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Perspectives Series: Cell Adhesion in Vascular Biology

Biomechanical Activation: an Emerging Paradigm in Endothelial Adhesion Biology

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Introduction

The adhesive properties of the endothelium, the single-cell-thick lining of the cardiovascular system, are central to its biology and pathobiology. In health, the luminal endothelial surface provides a relatively nonadhesive, nonthrombogenic container for the cellular and macromolecular constituents of the blood. Specialized adhesive molecules localized at the lateral cell-cell junctions control transendothelial permeability and the movement of leukocytes from the blood into the tissue spaces of the body. Along its basal aspect, focal adhesion complexes, consisting of transmembrane integrins and associated intracellular proteins, physically link the extracellular matrix to cytoskeletal elements, providing both stability and plasticity to the vascular lining. In disease, these various adhesive interactions can undergo dramatic changes. As is highlighted by the articles in this Perspectives Series, the molecular biological analysis of endothelial adhesion pathobiology has led to the discovery of novel families of molecules (e.g., the selectins), as well as a more dynamic appreciation of their mutual interactions (e.g., the leukocyte-endothelial adhesion cascade). This knowledge has added much to our basic understanding of vascular biology, as well as the pathophysiology of clinically important processes, such as acute and chronic inflammation, atherosclerosis, angiogenesis, vascular injury and repair, and developmental malformations. In certain instances, these insights have provided the basis for the rational design of promising new therapeutics for cardiovascular disease.

A central premise of modern vascular biology is that the endothelial lining is a dynamically mutable interface, locally responsive to various stimuli originating from the circulating blood and/or neighboring cells and tissues, and thus can actively participate in the physiological adaptation or pathophysiological dysfunction of a given region of the vasculature (1). From a teleological standpoint, the endothelium appears ideally suited to function in this capacity, given its unique anatomical position between blood and tissues, and its ability to generate an impressive repertoire of biological effectors, (e.g.,

nitric oxide, eicosanoids, cytokines, growth stimulators and inhibitors, vasoactive peptides, pro- and anticoagulants, and fibrinolytic factors). Early studies of the mechanisms underlying this plasticity of endothelial phenotype identified certain pro-inflammatory substances, such as cytokines and bacterial products, as important stimuli regulating the activity and expression of many of these effectors. The reproducibility of this humoral mode of stimulation, and its wide-reaching pathophysiological implications, has led to its extensive study, in both *in vitro* and *in vivo* experimental models, as a biochemical paradigm of endothelial activation (2, 3). In addition to these humoral stimuli, endothelial cells also are constantly exposed to a spectrum of hemodynamic forces generated by pulsatile blood flow. These forces include hydrostatic pressures, cyclic strains, and wall shear stresses. There is increasing evidence that these biomechanical stimuli can directly influence endothelial structure and function, acutely and chronically, thus constituting a novel paradigm of endothelial activation (Fig. 1). This brief editorial review will provide a perspective on the role of biomechanical forces, alone and in conjunction with humoral stimuli, in modulating the adhesive interactions of vascular endothelium in health and disease.

Activation of endothelium by humoral factors: an established paradigm

In the early 1980s, inflammatory cytokines such as IL-1 or TNF, and bacterial products such as gram-negative endotoxins were shown to act directly on cultured human endothelial cells to alter their adhesive properties for blood leukocytes (4). By comparison to previously studied leukocyte-directed activators, such as leukotrienes, activated complement components, or chemotactic peptides, the stimulatory effect of these agents was dramatic in amplitude and resulted in enhanced firm attachment and transmigration of leukocytes in various *in vitro* model systems. These adhesive changes in the cytokine-treated endothelial cell required *de novo* protein synthesis, which was manifested, in part, by the expression of activation antigens at the cell surface (5). Monoclonal antibodies to certain of these neoantigens were effective in blocking leukocyte adhesion and thus enabled the purification and molecular cloning of novel endothelial-leukocyte adhesion molecules (ELAMs),¹ such as ELAM-1 (now designated E-selectin) (6).

1. *Abbreviations used in this paper:* ELAMs, endothelial-leukocyte adhesion molecules; ELAM-1, endothelial-leukocyte adhesion molecule-1; E-selectin (CD-62E); HUVEC, human umbilical vein endothelial cells; ICAM-1, intercellular adhesion molecule-1; LSS, laminar shear stress; MCP-1, monocyte chemoattractant protein-1; SSRE, shear stress responsive element; VCAM-1, vascular cell adhesion molecule-1.

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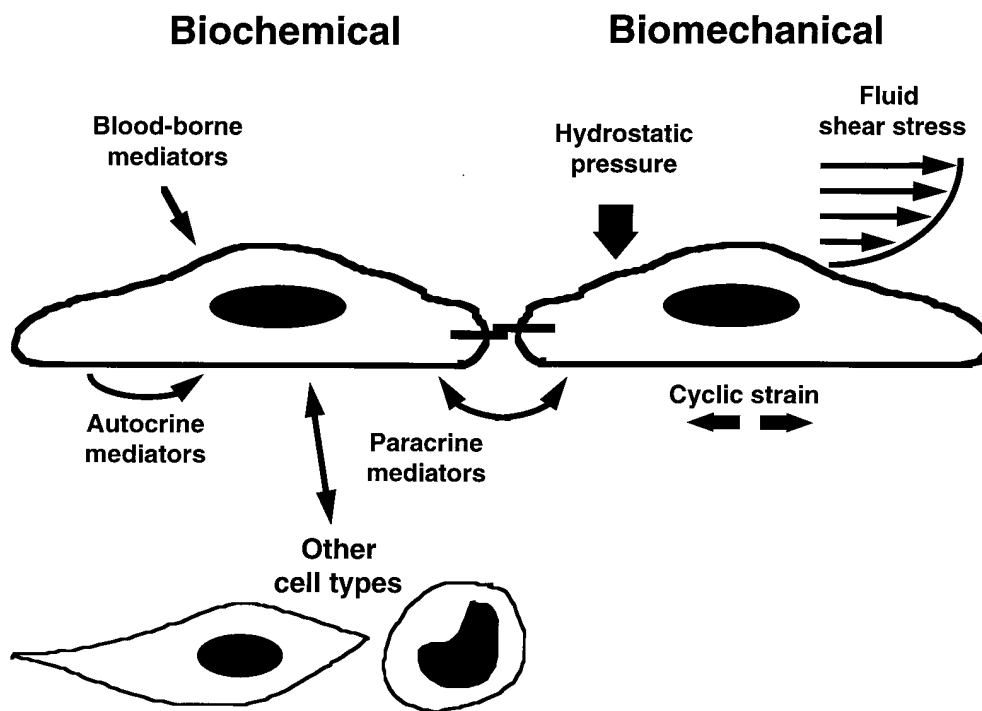


Figure 1. Two paradigms of endothelial activation: biochemical and biomechanical. Situated at the interface between flowing blood and the tissues of the body, vascular endothelium is exposed to both biochemical and biomechanical stimuli that can induce its activation. Biochemical mediators (e.g., cytokines, growth factors, hormones, bacterial products) are delivered via the blood, and/or are produced locally from endothelial cells as well as other cell types (e.g., smooth muscle, pericytes, leukocytes), and can act in an autocrine or paracrine manner. Biomechanical stimuli, generated by pulsatile blood flow, include fluid shear stress, hydrostatic pressure, and cyclic stretching. Both types of activating stimuli can result in altered gene expression in the endothelium. (Figure modified from reference 9.)

These cytokine-activated endothelial cells also expressed increased amounts of other adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), as well as chemoattractant cytokines such as IL-8 and monocyte chemoattractant protein-1 (MCP-1), involved in leukocyte recruitment (7). Concomitant changes in the expression of cell-associated procoagulant proteins (e.g., tissue factor), and fibrinolytic activators and inhibitors, indicated further implications of endothelial activation for hemostasis and thrombosis. Taken together, these *in vitro* studies thus provided a dynamic working concept of the modulation of endothelial phenotype by cytokines and other humoral factors. Immunohistochemical demonstration of endothelial activation antigens in human and animal tissues *in vivo*, in various acute and chronic inflammatory processes, provided further validation of this paradigm (8). In addition to recombinant cytokines and purified bacterial lipopolysaccharides, certain other substances, including polar phospholipids (e.g., lysophosphatidylcholine and related compounds), homocyst(e)ine, and advanced glycosylation end products associated with atherosclerotic and diabetic vascular disease, also have been defined as stimuli of endothelial activation *in vitro* (9). The induction of endothelial-leukocyte adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1), has been identified as a very early event in the development of atherosclerotic lesions in experimental animal models (10). Measurement of soluble ELAMs in circulating blood samples may provide a useful indirect index of endothelial activation in clinical and epidemiological studies (11).

In summary, phenotypic modulation of vascular endothelium by humoral factors, such as cytokines and bacterial endotoxin, has been dissected extensively at the cellular, molecular, and genetic regulatory levels, and at present constitutes the best studied paradigm of endothelial activation. While intricately related to the process of leukocyte recruitment in acute

and chronic inflammation, the biological implications of this process clearly extend beyond cell adhesion.

Activation of endothelium by biomechanical forces: an emerging paradigm

When cultured endothelial cells are exposed to quasiphysiological levels of fluid mechanical forces *in vitro*, in specially designed flow chambers, a spectrum of structural and functional changes is observed (12, 13). These include striking morphological changes in cell shape, cell alignment, and cytoskeletal architecture, as well as more subtle changes in membrane deformability and cell division rate. At a basic cell biological level, many of these changes can be viewed as the *in vitro* (re)adaptation of the cultured endothelial cell to certain of the biomechanical stimuli that are normally present in its *in vivo* environment within the vessel wall. This adaptation process involves various adhesive mechanisms. For example, real-time visualization of a cultured endothelial monolayer exposed to a unidirectional laminar shear stress (LSS) stimulus, using tandem scanning confocal microscopy, reveals a dynamic remodeling of the focal contact sites along its basal aspect (12). Changes also are observed in the phosphorylation state of cytoskeletal proteins associated with these focal adhesion complexes, as well as adhesion molecules such as platelet-endothelial adhesion molecule-1 that are localized to lateral cell-cell junctions (12, 14). These changes presumably are part of a generalized cellular adaptation to applied mechanical stresses, reflected internally in cytoskeletal architecture and externally in cell surface topography (15, 16). The net result of these structural adaptations is an endothelial cell which is in dynamic equilibrium with its ambient fluid mechanical environment.

In addition to these structural adaptations, biomechanical forces such as fluid shear stresses also stimulate the production in endothelium of a large and diverse array of potent biological

Table I. SSREs and Interacting Transcription Factors in Endothelial Expressed Genes*

Gene	Promoter element	Transcription factor(s)	Pattern of regulation	Reference
PDGF-B	5'-GAGACC-3'	NF-κB (p50-p65)	Transient upregulation	29, 30
PDGF-A	Egr-1 sites	Egr-1	Transient upregulation	31
MCP-1	TRE(AP-1) site	AP-1 (<i>c-fos</i> , <i>c-jun</i>)	Transient upregulation	32
Tissue factor	Sp1 sites	Sp1	Transient upregulation	33
TGF-β	ND	ND	Transient upregulation	34
ICAM-1	ND	ND	Transient upregulation	35
VCAM-1	ND	ND	Downregulation	20
Prepro-endothelin-1	ND	ND	Downregulation	36

*Biomechanical stimulus = uniform laminar shear stresses in physiological range; for the genes listed, the putative SSREs have been identified by analysis of mutated promoter-reporter genes in vitro. ND, not defined.

mediators (12, 13). Certain of these effects involve gene regulation at the transcriptional level and thus are analogous to endothelial activation by humoral factors. In this biomechanical paradigm of activation, the endothelial cell appears capable of responding not only to the magnitude of the applied forces but also their temporal and spatial fluctuations (e.g., steady versus pulsatile flow; uniform laminar, disturbed laminar, or turbulent flow regimens), thus suggesting the existence of primary flow sensors (receptors) that are coupled via distinct signaling pathways to nuclear events (12, 13). Considerable progress has been made recently in defining certain of the molecular mechanisms involved, including the identification of positive and negative shear stress responsive elements (SSREs) in the promoters of biomechanically responsive genes, and transcription factors that regulate their activation (9, 13) (Table 1).

In the context of vascular adhesion biology, fluid shear stress thus far has been the best studied biomechanical stimulus of endothelial gene regulation. When cultured human umbilical vein endothelial cells (HUVEC) are exposed to a physiologically relevant range (2.5–46 dyn/cm²) of steady, unidirectional LSS, there is a time-dependent induction of ICAM-1 expression that is evident at the mRNA level by 2 h, and is detectable as functional cell surface protein, by immunobinding and leukocyte adhesion assays, for as long as 48 h of continuous flow exposure (17). This LSS-induced upregulation, in part, reflects enhanced transcriptional activity as determined by nuclear runoff analysis (18). In the same endothelial monolayers, E-selectin (which is normally a silent gene in this cultured cell system) and VCAM-1 (which shows a relatively low level of constitutive expression) remained unchanged in response to LSS. This selective upregulation of ICAM-1, but not E-selectin or VCAM-1, by LSS is in contrast to the coordinated induction of these three ELAMs that is typically observed with humoral (cytokine and bacterial endotoxin) stimulation in the same cultured HUVEC system.

These experimental observations with ICAM-1 suggest that certain biomechanical stimuli might act as differential regulators of endothelial adhesion molecule expression, exerting additive, synergistic, or even antagonistic actions in conjunction with humoral stimuli. Further evidence supporting this working concept derives from the study of VCAM-1, another member of the immunoglobulin family of adhesion molecules, in cultured endothelial cells. This mononuclear leukocyte-selective endothelial adhesion molecule, which has been implicated in atherogenesis (10), can be induced in vitro by cyto-

kines and also components of oxidized lipoproteins such as lysophosphatidylcholine. In cultured murine endothelial cells which have a constitutively high level of VCAM-1, the application of steady LSS suppresses its expression (19). This effect, which is induced by relatively low shear stresses (0.7–7.1 dyn/cm²), is manifested at the level of steady state mRNA and cell surface protein and results in markedly decreased lymphocyte adhesion. Deletional analyses of the VCAM-1 promoter point to the presence of a negative SSRE that mediates this downregulation at the transcriptional level (20). In studies with cultured HUVEC, LSS preconditioning exerts an inhibitory effect on cytokine-induced VCAM-1 expression (21), mimicking the effects of intracellular antioxidants (22). In yet other studies with cultured human saphenous vein endothelial cells, nitric oxide, itself an LSS-regulated endothelial product (23), acts to decrease cytokine-induced VCAM-1 expression (24, 25). These observations serve to illustrate the potential complexity of the interplay of biomechanical and humoral stimuli in the induction and modulation of adhesion molecule expression in vascular endothelium.

To date, the majority of experimental studies of the effects of flow on adhesion molecule expression have used relatively simple in vitro fluid mechanical systems to generate uniform laminar shear stresses on cultured endothelial monolayers. By varying the viscosity of the perfusion medium, the effects of wall shear stresses, per se, can be distinguished from the influence of bulk flow on boundary layer diffusion in the vicinity of the endothelial surface (12). In most cases, however, the shearing force is being applied to a static culture and thus represents an abrupt transition in biomechanical loading of the system. In attempts to better model in vivo hemodynamics, investigators have devised unsteady flow systems that generate temporal and/or spatial fluctuations of fluid shear stresses (12, 13), and have even combined shear stress, pressure, and circumferential stretch in the same in vitro system to generate a quasiphysiological biomechanical environment for the endothelial cell (26).

(Patho)physiological relevance of biomechanical activation of vascular endothelium

As illustrated above, our current knowledge concerning the effects of biomechanical stimulation on endothelial gene expression has been derived largely from experiments in simplified in vitro model systems. This is attributable, in part, to practical limitations on experimental interventions in the in vivo setting. When surgical manipulations have been used to acutely modu-

late wall shear stresses in the rabbit carotid artery, significant flow-dependent changes in endothelial VCAM-1 and ICAM-1 expression were observed (27). However, the most striking example of flow-related changes in vessel wall biology is provided by an experiment of Nature — the nonrandom distribution of the early lesions of atherosclerosis in humans and experimental animals. Arterial bifurcations and curvatures, where disturbed flow patterns (flow separation, flow reversal, low amplitude, and fluctuating wall shear stresses) occur, typically are lesion-prone areas, whereas geometries associated with uniform laminar flow (pulsatile without flow reversal) and relatively constant (time-averaged) wall shear stresses, such as the straight tubular portions of the aorta and its primary tributaries, tend to be lesion-protected areas (9, 18). These patterns also are retained in genetically modified mouse models of atherogenesis, in which systemic risk factors such as markedly elevated levels of atherogenic plasma lipoproteins have been deliberately induced (28). A hallmark of these early lesions in the animal models is the localized upregulation of endothelial VCAM-1, an event that precedes mononuclear leukocyte recruitment (9, 10). These observations suggest that the endothelial cells in these lesion-prone areas are responding differentially to their fluid mechanical environment. Experimental evidence supporting this hypothesis comes from in vitro molecular biological experiments, using RT-PCR-based differential display technology, to examine the patterns of endothelial genes that are (up- or down-) regulated by various biomechanical and cytokine stimuli (18). Uniform laminar shear stress stimulation, characteristically associated with lesion-protected areas, selectively induces the sustained upregulation of a set of genes, including manganese superoxide dismutase, cyclooxygenase-2, and nitric oxide synthase (eNOS), the activities of which (antioxidant, antithrombotic, antiadhesive) are potentially atheroprotective. In contrast, turbulent shear stress, a nonlaminar fluid mechanical stimulus, does not induce these genes. Other flow-regulated endothelial genes with potential proinflammatory, proatherogenic activities, such as E-selectin, MCP-1, and ICAM-1, do not exhibit sustained LSS-selective upregulation. Interestingly, certain novel endothelial genes appear to be selectively induced by laminar shear stress but not by cytokine stimulation (18), thus further illustrating the differential responsiveness of the endothelial cell to biochemical and biomechanical activation (Fig. 1).

In the in vivo setting, a given endothelial cell is constantly being subjected to combinations of various biochemical and biomechanical stimuli, as well as information transduced via integrin-receptors from extracellular matrix components. The phenotype of a given endothelial cell thus represents an integrated response to its local (patho)physiological milieu. Cytokines, growth factors, and other mediators secreted by emigrating leukocytes or adjacent cells within the vessel wall can acutely modify this local environment as part of a response-to-injury program. Biomechanical stimuli, in a manner analogous to extracellular matrix components, appear to contribute in a more sustained way to the regulation of endothelial phenotype. This chronic mode of endothelial activation likely influences the vascular remodeling that occurs in diseases such as atherosclerosis and hypertension, as well as after interventions such as coronary artery bypass grafting and percutaneous angioplasty. Biomechanical modulation of endothelial gene expression, in particular the genes encoding adhesion molecules involved in cell-cell and cell-matrix interactions, may

also play an active role during embryonic development of blood vessels, and at times of hemodynamic transitions (e.g., in the neonatal period). Clearly, biomechanical forces have important implications for endothelial adhesion biology beyond their direct rheologic effects on leukocyte-endothelial interactions (37). The emerging paradigm of biomechanical activation of endothelial cells promises to be a conceptually rich and pathophysiologically relevant area for future investigation.

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