markedly elevated cholesterol levels (≈900 mg/dL). Because lipoproteins bind to endotoxin, inflammatory responses to endotoxin are dampened in severely hypercholesterolemic mice.3 Second, in the study by Bjorkbacka et al using apoE/CD14 double knock-out mice,7 the animals were raised in a pathogen-free environment. The extent of subclinical bacterial infection was likely very limited, although levels of endotoxin were not measured in that study. Third, and most important, humans are extremely responsive to endotoxin, whereas mice are far less sensitive, requiring much higher doses of endotoxin to elicit a response.4 Finally, structural differences in both TLR4 and MD2 protein between the species may contribute to the functional differences between human and murine responses to endotoxin.5–7

In addition to the points listed above, we believe that more comprehensive studies are warranted to definitively address the role of CD14 in atherosclerosis in mice. Thus, the lone study published to date examined atherosclerosis at a single time point (10 weeks) and location (aortic sinus).2 Most vascular biologists believe that it is important to examine the entire aorta for atherosclerosis, rather than just the aortic sinus. In this regard, it was initially reported that deletion of p47\(\text{phox}\), a subunit of the superoxide-generating NADPH oxidase enzyme, did not ameliorate atherosclerosis in hyperlipidemic mice.6 That study examined atherosclerosis only in the aortic sinus. However, a subsequent and more thorough inspection of the entire aorta showed a significant reduction in lesion area in apoE/p47\(\text{phox}\) double knock-out mice, thus revealing an important role for this enzyme in atherosclerosis.9

We would also like to point out a recent study that detected an independent association between periodontal bacterial burden and carotid intimal-medial thickening in humans.10 Although this does not specifically implicate endotoxin as a mediator of atherosclerosis, it is consistent with pathogen-associated molecules serving as ligands for innate immune activation in vascular disease. Further investigations in animal models and in humans are needed to test this hypothesis and to address the importance of alternative ligands in TLR signaling.

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