Pharmacological Reports
2008, 60, 139–143
ISSN 1734-1140

Review

Endothelium in health and disease

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Abstract:
The endothelium, which forms the inner lining of blood vessels and lymphatics, participates in many physiological functions. Endothelial cell phenotypes vary in structure and function, in space and time, and in health and disease. The goal of this review is to underscore the importance of phenotypic heterogeneity as a core property of the endothelium.

Key words:
Endothelial cells, endothelium, activation, dysfunction

Introduction

The endothelium forms the inner cellular lining of blood vessels. Endothelial cells are not inert but rather are highly metabolically active. The endothelium plays an important role in many physiological functions, including the control of vasomotor tone, blood cell trafficking, hemostatic balance, permeability, proliferation, survival, and innate and adaptive immunity. The endothelium is involved in most if not all disease states, either as a primary determinant of pathophysiology or as a victim of collateral damage. There exists a wide bench-to-bedside gap in endothelial biomedicine [15]. Clinicians are rarely attuned to the health of this cell layer. The endothelium is not amenable to traditional physical diagnostic maneuvers of inspection, palpation, percussion and auscultation. From a laboratory standpoint, the endothelium continues to elude convenient diagnostic interrogation. The endothelium has enormous untapped potential as a therapeutic target.

Endothelial cell heterogeneity

One of the difficulties in translating from bench to bedside relates to the ill-conceived notion that all endothelial cells are alike. The search for a common or universal phenotype has proved elusive and even defining the endothelium is challenging (reviewed in [2, 3]). From an anatomical standpoint, the endothelium represents the inner cellular lining of the vasculature. However, there are examples of vascular mimicry in which other cell types, e.g. trophoblasts, form the inner lining of blood vessels. Many of the characteristic ultrastructural features of the endothelium, such as Weibel-Palade bodies or fenestrae, are not present in every endothelial cell. Other structures, such as caveolae, are not specific to the endothelium. Developmentally, endothelium arises from mesoderm via the differentiation of hemangioblasts and/or angioblasts. However, other cell lineages may transdifferentiate into endothelial cells, and endothelial cells into other lineages. There are few, if any protein or

mRNA markers that are both specifically and uniformly expressed in the endothelium. From a functional standpoint, the endothelium displays a remarkable 'division of labor'. For example, endothelial cells that line the postcapillary venules are primarily responsible for mediating leukocyte trafficking, while arteriolar endothelial cells regulate vasomotor tone. Finally, when endothelial cell-specific promoters are targeted to the mouse genome, they invariably direct expression in specific subsets of endothelial cells (reviewed in [17]). In summary, each of the above definitions falls short of fully capturing the endothelium. The 'elusiveness' of the endothelium reflects its marked heterogeneity in structure and function.

In the final analysis, the endothelium is best defined not by a single marker or function, but rather by its enormous behavioral repertoire. Phenotypic heterogeneity is not simply a descriptor, but rather is, in and of itself, a core property of the endothelium. Future advances in diagnosis and therapy will rely on a detailed understanding of endothelial cell phenotypes. To that end three questions must be addressed:
1) What is the topography of endothelial cell phenotypes in health and disease? Various groups are presently employing proteomic and genomic strategies to uncover *vascular zip codes* which are amenable to vascular bed-specific targeting [18, 20].
2) What are the proximate mechanisms of EC heterogeneity? Work by our group and others have demonstrated that certain site-specific properties are governed by environmentally responsive (imminently reversible) differences in the extracellular environment, whereas other properties are epigenetically fixed (Fig. 1) [1].
3) What are the evolutionary mechanisms of phenotypic diversity? We have shown that phenotypic heterogeneity is an evolutionarily conserved feature of this cell lineage [26]. It will be important to determine the design constraints, trade-offs and mismatch to modernity that render the endothelium vulnerable to disease.

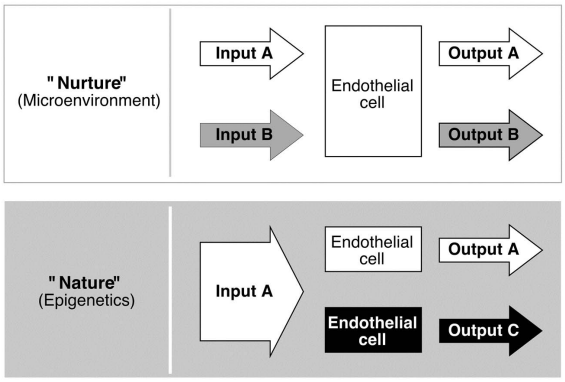

Fig. 1. Proximate mechanisms of endothelial cell heterogeneity. Each endothelial cell may be considered an input-output device. Input arises from the extracellular environment and may include biochemical and biomechanical forces. Output is manifested by the cellular phenotype. This model provides a valuable framework for understanding the molecular basis of endothelial cell heterogeneity. If endothelial cells are intrinsically identical (top), then spatial and temporal differences in input signals (e.g. input **A**, **B**) will result in spatial and temporal differences in output (output **A**, **B**), resulting in heterogeneity. If endothelial cells are epigenetically modified (bottom), then they may display heterogeneous phenotypes (output **A**, **C**) at rest and/or in response an identical input (input **A**). Both mechanisms are operative in the intact organism and contribute to generation and maintenance of endothelial cell heterogeneity

Endothelial cell activation and dysfunction

When considering the role of the endothelium in disease, the two most common terms that are used are *endothelial cell activation* and *endothelial cell dysfunction*. Each of these terms was introduced in the 1980s. Based on advances in the field in the past 20–30 years, the definitions have evolved.

Pober and Gimbrone were the first to demonstrate that a well defined stimulus could induce the expression of an endothelial cell marker [22]. Gimbrone and his colleagues identified the first inducible endothelial cell-specific leukocyte adhesion molecule (ELAM-1; later designated E-selectin) [6, 9, 21]. Subsequent studies by a number of groups demonstrated that numerous inflammatory mediators, including endotoxin, tumor necrosis factor (TNF)-α and interleukin (IL)-1, induced the expression of new antigens (so-called "activation antigens") on the surface of cultured endothelial cells, and was correlated with the expression of pro-adhesive, antigen-presenting and procoagulant activities [4–8, 12, 24]. In 1986, Ramzi Cotran et al. described activation of the endothelium *in vivo* [10].

Though not intended as such, these initial observations have given rise over the years to the notion of the endothelium as a toggle switch, in which the endothelial cells are either quiescent ("off") or activated ("on"). According to this view, quiescent endothelial cells express an anticoagulant, anti-adhesive and vasodilatory phenotype, whereas activated endothelial cells express procoagulant, pro-adhesive and vasoconstricting properties. However, endothelial cell ac-

tivation is not an all-or-none phenomenon. Rather, in keeping with the theme of heterogeneity, endothelial cells display a spectrum of response (Fig. 2). Moreover, *activated* and *active* are not synonymous. Normally, endothelial cells are highly active, sensing and responding to cues within the extracellular environment. Finally, the term *activation* does not address the cost of the phenotype to the host. In other words, activation may be adaptive or non-adaptive.

By contrast, endothelial cell dysfunction is by definition maladaptive. Early descriptions of endothelial cell dysfunction focused on structural changes or loss of anatomical integrity, particularly in the context of atherosclerosis. In 1973, Russell Ross and John Glomset proposed a response-to-injury hypothesis to explain the lesions of atherosclerosis [23]. Subsequent to Ross's hypothesis, there was a growing appreciation that the *intact* endothelium may actively contribute to disease initiation and/or progression [25]. Gimbrone employed the term *endothelial cell dysfunction* in 1980 to describe hyper-adhesiveness of the endothelium to platelets [13]. In 1986, Ganz and colleagues demonstrated paradoxical vasoconstriction of coronary arteries induced by acetylcholine in early and advanced human atherosclerosis, suggesting that abnormal vascular response to acetylcholine may represent a defect in endothelial vasodilator function [16].

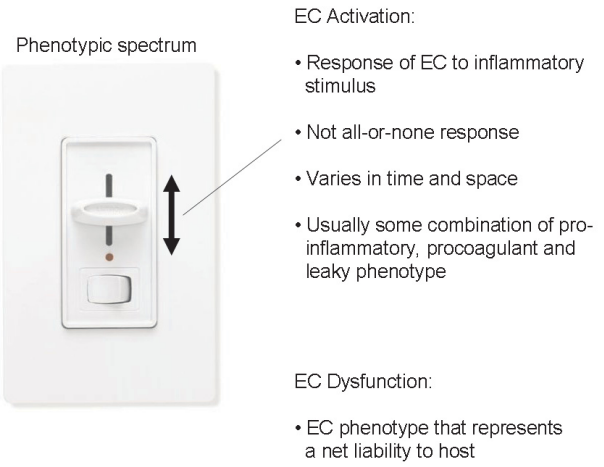

Fig. 2. Endothelial cell activation and dysfunction. Endothelial cell activation is defined as the phenotypic response to an inflammatory stimulus; endothelial cell dysfunction as any phenotype of the endothelium that poses a liability to the host. The endothelial cell is not a toggle (on-off) switch, but rather is more akin to a dimmer switch in which the phenotype displays a spectrum of response. EC, endothelial cell

Cybulsky and Gimbrone were the first to hypothesize a pathophysiological link between inducible endothelial-leukocyte adhesion molecules and atherosclerosis (so-called athero-ELAMs). Using a combination of in vitro cell culture and monoclonal antibody strategy, they identified an inducible endothelial cell-specific antigen that binds predominantly to monocytes [11]. Peptide sequencing revealed homology to the predicted sequence of human vascular cell adhesion molecule (VCAM)-1, which had been previously cloned as a cytokine-inducible protein in endothelial cells [19]. In support of their hypothesis, Cybulsky and Gimbrone localized VCAM-1 to the endothelium overlying atherosclerotic lesions in a hyperlipidemic rabbit model [11]. These latter observations not only emphasized the role of endothelial dysfunction as a primary determinant of atherosclerosis, but also helped to refocus research and development on the inflammatory nature of this disease process.

Based on their findings, Cybulsky and Gimbrone amended the definition of endothelial cell dysfunction as follows:

"Endothelial cell dysfunction has been implicated in the vasospastic and thrombotic complications that are evident in advanced atherosclerosis. Induction of an adhesion molecule early in atherogenesis may also be considered a manifestation of endothelial dysfunction, in that it results in an abnormally hyperadhesive endothelial cell surface" [11].

Given that the endothelium is multifunctional and highly distributed in space, it is safe to assume that endothelial cell dysfunction is not restricted anatomically to the heart, nor is it limited in disease scope to atherosclerosis. Endothelial cells residing in arteries, capillaries and veins or every tissue and organ other are prone to dysfunction. Gimbrone described endothelial cell dysfunction as:

"… non adaptive changes in endothelial structure and function, provoked by pathophysiological stimuli, (resulting in) localized, acute and chronic alterations in the interactions with the cellular and macromolecular components of circulating blood and the blood vessel wall" [14].

Indeed, the term endothelial cell dysfunction may be broadly applied to states in which the endothelial phenotype – whether or not it meets the definition of activation – poses a net liability to the host. Assigning liability scores is of course a subjective exercise. An

evolutionary biologist might argue that endothelial cell dysfunction is most relevant in its effect on an individual's reproductive capacity. A physician would surely expand the meaning of dysfunction to include a far broader spectrum of morbidity. An investigator interested in applying evolutionary principles to an understanding of endothelium in health and disease would point out that the endothelium evolved to a state of maximal fitness in the early ancestral environment, and is not adapted to withstand the rigors of high fat diet, epidemics associated with high density populations, sedentary lifestyle or old age.

Diagnosis and Therapy

The endothelium is a highly attractive therapeutic target. It is rapidly and preferentially exposed to systemically administered agents. Endothelial cells are malleable and thus amenable to therapeutic modulation. In establishing a dialogue with the underlying tissue, the endothelium provides the pharmacotherapist with a direct line of communication to the various organs of the body.

When applying the concepts of endothelial cell activation and dysfunction to a consideration of therapeutics, it is important to recognize that endothelial cells may be activated (i.e. adaptive) without being dysfunctional (i.e. maladaptive). Examples include wound healing, physiological angiogenesis, and local defense against pathogens and foreign bodies. Therapy is perhaps best reserved for cases in which the phenotype of the endothelium (whether or not it meets some arbitrary definition of activation) represents a net liability to the host. The notion that the endothelial cell is not merely a toggle switch has important therapeutic implications. The goal in treating the endothelium is not to reset the switch to the "off" position, but rather to fine-tune and recalibrate the cell, nudging it back to its ideal state. An important challenge is to learn how to determine the nature of that ideal state. Endothelial cell dysfunction usually arises from otherwise adaptive responses (or at least ones that were adaptive in the ancestral environment) that are now excessive, sustained, or spatially and/or temporally misplaced. The transition between endothelial cell function and dysfunction is not always clear. As more effective treatments become available for attenuating dysfunctional endothelium, it will be important to avoid overshooting the desired effect or "lobotomizing" the cells. In this respect, it will serve us well to remember that an active endothelium is a healthy endothelium. Finally, given that endothelial cell phenotypes vary according to time and location in the vascular tree – in both health and disease – it will be essential to target therapy to specific vascular beds.

Acknowledgments:
This work was supported in part by National Institutes of Health grants HL076540. The author thanks Steven Moskowitz for his art work.

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Received:
January 2, 2008; in revised form: January 9, 2008.