Toll in the vessel wall—for better or worse?

Göran K. Hansson[1] and Anna M. Lundberg

Center for Molecular Medicine L8:03, Department of Medicine at Karolinska University Hospital, Karolinska Institutet, SE-17176 Stockholm, Sweden

Pattern recognition receptors detect danger signals such as pathogen-associated molecular patterns. Among such detectors, the membrane-bound receptors of the Toll-like receptor (TLR) family can detect all kinds of pathogens and play an important role in host defense (1). Not surprisingly, TLRs are also involved in chronic inflammatory diseases such as arthritis, colitis, and atherosclerosis. A report in PNAS provides insights into the role of TLRs in vascular biology (2).

Atherosclerosis, the cause of myocardial infarction and stroke, is a chronic inflammatory disease elicited by cholesterol accumulation in the artery wall (3). Innate as well as adaptive immunity contributes to inflammation in atherosclerosis, and a spectrum of TLRs are expressed in the human atherosclerotic plaque (4). Experiments in gene-targeted models show that certain TLRs impact on atherosclerosis in hypercholesterolemic mice (5, 6). Specifically, TLR2 and TLR4 ligations promote disease development, whereas ablation of these receptors reduces lesion size (7, 8). Thus, one could envisage that endotoxins released during infections with Gram-negative bacteria would ligate TLR4 in lesions, leading to accelerated atherosclerosis. Similarly, peptidoglycans of Gram-positive bacteria could promote atherosclerosis by ligating TLR2. These findings are of obvious interest, because certain bacterial infections have been suggested to promote cardiovascular disease.

Viruses can be recognized by TLR3, which ligates dsRNA (9), TLR7 and TLR8, which bind certain ssRNA species, and TLR9, which is stimulated by unmethylated CpG DNA motifs. The effects of such signals are also of interest for cardiovascular disease, because certain viral infections have been associated with atherosclerosis. In addition, it is possible that endogenous danger signals could bind to TLR and trigger signaling cascades. For instance, TLR3 signaling can be activated by endogenous RNA molecules released during cell death (10, 11). However, little is known about the role of these TLRs in cardiovascular disease.

TLR3 Ligands Affect Macrophages and Smooth Muscle

The first indication that TLR3 signaling could be involved in atherosclerosis came from studies of cholesterol metabolism in macrophages (12). Accumulation of cholesterol in such cells is a hallmark of atherosclerosis and depends on the balance between receptor-mediated uptake and efflux through cholesterol transporters to high-density lipoproteins. The cholesterol transporter, ATP-binding cassette subfamily A member 1 (ABCA1), is expressed when intracellular sterols activate Lipid X receptor (LXR) transcription factors. The latter was found to be inhibited by TLR3 ligands signaling through the interferon regulatory factor-3 (IRF3) transcription factor. The consequence of TLR3 ligation was, therefore, impaired cholesterol efflux from macrophages, which is known to promote the formation of macrophage foam cells, the prototypic cell type of atherosclerotic lesions. In addition, TLR3 ligation stimulates secretion of proteases, potentially leading to plaque rupture, thrombosis, and myocardial infarction (13).

Cole et al. investigate the role of TLR3 in the arterial injury response and atherosclerosis.

In PNAS, Cole et al. (2) investigate the role of TLR3 in the arterial injury response and atherosclerosis. They report that treatment with the exogenous synthetic dsRNA poly(I:C) protects carotid arteries from neointimal scar formation after perivascular injury. Knocking out TLR3 in the system does not confer protection on its own, indicating that endogenous TLR3 ligands cannot mediate protection. In contrast, the effects of exogenously administered dsRNA depended on TLR3 ligation, because this protection was ablated in $Tlr3^{-/-}$ mice. To extend their findings to the human condition, Cole et al. (2) examine the response of human vascular smooth muscle cells to the TLR3 agonist. They registered a response of these cells to dsRNA, which induced expression of several adhesion molecules, chemokines, and inflammatory cytokines. This confirms the recent reports by Yang et al. (14) and Ahmad et al. (15) that showed that dsRNA induces a set of proinflammatory cytokines and chemokines and also stimulates the growth of smooth muscle cells derived from human coronary arteries.

Among mediators released on TLR3 ligation, several are known to contribute to the chronic inflammatory process of atherosclerosis. Interestingly, cells derived from atherosclerotic lesions responded more vigorously than cells derived from normal arteries in the study by Cole et al. (2). Although the two cell types may not be directly comparable because of differences in conditions of isolation, the data point to an increased responsiveness to TLR3 agonists in the atherogenic environment.

Ahmad et al. (15) went one step further and identified interferon-γ (IFN-γ) as a TLR3-inducing mediator that augments the response to nucleic acids (15). This cytokine is secreted by T cells in atherosclerotic lesions, acts on endothelial and smooth muscle cells, and is in itself proatherogenic in mouse models of atherosclerosis and transplant arteriosclerosis (3, 16). When human coronary arteries were grafted into SCID mice, IFN-γ induced TLR3 expression and a vivid response to dsRNA (15). It is interesting to speculate that the enhanced TLR3 expression in atheroma cultures registered by Cole et al. (2) may be because of the presence of IFN-γ derived from immune cells infiltrating plaques.

Unexpected Effects of TLR3 on Atherosclerosis

Cole et al. (2) surprisingly observe that TLR3-deficient $Apoe^{-/-}$ mice exhibited increased early atherosclerosis compared with TLR3-expressing mice under conditions where no exogenous TLR3 ligand was administered. Although the effect is relatively modest, it is puzzling. In view of the proinflammatory and growth promoting effects elicited by the TLR3 ligation, the opposite result would have been expected. It is also surprising given the effect of TLR3 signaling on cholesterol metabolism. Because TLR3 ligation inhibits cholesterol efflux by downregulating the atheroprotective ABCA1 transporter, loss of TLR3 should improve

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[1]To whom correspondence should be addressed. E-mail: Goran.Hansson@ki.se.

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elimination of cholesterol from macrophages in lesions and elsewhere. This would be expected to reduce rather than increase disease. Additional experiments will be important to clarify whether atheroprotective mechanisms counterbalanced the proatherogenic effects of TLR3 signaling in the *Apoe*$^{-/-}$ experiments. It will also be important to clarify whether natural TLR3 ligands, such as endogenous RNA molecules and commensal microbes, trigger different responses than the artificial ligand poly(I:C).

Is it possible that different cell types exert very different responses on TLR3 ligation? For instance, growth modulation in the SMC population may affect atherosclerosis in a different way than modulation of cholesterol metabolism in macrophages or modulation of immune activity in T cells. This would be in agreement with a previous report showing that human endothelial cells and fibroblasts exhibit a more vigorous response to dsRNA stimulation than macrophages and dendritic cells (17). It is also possible that endogenous TLR3 ligands presumed to act in the animal experiments target different cell types than the exogenous ligand poly(I:C), which seems to be the case for TLR2 ligands (8). Therefore, further studies will be needed to dissect the contribution of different cell types and downstream signaling pathways to clarify the role of TLR3 and dsRNA in atherosclerosis.

TLR3—A Virus Receptor in the Artery

The notion that TLR3, a virus sensor, has pathobiological effects in the vessel wall raises the interesting question whether viruses may contribute to or modulate vascular disease. Several viruses have been implicated in atherosclerosis (16), including Herpes family viruses, against which TLR3 confers protection (1). Others, such as HIV, are associated with various types of cardiovascular morbidity (16). dsRNA derived from many different pathogenic viruses can be detected by TLR3 and triggers pathways in smooth muscle cells, macrophages, and other cells that may modulate vascular disease. The studies on the role of TLR3 in the vasculature have opened up an interesting field of investigation.

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