Atherosclerosis is characterized by an accumulation of smooth muscle cells and a buildup of lipid-rich foamy macrophages within the arterial wall, which can lead to lumen narrowing and reduced blood flow or to a complete arterial blockage after plaque rupture. Atherosclerosis development is influenced by several factors, including age, sex, cholesterol level, and obesity. Despite the systemic nature of these risk factors, atherosclerotic plaque formation is remarkably localized, forming predominantly around arterial branches and the inner curvature of bends. Blood flow is a major influence on localized atherosclerosis development, because the location of plaques correlates with specific flow patterns. Regions of the artery wall where blood flow is low or disturbed are prone to atherosclerosis, whereas arterial regions exposed to high flow are protected. Flow affects molecular transport from the bloodstream to the endothelium, because different flow conditions directly influence the local concentration of molecules and their interaction time with the artery wall. In addition to modifying mass transport, blood flow exposes the endothelial layer to wall shear stress (WSS; the friction-like drag between the fluid and the arterial wall surface) and other mechanical actions. Flow-induced WSS varies over time in both magnitude and direction in a characteristic pattern that is dependent on the location within the arterial system (Figure 1). There are several metrics that can be used to characterize a stress pattern (Figure 1), including time-averaged WSS magnitude, oscillatory shear index (a figure of merit describing the fraction of the profile that the instantaneous WSS opposes the mean WSS direction during one oscillation), angular direction of shear stress, and harmonic frequency components. There is ongoing debate on the relative importance of these parameters in endothelial responses to WSS; however, it is plausible that mechanoreceptors integrate multiple mechanical parameters to convert information on WSS magnitude, frequency, and direction into biochemical signals. Although many interrelated signaling networks have been identified, understanding of mechanically triggered signaling is still developing. Here, we review established, biochemically triggered, inflammatory mechanisms before discussing recent progress in uncovering the role of mechanical cues in inflammatory pathways and possible future directions of research.

**Key Words:** endothelium • inflammation • shear stress

Atherosclerosis is promoted by the recruitment of monocytes from the blood to the vessel wall via interactions with...
activated endothelial cells (EC). Proinflammatory mediators (eg, monocyte chemotactic protein-1, interleukin-8, tumor necrosis factor-α, and interleukin-1) and selectins orchestrate the capture, rolling, and activation of monocytes by EC. As a result, monocytes undergo firm adherence in response to integrin activation and interaction with EC adhesion molecules (eg, vascular cell adhesion molecule-1 [VCAM-1]) before subsequent transmigration of monocytes through the endothelium to the intima. Studies using cultured EC or in vivo models demonstrated that WSS is an important regulator of the leukocyte adhesion cascade. Cultured EC exposed to physiological levels of unidirectional WSS exhibit reduced monocyte adherence and expression of inflammatory adhesion molecules and promote monocyte transmigration. Similarly, VCAM-1 is expressed in a porcine aortic arch, showing the wall shear stress (WSS) profile to the intima. Studies using cultured EC or in vivo models demonstrated that WSS is an important regulator of the leukocyte adhesion cascade. Cultured EC exposed to physiological levels of unidirectional WSS exhibit reduced monocyte adherence and expression of inflammatory adhesion molecules and promote monocyte transmigration. Similarly, VCAM-1 is expressed preferentially in EC at atherosusceptible sites exposed to low WSS and the induction of low WSS conditions in experimental arteries (via ligation or application of a constructive cuff) potentiated vascular inflammatory processes.

Flow-Responsive Inflammatory Mechanisms

The inflammatory mechanisms underlying the atherogenic process include the mitogen-activated protein kinase (MAPK) pathways that regulate the activity of activator protein 1 (AP-1) superfamily transcription factors and the nuclear factor-kB (NF-κB) pathway (Figure 2). The role of NF-κB and MAPKs in atherogenesis has been established by the observation that selective ablation of NF-κB or deletion of c-Jun N-terminal kinase 2 (JNK2) significantly reduced atherosclerotic lesion development in murine models. Because of these considerations, there has been extensive research on the effects of various stimuli/stresses on these pathways; this review focuses on the effects induced by WSS (summarized in Figure 2).

Signaling to NF-κB

Activation of NF-κB is essential for inflammatory activation of EC under various stress conditions, including hypoxia and ischemia. It is therefore not surprising to find that WSS has profound effects on NF-κB activation. The proinflammatory transcription factor is normally sequestered within the cytoplasm by its inhibitor IκB (inhibitor of κB), which on stimulation is phosphorylated by IκB kinase (IKK), an enzyme that leads to the nuclear translocation of NF-κB subunits, including p50 and p65, which then bind to DNA and regulate the expression of genes involved in inflammatory processes. NF-κB activation is crucial for the expression of inflammatory genes, including adhesion molecules, cytokines, and chemokines, which are involved in the recruitment and activation of inflammatory cells.

Pioneering studies from Martin Schwartz’s laboratory revealed that activation of the platelet endothelial cell adhesion molecule-1/vascular endothelial cadherin/vascular endothelial growth factor receptor 2 mechanosensory complex by the acute induction of flow leads to conformational activation of integrins that subsequently bind to cognate extracellular matrix molecules. This process induces outside-in integrin signaling that enhances focal adhesion kinase–dependent phosphorylation and transcriptional activation of NF-κB. NF-κB activation in response to acute flow is also dependent on degradation of IκBα by Ral GTPase-induced reactive oxygen species (ROS). Therefore, both ROS and focal adhesion kinase signaling events are essential for nuclear translocation and transcriptional activity of NF-κB in response to acute flow. Although it has been shown that the content of extracellular matrix differs between athoprotective (fibronectin-rich) and atheroprotected (collagen-rich) regions, disturbed flow in combination with integrin–fibronectin interaction is required for sustained NF-κB and JNK activation. Furthermore, a signaling pathway involving the adaptor protein Shc has been suggested as the molecular switch that coordinates interaction between cell–cell and cell–extracellular matrix, resulting in the initial extracellular matrix–independent or matrix–dependent activation of extracellular-signal–regulated kinase (ERK) on acute onset of flow to follow by extracellular matrix–dependent NF-κB activation.

Physiological mechanisms are complex, and arterial WSS varies in terms of magnitude, direction, spatial gradient, and frequency. In vitro studies showed that NF-κB is highly sensitive to variation in both the magnitude and direction of flow. For example, the activation of inflammatory pathways is dependent on the angle that the shear stress is applied to the endothelial cell and is sensitive to oscillations in magnitude, even when the shear stress direction is constant. However, nonidentical shear stress patterns with similar WSS and oscillatory shear index trigger divergent NF-κB activation, indicating that other characteristics of the stress profile, such as the harmonic frequencies, play an important role in mechanoresponsiveness. Complexity in the characteristics of the mechanical stimulus has hindered the understanding of how divergent mechanoresponses are triggered. It is only recently that single-interfering RNA knockdown in vitro studies have indicated that platelet endothelial cell adhesion molecule-1 differentially modifies NF-κB activation between high- and low-amplitude bidirectional oscillatory flow. This indicates that a single mechanoceptor may discriminate between different stimuli, although the molecular mechanism behind this process is unclear and it is uncertain how the activation level relates to different flow characteristics, especially the high unidirectional flow conditions linked to atheroprotection.

Chronic exposure of EC to WSS has strikingly different effects on inflammatory signaling compared with acute exposure. High unidirectional WSS (mimicking flow at atheroprotected sites) reduces signaling to NF-κB by inducing the transcription factor Krüppel-like factor 2 (KLF2) that inhibits the expression of adhesion molecules and E-selectin in response to proinflammatory stimuli through sequestering the transcriptional coactivator CBP/p300.

Sustained uniform flow also limits NF-κB activation by activating endothelial nitric oxide synthase that produces...
nitric oxide to reduce activation of the positive regulator IkB kinase. The function of NF-κB is also altered by flow because unidirectional WSS suppresses NF-κB–dependent inflammatory responses while simultaneously priming EC for NF-κB–dependent cytoprotective and anti-inflammatory responses. A stress-sensitivity regulator HuR (Hu-antigen R), which normally increases the stability and translation of certain mRNAs through high-affinity binding, has been shown to be upregulated in atheroprone regions in mouse en face preparation. Interestingly, the protein downregulates KLF2 to promote NF-κB activation via a mechanism independent of its mRNA stabilization function. Consistent with these observations, en face staining and microarray studies revealed higher expression of NFκB/IκB components at atherosusceptible regions of arteries. Transcriptional profiling of adult porcine aorta also identified the deubiquitinating enzyme cezanne to be enriched in EC at a protected site compared with an atheroprone region. Because cezanne is a negative regulator of NF-κB, future work examining its potential role in atherogenesis is warranted.

Apart from WSS, stretch may also induce activation of NF-κB at atheroprone sites through ROS production. NADPH (nicotinamide adenine dinucleotide phosphate-oxidase) oxidases (Nox) and Nox4 are responsible for ROS production in the endothelium, with the latter being more dominantly expressed. Although they are localized to different subcellular compartment in EC and respond differentially to stimuli such as tumor necrosis factor, angiotensin II, and insulin, their expression is upregulated by oscillatory flow and downregulated by pulsatile laminar flow in vitro. The use of EC-specific Nox2 knockout mice has confirmed the contribution of Nox2-produced ROS in atherogenesis; however, the role of Nox4 remains uncertain.

**Signaling to MAPK**

Two key shear-responsive transcription factors in the endothelium, KLF2 and nuclear factor erythroid 2–related factor 2 (Nrf2), are known to modulate MAPK signaling. High WSS induces KLF2 expression via the MAPK kinase 5/ERK5/myocyte enhancer factor 2 signaling cascade. KLF2 has been shown to inactivate proinflammatory AP-1 family transcription factors by inhibiting phosphorylation and nuclear localization of c-Jun and ATF2. The underlying mechanism involves formation of actin fibers in a RhoA-dependent, Rho kinase–independent manner, which results in inhibition of JNK. On phosphorylation and subsequent nuclear export of histone deacetylases 5 under high WSS, transcriptional activity of myocyte enhancer is enhanced to promote KLF2 and endothelial nitric oxide synthase expression. In addition, high WSS leads to dissociation of cytoplasmic Nrf2 from its suppressor kelch-like ECH-associated protein-1 and translocation of Nrf2 into the nucleus in a phosphoinositil 3-kinase/Akt-dependent pathway. In addition, Nrf2 activity is enhanced by KLF2 that promotes its nuclear localization. Nrf2 affects MAPK signaling and exerts its anti-inflammatory...
effect at protected sites by 2 distinct mechanisms. First, Nrf2 suppresses upstream activators of p38, MAPK kinases 3 and 6. Second, Nrf2 enhances activity of MAPK phosphatase-1 (MKP-1), a negative regulator of p38 and JNK, by altering its redox state and promoting the catalytically active, reduced form of MKP-1. This leads to suppressed expression of the adhesion molecule VCAM-1 at atheroprotected regions of the aorta. Interestingly, lack of MKP-1 protects against atherosclerosis in the apolipoprotein E–deficient mouse model via its effect on macrophage migration and activation of ERK pathway. This suggests that MKP-1 can have both atheroprotective and atherospromoting roles, depending on the cellular context. Therefore, generation of EC–specific knockouts of Nrf2 and MKP-1 is warranted to elucidate the role of endothelial Nrf2 and MKP-2 and MKP-1 in atherosclerosis. A mechanism of thioredoxin interacting protein, a scaffold protein belonging to the α-arrestin family whose expression in EC is increased by disturbed flow both in vitro and in vivo, showed that thioredoxin interacting protein has an essential role in mediating the expression of cell adhesion molecules (eg, VCAM-1) and in EC–leukocyte adhesion under disturbed flow. The mechanism involves transcriptional corepression of RELA by thioredoxin.55 It has been shown that protein kinase C activity is increased in atheroprotective regions of the porcine aorta and in murine atherosclerotic lesions.56 High WSS prevents cleavage of protein kinase Cζ into a truncated form (CATζ [catalytic domain of PKCζ]), which is associated with a higher kinase activity and enhanced tumor necrosis factor-α–mediated JNK activation.57 In addition, a recent study has demonstrated that protein kinase Cζ negatively regulates high shear–induced expression of endothelial nitric oxide synthase via inhibitory phosphorylation of ERK5 MAPK.58 Finally, WSS also regulates cross-talk between the MAPK and NF-κB pathways. For example, using a flow-altering, constractive cuff model, it was shown that low or oscillatory shear stress enhances RelA (v-rel avian reticuloendotheliosis viral oncogene homolog A) expression and promotes inflammation in murine arteries by activating JNK signaling.59

Flow-Responsive MicroRNAs

There has recently been a great interest in the role of microRNAs in atherogenesis. Of note, numerous flow-sensitive microRNAs have been identified, which modulate endothelial inflammatory processes. MicroRNA-10a is a documented negative regulator of canonical NF-κB signaling. This microRNA is suppressed by disturbed WSS, and therefore NF-κB signaling is promoted under such conditions, with subsequent expression of inflammatory molecules (Figure 2).60 Furthermore, microRNA-633 promotes monocyte adhesion in EC exposed to oscillatory shear stress.61 Conflicting studies propose alternate roles for microRNA-21 in endothelial inflammation. Human umbilical vein endothelial cells exposed to oscillatory WSS exhibited increased AP–dependent microRNA-21 expression, leading to inflammatory activation.62 However, microRNA-21 has also been shown to be induced in human umbilical vein endothelial cells in response to high WSS, where it enhances endothelial nitric oxide synthase activity and reduces apoptosis.63 Numerous studies have described a role for microRNA-92a in flow-mediated inflammatory processes. Sites of disturbed flow in the aorta of both mice and pigs exhibited increased expression of microRNA-92a,64,65 which inhibited the expression of anti-inflammatory factors such as KLF2, KLF4, and suppressor of cytokine signaling 5 (Figure 2).66 Despite these insights, the role of microRNAs in flow-mediated inflammation is an emerging field, and further studies are required to assess the roles of these molecules in NF-κB and MAPK regulation.

Flow-Induced Endothelial Microparticles

Endothelial microparticles (EMPs) are circulating small membrane vesicles released from endothelia in response to apoptosis, cell injury, or activation, where their content can be transferred to target cells to initiate signaling events.67,68 Although their unique compositions are determined by the stimuli triggering their release in vitro,69,70 hemodynamic forces have been proven to be a major determinant of EMP release in vivo.71 The inversed relationship between WSS and EMP level was observed and potentially regulated via 2 mechanisms: first, LSS (laminar shear stress) activates ROCK (Rho-associated protein kinase) and ERK1/2, leading to cytoskeletal reorganization and EMP release, and second, down-regulation of nitric oxide suppresses ABCA1 (ATP-binding cassette, sub-family A, member 1) that mediates the phosphatidylinerine exposure at the outer layer of plasma membrane, a hallmark of EMP production. Because EMPs can be found in healthy individuals, their level increases in certain diseases with endothelial dysfunction, thus emerging as a potential biomarker.72 In addition, although EMPs have been suggested to promote cardiovascular disease progression, their protective roles in tissue repair and cell survival have been also reported.70 Therefore, additional studies are required to understand the detailed mechanisms underlying the complex role of EMPs, including the effect of shear stress on their production and function.

Future Perspectives and Outstanding Questions

Systems Biology Approaches

Proteomics and microarray techniques have proven to be powerful tools for the unbiased identification of differentially expressed proteins, mRNA, and noncoding RNAs.73,74 Such systems biology approaches have provided novel insight into the molecular mechanisms underlying complex diseases such as atherosclerosis.75 However, although omics techniques have been used to identify WSS-regulated genes, technical (eg, sampling techniques, bioinformatics tools) and biological variation (eg, cell types, species, age) has led to

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inconsistencies between studies. Therefore, a systemic meta-
analysis of omics data from multiple studies should be per-
determined to determine which factors are consistently regulated by WSS. Genome-wide association studies have identified numerous loci that are associated with atherosclerosis risk,69 and it will be of interest to examine which genes at these loci are also regulated by flow. Because transcriptome studies have identified several hundred flow-regulated genes, there is also a requirement to develop high-throughput screening systems so that hits from array studies can be screened for the abil-
ity to regulate cellular responses to flow. Thus, further work is required to develop high-throughput in vitro systems in which vascular cells can be exposed to flow. Recent studies suggest that zebrafish embryos may also be useful for func-
tional screening of flow-sensitive genes. Zebrafish, which share >80% genes with humans,77 have several advantages for screening studies, including transparency that enables analy-
sis of biological processes in the single cell level. In addition, rapid protocols are available to generate genetically modified zebrafish (eg, using morpholinos,78 transcription activator–
like effector nucleases79 or clustered, regularly interspaced short palindromic repeats).80 Of particular relevance, several shear responsive pathways are conserved between mammals and fish. For instance, shear-dependent expression of KLF2 is conserved in zebrafish and mammals,41 and zebrafish models were used to study the role of primary cilium as a mecha-

nosenor of WSS.41 Thus, zebrafish embryos have potential for large-scale screening of flow-sensitive genes to assess their role in EC homeostasis and dysfunction. In summary, hypothesis-based studies during the past decade have revealed multiple mechanisms that partially explain the effects of WSS on inflammation. Unbiased systems biology approaches are now required to further our understanding of the complex interplay between fluid mechanics and inflammatory signal-

ating networks.

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