State of the Art Review

Journal of Veterinary Emergency and Critical Care **14**(2) 2004, pp 84–99

Critical role of the vascular endothelial cell in health and disease: a review article

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Abstract

Objective: To review the human and veterinary literature on the role of the vascular endothelial cell in health, as well as during hypoxic and inflammatory disease states.

Data sources: Data from human and veterinary literature were reviewed through a Pubmed search and a manual search of the references listed in articles covering some aspect of vascular endothelial cell function.

Human data synthesis: The development of techniques that allow the maintenance and growth of endothelial cells in culture has produced an explosion of new research in the area of endothelial cell physiology. This plethora of data has revealed the critical role that vascular endothelial cells play in both health and disease states. Interspecies variations can occur with respect to the vascular endothelial cell physiology and its response to pathologic conditions.

Veterinary data synthesis: There is a paucity of information regarding the role of the vascular endothelial cell in health or disease of small animals. Many human studies use species cared for by veterinarians, providing information that may be applied to small animals and that may be used to construct future studies.

Conclusion: An organ system itself, the vascular endothelium is an essential component of all organs in the body. The endothelial cell lining functions to maintain selective permeability between the blood and the tissue it supplies, regulate vascular tone, sustain blood fluidity through regulation of coagulation, and modulate interaction of leukocytes with the interstitium and inflammatory reactions. During disease states, the endothelial cell functions locally to limit the boundaries of the disease process. If these functions are not controlled, they can become a part of the pathogenic process, contributing to blood stasis and thrombosis, potentiation of local inflammation and interstitial edema formation, subsequent tissue hypoxia, and multiple organ dysfunction. Pharmacological investigations targeting the modulation of endothelial function during disease states have not yet advanced treatment protocols. Since all critically ill animals are at risk for some degree of endothelial cell dysfunction, treatment regimens should focus on promoting capillary blood flow and tissue oxygen delivery.

(J Vet Emerg Crit Care 2004; 14(2): 84–99)

Keywords: coagulation, inflammation, vascular endothelium, vascular permeability, vasomotor tone

Introduction

Once thought to be an inert lining to the vasculature, the vascular endothelium is now appreciated as a highly specialized, multifunctional organ. This single cell layer, which covers the entire inner surface of the circulatory system, forms a conduit for blood. Each endothelial cell is a dynamic, intimate interface between the circulating blood and the underlying

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tissues. This strategic location provides the endothelium with the unique opportunity to monitor biohumoral (chemical) and biomechanical (physical) stimuli from systemic and local origin. As a result, the endothelial cell can adapt its function as required to maintain homeostasis.

The vascular endothelium is intimately involved with many interdependent processes significant to the critically ill patient. These include (1) maintenance of a selectively permeable blood–tissue barrier, (2) modulation of vascular tone, (3) regulation of hemostasis, and (4) regulation of inflammation. The endothelium also actively participates in angiogenesis, wound healing, and tumor metastasis, each of which have a long-term impact on the patient. These functions are interconnected and can change in response to various stimuli, including inflammatory mediators, hypoxia, elements of coagulation, oxidative and fluid sheer stresses, as well as hormones and intracellular infections.

The interaction of specific endothelial receptors with chemical mediators or physical forces leads to second-messenger stimulation and initiation of intracellular metabolic or mechanical responses. Focal thrombosis, edema formation, and inflammation are beneficial responses to local injury, establishing boundaries for the disease process. When these focal adaptations participate in the development of systemic inflammation, then generalized vascular stasis, tissue edema, and tissue hypoxia can lead to multiple organ failure and death.

A major goal in critical care medicine is improving the outcome of patients with multiple organ dysfunction or failure. Advances in endothelial cell research have sparked a renewed interest in the integrated role that the endothelial cell plays in health and disease. As future research unfolds the intricacies of these processes, therapeutic modalities targeting endothelial changes that contribute to disease may become available.

Organization and ultrastructure of the vascular endothelial cell

The endothelial cell has several unique properties specific for its function (Figure 1). The anatomic location of endothelial cells creates three specific cell surfaces: the luminal, abluminal, and lateral surfaces. The luminal surface provides an interface between the circulating blood and the endothelial cell. Multiple luminal surface receptors and adhesion molecules stimulate second messengers, triggering cellular structural and functional changes. Vascular permeability, coagulation, anticoagulation, and inflammatory responses are affected by luminal surface stimulation.

The abluminal surface rests upon the subendothelium. The subendothelium is an extracellular matrix composed of proteins, including collagen type IV, elastin, laminin, fibronectin, thrombospondin, and von Willebrand factor (vWf), secreted predominantly by the abluminal surface of the endothelial cell.[1] The luminal and abluminal aspects of the endothelium are connected by the lateral surfaces.

The lateral surfaces of the endothelial cell provide an area for cell-to-cell bridging. An intercellular cleft forms a thin slit between adjacent cells. The cleft width is approximately 6–7 nm (60–70 Å), slightly smaller than the diameter of an albumin molecule.[2] The lateral cell surfaces are bridged within the cleft by intercellular adhesion sites or junctions.

The number and combination of endothelial intercellular junctions on the lateral surfaces allow the capillary to function differently in different organs.[3,4] Tight junctions and adherens junctions provide contact with the actin of the cytoskeleton, allowing changes in endothelial cell shape and size to occur. They also provide adhesive connections, strengthening the endothelial barrier and affecting cell polarity.[3] Distribution varies along the vascular tree, with tight junctions being more frequent in the larger arteries, correlating with a more stringent requirement for permeability control. Adherens junctions establish a communication between cell-to-cell contact points. Gap junctions enable communication between adjacent endothelial cells and cells within the subendothelial matrix (i.e., pericytes, smooth muscle cells).

The entire endothelial cell membrane (luminal, abluminal, and lateral cell surfaces) is coated with glycoproteins. The endothelium exhibits polarity manifested by asymmetric distribution of these cell surface glycoproteins. The glycocalyx lining the luminal surface contains a layer of immobile plasma and is thought to adsorb plasma proteins.[5] Together, the glycocalyx and the adsorbed proteins are referred to as the endothelial surface layer.[6] This endothelial surface layer may help regulate many different physiologic responses, including shear stress mechanotransduction,[7] oxygen diffusion across capillaries,[8] capillary permeability,[9–11] coagulation,[12,13] and interaction with immune cells.[14,15]

The tethering forces of cell–cell and cell–extracellular matrix junctions exist in equilibrium with contractile forces maintained by the endothelial cytoskeleton. Actin must interact with myosin for contraction to occur. Constitutive tension exists within the cell with a basal actin–myosin interaction, allowing the cell to be poised and ready to respond to its biologic environment.[16] When the endothelial cell is stimulated during disease (i.e., cytokines, shear forces, hypoxia, thrombin),

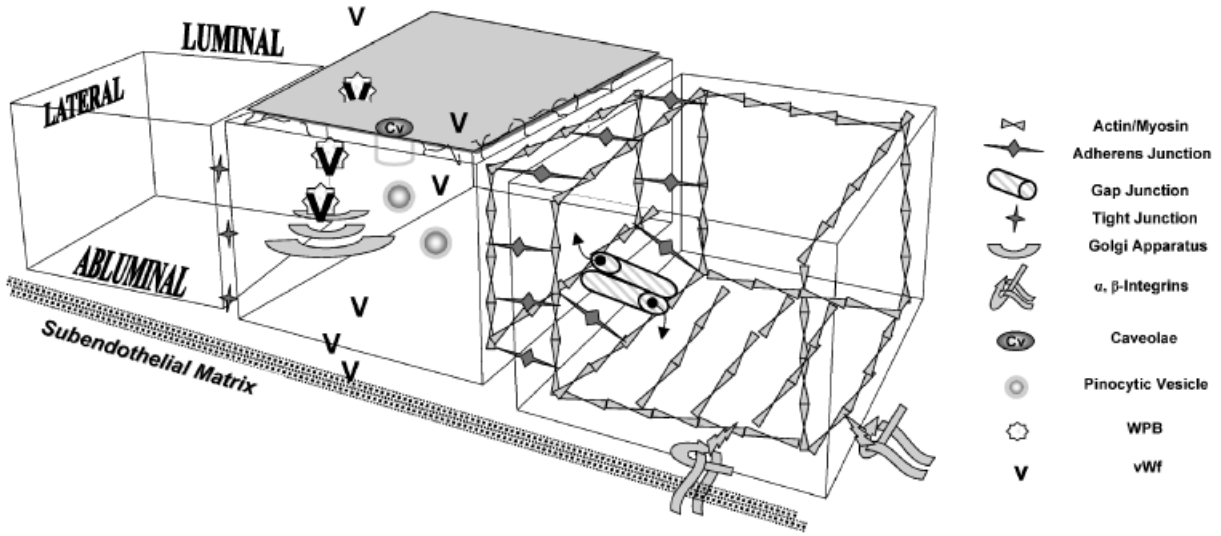

Figure 1: Basic vascular endothelial cell anatomy. The endothelial cell has intercellular junctions specific for the function of the vessel or organ, intracellular contractile proteins actin–myosin, transport and storage mechanisms that include caveolae and Weibel–Palade bodies (WPBs) and three functional cell surfaces. Caveolae are part of a sophisticated system that provides a transport mechanism for substances within pinocytic vesicles into and out of the cell and is a site for receptor–compound interactions. WPBs function as a storage organelle for highly multimeric von Willebrand factor (vWf) constituting virtually all of WBPs' protein content. The side in contact with the circulating blood is called the luminal surface, which is lined by a layer of macromolecules (proteins, glycolipids, glycoproteins, and proteoglycans), called the glycocalyx. The lateral surface encompasses the circumference of the cell and establishes endothelial cell-to-cell contacts. Three types of intercellular junctions predominate: tight junctions, adherens junctions, and gap junctions. The third surface is the abluminal surface, which rests upon the subendothelial matrix. The cell is fixed to the matrix by adhesion receptors called integrins. Integrins form focal adhesions by binding their extracellular α and β subunits to the extracellular matrix. On the intracellular surface, the integrins create a focal adhesion plaque with the cytoskeleton, mediating interaction between the cytoskeleton and subendothelial matrix.

a reorganization of the contractile proteins occurs to alter cell shape.

In addition to contractile proteins, the endothelial cell has a specialized structure called ''caveolae''. Caveolae are flask-shaped luminal membrane invaginations, 50–100 nm in diameter,[17,18] involved in endocytosis and transcytosis of macromolecules. It is speculated that these specialized membrane domains represent localized sites of plasminogen activation. They contain urokinase plasminogen activator (uPA) receptors and cell surface receptors for plasminogen and tissue plasminogen activator (tPA).[19] Endothelial nitric oxide synthase (eNOS) is also associated with caveolae.[20,21] The activity of eNOS is regulated by key structural components of caveolae, known as caveolin and calmodulin.[22,23] Caveolae have also been implicated in directly transmitting luminal forces to the cytoskeleton through signaling molecules that associate with actin.[24]

Weibel–Palade bodies (WPBs) are rod-shaped lysosome-related secretory organelles that are unique to the endothelium, although they are not a universal feature of all endothelial cells. WPBs are regulated secretory organelles[25,26] that ''flow'' to the luminal surface, where their contents are secreted into blood when their membranes fuse with the cell surface membrane.[27,28] P selectin is a transmembrane protein of WPBs, which is involved in the recruitment of leukocytes. WPBs, therefore, play a functional role in the initiation of both coagulation and inflammation.

The endothelium exhibits considerable physiologic and anatomic heterogeneity throughout the different tissues of the body as well as in different locations within an organ.[29] These site-to-site variations are largely determined by cues present in the microenvironment and may have an important impact on the dynamic function of the endothelium at that location.

Role of the vascular endothelial cell in health: its dynamic functions

Selective permeable barrier

The endothelium provides a semipermeable barrier that allows specific substances to move between the blood and the interstitium (Figure 2). With the larger vessels serving the role of conduits, the exchange of water, gases, solutes, and cells between the vascular and interstitial compartments occurs primarily across

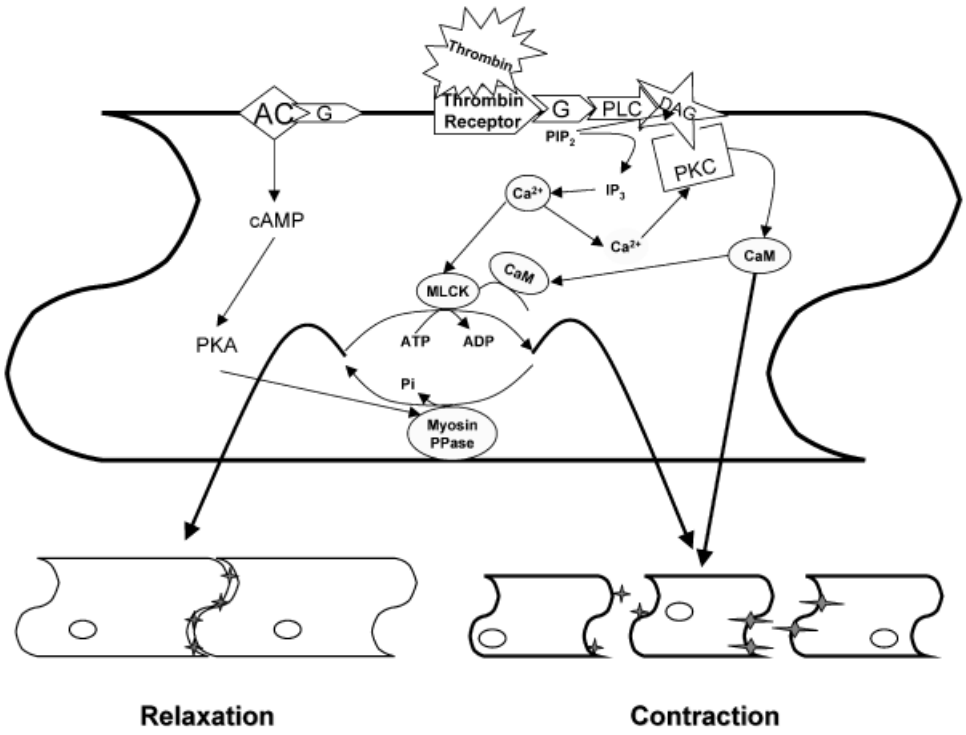

Figure 2: Endothelium and vascular permeability. Endothelial activators (e.g., thrombin) bind with specific receptors resulting in stimulatory G-protein/GTP activation. Receptors activate phospholipase C (PLC) to catalyze the production of inositol bisphosphate (PIP_2) to inositol trisphosphate (IP_3). IP_3 stimulates an internal release of calcium (Ca^{2+}) from the endoplasmic reticulum and also an influx of extracellular Ca^{2+}. Activation of protein kinase C (PKC) provides further signaling leading to increased microvascular permeability via the binding of Ca^{2+} with calmodulin (CaM) and eventual activation of myosin light-chain kinase (MLCK). MLCK promotes actin–myosin interaction and cell contraction, which can enhance paracellular transport. Adenyl cyclase (AC) activation by the G-protein/GTP complex catalyzes the synthesis of cyclic adenosine monophosphate (cAMP). Protein kinase A (PKA) and cAMP interaction promote actomyosin dissociation through myosin specific phosphatases (PPase), endothelial cell relaxation, and restoration of barrier function.

the capillaries and postcapillary venules. It is rare for any cell of the body to be more than 20–30 μm away from a capillary.[2]

Lipid-soluble substances such as carbon dioxide and oxygen diffuse directly across the capillary endothelial cell membrane. Low-molecular-weight hydrophilic substances move virtually unimpeded between endothelial cells through intercellular clefts.

The endothelium maintains its selectivity to macromolecular permeability through several distinct mechanisms.[16,30] The highly negatively charged glycocalyx provides an electrostatic repellant, confining negatively charged macromolecules to the vascular space. Some data suggest that serum albumin crosses through the endothelium via an extensive vesicular transport system of the endothelia.[31–37] In addition, interendothelial adhesion molecules (tight junctions and adherens junctions) regulate the size of the intercellular cleft and hence solute permeability (Figure 1). The integrity of these intercellular junctions is regulated by cytoskeletal tension, alterations in junctional protein binding, and linkage between junctional proteins and the cytoskeleton.[38]

In addition to clefts, some of the more porous capillaries contain fenestrations 20–100 nm wide. Fenestrae may be open perforations or closed by a thin membranous diaphragm approximately 25 nm thick. The fenestrations increase the passage of water and solutes across the capillary surface with no change in the passage of macromolecules. Other capillaries have a discontinuous endothelium, permitting the passage of molecules too large to pass through the intercellular clefts of the endothelium. These vessels have distinct interendothelial gaps 100–150 nm in diameter and tend to be clustered to form sieve plates.[39,40]

Junctions, fenestrations, and discontinuous endothelium are responsible for normal differences in capillary permeability in different body tissues. For example, blood–brain barrier capillary endothelium represents highly specialized abundant intercellular tight junctions impermeable to solutes greater than 2.5 nm.[41] Fenestrations are present in the capillaries of the renal

glomerular tufts. Discontinuous endothelium can be found in the bone marrow, liver, and spleen. Blood cells are typically confined to the intravascular space except in the lymph nodes, liver, spleen, and bone marrow.

Molecular exchange between the circulating blood and the interstitial fluid environment of tissue cells can be distorted during disease states. Increased microvascular (predominantly venular) permeability to macromolecules and water is the basis of edematous tissue injury and a hallmark of acute inflammation. Although a portion of this macromolecular efflux may be secondary to transcytosis via endothelial vesicles, evidence strongly suggests that the flux is predominantly paracellular,[16,30,42] involving the reorganization and widening of interendothelial junctions.[43] The widening of the junctions can be triggered by receptor-mediated (leukocyte-independent) or leukocyte-mediated (leukocyte-dependent) mechanisms.

The formation of gaps between adjacent endothelial cells in response to inflammatory mediators (leukocyte-independent process) likely reflects contraction of the cytoskeleton and recoil of cell–cell borders by altering intercellular junctions.[44] Reorganization of the cytoskeleton and cellular contraction are thought to be involved.[45] This is due to direct links between actin and one or more of the components making up the junctional complexes.[4] This can occur without a loss of cell adhesion to the underlying stratum.

Leukocyte proteases (leukocyte-dependent process), such as elastase[46] and metalloproteinases,[47] may break down junctional elements during leukocyte extravasation, contributing to leukocyte-dependent injury to the endothelial barrier. Thus, in addition to pharmacological regulation of barrier structure, inflammatory permeability during injury *in vivo* probably incorporates hormonal, cytokine, and protease effects on junctional integrity.[38]

Modulation of vascular tone

Vascular permeability and vascular tone are key determinants of blood flow. Vascular tone in a normal microenvironment alternates between vasodilation and vasoconstriction for optimal flow (Figure 3). Vascular tone is directed and maintained by the sympathetic nervous system. However, the specific needs of individual vascular beds are directed by local mediators produced by the endothelial cell.

Capillaries are devoid of smooth muscle and are, therefore, incapable of active constriction. Changes in capillary diameter are passive, caused by alterations in pre- and postcapillary resistance. Local microvasculature maintains a constant state of vasodilation to promote blood flow.[48]

Endothelial cells constitutively express cyclooxygenase-1 (COX-1) and prostacyclin synthase, which catalyze the production of prostacyclin (PGI2), a major vasodilating prostaglandin end product.[49,50] Both PGI2 and nitric oxide (NO; produced by endothelial NO synthase) production increase in response to agonists such as thrombin, shear stress, histamine, and hypoxia.[51–56]

Endothelial cells produce the most potent vasoconstrictor identified to date, endothelin-1 (ET-1).[57–60] The ET-1 isoform is produced constitutively and is not stored.[61] ET-1-mediated vasoconstriction occurs through its interaction with endothelin-receptor subtype-A, expressed on vascular smooth muscle cells. Endothelin-receptor subtype-B (ET_b) is expressed predominantly on endothelial cells and produces vasorelaxation with binding ET-1. Vasorelaxation mediated by ET-1 interaction with ET_b occurs through the production of the aforementioned vasodilators, NO and PGI2. The expression of messenger RNA and release of ET-1 are stimulated by interleukin-1 (IL-1), angiotensin II, hypoxia, and shear stress.[62–64] Local vasoconstriction can also occur due to circulating mediators (e.g., thrombin, angiotensin I, and vasopressin).

Hypoxia and sepsis can trigger the endothelium to alter normal vascular tone. During hypoxemia, the endothelium initiates an immediate but transient vasoconstriction, which is followed by relaxation.[65] When hypoxic exposure is prolonged, blood vessels react by gradual and sustained vasoconstriction. It is thought that this is the result of vasoconstrictors derived from the endothelium.[65,66] In the pulmonary circulation, regional hypoxia results in vasoconstriction as well; this ensures that blood flow is diverted to well-ventilated areas and that regional ventilation and perfusion are maintained.

In the presence of pro-inflammatory cytokines, as can occur in septic patients, the endothelial cells can produce an excess of NO when an inducible form of NO synthase (iNOS) is formed. In contrast to eNOS, iNOS activity is not calcium-dependent and is a major contributor to vasodilation during inflammation.

Sepsis or endotoxemia has a multifaceted effect on vascular tone. It is now well accepted that microcirculatory dysfunction is a major contributing factor to multiple organ dysfunction in sepsis. Arterioles lose normal vasoreactivity, resulting in decreased peripheral resistance and hypotension. This has been reported to be due to a hyporesponsiveness to vasoconstrictors, possibly mediated by NO.[67] In contrast to the systemic circulation, sepsis can induce a hypertensive state within the pulmonary circulation. Endothelins have been implicated as the causative agents. The role that the endothelial cell plays in these pathologic changes is

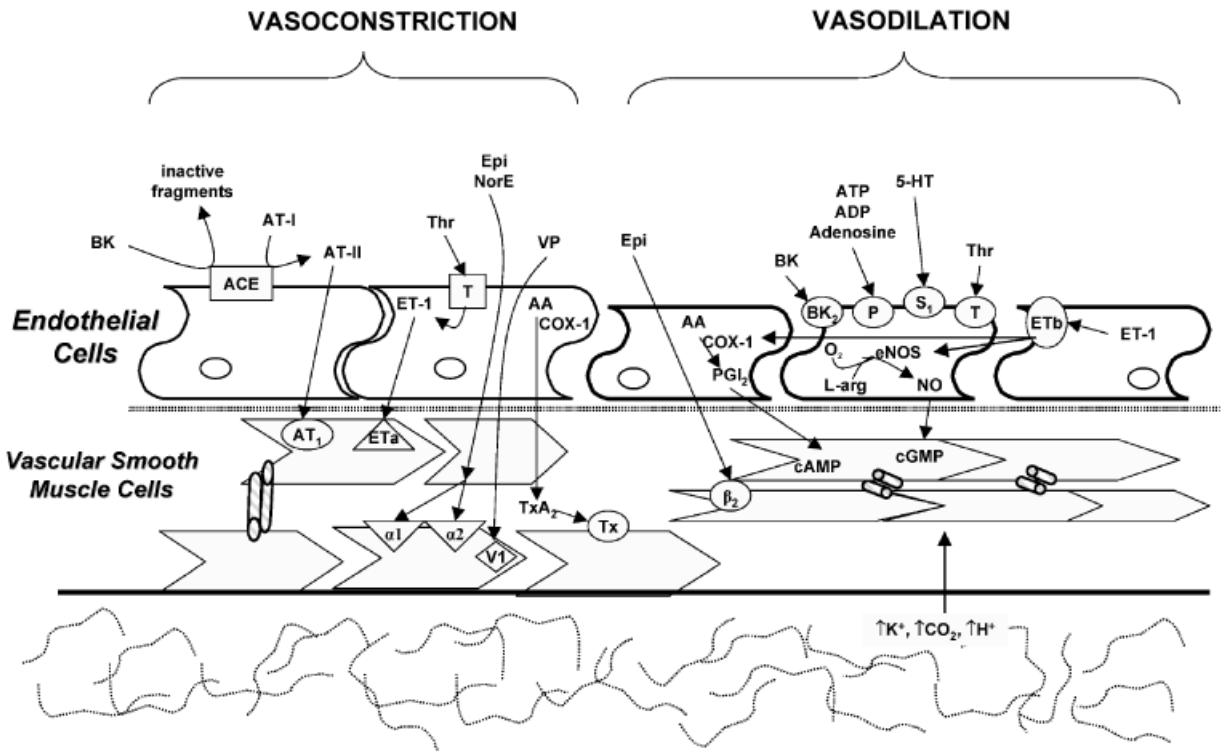

Figure 3: Regional vasoactive properties of the endothelium. The endothelial cells activate and are stimulated by circulating vasoactive substances, and they produce local vasoactive agents. Some, like thrombin (Thr), can produce both vasoconstricting and vasodilating effects depending on the receptor (T) activated. Local vasodilation occurs due to the production of nitric oxide (NO) and prostacyclin (PGI2). PGI2 is produced through the action of cyclooxygenase-1 (COX-1) on arachidonic acid (AA). Circulating mediators such as adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine, thrombin (Thr), and bradykinin (BK) will stimulate vasodilation. A low, basal level of constitutive NO is produced by the endothelium by the action of endothelial nitric oxide synthase (eNOS) on molecular oxygen and L-arginine (L-arg), which acts locally on the vascular smooth muscle. Constitutive NO production is calcium dependent and increases in response to stimuli such as shear stress, bradykinin (BK), Thr, histamine, vasopressin, and serotonin (5-HT). Additional vasodilating effects come from the action of epinephrine (Epi) on vascular smooth muscle β-2 receptors and ET-1 activation of endothelin-b receptor (ET-b), which stimulates the production of NO and PGI2. Finally, increased subluminal concentrations of potassium ions (K^+), hydrogen ions (H^+), and carbon dioxide (CO_2) decrease norepinephrine release from the sympathetic nerve terminals. Vasoconstriction is mediated locally by the endothelium by the action of endothelin-1 (ET-1) on smooth muscle endothelin A receptors (ETa) and, in selected sites, thromboxane (TxA2) on thromboxane receptors (Tx). A potent local vasoconstricting agent, angiotensin II (AT-II), is produced by the action of angiotensin converting enzyme (ACE; an endothelial ectoenzyme) on circulating angiotensin I (AT-I). This conversion enzyme is also important for degradation of the potent vasodilator, BK. Additional vasoconstricting actions are produced by the action of norepinephrine (NorE) and Epi on vascular smooth muscle α-1 and α-2 receptors, and vasopressin (VP) on V1a receptors.

yet to be defined. Important mechanisms may include increased ET-1 production or angiotensin conversion enzyme (ACE) expression.

Hemostasis

To maintain normal blood flow, a balance must exist between the activation and inhibition of coagulation within the circulation. There is a dynamic ongoing interaction between the vascular endothelium, blood cells, plasma coagulation factors, fibrinolytic factors, and their inhibitors (Figure 4). Under normal conditions, the endothelium slightly favors anticoagulant mechanisms to maintain blood fluidity. In times of vascular damage or disease, prothrombotic mechanisms predominate.

Anticoagulant mechanisms: The vascular endothelium provides a smooth covering to prevent contact activation of the intrinsic clotting cascade by the highly thrombogenic subendothelium. The negative charge of the glycocalyx layer, under normal conditions, repels coagulation factors and platelets as they flow through the vessels. Normal blood flow is laminar and the highly cellular central portion flows more rapidly than the constituents in the outer layer. Through the maintenance of vascular tone, the circulation of blood elements

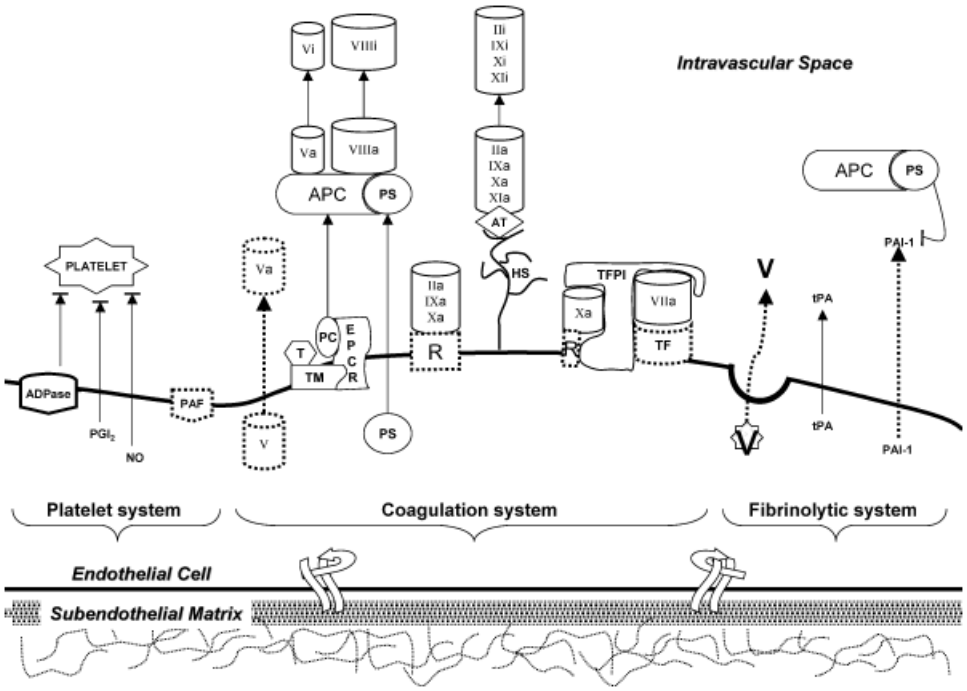

Figure 4: Endothelial modulation of hemostasis. The endothelial cell luminal membrane has pro- and anticoagulation properties. Anticoagulation nitric oxide (NO) stimulates guanylyl cyclase in platelets, resulting in the inhibition of platelet activation, aggregation, and adhesion. Prostacyclin (PGI2) increases platelet cAMP and is a potent inhibitor of platelet activation, shape change, aggregation, and adhesion. Platelet endoperoxidases are also converted to PGI2 by endothelial cells, suggesting a potential negative feedback regulatory mechanism inhibiting further platelet activation. Membrane-derived adenosine diphosphatase (ADPase) directly inactivates platelet-released adenosine diphosphate (ADP), limiting platelet recruitment and activation. The glycocalyx contains heparin sulfate (HS), which will accelerate AT binding and inactivation of several coagulation serine proteases. Thrombomodulin (TM) converts thrombin (T) from a procoagulant to an anticoagulant protease. Endothelial cells express a receptor for protein C (EPCR) enhancing protein C (PC) activation by presenting it to the thrombin–thrombomodulin complex. Activated protein C (APC) down-regulates thrombin generation by greatly accelerating the proteolytic destruction of the major cofactors of the coagulation cascade, factors Va and VIIIa. Protein S is synthesized and secreted by endothelial cells, binding to the surface of endothelial cells, where it forms a macromolecular complex with APC. This binding accelerates the rate of inactivation of factors Va and VIIIa. Protein S may directly inhibit prothrombinase in the absence of APC through direct interactions with factor Xa. Tissue factor pathway inhibitor (TFPI) is also synthesized and expressed by the endothelium. It is the major inhibitor of coagulation factor VIIa and does so in a two-step sequence. The end result is a quarternary structure consisting of TFPI–factor Xa-tissue factor–factor VIIa. When surface-bound, tissue plasminogen-activator (tPA) retains its enzymatic activity and is protected from inhibition by plasminogen activator inhibitor-1 (PAI-1). Procoagulant properties of the endothelial cell include intravascular platelet and serine protease activation. Circulating serine proteases are activated by tissue factor (TF) and other activated proteases. Von Willebrand factor (vWf) released at the endothelial membrane from Weibel–Palade bodies (WPBs) is the largest multimeric species and the most effective in promoting platelet adhesion and aggregation. TF is a predominant procoagulant produced by and contained on the endothelial surface, where it activates factor VII.

removes activated products from the local area, eliminating a microenvironment conducive to thrombosis.

Endothelial-derived vasodilators also have a direct inhibitory effect on platelets (Figure 4).[68–75] Microvascular endothelial cells produce about five times the amount of heparin-like activity compared to cells from the macrovascular tissue, accelerating antithrombin activity.[76,77] Tissue factor pathway inhibitor (TFPI) is also synthesized and expressed by the endothelium.

Thrombomodulin is a membrane glycoprotein constitutively expressed by endothelial cells of most vascular beds and has dramatic inhibitor influences on thrombin catalytic activity.[78,79] Thrombomodulin removes thrombin from plasma and concentrates it at the endothelial cell surface downstream from the site of production. The thrombomodulin–thrombin complex is internalized, enabling the degradation of thrombin, while thrombomodulin is recycled to the cell surface. The thrombomodulin–thrombin complex also accelerates the activation of protein C and its binding with protein S.[80,81]

The anticoagulant activity of the endothelium includes fibrinolysis as well as inhibition of platelets and serine proteases. Plasminogen can be converted to the

active enzyme, plasmin, by plasminogen activators. Endothelial cells synthesize 2 forms: tPA and uPA.[82,83] Endothelial cells are the main source of tPA, typically produced in a constitutive manner, and protected from inactivation by plasminogen activator-inhibitor-I.[84] The resultant plasmin subsequently degrades fibrin into fibrin degradation products.

Procoagulant mechanisms: Fibrin formation is the end product of thrombogenesis. Destruction of the endothelial cell lining allows direct contact of the platelets to the subendothelial collagen, stimulating platelet adherence and aggregation (primary hemostasis). Activated circulating serine proteases initiate a cascade of reactions resulting in fibrin formation (secondary hemostasis). Under normal conditions, the fibrin clot, in combination with transient vasoconstriction, will decrease blood flow through the damaged area and set the stage for vascular repair.

vWf is a large multimeric glycoprotein produced by platelets and endothelial cells that promotes platelet adhesion and thrombus formation at sites of vascular injury.[85,86] Most vWf of endothelial cell origin is dimeric, produced constitutively, and is released from both luminal and abluminal surfaces. Constitutive vWf synthesis is continuous, unaltered by stimulation.[87] Endothelial cell storage of the largest multimeric forms of vWf occurs in WPBs, which is relevant since these large oligomers are the most effective in promoting platelet adhesion and aggregation. The concentration of vWf varies throughout the vasculature, and is directly dependent upon the number of WPBs within the endothelial cell.[88] Secondary hemostasis is indirectly affected by vWf, which prolongs the half-life of factor VIII in circulation (Figure 4).[89] The release of vWf from WPBs occurs in response to agonists such as histamine, thrombin, complement, cytokines, and mechanical injury.[90–96]

Tissue factor (TF) is a protein embedded in the phospholipid bilayer by a variety of cells and, when expressed, it acts as the predominant procoagulant during secondary hemostasis (Figure 4). Normal endothelial cells express little to no TF, although TF is revealed at sites of vascular damage.[97,98] Perturbation of cultured endothelial cells by various agents, including endotoxin, thrombin, interferon-γ (IFN-γ), IL-1, hypoxia, tumor necrosis factor α (TNF-α), or bacterial cell walls, will result in an induction of TF production by the endothelial cell.[97,99–105] Inflammation enhances TF synthesis by endothelial cells *in vitro*, whereas only a slight increase in TFPI synthesis occurs.[106]

The endothelial cells will produce specific coagulation serine proteases in response to specific stimuli. Factor V is produced by endothelial cells (Figure 4)[107] and hepatocytes, and its concentration increases in response to mechanical injury.[108] Activated factor V acts as a nonenzymatic cofactor in the prothrombinase complex to activate prothrombin to thrombin. Endothelial cells bind factor IXa and express a cellular receptor for factor Xa. Endothelial-bound factor IXa is more active than soluble factor IXa.[109]

Any process that inhibits plasmin-induced fibrinolysis is a procoagulant process. Plasminogen activator inhibitors (PAI-1 and PAI-2) neutralize the activity of both tPA and uPA.[110] Endothelial cells have constitutive secretion of PAI-1, which is up-regulated in response to lipopolysaccharides (LPS), IL-1, TNF-α, and thrombin.[12] PAI-1 also inactivates factor XIa.[111] When complexed with activated protein C and protein S in the circulation or on the endothelial surface, PAI-1 loses its inhibitory activity (Figure 4).[112,113] Endothelial cells also synthesize PAI-2, which is secreted in small amounts under normal conditions, but may be released in high concentration from damaged endothelial cells. IL-1 and TNF-α will up-regulate endothelial cell-associated PAI-2 levels.[114] Under normal conditions, the balance favors the secretion of plasminogen activators over inhibitors and trace amounts of active plasmin are present on the endothelial surface.[115]

Inflammation

Damage to the vasculature not only stimulates hemostatic processes but also triggers a local inflammatory response to potentiate healing. The inflammatory response is initiated by chemotactic mediators released from damaged tissue, white blood cells, endothelial cells, or pathogens (either local or systemic). Subsequent leukocyte recruitment and adhesion to the vascular endothelium allow the leukocytes to respond to a mediator ''signal'' (Figure 5). Under normal physiologic conditions, the vascular endothelium expresses only a small number of leukocyte adhesion molecules. Therefore, circulating blood cells do not routinely adhere to or penetrate the microvascular endothelium, except in specialized organs such as the lymph nodes, spleen, and liver.

Endothelial cells and leukocytes express complementary adhesion molecules responsible for the control of leukocyte trafficking and distribution. Under normal conditions, leukocytes circulate freely and do not display significant interaction with the microvascular endothelium. Leukocyte recruitment can be dramatically increased, however, in response to chemotactic stimuli.

Leukocyte recruitment into sites of inflammation involves a dynamic series of events between the endothelial cell and leukocyte. Adhesion molecules, ligands, and the inflammatory process orchestrate these

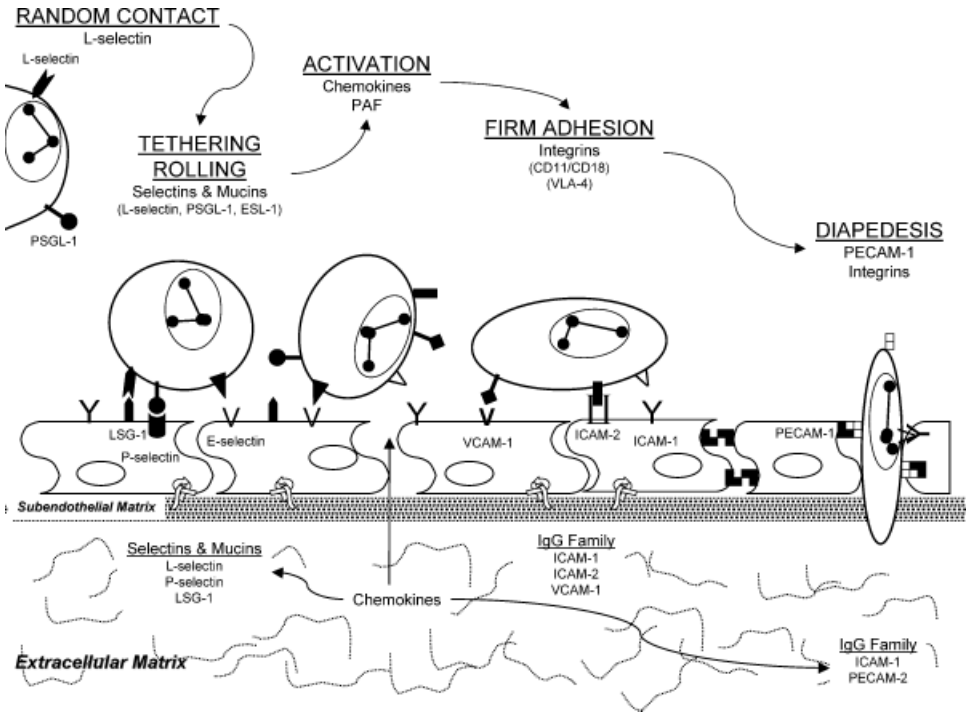

Figure 5: Endothelium and inflammation. Leukocyte attachment, adhesion, and diapedesis are orchestrated principally by three different types of adhesion molecules and their ligands (selectins on the endothelial cell and leukocytes, integrins on leukocytes, and immunoglobulins on endothelial cells). Initially, rolling is mediated by endothelial P-selectin and L-selectin glycoconjugate (LSG-1) and leukocyte L-selectin, P-selectin glycocongugate (PSG-L-1), and E-selectin ligand (ESL-1). Triggering molecules (cytokines and platelet activating factor (PAF)) activate integrin receptors that engage endothelial ligands of the immunoglobulin gene superfamily (IgSF). These consist of intercellular adhesion molecules (ICAM) 1 and 2 and a vascular cell adhesion molecule (VCAM-1), which irreversibly connects the leukocyte to the endothelium and initiates leukocyte spreading. Platelet-endothelial adhesion molecules (PECAM-1) expressed on both leukocytes and endothelial cells participate in transendothelial migration of the leukocyte into the extracellular matrix.

events, which have been described in detail in the veterinary and human literature.[116–133] Adhesion molecules include (1) selectins located on the endothelium and leukocytes (2) integrins primarily located on leukocytes and (3) immunoglobulins primarily located on endothelial cells.

Initially, leukocytes rapidly roll along the endothelial surface of the postcapillary venules adjacent to sites of inflammation. As the leukocyte rolls, it will weakly bind to surface selectins. Because of shear forces continuously agitating the adhesive bonds, some of the leukocytes are released and returned to the circulation. If the leukocytes adhere and become activated, they stick to the luminal surface of the endothelium until they migrate by diapedesis through the interendothelial junction into the subendothelium.

Rolling is mediated by endothelial P selectin, which is rapidly mobilized to the luminal surface from WPB[134–137] and L selectin of leukocytes. Later, E selectin is expressed on the endothelial cell surface after *de novo* synthesis.[138–141] Margination of leukocytes through attachment to P selectin and E selectin slows their velocity to about two orders of magnitude slower than that of main flow. P selectin can be internalized after release and returned to the surface of WPBs.[142]

Once leukocytes have initiated the rolling process and become ''tethered'' to the endothelium, they become activated by signals either originating from the intra- or extravascular space or at the endothelial surface.[143] The activation of leukocytes increases the affinity of integrins for their endothelial ligands of the immunoglobulin superfamily (IgSF). The IgSF adhesion molecule, intercellular adhesion molecule (ICAM-1), is constitutively expressed on endothelial cells,[140] with marked up-regulation occurring following stimulation with pro-inflammatory cytokines.[144,145] Expression of the vascular cell adhesion molecule (VCAM-1), which supports the adhesion of all leukocytes other than neutrophils,[146,147] is up-regulated by cytokines as well.

Once adherent leukocytes encounter the interendothelial junction, diapedesis occurs between the cells and through the subendothelial matrix to the site of inflammation. Leukocyte migration takes place across the venules where the surface charge is lowest,

hemodynamic shear is low, and adhesion molecules are selectively expressed. Diapedesis involves leukocyte signaling to the endothelial cell through a disruption of junction integrity.[148,149] Platelet-endothelial adhesion molecules (PECAM-1) and ICAM-1 serve an important role in mediating transmigration of neutrophils from the vascular space. PECAM-1 is constitutively expressed and localized to the lateral junctions of endothelial cells. ICAM-1 is expressed over the entire surface of the endothelium.[150] VCAM-1, in contrast, is confined to the luminal surface of the endothelium. In some vascular beds, leukocytes may emigrate by traversing through, rather than between, endothelial cells.[151]

Role of the vascular endothelial cell in disease

Most disease processes will have an effect on the vascular endothelium. Trauma (mechanical forces), hypoxia, malnutrition, microorganisms, chemical agents, and temperature extremes can stimulate changes within the endothelial cell. When the response is appropriate and focal to the area of injury, the result is protective. Should the stimulant or the response become systemic, normal endothelial function can produce pathological consequences.

Small animals can present with a variety of diseases that lead to systemic inflammation (systemic inflammatory response syndrome). Diseases include pancreatitis, severe trauma, generalized neoplasia, immune-mediated diseases, hypoxia, anaphylaxis, heat stroke, parvovirus enteritis, and sepsis. Hallmarks of these syndromes can be directly related to changes induced by the endothelial cell. Increased vascular permeability contributes to hemoconcentration, hypotension, impaired blood rheology, hypoalbuminemia, and tissue edema formation. Impaired vascular tone results in hypotension and contributes to inefficient cardiovascular compensatory responses. Microvascular stasis can lead to increased procoagulant activity and disseminated intravascular coagulation. The inflammatory response can be damaging to normal tissues. These ongoing physiologic processes will potentiate one another until multiple organ dysfunction occurs, leading to death. Investigations are ongoing for therapies aimed at modulating endothelial cell reactions during the progression of disease under pathological conditions. Studies that have focused almost exclusively on the inflammatory cascade, such as anticytokine therapy (i.e., monoclonal antiendotoxin antibody,[152] anti-TNF-α monoclonal antibody[153]) to antiadhesive therapy,[154] have yielded disappointing results. There has also been a lack of efficacy of early, high-dose corticosteroid therapy[155] or ibuprofen therapy,[156] medications known to reduce the levels of a number of inflammatory mediators. Failure of clinical trials using immunomodulating agents may be due to several factors: (1) the agents are each targeted towards single mediators and are ineffective against the complex, multiple-mediator-driven inflammatory and coagulation cascade; (2) the agents themselves may be ineffective; (3) the dosing or timing of the agent may be inadequate; and (4) the patient populations may be too heterogenous.[157] There is also the concern that these modalities could leave the patient immunosuppressed.

Studies have also focused on altering the vasoregulatory changes that can occur secondary to endothelial injury. While nonspecific inhibitors of NOS improve blood pressure, there is increased risk of reducing capillary blood flow, increased platelet and leukocyte adhesion, and increased coagulation activation and organ injury.[158] More recently, therapies aimed at alterations in the coagulation system have yielded favorable results in early clinical trials[159–164] and variable results in phase III clinical trials.[165,166]

At this time, there are no individual pharmacologic agents that specifically treat endothelial cell alterations caused by hypoxia and systemic inflammation. A few pharmacological agents may have multimodal effects that include actions on the endothelial cell during inflammation. Pentoxifylline has been shown to alter pathology at the microcirculatory level by impairing leukocyte adherence to the endothelium in addition to its classical role of increasing erythrocyte deformability.[167] Pentoxifylline has also been shown to prevent the activation of coagulation by endotoxins,[168] and is thought to reduce the direct toxic effects of TNF-α on the endothelial cell.[169] There has been recent evidence that the class of drugs called ''statins'' (cholesterol-lowering agents used in humans) may increase cerebral blood flow and attenuate inflammation at the microvascular level. The increase in blood flow is attributed to increased eNOS activity, with subsequent increased availability of NO. Statins inhibit inflammation by inhibiting leukocyte trafficking into inflamed areas and down-regulate proadhesive cell adhesion molecules.[170]

Until future research uncovers therapeutic modalities that specifically modulate unwelcome endothelial cell responses, basic management techniques used to promote microvascular blood flow and tissue oxygen delivery will still hold true for the management of disease states involving endothelial dysfunction. Intravenous crystalloids and colloids are administered to improve rheology and maintain intravascular volume. Administering colloid molecules larger than the size of the interendothelial cell cleft (e.g., hetastarch) may promote intravascular volume retention during situations of increased capillary permeability. Red blood cell

transfusions or hemoglobin-based oxygen carriers are used when hemoglobin levels drop to a precipitous level. Vasomotor tone can be manipulated using agents that affect endothelial smooth muscle constriction and dilation. Finally, hypercoagulable conditions are managed using anticoagulants and antithrombin replacement therapy as needed.

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