Role of Toll-Like Receptors in Atherosclerosis

To the Editor:

We read with interest both the recent paper by Dunzendorfer et al1 and the accompanying commentary.2 We wish to clarify some key points. Dunzendorfer et al report that under antiatherogenic conditions of laminar flow, human coronary artery endothelial cells (ECs) express low levels of Toll-like receptor 2 (TLR2) mRNA and protein. Dunzendorfer et al also reported that TLR2 mRNA and protein expression was increased under conditions of low shear stress and that laminar flow resulted in protein kinase CK2 phosphorylation of SP1, which inhibited binding of SP1 to the TLR2 promoter. These results conflict in part with data published 2 years previously by Liang et al,3 although the authors did not cite this work. That study showed low levels of TLR2 mRNA expression in ECs under laminar flow. There was no change in TLR2 expression but increased TLR4 expression and nuclear factor κB activation with low shear stress in ECs.

Moreover, the totality of data reported to date do not unequivocally demonstrate TLR2 expression by ECs that is functionally relevant and, instead, support a much more prominent role for TLR4-mediated signaling in ECs in diseases such as atherosclerosis. It is conceivable that coronary artery ECs, which are exposed to somewhat different hemodynamic forces from microvessel or venous ECs, might express a different repertoire of TLRs. However, human aortic ECs are exposed to similar hemodynamic stresses as coronary artery ECs, and Walton et al were unable to detect transcripts encoding TLR2 in these cells.4 Furthermore, in our hands, human coronary artery ECs, like human dermal microvessel ECs, do not express TLR2 mRNA and are unresponsive to a variety of TLR2 ligands (K.S.M. and M.A., unpublished data).

Dunzendorfer and colleagues used immunostaining in combination with fluorescence microscopy and fluorescence-activated cell sorting (FACS) analysis to show TLR2 protein expression in coronary artery ECs. Identical monoclonal antibody was used for both studies. The images depicted in Figure 2B of the article1 show weak spotty TLR2 immunofluorescence. From these images, it is impossible to determine the subcellular localization of immunofluorescence signals. They might be localized to the cytoplasm, membrane, nucleus, or some combination of these. This staining pattern is commonly found with nonspecific binding of primary antibody. The study does not show or mention control experiments to exclude this possibility. Control experiments with blocking peptide and isotype control antibody staining would resolve this question. Co-localization studies using confocal microscopy would clarify the subcellular localization of TLR2 protein in ECs. Specificity problems of the TLR2 antibodies used also raise doubts about the FACS analyses. Scatter plots from FACS analysis would indicate the quality of the data, but were not included. Western blotting would establish the significance of TLR2 protein expression in ECs and would corroborate immunofluorescent microscopy and FACS. In the absence of controls, and given the uncertainties in the data presented, it is difficult to evaluate the validity of the TLR2 protein expression results in coronary artery ECs.

One of the purposes of the studies reported by Dunzendorfer et al was to indirectly evaluate the possibility that TLR2 might have relevance to the development of atherosclerosis. The sparse data currently available by no means establish such a role, and some data suggest otherwise. For example, we reported that TLR2 is not expressed in human and mouse atherosclerotic plaques.5 However, other studies indicate that TLR2 may be expressed at very low levels in normal arteries, but that upregulation of TLR2 may occur in atherosclerotic plaque. If so, this also does not necessarily implicate a role for TLR2 signaling in development of atherosclerosis, because any TLR2 expression in plaque may be a response or epiphenomenon rather than a cause.

In contrast to the uncertain role of TLR2, there is a compelling case for TLR4-mediated signaling in vascular disease (reviewed by Michelsen et al). TLR4 is highly expressed in plaque by a number of different cell types known to be involved in development of atherosclerosis. Furthermore, we recently reported that TLR4-null mice crossed onto the apolipoprotein E (apoE) background showed significant inhibition of atherosclerosis (24% reduction of atherosclerotic lesions in whole aortas, 55% reduction of lipid content in aortic sinuses plaques, 65% reduction of macrophage infiltration in aortic sinus plaques).7 The role of TLR4 in atherogenesis was further confirmed in mice with genetic deficiency of both MyD88 and apoE.2 Our studies directly indicate a role for TLR4-mediated signaling in atherogenesis, but because inhibition of atherosclerosis was greater in MyD88/apoE double knockout mice compared with TLR4/apoE double knockout mice,7 and because MyD88 is a common adapter protein used by a number of TLRs, it is likely that signaling by other TLRs in addition to TLR4 might lead to development of atherosclerotic plaque. Alternatively, a simpler explanation of the discrepancy in the degree of reduction of atherosclerosis would be the documented role of interleukin-1 and -18, which also signal through MyD88. Additional evidence helps establish a role for TLR4 signaling in vascular pathology. For example, local activation of TLR4 by LPS augmented neointima formation in a murine model of arterial injury. Outward remodeling induced by lipopolysaccharide and arterial injury did not occur in TLR4-null mice. Furthermore, a number of genetic studies link TLR4 polymorphisms and vascular pathology, but no such studies have been reported for TLR2 mutations.6 Additionally, TLR4 has been demonstrated to recognize minimally modified LDL leading to activation of pro-inflammatory mediators (reviewed by Michelsen et al).

Collectively, these considerations are most consistent with the conclusion that there is currently only indirect evidence of uncertain significance linking TLR2-mediated signaling with vascular pathology, but a prominent role for TLR4 signaling in the development of atherosclerotic plaque. If, as the study by Dunzendorfer implies, TLR2 signaling is important to vascular pathology, that conclusion must await the results of more definitive and direct investigations.

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