The recognition that atherosclerosis is a chronic inflammatory disease has emphasized the fundamental link between the innate immune system and disease pathogenesis. Atherosclerotic lesions develop at vulnerable locations throughout the vasculature as a result of detection of endogenous or microbial ligands by germ-line encoded pattern recognition receptors, including the innate immune Toll-like receptors (TLRs). Certain dietary lipids that contribute to atherosclerotic disease resemble on a molecular level lipids expressed in the microbial cell wall and are recognized by TLRs. Engagement via TLRs of dietary lipid molecules on both immune and nonimmune cells initiates inflammatory responses that persist chronically and culminate in the deposition of fatty streaks, which progress to occlusive atherosclerotic plaques.

A central question that has recently been debated is whether the recognition and processing of TLR ligands and downstream signaling is similar or distinct in professional phagocytic macrophages versus nonprofessional phagocytic endothelial cells. Indeed, both cell types express TLRs and respond to TLR-specific ligands to sustain an inflammatory response. In both cell types, ligand activation recruits the adaptor, MyD88, to TLR dimers, resulting in a complex composed of interleukin receptor-associated kinases and the second adaptor, tumor necrosis factor (TNF) receptor–associated factor-6 (TRAF6). TRAF6 is an E3 ubiquitin ligase required for Smad-independent c-Jun N-terminal kinase activation. Thus, although overlap and sharing of signaling molecules exists between endothelial cells and macrophages, differences lie in effector functions of the 2 cell types.

Although TRAF6 is expressed in atherosclerotic aortic tissue of low-density lipoprotein–null mice, recent studies addressing the role of TRAF6 signaling in diet-induced atherosclerosis have yielded conflicting results. The perinatal lethality of systemic TRAF6 ablation has precluded the study of TRAF6 knockout mice in classic models of diet-induced atherosclerosis. A recent study by Lutgens et al used an apolipoprotein E (ApoE) transgenic mouse in which CD40 was mutated to disrupt its interaction with TRAF6 via expression of the chimeric transgene under the control of the major histocompatibility complex class II promoter. As major histocompatibility complex class II molecule is expressed on antigen-presenting cells and lymphocytes, the CD40–TRAF6 interaction and consequent downstream signaling were disrupted mainly in leukocytes. These investigators demon-
strated that mice fed a normal chow diet with defective CD40–TRAF6 signaling exhibited markedly diminished atherosclerosis accompanied by impaired activated (Ly6C<sup>hi</sup>) macrophage recruitment to the vessel wall. However, this study could not conclusively attribute the reduction in atherosclerosis to TRAF6 per se, because CD40-independent TRAF6 signaling remained intact. A separate study reported no effect of TRAF6 deficiency on the development of atherosclerosis to TRAF6 per se, because CD40-independent TRAF6 signaling remained intact. A separate study reported no effect of TRAF6 deficiency on the development of atherosclerosis after bone marrow ablation and reconstitution with fetal liver cells.

The study by Polykratis et al<sup>8</sup> in this issue of *Circulation* makes a major contribution to the understanding of TRAF6 signaling in atherosclerosis. To address the relative importance of endothelial cell versus macrophage TRAF6 signaling in the development of atherosclerosis, the authors used ApoE-null mice with conditional endothelial cell–specific or myeloid cell–specific TRAF6 deficiency, achieved using Cre recombinase-LoxP–mediated gene targeting. In addition to achieving cell specificity of TRAF6 deletion, conditional gene targeting of TRAF6 using tamoxifen in the diet starting at 6 weeks of age ensured that perinatal lethality attributable to TRAF6 deficiency would not occur. Mice were then fed a high-cholesterol Western diet for 10 weeks to accelerate atherosclerosis.

Polykratis et al<sup>8</sup> found that endothelial-specific TRAF6 deficiency was of no consequence to the development of atherosclerosis in male mice. However, atherosclerotic lesion size was significantly increased in female mice compared with their male counterparts. Endothelial-specific TRAF6 deficiency in females was associated with diminished atherosclerosis, decreased plaque macrophage burden, and, as expected given the histological phenotype, diminished expression of adhesion molecule, vascular cell adhesion molecule 1, and chemokines related to recruitment. Monocyte adhesion induced by oxidized low-density lipoprotein in TRAF6-null endothelial cells in culture was also reduced. These findings are all in keeping with a predominant proinflammatory response of the endothelium to TLR ligands through TRAF6. A hint at an anti-inflammatory effect of TRAF6 in the endothelium was found with the observation of increased transforming growth factor β expression at the mRNA level in aortas with endothelial-specific TRAF6 deficiency, although this requires further study.

Surprisingly, a different phenotype emerged in mice with TRAF6 ablation in macrophages. In both male and female mice, myeloid-specific TRAF6 deficiency was associated with greater atherosclerotic plaque area, with females again exhibiting increased lesions. Macrophage burden within the plaque was similar and was thus unaltered by TRAF6 deficiency, which is somewhat surprising and suggests that TRAF6 downstream signaling is not the only route to macrophage recruitment. A most revealing finding was the markedly reduced levels of anti-inflammatory IL-10 in the atherosclerotic lesion as well as in vitro experiments using cultured macrophages after stimulation with oxLDL. TRAF6 deficiency in macrophages was also associated with enlargement of the necrotic core and diminished apoptotic cell debris removal. These definitive results dissect out a significant role for macrophage-specific TRAF6 in mediating anti-inflammatory responses after chronic ingestion of proatherogenic dietary ligands.

A result that requires further clarification in this study is the difference observed in the role of cell-specific TRAF6 signaling in male versus female mice. Studies have shown that the endothelium is the cellular target of the beneficial effect of estrogen in humans and in mouse models of atherosclerosis.<sup>9,10</sup> In the present study the estrogen status of the female mice was not defined, and thus conclusions relevant to the role of estrogen in TRAF6 proinflammatory signaling in the endothelium cannot be made.

As stated above, TRAF6 is an intracellular adaptor that also participates in IL-1, TNF, and CD40 receptor signaling. At the mRNA level, expression in aortic tissue of TNF-α and IL-1β were not noticeably altered in mice with either endothelial-specific or myeloid-specific TRAF6 deletion (Figures 2 and 5 in Polykratis et al<sup>8</sup>). Thus, the cell-specific TRAF6 signaling observed in this study can most likely be attributed to TLR-mediated TRAF6 signaling with dietary lipids as the inciting TLR ligands, and not to secondary autocrine or paracrine IL-1 and TNF signaling. It is tempting to conjecture
that TLR activation via a microbial pathogen and downstream TRAF6 signaling would produce the same results with regard to TRAF6 signaling in the endothelium and in macrophages. However, recent studies have suggested that similar conclusions regarding the role of TLR signaling in high-fat diet and microbial-induced atherosclerosis models cannot be made.

Whereas studies using hyperlipidemic mice have shown reduced atherosclerotic development in TLR2−/−, TLR4−/−, and MyD88-deficient ApoE−/− mice on high-fat Western diet, we demonstrated no effect of TLR4-mediated signaling on mice fed a normal chow diet. Work from our laboratory has also demonstrated that infection with the oral pathogen Porphyromonas gingivalis results in diminished atherosclerosis in TLR2-deficient mice as compared with ApoE−/− fed a normal chow diet, in agreement with a major role for P. gingivalis fimbriae–mediated signaling via TLR2/MyD88. We also recently demonstrated a protective role for TLR4 in P. gingivalis induced chronic inflammatory atherosclerosis in ApoE−/− mice fed a normal chow diet, which results from P. gingivalis–specific mechanisms to evade TLR4-mediated signaling. Thus the unique ability of this microbial pathogen to evade TLR4 signaling while inducing TLR2-dependent proinflammatory signaling points to a critical role for microbial specificity in TLR-mediated inflammatory responses that contribute to atherosclerosis. This highlights the specificity of TLR signaling in lipid versus microbial induced atherosclerosis.

The provocative study by Polykratis et al has highlighted not only the importance of TRAF6 signaling, but also the importance of cell-specificity of TRAF6 signaling in lipid-induced atherosclerosis (Figure). An important next step is to dissect the commonalities and differences in cell-specific TRAF6 signaling arising from various endogenous and microbial TLR ligands.

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References

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