1 • The vascular endothelium: structure and function

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INTRODUCTION

The endothelium provides a cellular lining to all blood vessels in the circulatory system, and forms a structural barrier between the vascular space and the tissues. This cellular layer is no longer viewed as an inert structure, but rather has been recognized to be a dynamic organ, important in several house-keeping functions in health and disease. In adults, the endothelium weighs approximately 1 kg, comprises 1.6×10^{13} cells and has a surface area between 1–7 m^2 [1]. The endothelial cell (EC) is between 25–50 μm in length, 10–15 μm in width and up to 5 μm in depth. Each EC comes into contact with numerous smooth muscle cells and vice versa.

The location of the endothelium at the interface between the blood and vessel wall endows upon it an obligatory role in vasoregulation, the provision of an anti-thrombotic surface facilitating laminar blood flow, and selective permeability to haematopoietic cells and nutrients. These activities are most evident in the microcirculation where the endothelial cell surface area : blood volume ratio is maximal.

Nutrients and macromolecules may flow out of the bloodstream through intercellular spaces between ECs. These intercellular spaces are the result of cellular contraction and have been implicated in local oedema formation. Alternatively, nutrients may be actively transported by transcytosis through the cells themselves.

The endothelium is responsible for regulating the growth of the surrounding connective tissue. In its basal unactivated state, it prevents the proliferation of smooth muscle by the secretion of transforming growth factor-β (TGFβ) and by the surface expression of haparan-like molecules. When activated, however, cytokine/growth factor production by the deranged endothelium results in unchecked smooth muscle proliferation. Enhanced secretion of platelet-derived growth factor (PDGF) along with insulin-like growth factor (IGF) and basic fibroblast growth factor (bFGF) by the dysfunctional endothelium has mitogenic effects on smooth muscle cells, and plays a role in atherosclerotic plaque formation.

Endothelial activation may result from diverse insults such as disordered local cytokine production, viral infection, free radical formation or oxidation of lipids. Perturbations in endothelial function have been implicated in several diseases including atherosclerosis, cancer metastasis, inflammatory diseases and hypertension. Indeed anti-endothelial antibodies have been detected in diabetes mellitus, Raynaud's disease, scleroderma, Kawasaki's disease, vasculitides and in transplant rejection.

ANGIOGENESIS

Angiogenesis is the development of new blood vessels/capillaries from pre-existing vessels (as opposed to vasculogenesis which is the *de novo* formation of vessels important in embryogenesis) and is an essential process in normal growth. In the healthy adult, however, angiogenesis occurs only in select phases of the female reproductive cycle and as a protective mechanism in wound healing/tissue repair. Angiogenesis has been implicated as a key process in pathological conditions such as the proliferative phase of diabetic retinopathy, neovascularization of tumours and in inflammatory diseases such as rheumatoid arthritis. There has been considerable interest in the inhibition of angiogenesis as a therapeutic strategy and hence much research has focused on the underlying mechanisms regulating angiogenesis.

Recent evidence suggests that angiogenic ECs not only arise from contiguous ECs, but may also be derived from bone marrow derived EC precursors. These precursor cells are identified by characteristic cell surface antigen expression and have been demonstrated in the peripheral blood [2]. Bone marrow derived EC precursors have been shown to home to sites of angiogenesis and to be active participants in neovascularization in animal models of limb ischaemia [3; 4].

Angiogenesis involves the degradation of extracellular matrix (ECM) by EC, migration to the perivascular space, proliferation and formation of tubes to line the patent vessels, in association with pericytes. Survival of ECs is dependent on contact with like cells (cell–cell contact), as well as cell–matrix attachments.

Endothelial cell extracellular matrix attachments: the role of integrins

Attachments between the EC and surrounding ECM are mediated by the integrin group of cell surface adhesion receptors. Integrins provide adhesive and signalling functions between ECs and the ECM, and this interaction is critical in maintaining EC polarity and alignment along the vasculature. Integrins are heterodimeric proteins, comprising two non-covalently bound subunits, α and β. There are 18 α and 8 β subunits, which associate in various combinations to provide 24 different heterodimers [5]. The integrins present on ECs include β1, β3, β4, α1, α2, α3, α5, α6, αvβ3 and αvβ5.

Endothelial cell migration

The role of integrins

Migration of ECs involves the development of cell polarity and a leading edge (focal adhesion). The integrins link the cell with the ECM at the focal adhesion and then link with the actin cytoskeleton. This interaction stimulates cell contraction, thus allowing cell movement on adhesive contacts. The focal adhesion is of critical importance and is a site where regulatory proteins are identified. Focal adhesion kinase (FAK) is a cytoplasmic non-receptor tyrosine kinase which becomes phosphorylated on at least seven tyrosine residues when binding its ligands [6]. The FAK mediates its actions through various adaptor proteins and can ultimately activate cell movement via phosphatidyl-inositol-3-kinase (PI3) and Rac-1 (a G-protein).

Eventually integrin inactivation destroys the adhesive complex and allows detachment of the cell in its new location. Migratory deficits have been found in cells lacking FAK and reintroduction of FAK has been shown to restore migratory capacity in ECs [7].

Migration of ECs through the ECM is facilitated by matrix metalloproteinase (MMP) induced degradation of the ECM. Integrins play a role in the regulation of MMP activity. In ECs, α v β 3 not only stimulates MMP-2 production, but interacts with it, to activate the newly synthesized enzyme further [9]. This interaction is critical in EC migration, and indeed, inhibition of MMP-2/α vβ 3 binding has been shown to suppress angiogenesis [10].

The integrin αvβ3 is recognized as a key player in angiogenesis and is highly expressed on growing vessels. However, αvβ3 is barely detectable on quiescent endothelium [11; 12]. It can bind vitronectin, fibronectin, von Willebrand factor, fibrinogen and peptides containing the arginine-glycine-aspartate (RGD) motif. Which ligand binds αvβ3 in angiogenic states has not been established and although vitronectin is the ligand with greatest affinity for αvβ3, it is thought unlikely to be this. The integrin αvβ3 promotes extracellar signal-regulated kinase (ERK) activation and thus stimulates EC proliferation [13], and simultaneously inhibits p53 activity [14]. It promotes cell survival by activating NF-κ B [15]. By inducing cell motility [16] and facilitating ECM degradation (by interaction with MMP-2) [17], αvβ3 is critically important in angiogenesis. Furthermore, αvβ3 ligation promotes its association with vascular endothelial growth factor receptor-2 (VEGF-R2), thus augmenting VEGF-R2 signalling [18].

The use of a monoclonal antibody to αvβ3 (LM609) showed angiogenesis was inhibited in the chorioallantoic membrane assay for angiogenesis [12], thus further establishing the importance of this integrin in angiogenesis. The monoclonal antibody disrupted angiogenesis by promoting apoptosis, suggesting that in the absence of αvβ3 ligation by an endogenous ligand, protective signals are lost, with resultant EC apoptosis [19]. Consistent with this is the observation that disruption of αvβ3 ligation in ECs results in p53 expression in proliferating ECs [14].

The β1 integrins, expressed on both quiescent and growing blood vessels, are involved in cell adhesion and migration, and therefore are also important in angiogenesis. The α5β1 integrin is particularly important – α 5 negative teratocarcinomas have demonstrated reduced neovascularization and delayed tube formation *in vitro* [20]. Although α5β1 ligation enhances angiogenesis, this effect is thought to be secondary to regulation of α vβ 3 [21].

The role of sphingosine-1-phosphate

The bioactive sphingolipid metabolite sphingosine-1-phosphate (S1P), generated by the phosphorylation

of membrane associated sphingosine by sphingosine kinase, is involved in cell proliferation and survival, suppression of apoptosis, migration, and angiogenesis. It is a specific ligand for a family of G protein coupled receptors (GPCR), of which five members have been identified (endothelial differentiation gene EDG-1, 3, 5, 6, 8) [22; 23]. The EDG receptors are ubiquitously expressed and most is known about EDG-1, which has been implicated in migration of ECs [24; 25; 26] and smooth muscle cells [27; 28]

The metabolite S1P interacts with PDGF (a factor known to promote cell motility) to enhance cell migration. The receptor for PDGF is physically associated with EDG-1 [29] and indeed migration of cells towards PDGF is dependent on EDG-1 [30].

Cell motility is further regulated by the *Rho* family of small GTPases – Rac, Rho, Cdc42. Binding of S1P to EDG-1 stimulates Rac activity, with cortical actin fibre formation [25; 31], whereas EDG-3 is involved in stress fibre formation [32].

Cell-cell contacts

Endothelial cells must form cell-cell contacts in order to form capillary like networks, and cell-cell adhesion is mediated by cell surface receptors, including vascular endothelial cadherin (VE cadherin) and platelet endothelial cell adhesion molecule-1 (PECAM-1).

Platelet endothelial cell adhesion molecule-1 (PECAM-1)

Platelet endothelial cell adhesion molecule-1 (CD31) is a 130 kDa member of the immunoglobulin superfamily and is normally highly expressed on the vasculature [33]. Heterophilic ligands for PECAM-1 include $\alpha v\beta 3$ [34], glycosaminoglycans [35] and CD38 [36]. It can also form homophilic interactions with itself on adjacent cells via domains 1–2 [37]. It has been proposed that PECAM-1 acts like a docking molecule, which allows other proteins (including integrins) to provide further strength to vascular structures.

Vascular endothelial cadherin (VE cadherin)

Cadherins are transmembrane proteins which form homophilic interactions in a calcium dependent fashion [38]. They provide weak adhesive cell-cell forces, further stabilized by the catenins, which are intracellular proteins linking the cadherin cell surface molecule to the actin cytoskeleton. Vascular endothelial cadherin is abundantly expressed at cell junctions, thus it is thought to be involved in angiogenesis.

E selectin

E selectin is a cell surface adhesion molecule which is primarily involved in leucocyte adhesion to the activated endothelium and is also thought to play a role in angiogenesis. There are normally negligible levels of E selectin on quiescent endothelium; however, it has been detected in non-inflammatory angiogenic tissues such as human placenta [39]. Antibodies to E selectin have been found to inhibit tube formation *in vitro* [40], and conversely, the addition of exogenous E selectin stimulates angiogenesis in the rat cornea [41].

Regulation of angiogenesis: a balance of angiostatic versus proangiogenic factors

Vascular remodelling reflects a balance between angiogenic versus angiostatic factors. Proangiogenic factors include growth factors such as PDGF, epidermal growth factor (EGF), insulin-like growth factor-1 (IGF-1), vascular endothelial growth factor (VEGF), and cytokines such as tumour necrosis factor α (TNFα) and interleukin-2.

Vascular endothelial growth factor is involved not only in angiogenesis but also in vasculogenesis [42]. It interacts with specific tyrosine kinase receptors, receptor 1 (VEGF-RI/flt1) and receptor 2 (VEGF-RII/flk), stimulating receptor autophosphorylation, and EC replication and migration. Mice deficient in VEGF have impaired angiogenesis and vasculogenesis, and die by day 9 of gestation [43].

Also important in vasculogenesis is another family of receptor tyrosine kinases, Tie 1 and Tie 2. Angiopoietin-1 is a specific ligand for Tie 2. Angiopoietin-1/Tie 2 ligation has no effects on tube formation and does not have mitogenic effects on ECs. Instead, angiopoietin-1 regulates the assembly of other components of the vessel wall including smooth muscle cells [42; 44]. Receptor–ligand interaction results in growth factor secretion, which in turn, stimulates the differentiation of surrounding mesenchymal cells into smooth muscle cells. Indeed, Tie 2 mutations in two human families resulted in venous malformations with the absence of smooth muscle cells in the vasculature. Angiopoietin-2 is a naturally occurring antagonist of angiopoietin-1 [45]. The identification of this negative

regulator of the Tie 2 system indicates the need for extremely tight control of angiogenesis.

In this highly regulated fashion, therefore, VEGF is involved in the early assembly of the vascular tree and subsequent vessel maturation/stabilization is mediated by angiopoietin-1.

Chemokines are small secreted molecules with both chemoattractant and cytokine activity, which play a role in the regulation of leucocyte trafficking. Chemokines containing the ELR motif (Glu-Leu-Arg) are angiogenic (e.g. interleukin-8), whereas those lacking this critical motif are angiostatic (e.g. platelet factor-4). Angiostatic activity has been demonstrated in fragments of larger proteins, which themselves are not angiostatic [46], e.g. thrombospondin, fragments of fibronectin and prolactin. Angiostatin, a fragment of plasminogen, also inhibits angiogenesis [47] and is thought to be secreted by tumours. It has a long circulating half life and acts to control/inhibit the development of distant metastasis.

VASOREGULATION/ENDOTHELIAL REACTIVITY

Endothelial cells regulate vascular flow and basal vasomotor tone (hence blood pressure) by the highly controlled release of vasodilators (nitric oxide and the prostacyclin, PGI_2) and vasoconstrictors (endothelins and platelet activating factor). Nitric oxide and endothelins are the main regulators of basal vascular tone, and it is only when vascular function/haemodynamics are perturbed that prostaglandin I_2 (PGI_2) and platelet activating factor (PAF) come into play.

Nitric oxide

Nitric oxide (NO) is generated in ECs by the oxidation of L-arginine to L-citrulline by a family of enzymes, NO synthases (NOS) [48]. The endothelial NOS (eNOS) isoform is constitutively active, but is further induced by receptor dependent agonists such as thrombin, adenosine 5′-diphosphate, bradykinin and substance P [49]. Shear stress also stimulates eNOS activity, by virtue of a shear response consensus sequence GAGACC in the eNOS gene promoter [50].

Nitric oxide has pleiotropic effects on the vasculature. It causes vascular smooth muscle relaxation by binding to guanyl cyclase, hence maintaining basal vasomotor tone. It also plays a critical role in the inhibition of thrombosis by inhibiting platelet adhesion, activation and agonist-induced secretion, and in fact, it promotes platelet desegregation [51]. This is thought to be partly through a cyclic guanosine monophosphate (cGMP) dependent mechanism. By suppressing agonist-induced rise in intracellular calcium in platelets, it prevents the conformational change in glycoprotein IIb-IIIa [51; 52]. Nitric oxide also impairs phosphatidyl inositol 3-kinase (PI3-kinase) which normally facilitates conformational changes in glycoprotein IIb-IIIa. These two mechanisms act together to effectively prevent the binding of fibrinogen [53].

Endothelial cell-derived NO has effects beyond that on the vasculature. Nitric oxide inhibits leucocyte/EC adhesion [54; 55], and inhibits injury induced neointimal proliferation by inhibiting the proliferation [56] and migration [57] of smooth muscle cells.

Endothelins

The endothelins (ETs) are a family of 21 amino acid peptides, of which there are three members (ET-1, ET-2, ET-3). They are produced by diverse cell types, and serve to regulate vasomotor tone, cellular proliferation and hormone production. Endothelial cells produce only ET-1, which is also synthesized by vascular smooth muscle cells. Production of ET-1 is induced by hypoxia, ischaemia and shear stress, which induce the transcription of ET-1 mRNA, with prompt secretion of ET-1 within minutes. The kinetics of ET-1 synthesis allow sophisticated regulation of basal vascular tone, as the half life of ET-1 protein and mRNA is 4–7 minutes and 15–20 minutes, respectively [58]. The majority of plasma ET-1 (90%) is cleared by the lung during first passage [59]. The majority (up to 75%) of ET-1 secretion is towards the abluminal side of the EC and thus it acts in a paracrine manner by binding to specific receptors on smooth muscle cells, to cause vasoconstriction [60]. The endothelin ET-2 is produced in the kidney and intestine, while ET-3 has been detected in the brain, gastrointestinal tract, lung and kidney [61].

Synthesis of endothelins

The gene encoding ET-1 is on chromosome 6 [62]. The promoter contains regulatory sites for stimuli such as shear stress, thrombin, hypoxia, growth factors, catecholamines and angiotensin II, which act to induce transcription of ET-1 mRNA. Negative feedback is

provided by ET-3, prostacyclin and atrial natriuretic hormone, which inhibit transcription. These factors not only regulate transcription, but can also provide negative feedback at a translational level [63; 64].

Each endothelin is synthesized as a larger precursor, preproendothelin-1 (203 amino acids), which is degraded to the prohormone Big endothelin-1 (39 amino acids). Big endothelin-1 is secreted by ECs into the plasma and has approximately 1% of the potency of ET-1. Endothelin converting enzyme cleaves Big endothelin-1 to generate ET-1 [65].

Production of ET-1 is influenced by other vasoactive substances, vascular stress and numerous hormones (angiotensin II, vasopressin, thrombin, TGFβ, IGF-1, EGF, bFGF), and high and low density lipoproteins. In fact, it is thought that it may be the endothelins which exert the vasoconstriction which is seen in response to some of these stimuli. Conversely, NO and prostacyclin act to inhibit ET-1 production, and this has been shown to be mediated by production of cGMP [66; 67].

Endothelin receptors

There are two endothelin receptors (Type A and B), to which all three endothelins may bind. Type A receptors present on vascular smooth muscle and cardiac myocytes preferentially bind ET-1 with high affinity [68] and are thought responsible for the majority of ET-1 induced vasoconstriction. Ligand binding activates phospholipase C, with the subsequent formation of inositol 1,4,5 triphosphate and diacylglycerol, and a resultant increase in intracellular calcium and vasoconstriction [69]. The rise in intracellular calcium along with diacylglycerol activates protein kinase C, thus accounting for the mitogenic effects of ET-1 [70]. Dissociation of ET-1 from its receptor is outlived by the vasoconstrictive effect, due to the persisting elevated intracellular calcium concentration [71]. Importantly, NO acts to restore the intracellular calcium concentration, thus limiting the duration of the vasoconstrictive response [72]. In atherosclerosis, the protective effects of NO are abolished and thus the unopposed actions of ET-1 result in greatly heightened vasoconstriction [73]. After acute myocardial infarction, in which there is catecholamine induced reduction in the threshold for ventricular arrhythmias, the interaction of ET-1 with the Type A receptor is also important as a protective response and serves to suppress electrical excitability.

Type B receptors are mainly present on ECs [74], but are also detected on smooth muscle cells. Type B receptors are linked to G proteins, which may inhibit cyclic adenosine monophosphate (cAMP), but otherwise their effects are similar to Type A receptors, with phospholipase C activation. They bind ET-1 and ET-3 with similar affinity. The interaction of ET-3 with the Type B receptor is important in the development of cells from neural crest origin. In the absence of ET-3 or in the setting of Type B receptor dysfunction, ganglionic neurons in the intestine fail to develop [75]. Indeed, an inactivating mutation of the Type B receptor has been shown to be associated with a hereditary form of Hirschsprung's disease [76].

Expression of both endothelin receptors is under tight control and their expression often parallels that of the endothelins. Hypoxia induces ET Type A receptor expression and circulating ETs in an attempt to maintain local tissue perfusion.

Effects on the vasculature

The endothelin ET-1 is the most potent known endogenous vasoconstrictor and on a molar basis is 100 times more potent than noradrenaline [77]. Binding to Type A receptors mediates the majority of the vasoconstrictive effect [68; 78], and activation of Type B receptors in the coronary circulation and on other vascular smooth muscle, contributes to a lesser degree [79]. Catecholamines and noradrenaline act in concert with ET-1, each potentiating each other's effects.

Endothelins in disease

There is now convincing evidence that endothelins are involved in various pathological states. Plasma ET-1 levels are normal in essential hypertension but are raised in women with pre-eclampsia [1]. Elevated plasma concentrations of ETs are found in congestive cardiac failure and have prognostic value in this setting [80]. They are also shown to cause bronchoconstriction *in vivo*, an effect which may play a role in the development of pulmonary hypertension. Endothelins have been implicated in vascular diseases of the kidney and cyclosporin induced nephrotoxicity. Plasma ET levels are elevated after ischaemic cerebral infarction [81] and in patients with vasospasm occurring in the context of subarachnoid hemorrhage [82]. An endothelial receptor antagonist, bosentan is now available for the treatment of pulmonary hypertension.

Prostacyclin (PGI_2)

The prostacyclin PGI_2 is an eicosanoid which is not synthesized under resting conditions, but rather its production is induced by disturbances in endothelial function or vascular haemodynamics. It is released from ECs [83] and acts in a paracrine manner. It binds to a specific receptor on platelets and vascular smooth muscle cells to limit vasoconstriction and influence platelet deposition [84].

Platelet activating factor (PAF)

Platelet activating factor is a phospholipid which remains bound to the EC surface and acts in a juxtacrine fashion by binding to its receptor present on leucocytes. It is not constitutively produced and thus not a regulator of basal vasomotor tone. When infused intravenously it has variable effects on vascular dynamics ranging from vasodilatation to vasoconstriction, depending on the dose administered and the vascular bed involved. Its most important effect is in recruiting leucocytes to the EC surface, and its effects on vascular tone are indirect and exerted through the generation of other eicosanoids and leukotrienes [85].

ENDOTHELIUM IN INFLAMMATION

The endothelium plays a critical function in regulating the trafficking of leucocytes from the intravascular space to extravascular sites of inflammation. The mechanisms underlying leucocyte transmigration have been the focus of much attention and the role of adhesion molecules in this process is now well established. The main families of adhesion proteins involved in this process are the selectins, integrins, immunoglobulin supergene family and variants of the CD44 family.

The initial step in leucocyte transmigration is the arrest of leucocytes and random contact with the ECs. This step is mediated by the selectins, which allow the tethering and rolling of the leucocyte on the EC surface [86]. Increasing adhesion occurs with activation of the leucocyte integrins, leucocyte function-associated antigen-1 (LFA-1) and very late antigen-4 (VLA-4). The leucocytes then flatten and migrate along the endothelium, a process known as diapedesis. Extravasation occurs by the migration through EC junctions and subsequent attachment/migration on extracellular matrix components (fibronectin and collagen).

A normal quiescent endothelium does not bind leucocytes – it is only the activated endothelium, in response to cytokines including IL-1, TNFα and lipopolysaccharide, which expresses adhesion molecules and thus can bind leucocytes. Indeed inhibitors of IL-1 (anakinra, an IL-1 receptor antagonist) and TNFα (infliximab, a chimeric monoclonal antibody to TNFα, or etanercept, a Fc- receptor fusion protein) have shown profound therapeutic benefit in inflammatory conditions such as rheumatoid arthritis.

Selectins

All three members of the selectin family (E-selectin L-selectin and P-selectin) are characterized by a C-terminal lectin like domain which binds complex carbohydrates and all are involved in leucocyte recruitment to sites of inflammation. E-selectin (CD62) is a 115 kD antigen which is absent in normal tissues, but is found on the endothelium of post-capillary venules in inflammatory conditions, e.g. rheumatoid arthritis, and the skin in scleroderma. It is induced on ECs after IL-1 or TNFα stimulation. Expression of E-selectin depends on *de novo* protein synthesis, and expression at the cell surface peaks between four and six hours post stimulation. Expression is only transient and levels return to basal levels after 24 hours. It has a C type lectin binding domain, an epidermal growth factor (EGF)-like domain and six complement regulatory protein regions. E-selectin binds a surface glycoprotein, sialyl-Le^x or sialy-Le^y on neutrophils, monocytes and selected subpopulations of lymphocytes.

L-selectin is constitutively expressed on leucocytes and functions in leucocyte recirculation to the lymph nodes, and hence is known as the 'peripheral node homing receptor'. It is also important in leucocyte recruitment to inflammatory sites.

P-selectin (ELAM-1) is stored in the Weibel–Palade bodies (secretory granules) of ECs and platelets. Upon endothelial activation, it undergoes rapid translocation to the cell surface, where it binds cell surface mucin on neutrophils and monocytes.

Integrins

Integrins relevant to leucocyte recruitment are β1 (VLA family) and the β 2 integrins. The β 1 integrins α 5β 1 and α 6β 1 mediate binding to the extracellular

matrix (fibronectin and laminin, respectively), whereas α 4β 1 principally binds cell surface VCAM-1. The β 2 integrins are present only on leucocytes and their activity depends on conformational changes that occur on leucocyte activation. The β 2 integrins, LFA-1 and Mac-1, both bind intercellular adhesion molecules (ICAMs). The presence of chemokines in the vicinity of the activated endothelium, in conjunction with engagement of selectins, results in leucocyte activation. When activated, the β 2 integrins on leucocytes that are slowly rolling along the endothelium bind ICAM-1 and ICAM-2 on the EC surface. It is this integrin – immunoglobulin superfamily interaction which mediates firm adhesion and is essential for extravasation to occur.

Immunoglobulin gene superfamily

The immunoglobulin gene superfamily comprises numerous cell surface molecules including T cell receptors (CD4, CD8, CD3 and major histocompatibility complex (MHC) class I and II) and adhesion molecules (ICAM-1, ICAM-2, ICAM-3 and VCAM-1). Of the numerous immunoglobulin gene superfamily members, ICAM-1 and VCAM-1 are most relevant to leucocyte transmigration. The adhesion molecule ICAM-1 is constitutively expressed but is further induced by the cytokines IL-1, TNFα and PAF. It is highly expressed on the activated endothelium and is particularly important in mediating the firm adhesion of neutrophils on ECs by acting as a ligand for leucocyte β 2 integrins, and in transendothelial migration by binding LFA-1 and Mac-1 on neutrophils. It has also been implicated in eosinophil migration into the lung in experimental models of allergen induced asthma. Administration of IL-1 or TNFαcauses a gradual rise in ICAM-1 expression, peaking at 24 hours, with continued upregulation for 24–72 hours. This relatively delayed expression pattern is again consistent with its role in the later rather than the initial stages of neutrophil transmigration.

There are two isoforms of VCAM-1 containing six or seven extracellular immunoglobulin like domains and it is the isoform containing seven domains which is expressed on the vascular endothelium. Stimuli for the induction of VCAM include IL-1, TNFα and lipopolysaccharide. Maximum up-regulation takes several hours. The isoform VCAM-1 is a ligand for α 4β 1 (VLA-4) and leucocyte α 4β 7 integrin.

CD44 family

CD44 is the principal receptor that binds hyaluronate, and lymphocytes may use CD44 to form adhesions while rolling on EC surfaces exposing hyaluronate.

The endothelium in cell-mediated immunity

Another function of ECs is in antigen presentation to specific T cells in peripheral tissues. This is important in cell-mediated immunity. Both class I and class II MHC are constitutively expressed by microvascular ECs, but can be induced further by cytokines such as interferon gamma. Endothelial cells can activate only memory T cells [87], in contrast to classical antigen presenting cells which can induce both naïve and memory T cells. In fact, ECs have been described to induce clonal anergy (the prevention of naïve T cells from responding to stimulation) [88].

COAGULATION

The quiescent endothelium possesses anticoagulant activity, and indeed, a pivotal function of the endothelium is to provide an anti-thrombotic surface which inhibits the coagulation cascade. One of the major strategies used by ECs to maintain anticoagulant activity is to prevent activation of thrombin which, if activated, stimulates coagulation by causing platelet activation and the activation of several coagulation factors. Endothelial cells express haparan sulphate which along with glycosaminoglycans in the ECM, stimulates antithrombin-III [89]. They express tissue factor pathway inhibitor (TFPI), which prevents thrombin formation [90], and express thrombomodulin [91]. Thrombin–thrombomodulin interaction activates protein C, which has strong anticoagulant activity. Endothelial cells in fact synthesize protein S, a cofactor for activated protein C. Hence in the healthy endothelium, the balance is towards anticoagulant factors. Endothelial damage, however, results in the endothelium acquiring procoagulant activity in its own right.

The procoagulant activity of the deranged endothelium

An activated endothelium may promote coagulation. Bacterial endotoxin, inflammatory cytokines (e.g. IL-1) and glycosylated proteins may activate the endothelium

and promote procoagulant activity. The critical change which occurs on the ECs in the transformation from an endothelial surface with anticoagulant phenotype, to one with procoagulant effects, is the expression of tissue factor (TF). Tissue factor is not detected on the normal endothelium [92]. *In vitro*, agonists capable of inducing TF include thrombin, endotoxin, cytokines, hypoxia, shear stress and oxidized lipoproteins [93]. Tissue factor stimulates the activation of factor IX and factor X, and stimulates prothrombinase activity, with subsequent fibrin formation [94]. The thrombin receptor (protease activated receptor-1, PAR-1) is an EC surface coagulation protein binding site. Binding of thrombin to its receptor causes alterations in the EC surface expression of PAI-1, TF, NO, PAF, ET and PGI_2 [95], and also induces EC permeability by disrupting tight junctions [96]. Tissue factor expression is induced after vascular injury [97] and it has been found in association with ECs in atherosclerotic plaques [98; 99].

Two additional thrombin receptors have been identified – PAR-2 and PAR-3. Some ECs express both PAR-1 and PAR-2, however, PAR-3 has not been identified on ECs.

Endothelial cells and fibrinolysis

Plasminogen activators include tissue plasminogen activator (tPA), urokinase-type plasminogen activator (uPA) and kallikrein. Tissue plasminogen activator is synthesized by ECs and is released as a single chain zymogen, which undergoes proteolytic cleavage to form a two-chain structure, tPA. Stimuli for the formation of tPA include exercise, venous occlusion, vasodilation, thrombin and cytokines. In the plasma, tPA binds the inhibitor, plasminogen activator inhibitor-1 (PAI-1). In healthy states, PAI-1 is in excess, and hence tPA fails to activate plasminogen.

Plasminogen activator inhibitor-1 circulates in much higher concentration than tPA in normal conditions with more than 90% of the total blood PAI-1 being contained within platelets. However, the endothelium and liver are thought to be the main sources of plasma PAI-1. Indeed it is the plasma form of PAI-1 which is mainly active, as opposed to the platelet form which is essentially latent. Synthesis of PAI-1 is stimulated by thrombin, cytokines, lipoprotein a and oxidized low density lipoprotein [100].

Urokinase-type plasminogen activator is not constitutively expressed, but rather it is found in sites of wound repair or angiogenesis [101].

Thrombin–thrombomodulin interactions result in activation of the protein thrombin activatable fibrinolysis inhibitor (TAFI) [102]. This results in a loss of plasmin and tPA binding sites on fibrin, with impaired fibrinolysis. Thus ECs cells reduce the rate of intravascular fibrinolysis through the cell surface expression of thrombomodulin.

CONCLUSIONS

Advances in the understanding of endothelial function/physiology have been the basis for many therapeutic strategies. Many pharmacological interventions have been targeted to the endothelium with the intent of restoring it to its quiescent state. It is foreseeable that expanding the understanding of endothelial function further will lead to targeted therapies to a myriad of diseases, including cancer, cardiovascular disease and inflammatory conditions.

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