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Phenotypic Heterogeneity of the Endothelium

I. Structure, Function, and Mechanisms

William C. Aird

Abstract—Endothelial cells, which form the inner cellular lining of blood vessels and lymphatics, display remarkable heterogeneity in structure and function. This is the first of a 2-part review focused on phenotypic heterogeneity of blood vessel endothelium. This review provides an historical perspective of our understanding of endothelial heterogeneity, discusses the scope of phenotypic diversity across the vascular tree, and addresses proximate and evolutionary mechanisms of endothelial cell heterogeneity. The overall goal is to underscore the importance of phenotypic heterogeneity as a core property of the endothelium. (*Circ Res.* 2007;100:158-173.)

Key Words: endothelium ■ endothelial cells ■ heterogeneity

The endothelium forms the inner cellular lining of blood vessels. Endothelial cells (ECs) 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. Endothelial cell phenotypes are differentially regulated in space and time, giving rise to the phenomenon of “EC heterogeneity.” The endothelium has enormous yet largely untapped diagnostic and therapeutic potential. The goal of this review is to underscore the complexity of the endothelium and to emphasize the importance of approaching the endothelium as an integrated system.

History

Hippocrates and Galen viewed the vasculature as consisting of 2, unlinked systems of veins and arteries. Galen held that arteries contained air and vital spirits, whereas veins carried blood that was continuously formed in the liver. This erroneous theory of the circulation would hold sway for some 1500 years until William Harvey disproved Galen’s hypothesis in 1628. Through a series of elegant physiological experiments in dogs, Harvey showed that the arteries and veins are in fact connected and that blood is contained within a closed circulation. Although he could not directly visualize the capillaries, Harvey surmised their existence. With the benefit of compound microscopy, Marcello Malpighi was the first to actually observe blood capillaries in 1661. The term endothelium was coined by the Swiss anatomist Wilhelm His in 1865, to differentiate the inner lining of body cavities from epithelium. The original definition included the cell lining of blood vessels, lymphatics, and mesothelial-lined cavities. The

definition would later be narrowed to include only the inner cell layer of blood vessels and lymphatics.

In the 1950s and 1960s, the use of electron microscopy (EM) provided a powerful new window into the endothelium. Early EM studies revealed the presence of characteristic organelles, including plasmalemmal vesicles (which are now called caveolae) and Weibel–Palade bodies.¹ In addition, these investigations revealed—for the first time—the existence of structural heterogeneity. For example, in some vascular beds, ECs were tightly connected to one another and were surrounded by a continuous basement membrane (continuous endothelium). In a subset of these vascular beds, the ECs were permeated with holes or fenestrae (fenestrated endothelium). A third type of endothelium was characterized by the presence of fenestrae, frank gaps, and a poorly formed underlying basement membrane (discontinuous endothelium). The use of EM, together with tracers, led to revised theories of permselectivity and provided insights into the venular-specific regulation of permeability and leukocyte trafficking. These and other ultrastructural observations led Florey to conclude in 1966²:

Now it is recognized that there are many kinds of endothelial cells which differ from one another substantially in structure, and to some extent in function.

The 1970s and 1980s ushered in a new era of cell biology. This was made possible by the first successful isolation and characterization of ECs in culture. In 1973 and 1974, Jaffe and colleagues³ and Gimbrone and colleagues⁴ independently reported the isolation of human ECs from the umbilical vein. The ability to culture ECs allowed investigators to manipulate—in a controlled manner—the extracellular environment and to study cell biology in far greater detail. Among the seminal findings of that time was the observation that

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incubation of cultured ECs with inflammatory mediators or bacterial products induced proadhesive, antigen-presenting and procoagulant activities, a phenomenon that was termed “EC activation.”^{5–11}

Although the majority of research groups in the 1980s focused on cultured human umbilical vein ECs (and to a lesser extent, bovine aortic ECs), a small cadre of investigators used immunohistochemistry to characterize the endothelium *in vivo*. They reported that different vascular beds express different proteins.^{12–14} In other words, the intact endothelium displayed not only ultrastructural diversity, but also molecular heterogeneity. Implicit in these descriptive studies was a critical—if not largely overlooked—message, which was articulated by Auerbach and colleagues¹⁵:

The concept that vascular endothelial cells are not all alike is not a new one to either morphologists or physiologists. Yet laboratory experiments almost always use endothelial cells from large vessels such as the human umbilical vein or the bovine dorsal aorta, since these are easy to obtain and can be readily isolated and grown in culture. The tacit assumption has been that the basic properties of all endothelial cells are similar enough to warrant the use of the cells as *in vitro* correlates of endothelial cell activities *in vivo*. Recognizing the importance of heterogeneity, Auerbach championed the use of cell cultures from multiple organ beds to study the biology of the endothelium. Such an approach would make most sense if vascular bed-specific phenotypes maintained their identity *in vitro*. As is discussed in a following section (Mechanisms of Endothelial Cell Heterogeneity), this assumption is only partially correct.

Defining the Endothelium

How do we define the endothelium? From an anatomical standpoint, the endothelium represents the inner cellular lining of the blood and lymphatic vessels. However, there are examples of vascular mimicry in which other cell types, eg, 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 EC. Other structures, such as caveolae, are not specific to the endothelium. Developmentally, endothelium arises from mesoderm via the differentiation of hemangioblasts and/or angioblasts (reviewed elsewhere^{16,17}). However, other cell lineages may transdifferentiate into ECs, and ECs into other lineages.^{18,19} There are few, if any, protein/mRNA markers that are both specifically and uniformly expressed in the endothelium (reviewed elsewhere²⁰). Of the leading candidates, platelet/endothelial cell adhesion molecule (PECAM)-1 (also known as CD31) is also expressed in monocytes; thrombomodulin in keratinocytes, trophoblasts, and leukocytes; and vascular endothelial (VE)-cadherin in trophoblasts and fetal stem cells. From a functional standpoint, the endothelium displays a remarkable “division of labor” (Table) (reviewed elsewhere^{21,22}). Endothelial cells are typically “quiescent,” in the sense that they are not actively proliferating (the average lifespan of an EC is more than 1 year). However, the endothelium of the corpus luteum and uterus undergo cyclical episodes of intense physiological proliferation.²³ Finally, when endothelial-specific promoters are targeted to the mouse genome, they invariably fail to direct expression throughout the endothelium. Rather, they

promote expression in specific subsets of ECs (reviewed elsewhere²⁴). In summary, each of the above definitions falls short of fully capturing the endothelium. The “elusiveness” of the endothelium undoubtedly reflects its marked heterogeneity in structure and function.

Structural Heterogeneity

The shape of cells varies across the vascular tree. Although ECs are typically flat, they are plump or cuboidal in high endothelial venules (reviewed elsewhere^{25,26}). Endothelial cell thickness varies from less than 0.1 μm in capillaries and veins to 1 μm in the aorta (reviewed elsewhere²). Endothelial cells (and their nuclei) are aligned in the direction of blood flow in straight segments of arteries but not at branch points.^{27,28} When canine arterial vessel segments were excised, rotated 90°, and reimplanted, the nuclear pattern of ECs realigned in the direction of blood flow within 10 days after surgery.²⁹ Thus, flow-dependent alignment of ECs represents reversible endothelial structural remodeling in response to hemodynamic shear stress. In a study of rat blood vessels, aortic ECs were reported to be long and narrow (55×10 μm) with their long axes oriented in the direction of blood flow; ECs of the pulmonary artery were broader and shorter (30×14 μm), forming a rectangular shape; pulmonary vein ECs were large, and round in shape; and ECs of the inferior vena cava were long, narrow and rectangular.³⁰ In the cremasteric muscle of mice, ECs of arterioles are longer than their counterparts in veins (length-to-width ratio 7.45 and 2.66, respectively) and have a higher surface area (1200 versus 600 μm^2 , respectively).³¹ In a study of the tracheal microcirculation in rats, ECs were elongated and spindle shaped in arterioles; irregularly shaped in capillaries; large, elliptical, or irregularly shaped in postcapillary venules; and rounded in collecting venules.³² In scanning EM of the rat penis, EC nuclei in the deep artery were shown to leave elliptical depressions, whereas those in the helicine artery left deeper and more rounded depressions.³³

ECs possess clathrin-coated pits, clathrin-coated vesicles, multivesicular bodies and lysosomes, which represent the structural components of the endocytotic pathway (reviewed elsewhere³⁴) (Figure 1). Endocytosis targets macromolecules to the lysosomal compartment for degradation. In some cases, endocytosed substances are recycled to the cell surface, or sorted to other subcellular compartments such as the Golgi and endoplasmic reticulum. Endocytosis takes place either by a nonspecific (fluid-phase) process or via receptor-dependent pathways. The latter process is mediated by so-called scavenger receptors, which are responsible for uptake of low-density lipoprotein (LDL), transferrin, albumin, ceruloplasmin, and advanced glycosylation end products. Liver sinusoidal ECs demonstrate particularly high rates of clathrin-mediated endocytosis.

In addition to endocytosis, ECs actively engage in transcytosis, which governs the transcellular transfer of molecules across the endothelium. Transcytosis is mediated by specialized structures, including caveolae and vesiculo–vacuolar organelles (VVOs) (Figure 1). Caveolae are 70-nm membrane-bound, flask-shaped vesicles that usually open to the luminal or abluminal side but are occasionally free in the

Examples of Functional Heterogeneity of Endothelium in Normal Adult Vasculature

Function	Primary Site	Mechanism	Comments
Permeability			
Basal	Capillaries	Intercellular clefts, vesicle-mediated transcytosis, transendothelial channels	Constantly occurs across the endothelium, albeit at different rates between different vascular beds
Inducible	Postcapillary venules	Intercellular clefts, vesicle mediated transcytosis, transendothelial channels	May be physiological (localized, transient) or pathological (systemic, sustained, and/or excessive)
Leukocyte transmigration	Postcapillary venules in skin, mesentery, muscle; capillaries in lung and liver; HEV in lymph nodes	Site-specific repertoire of EC selectins, chemokines, and integrin ligands	Mechanisms differ according to site, leukocyte subset
Hemostasis	Panvascular	Site-specific repertoire of procoagulants and anticoagulants	Systemic imbalance in soluble factors will influence hemostatic balance in ways that differ between vascular beds, leading to focal thrombosis
Vasomotor tone	Arterioles	Release of site-specific vasodilator and vasoconstrictor molecules	
Humidification	Bronchial microcirculation	High surface area and close approximation to airways	
Thermoregulation	Bronchial and skin microcirculation	High surface area and close approximation to airways (bronchial microcirculation) and external environment (skin microcirculation)	Vasoconstriction of cutaneous vessels conserves body heat; vasodilation and secondary elevation of blood flow to the skin promotes convective heat transfer from the core to the periphery of the body
Sieve function	Liver sinusoids	Fenestrae	Important for chylomicron clearance
Scavenging	Liver sinusoids	Receptor-mediated endocytosis	Important for clearance of gastrointestinal-derived bacterial products and particles
Immune tolerance	Liver sinusoids	MHC class I and II molecules, costimulator molecules	Tolerance to oral antigens
Proliferation/angiogenesis	Reproductive system (eg, ovary, uterus)	Cyclical changes in expression of growth factors	

MHC indicates, major histocompatibility complex.

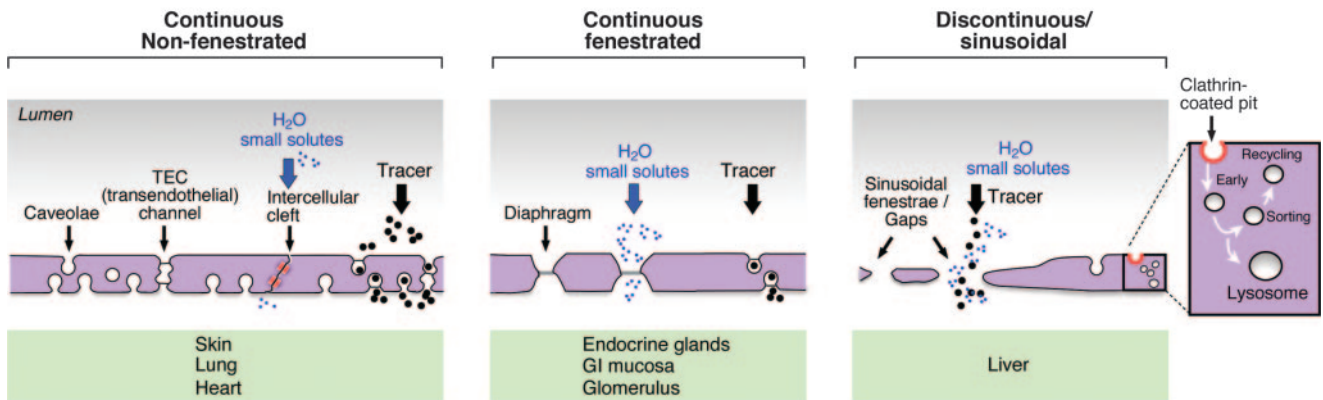
cytoplasm. Some, but not all, caveolae possess a thin non-membranous stomatal diaphragm that contains a protein termed plasmalemma protein-1 (PV-1) (also present on fenestral diaphragms).³⁵ Whereas clathrin-coated pits have a thick electron-dense coat, caveolae have a smooth inner surface. With the exception of liver sinusoids, caveolae are more numerous than clathrin coated pits in ECs. The density of caveolae is far greater in capillary endothelium (up to 10 000 per cell) compared with arteries, arterioles, veins, or venules. The number of caveolae is highest in continuous nonfenestrated endothelium, particularly in heart, lung, and skeletal muscle (reviewed elsewhere³⁶). A notable exception is the blood brain barrier, where caveolae are rare.³⁷ In ultrastructural studies of rat capillaries, the fractional area occupied by caveolae was estimated to be 5% in capillaries of the diaphragm, 7.3% in those of the myocardium, 2.5% in those of the pancreas, and 0.8% in those of the jejunal mucosa.³⁸ Although caveolae are present in many non-ECs, there is evidence that expression of caveolin-1, the main structural protein of caveolae, is regulated by distinct transcriptional mechanisms in ECs.³⁹

VVOs, which represent focal collections of membrane-bound vesicles and vacuoles, are most commonly observed in

venular endothelium, where the cytoplasm is thicker compared with capillaries (reviewed elsewhere^{40,41}). The complexity of VVOs varies between venules according to the thickness of the endothelium. For example, in skin venules, in which ECs are tall, VVOs occupy nearly one-fifth of the venular endothelial cytoplasm and consist of groups of more than 100 individual vesicles and vacuoles, whereas in the shorter ECs of the mesenteric venules, VVOs consist of smaller aggregates of vesicles. VVOs contain caveolin-1 and are believed to arise from the fusion of individual caveolae.

Two main types of intercellular junctions are recognized in endothelium: tight junctions (also termed zona occludens), which are usually found at the apical region of the intercellular cleft; and adherens junctions (also termed zona adherens) (reviewed elsewhere^{42,43}). Tight junctions form a barrier to transport between ECs (so-called paracellular transport) and help to maintain cell polarity between the luminal and abluminal side of the EC. The junctional composition of intercellular clefts varies across the vascular tree. Large artery ECs display a well developed system of tight junctions, as might be predicted by the conduit function of these vessels and their exposure to high rates of pulsatile blood flow. Within the microvasculature, junctions are tighter in arte-

A Capillary



B Post-capillary venule

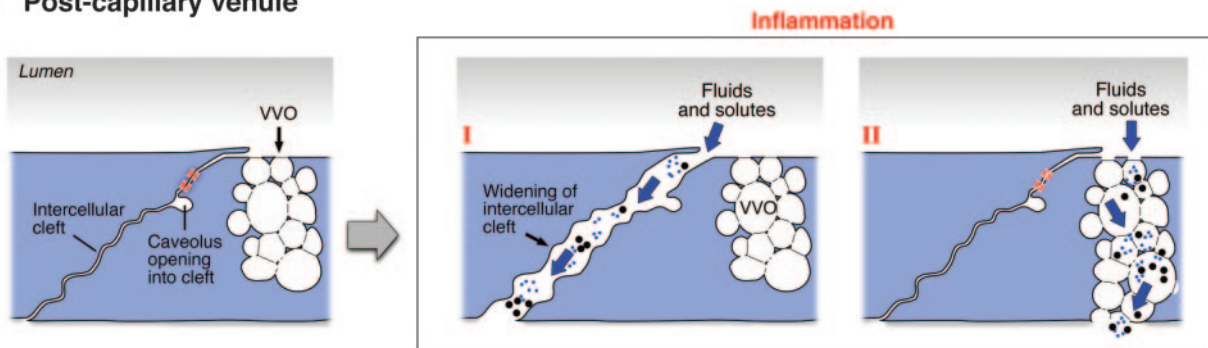


Figure 1. Endothelium and permeability. A, Capillaries mediate constitutive (albeit physiologically regulatable) transfer of solutes and fluids between blood and underlying tissue. In continuous nonfenestrated endothelium, water and small solutes (molecular radius, <3 nm) pass between ECs, whereas larger solutes (depicted as back tracer) pass through ECs either via transendothelial channels or transcytosis, the latter process being mediated primarily by caveolae. Caveolae are particularly prevalent in capillaries of heart and skeletal muscle and rare in blood-brain barrier. Compared with their nonfenestrated counterpart, continuous fenestrated endothelium demonstrates greater permeability to water and small solutes but similar reflection coefficients to albumin and larger macromolecules (the diaphragms of the fenestrae act as molecular filters). Discontinuous endothelium is characterized by fenestrae (without diaphragms), gaps, and poorly organized basement membrane. These ECs contain many clathrin-coated pits, which play an important role in receptor-mediated endocytosis (although they may also take part in transcytosis). The endocytic pathway includes endosomal and lysosomal compartments (shown on the right). B, In response to inflammation, postcapillary venules demonstrate increased permeability. Inducible transfer of water and solutes occurs between ECs (paracellular route) (I) and/or through ECs (transcellular route) (II). The paracellular route involves formation of gaps between ECs; the transcellular route involves VVO-mediated formation of transcellular pores. VVOs are enriched in the perijunctional regions of the cell. Red shapes in intercellular cleft represent tight junctional complexes.

rioles compared with capillaries and are quite loose in venules. The “disorganized” nature of tight junctions in post-capillary venules likely reflects the role of this blood vessel type in mediating inflammation-induced extravasation of leukocytes and plasma constituents. In contrast, the blood brain barrier, which protects neural tissue from fluctuations in blood composition, is particularly rich in tight functions.

Endothelium may be continuous or discontinuous (Figure 1). Continuous endothelium, in turn, is fenestrated or non-fenestrated. Nonfenestrated continuous endothelium is found in arteries, veins, and capillaries of the brain, skin, heart, and lung. Fenestrated continuous endothelium occurs in locations that are characterized by increased filtration or increased transendothelial transport. These include capillaries of exocrine and endocrine glands, gastric and intestinal mucosa, choroid plexus, glomeruli, and a subpopulation of renal tubules. Fenestrae are transcellular pores (≈ 70 nm in diameter) that extend through the full thickness of the cell. The majority of fenestrae possess a thin 5- to 6-nm nonmembra-

nous diaphragm across their opening. (It was long held that the fenestrae of glomerular ECs lacked a diaphragm; however, more recent evidence suggests that they do indeed possess such a structure.⁴⁴) With the exception of the glomerulus, fenestral diaphragms contain the integral membrane glycoprotein PV-1.³⁵ The presence of a diaphragm may provide fenestrae with increased size selectivity. Some ECs are polarized with respect to fenestral distribution. For example, in the lamina propria of the human small intestine, capillaries are fenestrated on the side that faces the absorptive epithelial layer of the mucosa.⁴¹ The density of fenestrae varies between vascular beds. For example, in rats, fenestral density is almost twice as high in the jejunal versus pancreatic capillaries, accounting for an aggregated area of 9.5% and 6%, respectively, of the total endothelial surface.³⁸

Discontinuous endothelium is found in certain sinusoidal vascular beds, most notably the liver. In contrast to fenestrated continuous endothelium, liver sinusoidal ECs possess larger fenestrations (100 to 200 nm in diameter) that lack a

diaphragm and contain gaps (or large circular pores) within individual cells.⁴⁵ The underlying basement membrane is poorly formed. Fenestrae demonstrate spatial heterogeneity within the liver; they are larger and less frequent in the periportal region compared with the centrilobular region of the liver lobule.⁴⁶

The thickness of the luminal glycocalyx varies across the vascular tree,⁴⁷ as does the continuity and thickness of the underlying basement membrane and the degree of investiture with smooth muscle cells or pericytes.

There is increasing evidence that site-specific structural properties are not fixed but, rather, are dynamically regulated during embryogenesis and in the postnatal period. For example, during rodent development blood vessels in the brain are fenestrated and relatively devoid of tight junctions until embryonic day 11 (E11) to E13.^{48,49} At E10 to E12, liver capillaries in rodents possess a basement membrane and small diaphragm-subtended fenestrae that resemble those in typical fenestrated continuous endothelium. Only later during development ($\approx$ E17) do these cells begin to acquire the adult phenotype.⁵⁰ Recent studies suggest that vascular endothelial growth factor (VEGF) plays a key role in the generation and maintenance of fenestrae. For example, in animal and human models, deficiency of VEGF is associated with loss of glomerular fenestrations, increased permeability, and proteinuria.^{51,52} Similarly, conditional liver-specific knock out of VEGF activity resulted in loss of fenestrae in liver sinusoidal ECs and secondary impairment in lipoprotein clearance.⁵³ Exogenous administration of VEGF has been shown to induce fenestrations in vascular beds, which do not normally have fenestrae, including the capillaries and venules of skin and cremasteric capillaries.⁵⁴ (In contrast to these latter findings, overexpression of VEGF in the skin of transgenic mice did not induce fenestrations in the subcutaneous vessels.⁴¹) The structural properties of the endothelium are also modulated in disease. For example, in a rabbit model of interstitial pulmonary edema, lung capillary ECs demonstrated a 2-fold increase in caveolar density.⁵⁵ As another example, many liver diseases are associated with defenestration of sinusoidal endothelium and acquisition of a continuous basement membrane.⁵⁶

Functional Heterogeneity

Endothelial cells perform many functions, most of which are performed by specific subsets of blood vessel types or vascular beds. Below, 3 representative examples are discussed: permeability, leukocyte trafficking, and hemostasis. Other functions, such as endothelial regulation of vasomotor tone, angiogenesis, and innate and acquired immunity, are no less important but owing to space limitations they will not be discussed here. Rather, they are referred to in subsequent sections dealing with representative vascular beds (see the second part of this review⁵⁷).

Permeability

The endothelium is semipermeable; it must allow for regulated transport of fluids and solutes into and out of the blood. For purposes of discussion, permeability may be separated into 2 types: basal and inducible (Figure 1).

Under basal conditions, there is a continuous (although physiologically regulatable) flux of material between blood and underlying interstitium. Such activity takes place primarily in the capillaries, the major exchange vessels of the circulation. As noted by Pappenheimer in 1953⁵⁸:

There are good reasons for supposing. . . that the visible flow of blood through the capillaries is, in fact, very small in comparison with the *invisible* flow of water and dissolved materials back and forth through the capillary walls. . . this invisible component of the circulation takes place at a rate which is many times greater than that of the entire cardiac output. Indeed it is by means of this 'ultramicroscopic circulation' through the capillary wall that the circulatory system as a whole fulfills its ultimate function in the transport of materials to and from the cells of the body.

The study of vectorial transport has interested physiologists and electron microscopists alike for decades, and the field has engendered considerable controversy (reviewed elsewhere^{36,37,59,60}). There is now a general consensus that fluids and small solutes move passively across the barrier via the paracellular route, whereas macromolecules use a transcellular route, shuttling across the endothelial barrier in membrane-bound vesicular carriers, including caveolae and VVOs, and/or passing through vesicle-derived transendothelial channels. Transcellular transport of macromolecules may involve receptor-dependent (receptor-mediated transcytosis) or receptor-independent (fluid-phase transcytosis) mechanisms. The importance of caveolae in mediating transcellular transport of albumin was confirmed in studies of caveolin-1^{-/-} mice.⁶¹

Spatial heterogeneity in basal permeability may be explained by differences in junctional properties, the presence or absence of fenestrae, and/or differential activity of the transcytotic machinery. Indeed, the number and complexity of tight junctions is inversely proportional to permeability. For example, arteries and blood brain barrier have highly developed tight junctions and low permeability. Mice that are null for claudin-5, a major component of the tight junctional region, have selective impairment in blood brain barrier function.⁶² The importance of adherens junctions in mediating site-specific permeability is evidenced by studies in which the systemic administration of anti-VE-cadherin antibodies in mice resulted in preferential changes in vascular permeability in the lung and heart.⁶³ The presence of fenestrae in continuous endothelium is associated with increased permeability of fluids and small solutes, but not macromolecules.⁶⁴ Although there is a poor correlation between the number of caveolae and segmental permeability, differences in the repertoire of caveolae-associated membrane receptors may underlie site-specific transcytosis.⁶⁵

In addition to the basal, constitutive transfer of substances across the capillary beds, the endothelium is capable of mediating inducible permeability in states of acute and chronic inflammation. The predominant site of inducible permeability is the postcapillary venule. According to the conventional view, agonists (eg, histamine, serotonin, bradykinin, substance P, and VEGF) induce EC retraction and the formation of intercellular gaps.^{66–68} Others have argued permeability-enhancing agents do not cause gap formation but result in increased transcellular vascular leakage of macromolecules via VVO-derived transendothelial pores.^{41,69}

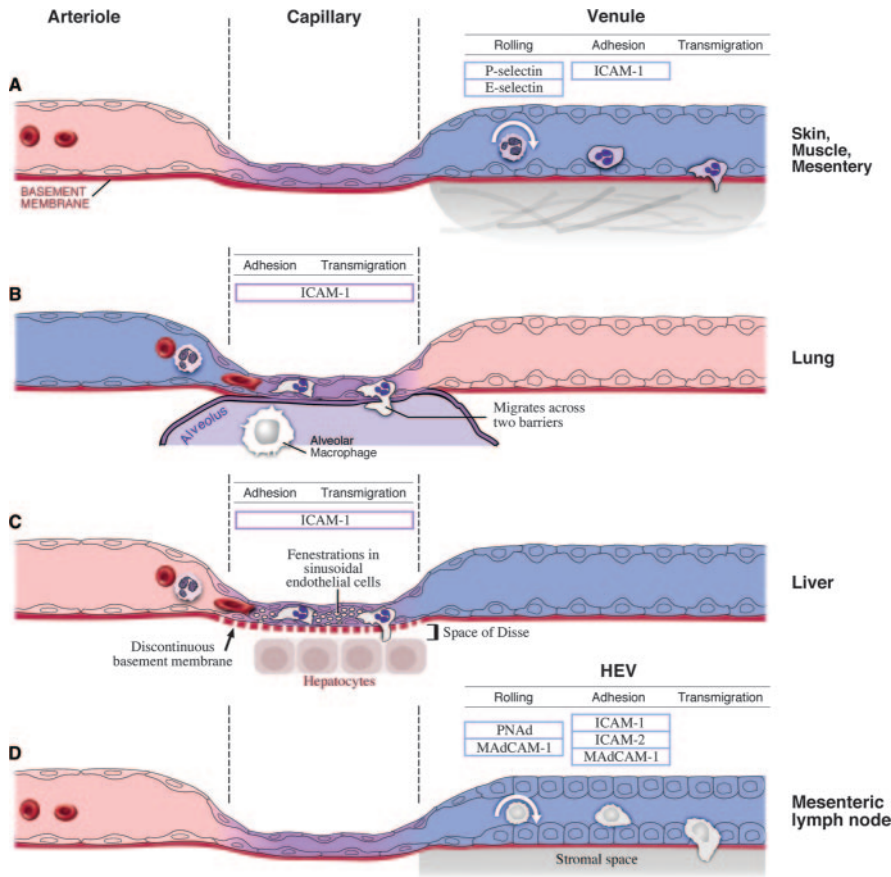


Figure 2. Endothelium and leukocyte trafficking. A, According to the classical multistep paradigm for leukocyte recruitment (based on studies of skin, skeletal muscle, and mesentery), leukocytes (neutrophils are shown) undergo initial attachment, followed by rolling, firm adhesion, and transmigration through activated endothelium in postcapillary venules (labeled as venule). Rolling and adhesion are mediated by interactions between EC receptors (P- and E-selectin and ICAM-1) and leukocyte counter-receptors/ligands (not shown). Leukocytes may transmigrate between or through ECs. The molecular details of transendothelial cell migration (also known as diapedesis or extravasation) are less well understood but appear to involve PECAM-1/CD31, junctional adhesion molecule-1 (JAM-1), and CD99. Activated ECs present chemokines that induce integrin activation on the surface rolling leukocytes (not shown). B, In the lung, the majority of leukocyte trafficking takes place in pulmonary capillaries. Leukocyte trafficking at this site is dependent on ICAM-1 but not E- or P-selectin. Other contributing mechanisms include reduced deformability of activated neutrophils and activation state of the endothelium. Note that leukocytes must cross 2 barriers (endothelium and epithelium) to reach the airspace. C, In the liver, leukocyte trafficking occurs primarily in sinusoids by an ICAM-1-dependent mechanism. Cytoplasmic projections of the neutrophil may reach through fenestrations and gaps into the space of Disse and thus sense and respond to signals from hepatocytes and Ito cells. D, In HEVs of mesenteric lymph nodes, lymphocytes constitutively roll on, adhere to and transmigrate across specialized cuboidal endothelium via distinct mechanisms that involve binding of lymphocyte L-selectin to EC PNAd and mucosal addressin cell adhesion molecule (MAdCAM-1), and leukocyte integrins to ICAM-1, ICAM-2, and MAdCAM-1. Not shown in the figure (but of importance in health and disease) is the transmigration of monocytes or lymphocytes in peripheral tissues, as well as leukocyte trafficking in large veins and arteries.

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The predilection for postcapillary venules as a site for inducible permeability may be explained by the relative abundance of VVOs, the relative paucity of tight junctions (hence weak intercellular contacts), and the preferential expression of histamine H₂, serotonin, and bradykinin receptors. Whether or not all postcapillary venules in the body share these attributes is unknown. Moreover, inflammation, when severe, may cause barrier dysfunction in other segments of the vascular tree, including large veins, arterioles, and capillaries. For example, tumor necrosis factor (TNF)- α treatment resulted in increased permeability of mouse inferior vena cava, but not the aorta, as measured by accumulation of fluorescein isothiocyanate-dextran in the blood vessel wall.⁷⁰ In EM studies, TNF- α was shown to promote intercellular gap formation in the inferior vena cava.⁷⁰ In a rat model of diabetes, paracellular permeability was increased first in arterioles and venules, and then in capillaries.⁷¹ VEGF has been shown to induce intercellular gaps and fenestra in both postcapillary venules and capillaries.⁵⁴

Leukocyte Trafficking

Passage of leukocytes from blood to underlying tissue involves a multistep adhesion cascade that includes initial attachment, rolling, arrest and transmigration (Figure 2A).

These steps take place almost exclusively in postcapillary venules. Rolling is mediated primarily by interactions between leukocyte carbohydrate-based ligands and endothelial E- and P-selectin, and firm adhesion by interactions between leukocyte integrins and endothelial intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 (reviewed elsewhere^{72–74}). The molecular basis of transmigration is less well understood but is believed to involve CD99, PECAM-1/CD31, and junctional adhesion molecule-1 (reviewed elsewhere^{72–74}).

E-selectin, among the very few genes that is highly restricted to ECs, is expressed in activated, but not “resting” (ie, nonactivated), endothelium (a possible exception is in the bronchial circulation⁷⁵). In activated endothelium, E-selectin expression is largely confined to postcapillary venules.^{76,77} In contrast to the usual situation in adult endothelium, E-selectin is not inducible in early embryonic endothelium (before E12.5).⁷⁸ P-selectin is expressed in megakaryocytes and ECs. In “resting” endothelium, P-selectin is stored intracellularly in preexisting Weibel–Palade bodies and is expressed preferentially in postcapillary venules.⁷⁹ In mice, constitutive expression of cell surface P-selectin is highest in lung and mesentery, compared with heart, brain, stomach, pancreas, intestine, and muscle.⁸⁰ Systemic administration of histamine

in mice resulted in rapid increases in cell surface P-selectin, but not E-selectin, in all tissues examined except the brain.⁸⁰ Endotoxemia in mice resulted in increased cell surface P-selectin and E-selectin in all tissues examined, including lung and mesentery, heart, brain, stomach, pancreas, intestine, and muscle, with largest increments of both selectins in lung, small intestine, and heart.⁸⁰ Induction of cell surface P-selectin in multiple tissues was sustained at 24 hours, whereas E-selectin levels had returned to baseline.⁸⁰ In another study, lipopolysaccharide administration to mice was shown to increase P-selectin mRNA expression 87-fold in the heart, 12-fold in the lung, 15-fold in the liver, 12-fold in kidney, 33-fold in brain, and 2.5-fold in the spleen.⁷⁷ In contrast, E-selectin was induced 14.6-, 5.6-, 3.8-, 18-, 5.3-, and 0.6-fold, respectively.⁷⁷ Cecal ligation puncture in mice resulted in increased P-selectin protein expression in brain, kidney, stomach, small bowel, and large bowel, an effect that was greatly accentuated in obese animals.⁸¹ In a mouse model of sickle cell disease, constitutive expression of P-selectin was increased in several tissues, whereas E-selectin was increased in only the penis.⁸² Interestingly, inflammatory mediators such as interleukin-1 and TNF- α induce P-selectin mRNA expression in mice, but not primates,⁸³ underscoring the importance of interspecies differences in transcriptional control.

Unlike E-selectin and P-selectin, ICAM-1 and VCAM-1 are expressed in many vascular and nonvascular cell types. Constitutive expression of cell surface VCAM-1 in mice is generally lower than that of ICAM-1, with the exception of the heart, where both are equally expressed; and the brain, where VCAM-1 density is 4-fold higher than that of ICAM-1.⁸⁴ In normal mice and rabbits, ECs at sites predisposed to atherosclerosis express ICAM-1 and VCAM-1.⁸⁵ Both ICAM-1 and VCAM-1 are induced by activation agonists. For example, in mice administered lipopolysaccharide, cell surface expression of ICAM-1 and VCAM-1 was increased in heart and small intestine (ICAM-1 was also induced in mesentery and brain) at 5 hours, with highest induction of ICAM-1 occurring in heart, and VCAM-1 in small intestine (VCAM-1 levels were actually reduced in the spleen).⁸⁶ In another study of mouse endotoxemia, ICAM-1 mRNA expression was induced 6-fold in the heart, 1.9-fold in the lung, 2.2-fold in the liver, 18.3-fold in kidney, 6.8-fold in brain, and 2.1-fold in the spleen.⁷⁷ In the same report, VCAM-1 was induced 2.0-, 1.1-, 0.6-, 1.3-, 2.1-, and 0.6, respectively. In contrast to P-selectin and E-selectin, which were localized to postcapillary venules, VCAM-1 and ICAM-1 were also induced in the capillaries of the heart.⁷⁷ In a cecal ligation puncture model of sepsis, ICAM-1 protein was induced in lung, kidney, liver, and heart, whereas VCAM-1 levels were induced in kidney, liver, and heart, but not lung.⁸⁷

There are 2 routes for leukocytes to pass through the endothelium. They may pass between ECs (the paracellular route), or they may pass through the EC itself (the transcellular route). Recent studies have provided compelling evidence that leukocytes may indeed transmigrate through ECs.^{41,88,89} An interesting question is whether different vascular beds use different rates of paracellular versus transcellular transport, and/or whether this ratio differs with different

subsets of leukocytes or different activation agonists. For example, it is tempting to speculate that the blood-brain barrier—with its abundance of tight junctional complexes—relies primarily on the transcellular route (as it does for solute and fluid transport).

Although leukocyte-endothelial interactions are generally observed in postcapillary venules, they may also occur in other segments of the vascular tree, including large veins, capillaries, and arterioles. For example, perivascular exposure of mouse inferior vena cava to TNF- α in vivo resulted in the induction of ICAM-1, VCAM-1, E-selectin, and P-selectin.⁷⁰ This pattern was similar to that observed in postcapillary venules and was more pronounced compared with the aorta and iliac arteries. Moreover, the increased cell adhesion molecule expression in TNF- α -treated inferior vena cavae correlated with increased firm adhesion of leukocytes. Data suggest that leukocyte sequestration and transmigration in the pulmonary circulation occurs primarily in alveolar capillaries (reviewed elsewhere^{90,91}) (Figure 2B). The mechanism involves trapping of poorly deformed activated leukocytes on activated endothelium (reviewed elsewhere⁹²). In liver inflammation, the sinusoidal endothelium accounts for 70% to 80% of leukocyte adhesion.⁹³ Previous studies have shown that certain mediators, such as TNF- α , interleukin-1, cigarette smoke, and oxidized LDL, induce leukocyte rolling in arterioles and arteries.^{94–97} Inflamed arterial endothelium may support transmigration of different subsets of leukocytes, compared with postcapillary venules. For example, systemic administration of angiotensin II to rats resulted in increased adherence and transmigration of lymphocytes and monocytes in arterioles, and of neutrophils in postcapillary venules.⁹⁸

Just as leukocyte trafficking does not always take place in postcapillary venules, the multistep cascade does not apply universally to all vascular beds. For example, in the lung and the liver, leukocytes adhere to capillary endothelium independent of a rolling motion⁹³ (Figure 2B and 2C). Neutrophil recruitment in these vascular segments does not require E- or P-selectin but is partially dependent on ICAM-1 (arguing against a role for simple trapping of white blood cells).^{93,99,100} Together, these data strongly suggest that the universal cascade, although perhaps relevant to the microvasculature of those tissues in which the majority of studies have been performed, does not apply to all organs or vascular beds.

Another form of leukocyte trafficking, which is distinct from those described above, takes place in secondary lymphoid organs, including peripheral lymph nodes, mesenteric lymph nodes, Peyer's patches, appendix, and tonsils. Here, lymphocytes transmigrate across specialized postcapillary venules, termed high endothelial venules (HEVs) (reviewed elsewhere^{25,26,101}). In contrast to nonlymphoid postcapillary venules, which mediate appreciable levels of leukocyte transmigration only during inflammation, HEVs support the constitutive recirculation of lymphocytes between blood and lymph nodes (Figure 2D). Endothelial cells lining HEVs are morphologically distinct and express a unique repertoire of adhesion molecules. Lymphocyte homing in peripheral lymph nodes depends on three distinct molecular steps: (1) rolling, mediated by interactions between lymphocyte L-selectin and its ligand on HEV ECs, namely peripheral

node addressin (PNAd); (2) chemokine-mediated activation of lymphocyte integrins (eg, constitutively expressed CCL21 on HEV ECs binds to CCR7 on lymphocytes, resulting in activation of lymphocyte function-associated antigen (LFA)-1; and (3) firm adhesion, mediated by lymphocyte LFA-1. The fact that neutrophils express L-selectin and LFA-1, but not CCR7, may explain why granulocytes roll but do not arrest (or transmigrate) in HEVs. In mesenteric lymph nodes, ECs lining HEVs express not only PNAd but also the mucosal addressin cell adhesion molecule-1, which binds to $\alpha_4\beta_7$ on the surface of lymphocytes.

The extent to which differential expression of selectins and other adhesion molecules can be translated into regional differences in the regulation of leukocyte adhesion and transmigration is presently unknown. Clearly, many factors are involved in regulating leukocyte trafficking in space and time. Thus, at present, it is more instructive to consider the remarkable heterogeneity in molecular profiles and site-specific endothelial-leukocyte interactions than it is to infer precise cause-effect relationships between them.

Hemostasis

A common function of the endothelium is to maintain blood in a fluid state, and to promote limited clot formation when there is a breach in the integrity of the vascular wall. On the anticoagulant side, ECs express tissue factor pathway inhibitor (TFPI), heparan, thrombomodulin, endothelial protein C receptor (EPCR), tissue-type plasminogen activator (t-PA), ecto-ADPase, prostacyclin, and nitric oxide. On the procoagulant side, ECs synthesize tissue factor, plasminogen activator inhibitor (PAI)-1, von Willebrand factor (vWF), and protease activated receptors (reviewed elsewhere¹⁰²). Importantly, endothelial-derived anticoagulant and procoagulant molecules are unevenly distributed throughout the vasculature (reviewed elsewhere^{22,102}) (Figure 3).

EPCR is expressed predominantly in large arteries and veins,¹⁰³ whereas thrombomodulin is highly expressed in blood vessel types of every caliber in all organs, with the exception of the brain, where levels are low.¹⁰⁴ In mice, EPCR transcripts are highest in the placenta, lung, liver, and heart.¹⁰⁵ Expression of EPCR and/or thrombomodulin is decreased in some but not all models of inflammation. For example, endotoxemia resulted in reduced thrombomodulin expression in rat liver sinusoidal ECs.¹⁰⁶ In patients with meningococemia, thrombomodulin and EPCR protein levels were decreased in the endothelial lining of dermal microvessels.¹⁰⁷ However, in rats injected with *Escherichia coli*, there were no changes in thrombomodulin antigen levels or activity.¹⁰⁸ Moreover, in a baboon model of *E coli* sepsis, thrombomodulin protein levels were unaltered.¹⁰⁹ In mice, systemic administration of lipopolysaccharide, but not TNF- α , resulted in increased EPCR mRNA expression in lung and heart, with protein expression still limited to large vessel endothelium.¹⁰⁵ In humans, thrombomodulin and EPCR were shown to be downregulated in atherosclerotic coronary arteries.¹¹⁰

vWF is expressed predominantly on the venous side of the circulation.^{102,111} In mice, basal vWF expression mRNA was reported to be highest in lung and lowest in liver and muscle.¹¹¹

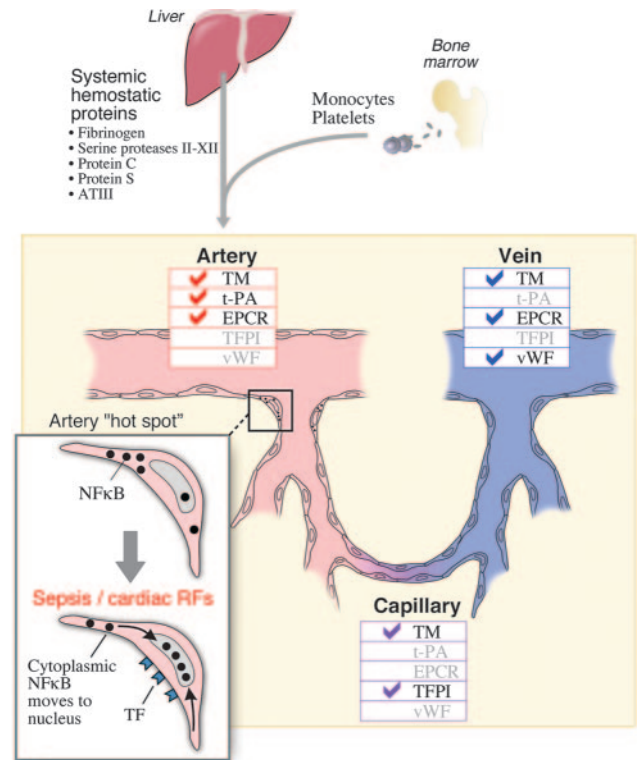


Figure 3. Endothelium and hemostasis. The liver synthesizes a relatively constant supply of clotting factors (serine proteases II through XII) and fibrinogen. In addition, hepatocytes synthesize several natural anticoagulants including protein C and S and antithrombin III (ATIII). The bone marrow produces 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 also express procoagulants and anticoagulants. However, the repertoire of EC-derived hemostatic factors varies between vascular beds (shown are reported differences among arteries, veins, and capillaries, although differences also exist among 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 hemostatic 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 or S or ATIII, 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 nuclear factor κ B [NF- κ B] in their cytoplasm) and that systemic imbalances (eg, associated with sepsis or cardiac risk factors [RFs]) may result in the translocation of nuclear factor κ B to the nucleus and increased expression of procoagulants such as tissue factor and adhesion molecules. TM indicates thrombomodulin.

In en face Hautchen preparations of rat aorta, only 20% to 50% of ECs were shown to be positive for vWF as detected by immunohistochemistry.¹¹² vWF positivity occurred in groups of cells orientated along the longitudinal axis of the aorta but was scant at sites of intercostal orifices.¹¹² Systemic administration of lipopolysaccharide in mice resulted in upregulation of vWF mRNA in heart and kidney but decreased expression in the lung, aorta, brain, and adipose tissue.¹¹¹

In mice, t-PA expression in the endothelium was reported to be restricted to arteries of the pulmonary system and central nervous system.¹¹³ As mice reached maturity, expression of t-PA in the brain decreased in large arteries, yet persisted in smaller vessels.¹¹³ PAI-1 expression in mice is

highest in lung, followed by heart, brain, spleen, liver, and kidney.⁷⁷ Endotoxemia resulted in 187-fold increase in PAI-1 in the liver but only a 3-fold increase in spleen.

TFPI expression in human tissues is highest in placenta and lung and lowest in the brain.¹¹⁴ In the mouse, TFPI mRNA expression is highest in lung and undetectable in liver.¹¹⁵ Under normal conditions, TFPI is expressed primarily by microvascular endothelium.^{114,116} Tissue factor is not detectable in normal intact endothelium. However, tissue factor is expressed by ECs in certain pathophysiological states. For example, in a baboon model of *E coli* sepsis, the gene was upregulated in a subset of ECs in the marginal zone of splenic follicles¹⁰⁹ and in regions of disturbed flow in the aorta.²⁸ Tissue factor was also detected in pulmonary vein endothelium in a mouse model of sickle cell disease.¹¹⁷ Human atherosclerotic coronary arteries demonstrate increased tissue factor and TFPI levels, which colocalize in ECs.¹¹⁸ TFPI protein and mRNA expression and TFPI activity were shown to be increased in the ECs overlying plaque in human atherosclerotic carotid arteries.¹¹⁹ Tissue factor has also been detected in ECs in tumors (reviewed elsewhere¹²⁰), and in xenograft and allograft vasculopathy.^{121,122}

The differential distribution of procoagulants and anticoagulants in the endothelium suggests that ECs from different sites of the vascular tree use site-specific “formulas” of procoagulants and anticoagulants to balance local hemostasis.

In humans and animal models, systemic imbalance of clotting factors results in a local thrombotic phenotype (reviewed elsewhere¹²³). For example, factor V Leiden is associated with an increased risk for venous thromboembolism but not acute myocardial infarction or stroke.^{124,125} A similar propensity for thrombosis on the venous side of the circulation was demonstrated in mice that carry the factor V Leiden gene.¹²⁶ Mice with antithrombin III (ATIII) deficiency develop hepatic and cardiac thrombosis and die by E16.5.¹²⁷ Interestingly, when *ATIII*^{-/-} were crossed with genetically modified mice that express low levels of tissue factor, thrombosis was attenuated in the heart, but not the liver.¹²⁸ As a final example of organ-specific thrombosis, mice with low functional levels of thrombomodulin reveal increased fibrin deposition in the lung, heart, spleen, and liver but not brain or kidney.¹²⁹ Taken together, these studies provide indirect, yet compelling, evidence that systemic imbalances in natural anticoagulant activity may be “channeled” by the endothelium into local thrombotic phenotypes.

It is well established that different vascular beds express distinct repertoires of anticoagulants and procoagulants. Moreover, it is widely accepted that systemic imbalances in hemostatic factors lead to site-specific thrombus formation. However, despite significant advances in these areas, it is difficult to predict thrombotic phenotypes based solely on our knowledge of vascular bed-specific profiles of hemostatic factors. Rather, the dual approach of characterizing the clinical phenotype of hypercoagulable states in humans and mice, while continuing to finely map the patterns of basal and inducible hemostatic gene expression, provides an invaluable tool for further delineating mechanisms of vascular bed-specific hemostasis.

Documenting Endothelial Cell Phenotypes

The last several years have witnessed remarkable progress in documenting phenotypic heterogeneity of the endothelium. Among the approaches that have been used are lectin staining, immunohistochemistry, *in situ* hybridization, and real-time intravital microscopy (reviewed elsewhere¹³⁰). The use of sophisticated proteomic techniques, including antibody and phage approaches, has yielded a vast array of cell type-specific phenotypes.^{65,131} In some cases, ECs have been rapidly sorted from different blood vessel types (in health and disease) and assayed for gene expression by serial analysis of gene expression (SAGE), subtractive hybridization, or DNA microarray analysis.^{132–138} However, even with rapid harvest times, ECs may undergo phenotypic drift (examples are found elsewhere^{139,140}). A technique that holds promise for the future is laser capture microdissection of ECs from intact blood vessels, followed by RT-PCR and/or DNA microarray analysis.^{141–144}

A popular—although far less physiological—strategy for documenting EC heterogeneity is to study cultured ECs harvested from different vascular beds. Such an approach has been used to assay for differences in gene or protein expression, enzyme activity, or signaling pathways.^{145–153} Additional studies have examined the differential effects of 1 or more agonists on different types of ECs.^{154–159} These *in vitro* analyses, although interesting and potentially important, must be interpreted with caution. As discussed below, ECs when removed from their native microenvironment are uncoupled from critical extracellular cues and undergo phenotypic drift. (In one study, 40% of proteins expressed in lung endothelium *in vivo* were not detected *in vitro*.¹⁴⁰) Thus, the extent to which site-specific differences *in vitro* correlate with those in the intact endothelium is unclear, emphasizing the importance of validating these results *in vivo*.

One approach for approximating *in vivo* conditions, while maintaining the advantages of cell culture, is to recapitulate the microenvironment *in vitro*. For example, ECs may be grown in coculture with 1 or more non-EC types, allowing varying degrees of heterotypic cell–cell contact.^{160–163} Alternatively, ECs may be incubated with plasma/serum from patients or animal models,^{77,164} or medium that is conditioned during culture with another cell type. Culturing ECs under flow is a more physiological approach compared with static conditions. A major advance in this area is the adjustment of hemodynamic parameters *in vitro* to approximate *in vivo* flow patterns.¹⁶⁵

Mechanisms of Endothelial Cell Heterogeneity

Each EC is analogous to a miniature adaptive nonlinear input/output device (reviewed elsewhere²²). Input arises from the extracellular environment and consists of biomechanical (eg, shear stress and cyclical strain) and biochemical forces (eg, growth factors, cytokines, chemokines, hormones, complement, nitric oxide, oxygen, and reactive oxygen species). Output represents the cellular phenotype and may be measured as cell shape, calcium flux, protein expression, mRNA expression, migration, proliferation, survival/apoptosis, vasomotor tone, hemostatic balance, release of inflammatory mediators, and leukocyte adhe-

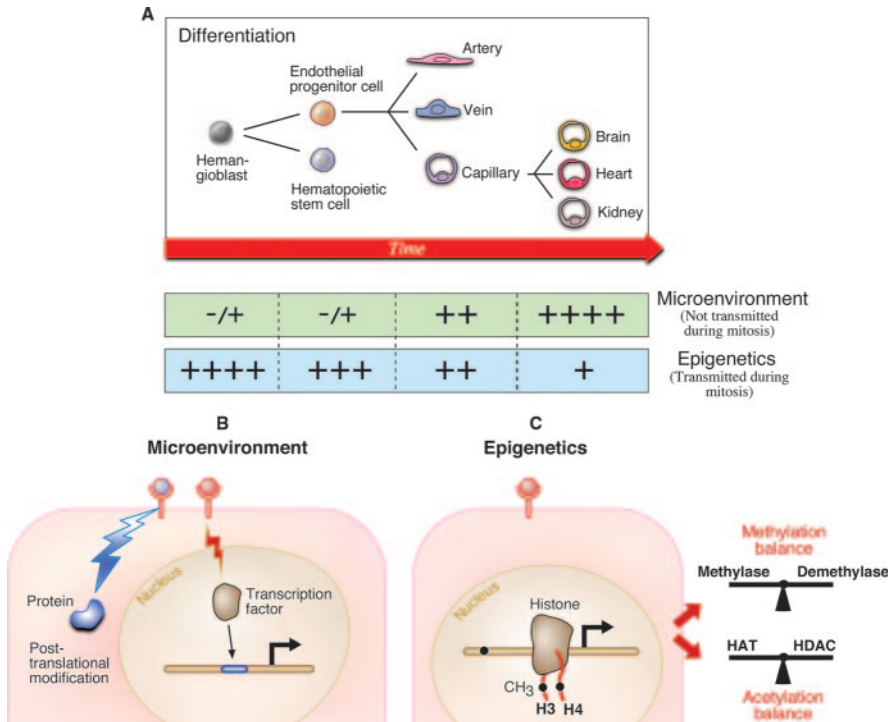


Figure 4. Mechanisms of EC heterogeneity. A, Hemangioblasts give rise to endothelial progenitor cells (angioblasts), which in turn differentiate into ECs of arteries, veins, and capillaries. Cell phenotypes are represented by color shades. Shown is the hypothetical relative role for microenvironment and epigenetics in mediating cell type-specific phenotypes. B, The role of the microenvironment in mediating nonheritable changes in EC phenotype is represented by receptor-mediated posttranslational modification of protein (eg, phosphorylation of a signal intermediate) and transcription factor-dependent induction of gene expression. Removal of the extracellular signal will result in eventual loss of translational/transcriptional effects, and residual effects will be “diluted out” with cell division. C, The role of epigenetics in mediating heritable changes in EC phenotype is represented by DNA methylation (●), histone methylation (CH₃, ●), and histone acetylation (red lines), which in turn negatively or positively influences gene expression. Methylation is regulated by a balance between methylases and demethylases, whereas acetylation of histones is mediated by a balance between histone

acetyltransferases (HAT) and histone deacetylases (HDAC). Although epigenetic modifications are triggered by extracellular signals and are dynamically regulated, they may persist on removal of the signals, and are transmitted during mitosis.

sion/transmigration. Input is coupled to output by signaling pathways that typically begin at the cell surface and end at the level of transcription or posttranscriptional modification. At any point in time, the net input of biomechanical and biochemical signals varies across the vasculature. For example, ECs in the brain are exposed to myriad astroglial-derived paracrine factors that are essential for maintenance of the blood brain barrier. In contrast, ECs lining capillaries in the heart are exposed to regional forces generated from the contracting heart, and paracrine factors derived from neighboring cardiomyocytes. At any single site of the vasculature, net signal input varies from one moment to the next. For example, liver sinusoidal endothelium is exposed to portal venous blood of vastly different composition in the pre- and postprandial period. Because signal input varies in space and time, and because ECs are capable of sensing and responding to the microenvironment, EC phenotypes display marked spatial and temporal heterogeneity.

In theory, spatial and temporal differences in the extracellular environment are sufficient to explain the existence of structural and functional heterogeneity of the endothelium. However, there is also evidence that certain site-specific properties of ECs are epigenetically programmed, such that their maintenance is no longer dependent on signals from the extracellular milieu (Figure 4). For example, DNA microarray studies of multiply passaged ECs cultured from different sites of the human vasculature revealed differences in transcriptional profiles between arterial and venous ECs, and between macrovascular and microvascular ECs.¹⁵² Although the data were not system-

atically validated *in vivo*, the shear number and reproducibility of these differences provide compelling evidence for the existence of site-specific epigenetic modification. Stimulation of human coronary artery ECs with oxidized LDL resulted in more pronounced changes in the expression of genes associated with adhesion, proliferation, and apoptosis pathways compared with human saphenous vein ECs, suggesting an inherent susceptibility of arterial versus venous ECs to atherosclerosis.¹⁶⁶ Pulmonary artery and microvascular ECs display site-specific barrier properties that are preserved in multiply passaged cells.¹⁶⁷

In a particularly revealing study, Lacorre et al harvested human ECs from HEVs of tonsils and umbilical veins and compared gene expression profiles in freshly isolated cells (as a surrogate for *in situ* endothelium) and cells grown in culture for 2 days.¹³⁹ They showed that some, but not all, site-specific transcripts were downregulated between the time of harvest and after 2 days of culture. These latter findings provide strong support for the dual role of microenvironment and epigenetics in mediating vascular bed-specific phenotypes.

Several mechanisms have been implicated in epigenetic modification including DNA methylation, methylation of histone proteins, and histone hyperacetylation (reviewed elsewhere¹⁶⁸). Methylation of DNA and histones is regulated by a balance between methylases and demethylases, whereas acetylation of histones is mediated by a balance between histone acetyltransferases and histone deacetylases. Recent *in vitro* studies have demonstrated a potential role for DNA methylation and/or histone acetylation/methylation in mediating EC-specific gene expression.^{169–172}

However, the extent to which epigenetic modification mediates vascular bed-specific phenotypes (ie, EC heterogeneity) is currently unknown and is an area that is ripe for study.

It is interesting to speculate that aging and/or disease is associated with increased epigenetic modification, hence less plasticity of the endothelium. For example, human intestinal microvascular ECs isolated from affected regions in inflammatory bowel disease demonstrate hyperadhesiveness to leukocytes compared with cells isolated from unaffected regions, or from patients without inflammatory bowel disease.¹⁷³ Remarkably, these differential properties, which were attributed to differences in inducible nitric oxide synthase activity, persisted during sequential passaging.¹⁷⁴

In addition to proximate mechanisms, biological traits (such as EC heterogeneity) require evolutionary explanations. Specifically, what is the phylogeny of the trait and how does it provide a fitness advantage? Endothelium is absent in invertebrates, cephalochordates, and tunicates, but present in the 3 major groups of extant vertebrates, including hagfish, lampreys, and jawed vertebrates. These observations predict that the endothelium evolved in a common ancestor of all extant vertebrates following the divergence of cephalochordates and tunicates (ie, between 540 and 510 million years ago). A recent study of hagfish endothelium revealed the existence of EC heterogeneity in structure, molecular markers, and function.¹⁷⁵ These data suggest that EC heterogeneity evolved as an early, perhaps obligate, feature of this cell lineage. Phenotypic heterogeneity is likely to provide at least 2 fitness advantages: (1) it allows the endothelium to conform to the diverse needs of the underlying tissues throughout the body; and (2) it provides the endothelium with the capacity to adapt to different microenvironments (eg, the profoundly hypoxic and hyperosmolar environment of the inner medulla of the kidney versus the well-oxygenated environment of pulmonary alveoli).

Implications for Diagnosis and Therapy

There are currently few assays for diagnosing endothelial dysfunction. The most common is the use of flow studies to assay for vasomotor reactivity. Future advances in diagnostics will likely rely on the development of multifaceted platforms that assay combinations of soluble mediators, circulating ECs and endothelial-derived microparticles. A limitation of assaying blood from a peripheral vein or artery is that the sample represents the sum average of activity from multiple vascular beds. Thus, information about individual vascular beds may be “diluted out.” One approach to overcome this limitation is the use of catheters to sample blood from one or another vascular bed. Another strategy, yet to bear fruition, is to use our knowledge about vascular bed-specific phenotypes to identify “footprints” of those EC subsets in circulating blood. For example, if dysfunctional ECs in the lung vasculature (but not other vascular beds) release factors X and Y into the blood, and/or express specific receptors on their cell surface, then one might localize disease to lung endothelium by per-

forming an ELISA for X and Y and/or sort for circulating ECs or microparticles that express those receptors. Finally, molecular imaging, which combines the power of proteomics and advanced labeling techniques, promises to revolutionize the diagnosis of endothelial-based disorders.

The endothelium is rapidly and preferentially exposed to systemically delivered agents. Given the capacity of ECs 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 EC 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. This idea is supported by the many knockout models (some of which have been detailed in the current review) that demonstrate vascular bed-specific phenotypes. In so far as treatment against one or another target creates a functional knock out of that target, the effects on the vasculature will be similarly heterogeneous. For example, a treatment aimed toward neutralizing P-selectin might reduce leukocyte trafficking in the gastrointestinal tract, but not the liver. A drug that is designed to promote revascularization in liver disease might also induce fenestrations (hence permeability) in vascular beds such as blood brain barrier, with potentially disastrous consequences.

Revisiting the Definition of the Endothelium

As underscored in the current review, ECs demonstrate remarkable heterogeneity in structure and function. Indeed, it is not a stretch to imagine that each 1 of our approximately 60 trillion ECs is phenotypically distinct. The existence of such profound heterogeneity raises the following question: what, if any, common or core properties unite the endothelium? A clue to the answer lies in the very phenomenon of phenotypic heterogeneity. The endothelium is analogous to a chameleon, constantly molding itself to the needs of the local tissue (some of the variation in “skin color” or phenotypes becomes irreversibly locked in through epigenetic modification). In addition to serving the needs of other cells, ECs must also adapt to many different microenvironments. Perhaps the definition of the endothelium lies not so much in any one structure or function, but rather in the vast, “exploratory-like (ie, plastic) behavior” of ECs. Stated another way, the endothelium may be defined as the inner cellular lining of blood vessels, which, through its remarkable capacity to sense and respond to its local environment, meets the physiological requirements of the underlying tissue, balanced with the need for self-survival, and/or capacity for self-renewal/regeneration, in that microenvironment.

Conclusions

Far from being an investigative curiosity, phenotypic heterogeneity is part of the very fabric of the endothelium. The term

“endothelial cell heterogeneity” is not merely a characterization of existing cellular phenotypes, but rather is itself a property, perhaps the principal core property, of the endothelium. The endothelium is an emergent system in which the whole is greater than the sum of the parts. As much as it is necessary to study and characterize the individual components in isolation, the endothelium should be viewed for what it is: an organ “teeming with life,” every bit as active and complex as any other organ in the body.

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