Atherosclerosis is a chronic inflammatory disease modulated by both genetic and environmental factors. Disease onset is thought to be triggered by hypertension, high plasma concentrations of low-density lipoprotein (LDL) cholesterol, diabetes mellitus, or even infection. Endothelial injury by these factors is central to atherogenesis. Lesion susceptibility is greatest in those vascular regions with altered hemodynamics, such as the outer edges of arterial branches or curvatures. In these low-shear sites, endothelial proliferation, apoptosis, and permeability are all increased. In addition, the expression of adhesion molecules and chemokines increases, which facilitates the recruitment of monocytes, lymphocytes, and platelets from the circulation into the artery wall, resulting in the formation of an advanced, complicated lesion. With progressive subendothelial accumulation of cholesterol-engorged macrophages (“foam cells”) and the accompanying formation of a fibrous cap encapsulating a necrotic core, atheromatous lesions can rupture, resulting in a thrombus that can cause myocardial infarction or stroke. Although what initiates and maintains this inflammatory state is unclear, it is intriguing that both myocardial infarction and stroke are increased during acute infections and numerous pathogens have been detected in human lesions. However, how pathogens contribute to atherosclerosis remains unclear.

The detection of microbial infection and the initiation of the innate immune response are mediated via germline-encoded, pattern-recognition receptors, including the Toll-like receptors (TLRs), which recognize highly conserved pathogen-associated molecular patterns. The ligation of these receptors with microbial ligands elicits a cascade of cytokines, chemokines, and other pro-inflammatory molecules that contribute to the inflammatory response and bacterial clearance.

In addition to their crucial role in innate immunity, TLRs have recently been associated with atherosclerosis. Indeed, Edfeldt et al have found elevated levels of TLR1, TLR2, and TLR4 in human atherosclerotic lesions. Furthermore, a polymorphism in the human TLR4 gene that impairs its ability to signal has also been associated with reduced development of carotid atherogenesis. However, the role of TLRs in murine atherosclerosis is less clear.

Atherosclerosis-prone apolipoprotein E (ApoE)-null mice that were also deficient in TLR4 had slightly reduced or no observable differences compared with ApoE−/− mice, suggesting a very limited role for TLR4 in atherogenesis. In contrast, two recent studies have reported that when mice were made deficient in the TLR adaptor MyD88 (downstream of most TLRs, as well as interleukin 1 [IL-1]), there was a profound reduction in disease compared with ApoE−/− mice. Although the reason underlying the greater reduction in atherosclerosis in MyD88−/− mice than in TLR4-deficient mice is unclear, a simple explanation could be the previously documented role for IL-1. However, an alternative explanation could be other MyD88-dependent TLRs (such as TLR2) that are also up-regulated in atherosclerotic lesions and may play an important role in atherogenesis.

Although there is a growing body of evidence to support an association between the TLRs and atherosclerosis, the connection between the TLRs and altered hemodynamic parameters, so crucial to atherogenesis, has never been addressed. In this issue of Circulation Research, Dunzendorfer et al provide very intriguing data that demonstrate flow-dependent regulation of TLR2 surface expression in cultured human coronary artery endothelial cells. Using an in vitro model of both laminar and disturbed (or static) flow over an extended period, this study addresses how a TLR could be involved in the etiology of a disease that is governed largely by hemodynamics. Dunzendorfer et al reported that TLR2 mRNA and surface expression, as well as the ability of TLR2 ligands to activate endothelial cells, are reduced under laminar flow conditions, whereas under “disturbed flow,” as would occur at atherosclerosis-prone arterial branches, endothelial TLR2 expression and functional responsiveness are increased. This effect appears to be TLR2-specific, as endothelial responses to TLR4 and TNF (tumor necrosis factor) receptor ligands (lipopolysaccharide [LPS] and TNF-α, respectively) were not altered by laminar flow.

How, then, is endothelial TLR2 surface expression regulated by flow? The human TLR2 promoter has previously been shown to contain SP1/SP3 elements. Dunzendorfer et al found that although SP1/SP3 mRNA levels were unchanged between static and flow conditions, there was a dramatic reduction in the ability of SP1 and SP3 to bind DNA.
under flow conditions. Laminar flow resulted in the serine phosphorylation of SP1 in a time-dependent manner, which was inversely proportional to its DNA-binding activity. Interestingly, a casein kinase 2 (CK2) inhibitor was able to restore both SP1 and SP3 DNA binding, as well as TLR2 surface expression, to levels observed under static conditions. Thus, the surface expression of endothelial TLR2 under disturbed flow, but not laminar flow, may explain how a TLR could be involved in atherogenesis with regional specificity of lesion development.

The research by Dunzendorfer et al also indirectly touches on the importance of the site origin of cultured endothelial cells. A recent study by this group showed that resting human coronary artery endothelial cells express TLR2 and are responsive to TLR2 ligands, including the mycoplasmal lipopeptide MALP-2. In contrast, resting human microvascular endothelial cells (HAEC), human aortic endothelial cells (HMEC), human umbilical vein endothelial cells (HUVEC), and mouse lung vascular endothelial cells (MLVEC) have all been shown to express weak, if any, TLR2 at the mRNA and/or protein levels. As would be expected, these cells were unable to respond to TLR2 ligands unless transfected with the TLR or challenged with proinflammatory stimuli that upregulate TLR2. This suggests that TLR2 may be differentially expressed by different endothelial beds under basal conditions. Indeed, we noted that only one or two microvascular beds responded to the TLR2 ligand lipoteichoic acid under noninflammatory conditions in vivo.

Although the current study proposes a mechanism whereby TLR2 could be involved in atherogenesis based on its flow-dependent expression, it remains unknown whether endothelial cell TLR2 is a primary initiator or a modulator of atherogenesis. Further work will be required to address whether or not atherosclerosis-prone mice deficient in TLR2 have similar atherogenic defects as MyD88−/− mice. Another important question that remains unanswered is what ligand might be responsible for TLR2 activation in atherogenesis. A study by Laman et al found peptidoglycan (a TLR2 ligand) in 19 of 31 coronary atherosclerotic segments. Chlamydia pneumoniae (a risk factor for atherosclerosis) also activated monocytes in a TLR2-dependent fashion. However, there are data that do not support a role for pathogens in atherogenesis. At first glance, the finding by Wright et al, that atherosclerosis in germ free mice was not measurably different from mice with ambient microbial challenge, seems to disagree with a possible role for TLRs in atherogenesis. However, there is growing evidence that modified endogenous molecules can be detected by TLRs. Indeed, minimally modified LDL is recognized by TLR4, leading to a proinflammatory state. Clearly, TLR2 could also detect various modified lipids, leading to a proinflammatory state independent of pathogens. This possibility also warrants some attention. In summary, the findings of Dunzendorfer et al provide an important new link between shear and TLR2 expression, lending further credence to the notion that TLRs contribute to vascular disease.

References


