Rounding up the usual suspects in atherosclerosis.
Focus on “Growth factors induce monocyte binding to vascular smooth muscle”

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In the film “The Usual Suspects,” a group of criminals find themselves thrown together in a holding cell, where they hatch an elaborate plot that none of them alone could have executed. In studying the mechanisms of atherosclerosis, we tend to look at the suspects one by one, but it is clear that they work together to cause the disease. In the current article in focus (Ref. 3, see page C707 in this issue), Cai et al. have rounded up a large cadre of the usual suspects: the cell types of monocytes and vascular smooth muscle cells (VSMC), the growth factors angiotensin II (ANG II) and platelet-derived growth factor (PDGF), and the arachidonic acid (AA)-metabolizing enzymes 12/15-lipoxygenase (12/15-LO) and cyclooxygenase-2 (COX-2). Importantly, they have placed their “suspects” in the context of vascular smooth muscle, not endothelium, to examine their potential role in monocyte retention (as opposed to recruitment) within the arterial wall. As the title of their article states, the interplay of these suspects in promoting monocyte binding in vascular smooth muscle has implications for atherosclerosis and warrants a reconsideration of the interactions between the key players as well as the anatomic locations of the pathological actions.

It is abundantly clear that monocyte-macrophages and VSMC participate in the pathophysiology of atherosclerosis. The role of ANG II has also been extensively studied and is the subject of several recent reviews (e.g., Ref. 6). Human trials with ANG II receptor antagonists and inhibitors of ANG II formation have demonstrated improved endothelial function and decreases in myocardial infarction and stroke. Although a reduction in blood pressure can explain some of the clinical benefits seen in disruption of ANG II signaling, we and others have shown that ANG II has significant effects on atherosclerosis independent of blood pressure (16). In apolipoprotein E null mice (ApoE−/−), ANG II treatment with a high-fat diet has a synergistic effect on lesion formation that is not shared by norepinephrine treatment at doses that produce the same degree of hypertension. The myriad effects of ANG II include the generation of oxidative stress via NADPH oxidases and the induction of a host of proinflammatory mediators. Cai et al. (3) propose additional effects on monocyte binding mediated by the release of soluble factors from VSMC, although further studies are required to establish the identity of these factors.

Whereas human and animal studies employing blockade of ANG II signaling leave little doubt about its key role in atherosclerosis, the case for PDGF is less clear. Although PDGF has long been recognized as a potent stimulus of VSMC proliferation and migration, its role is more established in neointima formation following allograft transplantation or angioplasty than in atherogenesis. The lethality of knocking out PDGF signaling in mice has hindered a definitive assessment of its role in atherosclerosis, but some recent studies have managed to partially circumvent this obstacle. It appears that PDGF signaling in VSMC is normally held in check by the interaction of ApoE with low-density lipoprotein (LDL)-receptor protein-1 (LRP1). Tissue-specific knockout of LRP1 in the VSMC of LDL receptor knockout mice leads to a dramatic increase in atherosclerotic lesions, which is partially reversible by the tyrosine kinase inhibitor STI571 (Gleevec) (2). The data reported by Cai et al. (3) suggest that PDGF could contribute to plaque formation not only through direct effects on VSMC but also by promoting the adhesion of monocytes to VSMC. Combined with a report that interaction of endothelial cells and monocytes leads to increased PDGF formation in both cell types (7), it is tantalizing to imagine a feed-forward loop in which transmigrating monocytes produce PDGF that stimulates VSMC to increase monocyte retention.

Enthusiasm for such a pathway must be tempered by data weighing against a critical role for PDGF in atherosclerosis. In a study in which ApoE−/− mice were used, administration of a PDGF receptor antagonist had no effect on lesion area and delayed, but did not prevent, fibrous cap formation (12). In the same study, PDGF production was targeted by using fetal liver cells from ApoE−/− PDGF-B−/− double knockouts to reconstitute the bone marrow of irradiated ApoE−/− mice. The resulting chimera had no PDGF-B production in their monocytes but again showed no effect on atherosclerotic lesion extent. While these results may reflect the ability of compensatory mechanisms to overcome PDGF blockade over long periods of time, they also raise the possibility that PDGF may not play a critical role in atherosclerotic lesion formation.

Cai et al. (3) report that the effects of ANG II and PDGF on monocyte binding are attenuated by blockade of AA metabolism. After its production by phospholipases, AA can be metabolized by several enzymes, such as 12/15-LO and COX-2. Although deletion of 12/15-LO attenuates atherosclerosis in both the LDL receptor and ApoE mouse models, some studies in rabbits suggest a protective effect for the enzyme (reviewed in Ref. 8). In the mouse studies, the proatherogenic effects have in the past been attributed to the ability of macrophage 12/15-LO to oxidize LDL. The key role of monocyte-macrophages in this pathway is highlighted by new data showing that ApoE mice given bone marrow transplants (BMT) from ApoE−/− 12/15-LO−/− double-knockout donors have the same reduction in lesions as the double knockouts themselves (10). In contrast, Cai et al. postulate that 12/15-LO

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metabolites produced in VSMC also may play a role in monocyte binding, as shown not only by inhibitors but also through the use of VSMC derived from 12/15-LO null mice. Even though macrophages appear to be a significant source of 12/15-LO in advanced lesions, the current study suggests that eicosanoids produced by 12/15-LO in VSMC or endothelium may further facilitate the recruitment and retention of monocyte-macrophages at earlier stages of atherosclerotic lesion development.

Just as the animal literature offers conflicting data regarding the role of 12/15-LO in atherosclerosis, so too has controversy surrounded the role of COX-2. The data that support a proatherogenic role for COX-2 mirror those for 12/15-LO, including expression of COX-2 in atherosclerotic plaques and the reduction of lesions in LDL receptor null mice after BMT from COX-2 null animals (reviewed in Ref. 13). Although several studies in which COX-2-selective inhibitors were used have shown improvement in atherosclerosis, a number of others show no effect (most recently, Ref. 1). Once again, additional BMT or tissue-specific knockout studies may be useful in resolving these disparate results.

COX-2-selective inhibitors are in general clinical use, and as a result, several human studies have addressed the effect of COX-2 inhibition on cardiovascular disease. These data have also been conflicting, perhaps in part because the selective inhibitors are often compared with nonselective drugs that may carry antithrombotic and other benefits (reviewed in Ref. 4). The discovery of a common polymorphism in an Sp1 site in the human COX-2 promoter (15) provides some nonpharmacological data that support a role for COX-2 in cardiovascular disease. This polymorphism reduces COX-2 expression by ∼30% in reporter assays (15) and has been associated with protection from myocardial infarction and stroke in a case-control study (5). Long-term, population-based studies of such polymorphisms may eventually offer the best evidence for a pathophysiological role for COX-2 in atherosclerosis, even if we are unable to target the pathway effectively using genetic or pharmacological approaches.

The work of Cai et al. (3) not only prompts consideration of the many suspects in atherosclerosis but also illustrates the importance of studying the interactions between these suspects and the location of the interactions. Many of these interactions have been examined in cell culture models, such as the ability of ANG II to stimulate PDGF (11), COX-2 (9), and 12/15-LO (14). However, animal studies are ultimately necessary to address the physiological importance of such interactions. For example, would knocking out COX-2 or 12/15-LO expression attenuate the lesion formation in ApoE−/− mice treated with ANG II? Such experiments in concert with the use of tissue-specific knockouts to assess the key cell types that mediate these pathways should help us to further unravel the web that links these suspects.

REFERENCES