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REVIEW

Normal endothelial cell function

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Endothelial cell functions, primarily involving regulated mediator secretion or altered surface protein expression, are vital for normal homeostasis. Endothelial cells secrete the potent vasodilator and anti-platelet agent prostacyclin and nitric oxide, and also the potent vasoconstrictor peptide endothelin-1; they control the selective adhesion and emigration of leukocytes from the bloodstream; and they are the source of circulating von Willebrand factor, tissue plasminogen activator and type 1 plasminogen activator inhibitor. The properties of healthy endothelium ensure that an antithrombotic and anticoagulant balance is maintained in the bloodstream, and provide a tonic vasodilator action that controls blood flow and pressure on a minute-to-minute basis. Disturbances of normal endothelial function are strongly implicated in the pathogenesis of atherosclerosis and autoimmune vasculitic diseases including lupus.
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Introduction

Normal endothelial cell function is now recognised to be critical for all aspects of vascular homeostasis (see Table 1). Thus the active metabolism of these cells is necessary for the continuous adjustment of vascular tone, and hence the control of blood pressure; for the physiological regulation of leukocyte traffic from blood to tissues; and for the maintenance of an antithrombotic and anticoagulant balance in flowing blood. These are summarised in the following sections of this brief review. More recent advances in developmental biology have additionally demonstrated that endothelial cells are most closely related to the hematopoietic cell lineage, with a common precursor, the hemangioblast, that differentiates into either blood cell precursors or the surrounding endothelial cell precursor (the angioblast) in primitive blood islands in the embryo.[1] Initial blood vessel development by this process of differentiation is referred to as vasculogenesis. Vessel growth after this point is predominantly or exclusively by angiogenesis, the process of capillary sprouting of endothelial cells from pre-existing vessels,[2] which is followed by vascular remodelling to form a mature vessel with the requisite pericyte or smooth muscle cell layers to become arterioles, veins, etc. It is now recognised that the endothelium directs the acquisition of the pericytes or smooth muscle cells in development by secreting signals that lead to the recruitment of undifferentiated mesenchymal cells and their maturation, and in the adult similar signals induce mitogenesis and recruitment of neighbouring pericytes and smooth muscle cells.[3] Hence endothelial cells are the primary cell type orchestrating the construction of mature blood vessels.

Control of vascular tone

Endothelial cells are responsible for the regulated synthesis and secretion of two potent, short-lived, vasodilator substances that together provide minute-by-minute control of vascular tone and blood pressure: prostacyclin (PGI_2), synthesised from arachidonic acid released from membrane phospholipids; and nitric oxide synthesised from arginine. The use of inhibitors of NO synthesis has proved that in virtually every vascular bed in many species, including humans, NO is an important vasodilator.[4] Lack of the endothelial isoform of NO synthase in mice produces a phenotype with reduced endothelium-dependent vasodilatation and elevated systemic blood pressure.[5] In some vascular beds one or more further endothelium-derived vasodilators are also produced

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Table 1 Endothelial cell properties that regulate vascular homeostasis

Control of vessel wall growth, development and differentiation
Control of vascular tone, regional blood flow and blood pressure
Control of leukocyte traffic to extravascular tissues
Control of solute flux and fluid permeability across the vessel wall
Control of platelet adhesion and aggregation
Control of blood coagulation
Control of fibrinolysis

known as endothelium-derived hyperpolarizing factors (EDHF) from their effect on smooth muscle cells. Current candidates include oxidized derivatives of arachidonic acid, but the identity of EDHF is still uncertain.[6] Failure of endothelium-dependent vasodilatation due to lack of NO synthesis (and/or increased NO destruction by reactive oxygen species) is an early feature of hypercholesterolemia and atherogenesis, and deliberate chronic inhibition of NO synthase exacerbates atherogenesis in animal models.[7] Intriguingly, the deficit in endothelium-dependent relaxation can be reversed by feeding extra arginine in the diet. The cause of the defect is not clear, but an increased production of endogenous inhibitors of NO synthase, such as asymmetric dimethylarginine, has recently been proposed.[8]

Both NO and PGI_2 synthesis can be triggered rapidly and transiently by a variety of agents that cause increased cytoplasmic concentrations of Ca^{2+}, acting via G protein-coupled receptors, and both NO synthase and phospholipase A_2 (the initial rate limiting enzyme in the PGI_2 synthetic pathway) are activated by Ca^{2+}.[9] These agents are predominantly molecules generated during coagulation or platelet activation (e.g. bradykinin, thrombin, ATP), reflecting the other important role of NO and PGI_2 as inhibitors of platelet function. However, there are dual controls on NO and PGI_2 synthesis, and each can also be activated by protein kinases.[10,11] This is currently believed to be of particular relevance in the control of NO synthesis by what is arguably its most important physiological activator—shear stress. Increasing shear stress induces endothelial NO synthesis in the absence of detectable changes in cytoplasmic Ca^{2+}. This requires activation of protein kinase B,[12,13] and is apparently dependent on the close physical association of NO synthase with the plasma membrane, notably within its abundant invaginations known as caveolae, which have been proposed to act as microenvironments specialised to respond to external signals such as shear forces.[14] Thus increased blood flow or pressure is sensed by the endothelial cell and leads to the feedback production of local vasodilators to maintain vascular homeostasis.

Endothelial cells are also the source of the potent vasoconstrictor peptide endothelin-1 (ET-1). The regulation of its synthesis and secretion remain less well understood than for NO or PGI_2, but there is *in vitro* evidence that ET-1 release can also be modulated by physical forces such as shear or stretch. Because of its long-lasting action, ET-1 is more likely to be a tonic modulator of vascular tone. There is evidence for increased circulating ET-1 levels in various forms of human hypertension, and ET receptor antagonists have definite clinical promise in the treatment of hypertension and heart failure.[15,16]

Control of leukocyte traffic

The importance of changes in the properties of the endothelium that lead to the increased adhesion and extravasation of leukocytes during the acute inflammatory response was first recognised over a century ago, but the identification of the molecular mechanisms involved has only advanced substantially in the last decade. It is now clear that activation of endothelial cells by thrombin, inflammatory cytokines (notably interleukin-1 or tumor necrosis factor) or bacterial endotoxins causes the increased expression of a series of adhesive molecules at the endothelial luminal surface, and the key molecules involved have been characterised in the multistep process by which leukocytes first transiently tether to and roll on the endothelial surface, then adhere more strongly and migrate over the endothelium, and finally squeeze between endothelial cells to emigrate from the blood vessel and move towards the site of tissue damage or infection.[17]

Resting endothelial cells in most blood vessels do not constitutively express molecules that can attract leukocytes to bind from flowing blood. However, thrombin (or histamine) selectively causes the rapid and transient translocation to the endothelial surface of preformed P-selectin, one of the three member of the selectin family of transmembrane proteins involved in leukocyte capture and rolling.[18] The second member, E-selectin, is expressed by endothelium within a few hours after exposure to inflammatory cytokines or endotoxin.[19] The counterligands for E- and P-selectin on leukocytes are certain sialylated and fucosylated oligosaccharides presented by particular glycoproteins.[20] The unusual chemical properties of the selectin–oligosaccharide interaction are well suited to their biological role, enabling rapidly reversible binding at quite high affinity. The third member, L-selectin, by contrast, is constitutively present on the majority of leukocytes, and counter-

ligands constitutively present on the specialised 'high' endothelium in lymph node venules are required for the efficient capture and physiological traffic of lymphocytes that underlies the process of leukocyte recirculation necessary for immune surveillance.[21] As yet undefined counterligands for L-selectin are also upregulated on endothelial cells at sites of inflammation, and there they contribute with the other two selectins to the initial phase of leukocyte attachment.[22]

The second phase, firm attachment of leukocytes to endothelium, is mediated by interactions between two other classes of transmembrane proteins, integrins on the leukocyte surface and members of the immunoglobulin superfamily on the endothelium, and is triggered by activation of the integrins. The activation process is due to exposure of the leukocyte to one or more chemokines (members of a large family of small proteins) or other chemoattractants (e.g. the bacterial peptide fMet–Leu–Phe, or the lipid mediator platelet-activating factor) that bind to a family of G protein-coupled receptors. This leads to an 'inside-out' signalling which changes the conformation of the integrins and enhances their affinity for their counterligands.[23] Members of the β_2 integrin family bind to intercellular adhesion molecule-1 (ICAM-1), present on resting endothelium and upregulated on cytokine-activated endothelium. Monocytes, but not neutrophils, express the integrin $\alpha_4\beta_1$ which binds to vascular cell adhesion molecule (VCAM), only expressed on activated endothelium.[24] Selective interactions of this type contribute to the programmed evolution of the inflammatory response, in which early neutrophil emigration is usually followed by monocyte emigration.

In the context of atherogenesis, it is also important to note that expression of VCAM is sensitive to oxidant stress, and both hyperlipidemia and hyperglycemia (as in diabetes, where atherogenesis is exacerbated) lead to the generation of pro-oxidant molecules that can upregulate endothelial synthesis of VCAM and the monocyte-selective chemokine MCP-1, providing a plausible link to the early accumulation of monocytes in the vessel wall in developing atherosclerotic lesions.[25,26]

The final phase of leukocyte emigration between endothelial cells involves another member of the immunoglobulin superfamily, platelet-endothelial cell adhesion molecule (PECAM). PECAM is concentrated at endothelial junctions, where it forms homodimers linking two endothelial cells. It is also present on leukocytes, and breaking of the dimers between endothelial cells to form dimers between the emigrating leukocyte and endothelium appears to be critical for diapedesis.[27]

Control of platelet function, coagulation and fibrinolysis

Endothelial cells are the source of circulating von Willebrand Factor (vWF). This highly multimeric glycoprotein has two separate biological functions: first as the carrier for coagulation Factor VIIII; and second as the cofactor required for platelets to bind to collagen exposed when the vessel wall is damaged, thus initiating formation of a hemostatic plug. vWF is secreted by two pathways, one constitutive and one by triggered exocytosis from storage granules (Weibel–Palade bodies) that contain high molecular weight multimers (with greater biological activity).[28] Secretion from granules is rapid, and initiated by a fairly small range of agonists, of which thrombin and histamine are the best described. The granule membrane contains P-selectin, and thus vWF secretion is accompanied by the transient expression of P-selectin at the endothelial cell surface as granule and plasma membranes fuse for exocytosis. The intracellular transduction pathways leading to granular secretion include elevation of cytoplasmic Ca^{2+}, but also other signals that are poorly understood.[29] Plasma vWF levels are quite stable in healthy individuals, but are transiently elevated several fold during infection, like other acute phase reactants, which is likely to contribute to increased prothrombotic risk.[30] The mechanism for this elevation is unclear, since pro-inflammatory cytokines do not directly induce vWF release, though they can enhance thrombin-induced secretion.[31] In disease states where there is vascular involvement (autoimmune vasculitic diseases, atherosclerosis, etc) plasma levels of vWF are chronically elevated, presumably reflecting ongoing endothelial activation or damage.[32] Again, the underlying mechanisms have not been elucidated.

Thrombin, generated during the clotting process, is a major physiological modulator of endothelial cell behaviour. As noted above, it triggers the release of NO and PGI_2, which in addition to their effects on vascular tone, are each powerful and physiologically relevant inhibitors of platelet activation, as demonstrated by the phenotypes of mice lacking endothelial NO synthase and humans with mutant defective PGI_2 synthase.[33,34] The main consequences of the interaction of thrombin with endothelium are summarised in Table 2. The secretory responses are predominantly a consequence of the interaction of thrombin with its specific G protein-coupled receptor (protease-activated receptor 1; PAR1), one of a growing family of these receptors that are activated by the protease clipping an N terminal extracellular peptide from the receptor itself, thus exposing a sequence that now binds as a 'tethered ligand' to another region of the receptor and self-activates it.[35]

Table 2 Interactions of thrombin with endothelial cells

Rapid synthesis and secretion of NO and PGI_2
Rapid synthesis and secretion of PAF
Rapid secretion of vWF with concurrent surface expression of P-selectin
Rapid and transient increase in solute permeability between endothelial cells
Increased tissue factor gene transcription and protein expression
Increased tPA and PAI-1 gene transcription and protein secretion
Conversion of thrombin to anticoagulant by binding to thrombomodulin
Inactivation of thrombin by binding to antithrombin

Two other endothelial surface interactions form the main physiological homeostatic systems designed to ensure that the effects of locally generated thrombin are spatially confined, preventing unwanted systemic procoagulant effects. The first of these is capture and inhibition of thrombin by antithrombin. Antithrombin is synthesised mainly in the liver, but binds from the circulation avidly to surface glycosaminoglycans on endothelium. When it binds thrombin, the inactive complex is released back into the circulation and cleared rapidly by the liver.[36] The second is the expression of an endothelial transmembrane protein, thrombomodulin.[37] When thrombin binds to thrombomodulin its conformation is changed such that it is far less efficient at cleaving fibrinogen, but far more efficient at cleaving and activating circulating protein C. Activated protein C is an anticoagulant that inactivates coagulant factors Va and VIIIa. Recently, a specific endothelial transmembrane protein has been characterised that binds protein C and may enhance its ability to interact with thrombomodulin-bound thrombin, known as the endothelial protein C receptor (EPCR).[38] Soluble forms of thrombomodulin are found in the circulation, and their levels, like those of vWF, may reflect aspects of endothelial cell activation or damage (e.g. ref. 39).

The properties of healthy endothelial cells, as described above, contribute to the maintenance of normal blood fluidity by their anticoagulant and antiplatelet effects. When activated by exposure to inflammatory cytokines, endotoxin or thrombin, however, the endothelial cell phenotype becomes less anticoagulant, in addition to the modulation of leukocyte adhesion and migration noted above. The two main elements controlling fibrinolysis, tissue plasminogen activator (tPA) and its physiological inhibitor, plasminogen activator inhibitor-1 (PAI-1), are each endothelial cell secretory products: both are released continuously into the bloodstream with PAI-1 normally in excess.[40] tPA can also be acutely released from small granular stores, notably in response to thrombin.[41] Following endothelial activation by inflammatory cytokines or endotoxin the rate of PAI-1 secretion is enhanced, whereas constitutive tPA secretion is unaltered or diminished.[42] It has also recently been shown that PAI-1 secretion can be enhanced by treatment of endothelium with glycated or oxidised lipoproteins, suggesting one mechanism for the increased procoagulant risk found in diabetes and atherosclerosis.[43]

All extravascular cells constitutively express the transmembrane protein tissue factor (known as thromboplastin when combined with cofactor membrane phospholipids) responsible for binding and activating Factor VII. Although this 'extrinsic' coagulation pathway for the activation of Factor X, and hence the conversion of prothrombin to thrombin, was for many years regarded as subordinate to the first described 'intrinsic' pathway, it is now clear that tissue factor is the primary physiological initiator of coagulation.[44] By contrast, and as required to prevent intravascular coagulation, blood cells and endothelium do not normally express tissue factor. *In vitro* both monocytes and endothelial cells can be induced to express tissue factor by exposure to inflammatory cytokines or endotoxin, and there is good evidence that monocyte expression of tissue factor occurs *in vivo* and contributes to intravascular coagulation seen, for example, in bacteremia (e.g. ref. 45). However, endothelial cell expression of tissue factor *in vivo* has been much less convincingly demonstrated, and seems to be under tighter control than in monocytes. Instead, endothelial cells are the source of the most important physiological inhibitor of the extrinsic pathway, tissue factor pathway inhibitor (TFPI, previously also known as EPI or LACI).[46] This protein is secreted, circulates in plasma, and is also found bound at the endothelial cell surface.

Conclusions

Healthy endothelial cells are vital for the correct maintenance of vascular homeostasis. By the secretion or surface expression of a series of specific molecules, these cells ensure that under normal conditions blood flow is appropriately regulated and that intravascular platelet activation and blood coagulation are avoided. In response to pathophysiological mediators, endothelial cell properties are dynamically modulated, to support vessel growth or repair and to guide the resolution of inflammatory or infections process. In many instances this temporary alteration of endothelial cell phenotype contributes to the successful restoration of vascular homeostasis. However, in certain disease states — notably systemic autoimmune diseases with a vasculitic component (including

systemic lupus erythematosus) and atherosclerosis—it appears that endothelial cell behaviour is perturbed chronically and that this is critical for disease progression. In the last twenty years exploration of endothelial cell biology has defined many endothelial functions at the molecular level, and several of the mechanisms causing acute changes. In future, efforts to understand what mechanisms underlie long-term changes in endothelial cell properties and how to correct them will be key to improving our treatment of these diseases. One recent development in particular may prove to be instrumental in our ability to modify endothelial cell function. It is now certain that endothelial cell precursors can be detected in the circulation of healthy individuals, and in mouse models it has been shown that these cells can contribute significantly to new endothelium found when bloods vessel grow or repair in response to a wide variety of stimuli.[47] Thus we may be entering an era when these precursor cells can be harvested, genetically modified and expanded, and re-introduced to modulate vascular properties at sites of damage.

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