Endothelium and haemostasis

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Summary
The endothelium is a widely distributed organ system that plays an important role in health and disease. The endothelium is remarkably heterogeneous in structure and function. One vital function of the endothelium is to maintain blood in its fluid state, and to provide controlled haemostasis at sites of vascular injury. In keeping with the theme of endothelial cell heterogeneity, endothelial cells from different sites of the vascular employ different strategies to mediate local haemostatic balance. These differences are sufficient to explain why systemic imbalances of haemostatic components invariably lead to local thrombotic phenotypes. An important goal for the future is to identify diagnostic markers that reflect phenotypic changes at the level of individual vascular beds, and to develop therapies that target one or another site of the vasculature.

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Content

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The endothelium, which forms the inner lining of blood vessels and lymphatics, is a systematically distributed organ. It reaches all expanses of the human body with the exception of avascular tissues (e.g., the epidermis, nails, cornea and cartilage). The endothelium was once considered to be little more than an inert layer of nucleated cellophane. However, endothelial cells are now understood to participate in a multitude of physiological functions, including:

- permeability,
- leukocyte trafficking,
- innate and acquired immunity, and
- vasomotor tone.

Moreover, endothelial cell phenotypes vary greatly between different vascular beds. Indeed, rather than forming one giant monopoly of homogeneous cells, the endothelium represents a consortium of smaller enterprises, each adapted to the needs of the underlying tissue.

As an important corollary, endothelial dysfunction leads to vascular bed-specific pathology.

The goal of this review is to consider the scope and nature of endothelial phenotypes and to apply these principles to an understanding of the endothelium in haemostasis and thrombosis.

Endothelial heterogeneity

Cells

The notion that endothelial cells are heterogeneous is by no means new. In 1966, Sir Howard Florey, who was awarded the 1945 Nobel Prize in Physiology or Medicine for the discovery of penicillin, wrote (1): “A few years ago the endothelial cell was thought of as a structure so thin that few details could be made out in its cytoplasm, while now it is recognized that there are many kinds of endothelial cell which differ from one another substantially in structure, and, to some extent, in function.”

During the 1960s, most of our information about endothelial cell heterogeneity was derived from ultrastructural studies. For example, electron microscopy revealed that endothelial cells of arteries and veins form a continuous uninterrupted monolayer, whereas endothelial cells that line certain capillaries are permeated by fenestrae or large gaps. Moreover, structural variation was shown to correlate with functional differences. For example, fenestrated endothelium was noted in those organs that are involved in secretion (e.g., endocrine glands, gastric and intestinal mucosa, and choroid plexus) or filtration (i.e., kidney glomeruli).

The introduction of endothelial cell culture in the early 1970s provided investigators with a powerful new tool to study relatively pure populations of living cells under tightly controlled conditions (2, 3). This technique led to an exponential in-

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crease in the number of publications related to endothelial cells and to enormous advances in our understanding of endothelial cell biology (4). Among these advances was the recognition that endothelial cells express a number of procoagulants and anticoagulants and that endothelial cell activation is associated with a shift in the haemostatic balance to the procoagulant side. A disadvantage of endothelial cell culture is that it encourages investigators to approach the endothelium as a monopoly of identical cells, rather than as a transcendent organ with emergent site-specific properties, whose whole is greater than the sum of the parts. Indeed, the very notion of endothelial cell heterogeneity was largely forgotten or ignored during the heyday of cell culture. The introduction of biochemical techniques in the 1980s and 1990s led to the discovery that endothelial cell proteins are differentially distributed in the intact vasculature. For example, in 1992, a survey of the distribution of von Willebrand factor (VWF) in human tissues found that expression was higher in large vessels compared with capillaries, leading the authors to conclude (5): “A surprising result from this study was the heterogeneous expression of VWF, which is thought to be produced in vascular endothelial cells as part of the coagulation system.”

The reason this finding was surprising: VWF was (and still is) widely touted as a universal marker for endothelial cells in culture. Such discordance in gene expression between in vitro and in vivo settings is not unique to VWF. Indeed, only a small minority of endothelial-restricted genes (e.g., CD31, VE-cadherin and Erg) are uniformly expressed in the endothelium. Previous in vivo phage display studies have uncovered site-specific repertoires of endothelial cell surface proteins, which have been referred to as “vascular zip codes” (6, 7). This metaphor nicely captures the therapeutic potential of targeting drugs to one or another vascular bed.

Mechanisms

Every biological trait, including endothelial cell heterogeneity, requires both a proximate and evolutionary explanation (8).

• Proximate explanations (how?) employ traditional approaches of cell biology and molecular biology to determine the anatomy, physiology and ontogeny (developmental history) of a trait at the level of a single organism.

• Evolutionary explanations (why?) draw on the fossil record and comparative morphology and DNA sequences to uncover the phylogeny (evolutionary history) of a trait as well as the fitness advantage that the trait provides at the level of a population or species.

Proximate mechanisms

Like all living cells, the endothelial cell may be considered an input-output device. Input arises from the extracellular environment and may include any number of biochemical and biochemical signals arising from the blood vessel lumen, the abluminal side and/or neighbouring endothelial cells. The output represents the phenotype of the endothelial cell and varies from single-cell-level changes (e.g., calcium flux, migration, proliferation, apoptosis) to larger scale changes (e.g., angiogenesis, fibrin deposition). Input is coupled to output by a non-linear array of signalling pathways that typically begin at the cell surface and end at the transcriptional or post-transcriptional level. Since endothelial cells are distributed throughout the body, they are exposed to enormously diverse input signals. For example, in the blood brain barrier, endothelial cells receive cues from astroglial cells that are critical for maintaining barrier function, whereas endothelial cells that line the hepatic sinusoids are exposed to nutrient-rich blood on the luminal side and stellate cell/hepatocyte-derived paracrine factors in the space of Disse. Since input varies across the vascular tree, and in so far as the endothelial cell is capable of transducing signals, then output must also vary throughout the vasculature. In other words, the broad distribution of the endothelium is sufficient to explain phenotypic heterogeneity.

So far, this description paints the endothelium as a blank slate, marching blindly to the tune of the microenvironment. However, endothelial cells also express properties that are epigenetically fixed and impervious to changes in the cell’s microenvironment. Viewed from this perspective, endothelial cell heterogeneity is mediated by a combination (9) of

• imminently reversible environmentally responsive signal transduction pathways (nurture) and

• stable epigenetic mechanisms that “lock in” gene expression regardless of the environment (nature).

Understanding the relative roles of nurture and nature has important therapeutic implications. If one wishes to target a phenotype that is mediated by the microenvironment, then treatment may be best directed towards that environment. By contrast, those properties that are more fixed may require targeting of the cell itself.

Evolutionary mechanisms

Comparative studies have revealed that endothelial cells are present in all vertebrates, and absent in all invertebrates (10). Thus, the endothelium evolved in a common ancestor of all extant vertebrates, which lived some time more than 510 million years ago. The hagfish is the oldest extant vertebrate. Previous studies have revealed that hagfish endothelium is heterogeneous in structure and function (11, 12). Thus, endothelial cell heterogeneity evolved as an early feature of this cell lineage. What is the selective advantage of having an endothelium in the first place, and why is it so heterogeneous? These are difficult questions to answer, but it seems likely that the endothelium evolved to optimize flow dynamics and barrier function, and/or to localize immune and coagulation functions, while phenotypic heterogeneity reflects the role of the endothelium in meeting the diverse needs of body tissues, as well as its need to adapt to, and survive in, many different extracellular environments.

Endothelial cell activation and dysfunction

The two most common descriptors for the endothelium in disease are activation and dysfunction. The term endothelial cell activation first appeared in the 1980s when it
was observed that exposure of cultured endothelial cells to inflammatory mediators resulted in 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; reviewed in (13). Today, the term activation is used more widely to describe the response of endothelial cells to an inflammatory stimulus in vitro or in vivo (some investigators in the angiogenesis field employ the term to describe proliferating endothelial cells). The activation phenotype varies according to the stimulus and the vascular bed of origin. However, it often includes some combination of increased permeability and net procoagulant and/or proinflammatory activity. It is important to recognize that while endothelial cell activation describes a phenotype, it does not refer to the cost of that phenotype to the host. In other words, endothelial cell activation may be adaptive or non-adaptive.

In contrast, endothelial cell dysfunction is by definition maladaptive. The term dysfunction was initially applied to the phenotype of the endothelium overlying atherosclerotic lesions in animal models of hyperlipidaemia. Today, the term is almost always used to describe abnormalities in endothelium-dependent vasomotor tone in atheromatous conduit arteries. However, because the endothelium is widely distributed, and because it is involved in so many different functions (over and above the control of arteriolar diameter), the term dysfunction should be used more broadly to describe situations in which the endothelial phenotype – whether or not it meets some predetermined definition of activation – represents a net liability to the host.

**Diagnosis and therapy**

The endothelium has remarkable, yet largely untapped diagnostic and therapeutic potential. From a diagnostic standpoint, the endothelium is hidden from view and difficult to access. Owing to its thinness and disseminated nature, the endothelium is not amenable to conventional radiological imaging. The most commonly used assays for the endothelium are flow studies, which measure endothelium-dependent vasodilation; reviewed in (14). Abnormalities in endothelium-dependent flow have been correlated with atherosclerosis and risk for acute coronary syndrome. However, these studies are highly operator dependent, and they measure only a single function of the endothelium. In recent years, progress has been made in developing novel diagnostic tools for assaying endothelial function, including

- ELISA-based quantitation of circulating biomarkers, e.g., endothelin-1, VWF, tissue-type plasminogen activator

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**Fig. 1** The liver synthesizes a relatively constant supply of clotting factors (serine proteases II through XII and fibrinogen). In addition, hepatocytes synthesize several natural anticoagulants including protein C, protein S and antithrombin IIII (AT). The bone marrow produces and releases a relatively constant number of monocytes and platelets. Monocytes are capable of expressing tissue factor (TF), whereas activated platelets provide a cell surface for assembly of clotting reactions. Endothelial cells (ECs) also express procoagulants and anticoagulants. However, the repertoire of EC-derived haemostatic factors varies between vascular beds (shown are reported differences between arteries, veins and capillaries, though differences also exist between different types of arteries, veins and capillaries). The systemic mix of liver-derived soluble mediators and bone marrow-derived blood cells is integrated into the unique haemostatic balance of each vascular bed. It follows that changes in the systemic balance (as occurs for example in patients with congenital deficiency of protein C, protein S or AT, or with factor V Leiden) will have different local effects, giving rise to site-specific thrombotic phenotypes. Recent evidence suggests that ECs in regions of disturbed flow in arteries are primed for activation (they have increased levels of NF-κB in their cytoplasm) and that systemic imbalances (e.g. associated with sepsis or cardiac risk factors) may result in the translocation of NF-κB to the nucleus and increased expression of procoagulants such as tissue factor (TF) and adhesion molecules (adapted with permission from Aird WC. Circ Res 2007; 100: 158–173). TM: thrombomodulin; t-PA: tissue-type plasminogen activator; EPCR: endothelial protein C receptor; TFPI: tissue factor pathway inhibitor; VWF: von Willebrand factor.
(t-PA), soluble thrombomodulin, soluble vascular cell adhesion molecule 1 (VCAM-1), soluble intercellular adhesion molecule 1 (ICAM-1), and soluble E-selectin.

- enumeration and phenotyping of endothelial-derived microparticles and circulating endothelial cells, and
- molecular imaging; e.g. (15).

However, these various assays remain investigational and have yet to reach the clinical mainstream.

The endothelium is a highly attractive therapeutic target: It is rapidly and preferentially exposed to systemically delivered agents.

Given the capacity of endothelial cells to sense and respond to the local environment, it is hard to imagine that there exists any treatment for any disease that does not affect endothelial cell phenotypes in one way or another. From a therapeutic standpoint, the combination of phenotypic heterogeneity and modulability offers both opportunities and challenges.

- On one hand, the identification and characterization of vascular bed-specific “zip codes” should provide a foundation for site-specific targeting.
- On the other hand, drugs that lack such specificity are likely to exert mixed effects on the vasculature with
  - protective effects in some vascular beds and
  - neutral or deleterious consequences in others.

Endothelium in haemostasis

The vast majority of thrombotic disorders in humans and genetically modified mice are associated with focal clots in characteristic sites of the vasculature (16–18).

How does a systemic change in a circulating procoagulant or anticoagulant lead to site-specific thrombosis?

The answer lies, at least in part, in the endothelium. Endothelial cells are mini factories for the production of anticoagulant and procoagulant molecules. For example, endothelial cells express on the

- anticoagulant side thrombomodulin, endothelial protein C receptor (EPCR), heparan, tissue-type plasminogen activator (t-PA), tissue factor pathway inhibitor (TFPI), endothelial nitric oxide synthase (eNOS), and CD39,
- procoagulant side tissue factor (at least in vitro), VWF, factor VIII, and plasminogen activator inhibitor.

In keeping with the theme of heterogeneity, each of these molecules is differentially expressed across the vascular tree (19–21), for example,

- TFPI is expressed predominantly in capillary endothelium,
- EPCR in large veins and arteries,
- eNOS on the arterial side of the circulation,
- VWF in veins,
- t-PA in pulmonary and cerebral arteries,
- thrombomodulin in blood vessel types of every caliber in all organs except the brain.

In mice, factor VIII is preferentially expressed in endothelial cells of the liver and kidney, compared with brain and heart (22). Thus, the picture that emerges is one of heterogeneity layered upon heterogeneity, such that endothelial cells from different sites of the vascular tree employ site-specific “formulas” of haemostatic proteins to maintain blood in its fluid state and to promote limited clot formation when there is a breach in the integrity of the vascular wall (Fig. 1). As a result, any change in the systemic balance of haemostatic factors (as occurs for example with hereditary hypercoagulable states, liver disease, warfarin-induced skin necrosis, and sepsis) is integrated into the local haemostatic balance in ways that differ between vascular beds. In other words, the normal spectrum of endothelial heterogeneity may explain why systemic hypercoagulable states are channeled into focal thrombotic lesions.

On top of these baseline differences in the expression of endothelial procoagulants and anticoagulants, pathological states may further alter local haemostatic balance. For example, in a mouse model of endotoxemia, VWF mRNA levels were decreased in lung, aorta, brain, and adipose tissue, but increased in the heart and kidney (19). Tissue factor is not detectable in normal endothelium. However, in a baboon model of sepsis, it was shown to be up-regulated in a subset of endothelial cells in the marginal zone of splenic follicles, and in regions of disturbed flow (23, 24). Moreover, in transgenic mice with moderate-severe sickle cell disease, tissue factor expression was reported to be selectively up-regulated in pulmonary vein endothelium (25). These various disease-associated changes in protein expression are likely to further influence the propensity to develop site-specific thrombi.

Conclusion

The endothelium is a widely distributed organ system that plays an important role both in physiology and disease. The endothelium has many functions, over and above its well-recognized role in mediating vascular tone. One of these critical activities is maintenance of blood fluidity and repair of injured blood vessels. The endothelium is remarkably heterogeneous in structure and function. Indeed, endothelial cells regulate haemostatic balance by site-specific formulas of anticoagulants and procoagulants. These different equations are sufficient to explain why systemic imbalances in coagulation factors invariably lead to local thrombotic phenotypes. The propensity to develop clots in specific vascular beds is further accentuated by local endothelial dysfunction. An important goal for the future is to identify diagnostic markers that reflect phenotypic changes at the level of individual vascular beds, and to develop therapies that target one or another site of the vasculature.

Conflict of interest

The author has no conflict of interest to declare.

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