The Role of Cellular Adaptation to Mechanical Forces in Atherosclerosis

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***Abstract*—**Atherosclerosis is a chronic inflammatory disease that originates at regions of arteries exposed to disturbances in fluid flow and results in progressive plaque formation in those areas. Recent work on cellular responses to flow has identified potential mechanosensors and pathways that may influence disease progression. These results led us to hypothesize that the same mechanisms that mediate adaptive responses in the vasculature become maladaptive at sites of disturbed flow. Subsequent changes in gene expression and matrix remodeling help to entrain these inflammatory pathways. These events synergize with systemic risk factors such as hyperlipidemia, smoking, and diabetes, leading to disease progression. **(*Arterioscler Thromb Vasc Biol*. 2008;28:2101-2107.)**

Key Words: fluid shear stress ■ endothelial ■ smooth muscle ■ inflammation ■ disturbed flow ■ laminar flow

Mechanical forces are among the many environmental cues that regulate cell behavior and function. However, the processes by which forces are transduced to biochemical signals and subsequently translated into downstream effects are poorly understood; indeed, are among the major outstanding questions in biology. The importance of mechanical signaling is especially clear in the vascular system, where forces required for blood circulation are critical determinants of not only morphogenesis and function, but also pathological states such as hypertension and atherosclerosis. Recent work on mechanotransduction in the vasculature suggests connections between normal physiological regulation and the development of atherosclerosis.

Vascular Physiology and Mechanical Forces

Endothelial cells (ECs) in the vasculature experience both tensile stretch circumferentially along the vessel wall from blood pressure, and shear stress, the frictional force per unit area from blood flow parallel to the vessel wall (Figure 1A). Whereas vascular smooth muscle cells (VSMCs) appear to respond mainly to stretch, ECs are highly sensitive to shear stress. To maintain vascular homeostasis, ECs respond to acute increases in shear by releasing vasoactive factors such as nitric oxide (NO) and prostaglandin (PG) I_2. These factors rapidly induce smooth muscle relaxation, thereby increasing vessel diameter to accommodate higher flows, which brings shear stress back to baseline levels.[1] Another group of dilators, termed endothelium-derived hyperpolarizing factors (EDHF), act by opening potassium channels that lead to endothelium-dependent hyperpolarization of VSMCs, also promoting vasodilation.[2] EDHF encompasses a variety of chemicals, including metabolites of arachidonic acid, potassium ions, and the C-type natriuretic peptide. Conversely, acute decreases in shear result in the release of factors such as thromboxane A_2, superoxide anion, hydrogen peroxide, endothelin-1, and angiotensin II that stimulate smooth muscle contraction.[3]

On longer time scales, VSMCs and ECs can adapt to changes in shear stress by altering vessel morphology. Surgical manipulations that increase laminar shear in arterial segments induce remodeling over several weeks to increase luminal diameter.[4] A study of flow-induced arterial remodeling in rat mesenteric arteries suggested that this response involved hyperplasia of both ECs and vascular smooth muscle cell (VSMCs), and increased synthesis of connective tissue in the vessel wall.[5] Conversely, reducing blood flow in the common carotid artery of rabbits stimulated an EC-dependent decrease in vessel diameter that was not relieved by a VSMC relaxant, suggesting structural changes to the vessel wall rather than increased VSMC contraction.[6] Interestingly, NO mediates not only acute responses to increased shear stress but also negatively regulates VSMC proliferation in response to remodeling stimuli.[7] Thus, ligation of the left carotid artery in endothelial NO synthase $(eNOS)^{-/-}$ mice did not induce decreases in arterial lumen diameter as seen in wild-type mice; instead, arterial wall thickness increased through proliferation of VSMCs. Further clues to remodeling were found in the canine heart, where surgical constriction of the left circumflex artery that increased flow in collateral arteries led VSMC to assume a synthetic phenotype, similar to areas of endothelial injury and early atherogenesis.[8,9] These vessels also showed increased deposition of fibronectin (FN)

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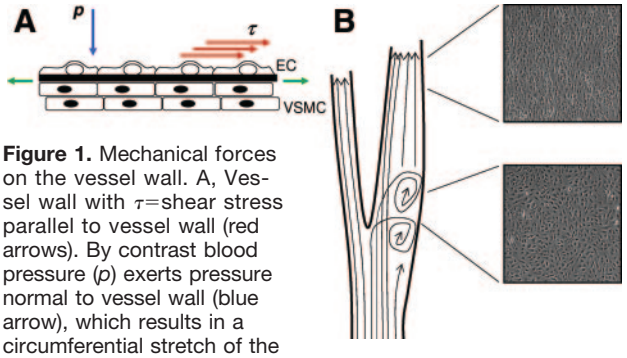

Figure 1. Mechanical forces on the vessel wall. A, Vessel wall with τ=shear stress parallel to vessel wall (red arrows). By contrast blood pressure (p) exerts pressure normal to vessel wall (blue arrow), which results in a circumferential stretch of the artery wall (green arrows). B, Vascular bifurcation with disturbed flow at outer walls where atherosclerotic plaques are found. Endothelial cells align in atheroprotected but not atheroprone regions where flow is disturbed.

into the extracellular matrix (ECM). Although the exact mechanisms behind arterial remodeling remain poorly understood, in addition to NO, a role for inflammation and cytokine production has been proposed. Genetic manipulation in mouse models identify matrix metalloproteinase (MMP)-9, t-ACE, dopamine β-hydroxylase, iNOS, TLR-4, nNOS, P2X type ATP-receptor, and p22 phox as potential mediators.[9] To summarize, complex mechanisms that operate on multiple time scales govern EC responses to mechanical forces. The net result of these effects is to return forces to baseline levels and maintain vascular homeostasis.

Atherosclerosis and Mechanotransduction

Atherosclerosis, a chronic inflammation of large and medium-sized arteries, is characterized by intimal plaques containing lipids, lipid-laden macrophages, leukocytes, smooth muscle cells and, at later stages, necrotic cores with cholesterol crystals and calcification.[10] Over time, plaques can gradually narrow vessels, limiting delivery of oxygen and nutrients, and causing pain. Plaques can also rupture, resulting in rapid thrombus formation and acute occlusion of arteries, often manifesting as stroke or myocardial infarction. Disease progression is heavily influenced by systemic risk factors such as diabetes, obesity, hyperlipidemia, and smoking history; however, these agents affect the vasculature in relatively uniform ways. By contrast, atherosclerosis originates mainly at arterial bifurcations, branch points, and regions of high curvature. These sites are associated with complex blood flow patterns that include low flow, flow separation, shear gradients, flow reversal and, in limited cases, turbulence, often grouped under the term "disturbed flow" (Figure 1B).[11,12] ECs appear to be unable to adapt to these shear forces and fail to maintain their normal quiescent phenotype. Instead they develop an activated or proinflammatory phenotype and fail to trigger relaxation of smooth muscle in response to increased flow or other agonists, so-called endothelial dysfunction. Both in vivo and in vitro, disturbed flow is associated with higher EC turnover (through both proliferation and apoptosis), increased junctional permeability, impaired alignment, increased oxidative stress, and the expression of cytokines and adhesion receptors such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule (VCAM-1).[13–17] ICAM-1 and VCAM-1 promote leukocyte adhesion to ECs and transmigration into the vessel wall, promoting inflammation.[18] Recent studies focusing on gene expression profiles in porcine aortas suggest that endothelial cells in regions of disturbed flow appear primed for inflammation, shifting to atherogenesis when protective antioxidant mechanisms are overcome.[19]

On the other hand, high levels of unidirectional laminar blood flow in straight segments of arteries exert a protective effect, decreasing EC turnover, promoting alignment, and increasing multiple antiinflammatory, antioxidative mechanisms.[20–22] Of great interest is the transcription factor Kruppel-like Factor 2 (KLF2), which is induced downstream of the mitogen-activated protein kinase (MAPK) Erk5 in atheroprotected regions and appears to play a central role in protective gene expression.[23] Overexpression of KLF2 in human ECs upregulates vasodilatory, anticoagulant, and antiinflammatory pathways, whereas reducing its expression via siRNA causes loss of these atheroprotective mechanisms.[23,24] Sustained laminar flow also downregulates Toll-like Receptor 2 expression, which has been reported to promote inflammatory gene expression in endothelial cells.[25] Thus, high laminar flow appears to promote a quiescent, protected endothelial phenotype through several pathways that modify gene expression. In addition to its effect on gene expression, high laminar flow is suggested to downregulate inflammatory cytokine-mediated signals that contribute to atherosclerotic progression.[26] Preexposure of ECs to high laminar flows can inhibit tumor necrosis factor (TNF)-mediated activation of the MAPK JNK and subsequent VCAM-1 expression through activation of Erk5 and thioredoxin.[27,28] In response to hydrogen peroxide, cells exposed to high laminar flow also no longer activate JNK, in this case via glutathione reductase.[29]

Interestingly, low but unidirectional laminar flow is also believed to be atherogenic.[30] One study reported that restricting flow through the carotid artery induced atherosclerosis in both the upstream region of low flow and the downstream region of disturbed flow; indeed, a more inflammatory phenotype was noted in the upstream, low flow region.[31] However, this difference should be interpreted with caution because one would predict that at a severe constriction, the upstream region should have higher blood pressure than the downstream region. Thus, one cannot interpret these results solely in terms of flow patterns. In any case, low flow is also associated with both failure of ECs to align and sustained inflammatory signaling.[32,33] These results suggest that alignment and the atheroprotective effects of shear require higher shear forces than those that stimulate inflammatory pathways. The mechanotransducers that mediate inflammatory effects may therefore be more sensitive to shear stress than the adaptive mechanisms used to maintain vessel integrity.

Shear stress also modulates the interactions between ECs and VSMCs, which are crucial in determining vascular stability, morphology, and function.[34] In a coculture model of ECs and VSMCs, atheroprone flow applied only to the EC layer resulted in proinflammatory priming in the VSMCs, resulting in expression of MCP-1 and VCAM-1 in these cells; by contrast, atheroprotective flow profiles promoted a quiescent VSMC phenotype.[35] Conversely, coculture with VSMCs increased MAPK signaling and E-selectin expression in ECs,

which could be abrogated by application of laminar shear stress to the EC layer.[36] Both results suggest that application of different types of shear can modulate the communication between these cells to determine vascular phenotype.

Hypertension, which results in circumferential stretch of the artery wall (Figure 1), is also a major risk factor that can substantially accelerate atherosclerotic plaque formation or progression.[37] In hypertension, thickening of the smooth muscle layer leads to decreased elasticity and narrowing of the vessel lumen and may contribute to atherosclerotic plaque size.[38,39] Similar to atherosclerosis, hypertension is associated with increased oxidative stress in ECs and VSMCs, perhaps contributing to atherogenesis.[40] Changes in VSMC behavior appear to be attributable to both direct responses to stretch and to signals from the endothelium. The latter include secretion of endothelin-1 and PDGF, a growth and migration factor for VSMC, and decreased secretion of NO and prostacyclin.[41,42]

Potential Mechanotransducers of Fluid Shear Stress

A number of cellular components have been proposed to participate in cellular responses to flow. Potential mechanotransducers on the cell surface include ion channels, integrins, receptor tyrosine kinases, G proteins, PECAM-1, and VE-cadherin.[14,43] The actin, tubulin, and intermediate filament cytoskeletal networks have long been postulated to mediate mechanical signaling by physically connecting different regions of the cell, allowing force transmission.[44] Although shear stress is applied to the apical domain of the cell, many mechanical signaling events occur at the basal or lateral domains. Imaging of intermediate filaments in cells exposed to shear demonstrated displacement of lateral and basal structures, suggesting force transmission to these sites.[45] Other studies have shown that disrupting cytoskeletal structures, most often actin, inhibits EC responses to flow.[46–48] Thus, force transmission by the cytoskeleton must be involved; however, a more direct role in mechanotransduction per se has not been convincingly demonstrated.

PECAM-1, VE-cadherin, and the VEGF receptor, Flk-1, have been implicated as part of a shear stress-responsive complex.[43] In this model, PECAM-1 functions as the true mechanotransducer that experiences the force and triggers activation of a Src family kinase. VE-cadherin acts as an adaptor protein that brings Flk-1 into the complex, allowing its transactivation by Src. Once activated, Flk-1 stimulates phosphoinositide 3-kinase (PI3K), resulting in downstream signaling events. One critical event downstream of PI3K is the conformational activation of integrins at the basal surface of cells, leading to increased binding to extracellular matrix (ECM) proteins under the endothelium. Signaling of these newly bound integrins stimulates polarized activation of Rho, Rac, and Cdc42 that mediate cell alignment in the direction of flow.[49–51] Integrin-dependent pathways also mediate activation of inflammatory signaling pathways that, in disturbed flow, contribute to atherosclerosis.[50]

Elements on the luminal side of the cell that are directly exposed to flow may also contribute. The plasma membrane shows a local increase in fluidity at the upstream side of the cell relative to the direction of flow.[52] It has been proposed that membrane fluidization activates heterotrimeric G proteins, either directly[53] or through ligand-independent conformational effects on G protein–coupled receptors (GPCR).[54] How these mechanisms are relevant in vivo is unclear; animal studies demonstrated that genetic deletion of kinin, a GPCR ligand, blocked endothelial responses to flow, contradicting the idea that these effects are ligand-independent or even direct consequences of flow acting on the lipid bilayer.[55]

In addition to GPCRs that may be activated by changes in lipid bilayer properties, mechanosensitive ion channels can be gated by tension within the lipid bilayer, suggesting that changes in fluidity or tension could trigger signaling events.[56] Indeed, opening of an inward rectifying potassium channel is detectable within seconds, one of the fastest known responses to shear.[57] These channels contribute to vessel dilation[58] and are inhibited by increasing plasma membrane cholesterol,[59] consistent with the idea that flow-induced membrane fluidization can trigger channel opening. This last effect could possibly contribute to the changes in vascular regulation that are seen in the presence of high cholesterol levels.

A role for caveolin or caveolae in shear-mediated EC responses has also been suggested. Caveolae are plasma membrane invaginations that are particularly abundant in ECs. Caveolin1 (cav1) is the major structural protein of caveolae, which are also enriched in cholesterol and sphingolipids. Caveolae have been proposed to mediate EC responses to flow in vitro,[60,61] and in mice, cav1 expression is required for dilation and Akt signaling in response to acute changes in shear, as well as long-term remodeling after carotid artery ligation to reduce blood flow.[62,63] Furthermore, cav1$^{-/-}$ mice in the ApoE$^{-/-}$ background show decreased atherosclerosis.[64] However, cav1 is also a critical negative regulator of eNOS, and cav1$^{-/-}$ ECs have constitutively active eNOS and elevated NO production.[65,66] Thus, effects of cav1 deletion could be explained through deregulated eNOS; alternatively, cav1$^{-/-}$ mice have elevated high density lipoproteins, reduced hepatic low density lipoprotein (LDL) production and decreased LDL transcytosis across ECs.[67] Thus, a role for cav1 in mechanotransduction per se has not been established.

The endothelial lumen is covered with a glycocalyx that is several hundred nanometers thick.[68] This layer appears to consist, at least partly, of long proteoglycans anchored to the plasma membrane, and may function in transferring force to the plasma membrane. Enzymatic disruption of the glycocalyx attenuates cytoskeletal reorganization and NO release in response to fluid shear stress.[68] However, these treatments would also disrupt proteoglycans at the basal surface of cells that are coreceptors with integrins for ECM proteins.[69] Indeed, these enzymatic treatments also block endothelial responses to osmotic shock, a distinct mechanotransduction response that does not involve shear.[70] Thus, a definitive role for the glycocalyx in flow sensing remains to be established.

Another luminal structure, the primary cilium, is a rod-like, microtubule-containing structure, initially implicated in sensing low levels of shear in the kidney.[71] Interestingly, the polycystin-1 and -2 proteins involved in flow sensing in the kidney are also found in endothelia, and both humans with

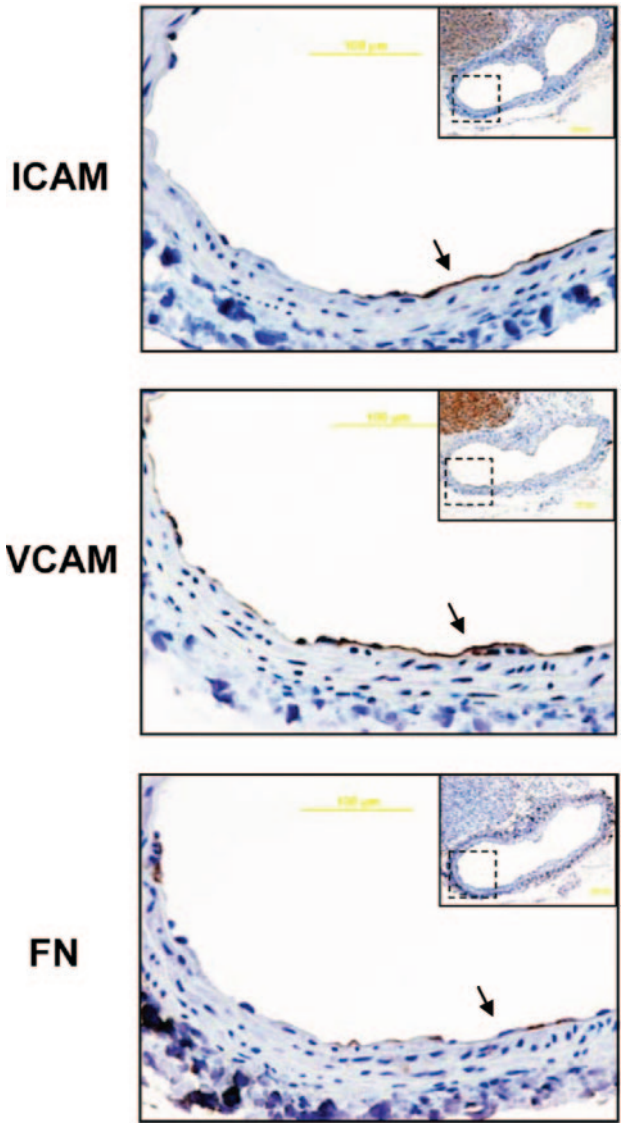

Figure 2. Matrix remodeling and inflammation. Sections through the carotid bifurcation stained for inflammatory markers (ICAM-1 and VCAM-1) and for fibronectin (FN). These markers are expressed only in the atheroprone region of the carotid sinus and correlate well with each other. Adapted from Orr et al.[76]

mutations in the polycystin genes and polycystin1$^{-/-}$ mice show vascular defects.[72] Primary cilia are observed on endothelia only rarely; in cultured ECs, high shear triggers their disassembly.[73] However, primary cilia were recently found on a fraction of ECs in vivo, specifically at atherosclerosis-prone sites, potentially acting as signal amplifiers.[74] Cilia may therefore contribute to sensing low levels of flow at these sites, but more work is required to understand their potential roles in atherosclerosis.

Extracellular Matrix

The subendothelial ECM at atherosclerosis-prone sites shifts from a normal basement membrane (BM) consisting mainly of collagen (Coll) IV and laminin (mainly laminins 9 and 10 in endothelial BM) to proteins characteristic of wounds and inflammation such as fibronectin (FN), fibrinogen (FG), and thrombospondin[75,76] (Figure 2). In vivo, FN and FG correlate with expression of inflammatory markers such as ICAM-1 and VCAM-1 in ECs, whereas in vitro, FN and FG promote expression of these proteins.[76] BM-binding integrins are commonly associated with a quiescent cell phenotype, whereas FN- and FG- binding integrins trigger signals that promote cell proliferation and activation.[69] The changes in ECM composition at atheroprone sites are therefore consistent with the locally activated endothelial phenotype. Because some shear signals are mediated by integrin activation and binding to ECM, the ECM should be able to influence subsequent signaling. Indeed, integrin $\alpha2\beta1$ binding to Coll specifically activates p38 MAPK during cell adhesion,[77] and in response to shear.[76] Integrin $\alpha5\beta1$ and $\alpha v\beta3$ binding to FN specifically activate the transcription factor NF-κB in both situations.[76,78] Shear stress also activates the kinase PAK in cells on FN, but not on Coll or BM protein, and this pathway is linked to increased endothelial monolayer permeability in response to shear in cells plated on FN.[79] The reasons for ECM remodeling in athero-prone regions are unknown, but in vitro, flow can modulate both the levels and organization of ECM proteins.[80,81] These data suggest that the initial changes to the ECM in atheroprone regions may sensitize the endothelium to the proinflammatory effects of disturbed flow, forming a positive feedback loop that further supports inflammation.

Downstream Signaling, ROS, and Inflammation

Laminar shear initially stimulates signaling events including activation of focal adhesion kinase, Shc, Rho family GTPases, and production of reactive oxygen species (ROS). Over time, as cells align, these events are downregulated and cells acquire a quiescent, noninflammatory state. Disturbed shear, by contrast, triggers sustained ROS production and expression of inflammatory genes, which correlate with the failure to align.[82,83]

ROS serve as second messengers that stimulate cell cycle progression, cytoskeletal remodeling and gene expression in many systems.[84] In ECs, ROS are important second messengers in regulation of cell growth, migration, proliferation, and survival.[85,86] ROS initially seen in response to laminar shear are produced mainly through the NADPH oxidase.[87] Eventually, sustained laminar shear downregulates ROS through the downregulation of Rac, which is upstream of the NADPH oxidase,[50] the upregulation of antioxidant genes such as MnSOD and the peroxiredoxin PRX1[88] and the downregulation of NADPH oxidase genes.[87,89]

Sustained ROS production in oscillatory shear also depends on the NADPH oxidase.[90] In addition, ROS-dependent signaling in disturbed shear is further enhanced by the downregulation of antioxidant genes[87] and upregulation of secreted factors such as BMP4.[91] Disturbed flow appears to produce sustained ROS through both a failure to downregulate the transient signals associated with laminar shear and through changes in gene expression that promote ROS production. Prolonged high production of ROS push ECs toward an oxidative state, with consequent activation of MAPKs and NF-κB, promoting endothelial dysfunction and inflammation.[92] Oxidant stress is also linked to the production

of oxidized LDL, which can stimulate activation of multiple inflammatory pathways,[93] further promoting atherogenesis.

Adaptive/Maladaptive Signaling

These results led us and others to hypothesize that the normal adaptive responses to shear that mediate vascular homeostasis become maladaptive under conditions where shear pathways are chronically stimulated and other risk factors are present.[94] Evidence suggests that the failure to align and adapt under low or disturbed shear results in sustained signaling through multiple mechanisms, including production of ROS and activation of MAPK and NF-κB. These pathways also promote expression of genes that sustain the activated, proatherogenic state, while downregulating antioxidant genes. Additionally, the deposition of ECM proteins such as FN and FG that promote proliferation, survival, and remodeling further contribute to maintenance of an activated state. Many of these events contribute to homeostasis in response to short-term changes in laminar shear. Some events, such as matrix remodeling, contribute to repair and remodeling of vessels under stress. However, in response to the chronic stimulation under disturbed flow, they can eventually lead to the formation and progression of atherosclerotic plaques.

Conclusion

A pressurized vascular system is a wonderful evolutionary advance for efficiently moving blood to the tissues to increase metabolic output. Optimal functioning of this system requires that the resultant mechanical forces modulate the structure and function of the cardiovascular system. Thus, ECs and VSMCs have multiple mechanisms that sense forces and promote homeostasis. However, evolution often has little concern for unfavorable developments later in life. Disturbed and low flow profiles are an unavoidable consequence of vessel anatomy. We propose that the same signaling responses that promote homeostasis in most of the vasculature may be maladaptive at these sites because of chronic stimulation of signaling pathways involved in tissue remodeling. A deeper understanding of the mechanisms involved in mechanosensing, downstream signaling events, and synergy with systemic risk factors is needed to identify potential therapeutic targets that specifically modulate the pathological aspects of these responses.

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Disclosures

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