

Physiology of the endothelium

H. F. Galley and N. R. Webster*

Academic Unit of Anaesthesia & Intensive Care, School of Medicine, University of Aberdeen AB25 2ZD, Scotland UK

*Corresponding author. E-mail: n.r.webster@abdn.ac.uk

In the past, the endothelium was considered to be inert, described as a 'layer of nucleated cellophane', with only non-reactive barrier properties, such as presentation of a non-thrombogenic surface for blood flow and guarding against pro-inflammatory insults. However, it is now becoming clear that endothelial cells actively and reactively participate in haemostasis and immune and inflammatory reactions. They regulate vascular tone via production of nitric oxide, endothelin and prostaglandins and are involved in the manifestations of atherogenesis, auto-immune diseases and infectious processes. They produce and react to various cytokines and adhesion molecules and it is now clear that they can mount anti- and pro-inflammatory and protective responses depending on environmental conditions and are key immunoreactive cells. Endothelial dysfunction or activation also contributes to a variety of disease states.

Br J Anaesth 2004; 93: 105–13

Keywords: cells, endothelial: physiology, endothelium

A single layer of endothelial cells lines the entire vascular system. In adults, approximately ten trillion (10^{13}) cells form an almost 1 kg 'organ'. Endothelial cell structure and functional integrity are important in the maintenance of the vessel wall and circulatory function, but the endothelium is by no means inert. As a barrier, the endothelium is semi-permeable and regulates the transfer of small and large molecules. Endothelial cells are dynamic and have both metabolic and synthetic functions (Fig. 1). They exert significant autocrine, paracrine and endocrine actions and influence smooth muscle cells, platelets and peripheral leucocytes.

Endothelial cells and haematopoietic cells arise from haemangioblasts, blast-like bipotential cells.⁹ Precursor cells are thought to arise from the ventral floor of the dorsal aorta within the aorto-gonad-mesonephros region. Splanchnopleuric mesoderm transforms into mesenchymal cells, which differentiate into the haemangioblasts. The haemangioblast then becomes an intermediate pre-endothelial cell, which can further differentiate into either a committed haematopoietic cell line, or an endothelial cell. Endothelial cells can also transdifferentiate into mesenchymal cells and intimal smooth muscle cells.

It is important to point out that there is marked phenotypic variation between endothelial cells in different parts of the vascular system, such that cells from different locations in the same person not only express

different surface antigens and receptors but can generate different responses to the same stimulus.²⁰ Even cells from the same part of the vasculature can have varied responses. It is also important to note that responses of cultured endothelial cells may not reflect responses seen in the same cells *in vivo*, and the immortalized endothelial cell lines used in many laboratory studies may, in particular, have altered expression patterns of key markers compared with cells studied *in vivo*.²¹

Endothelial cells have a role in maintaining a non-thrombogenic blood-tissue interface and regulate thrombosis, thrombolysis, platelet adherence, vascular tone and blood flow. The endothelium is indispensable for body homeostasis; an uncontrolled endothelial cell response is involved in many disease processes, including atherosclerosis, hypertension, pulmonary hypertension, sepsis and inflammatory syndromes. These diseases are related to endothelial injury, dysfunction and activation.

Selected functions of endothelial cells

Endothelium transport functions

The endothelium is an important barrier to the free passage of molecules and cells from the blood to the underlying interstitium and cells. Specific transport mechanisms transport essential circulating blood macromolecules across

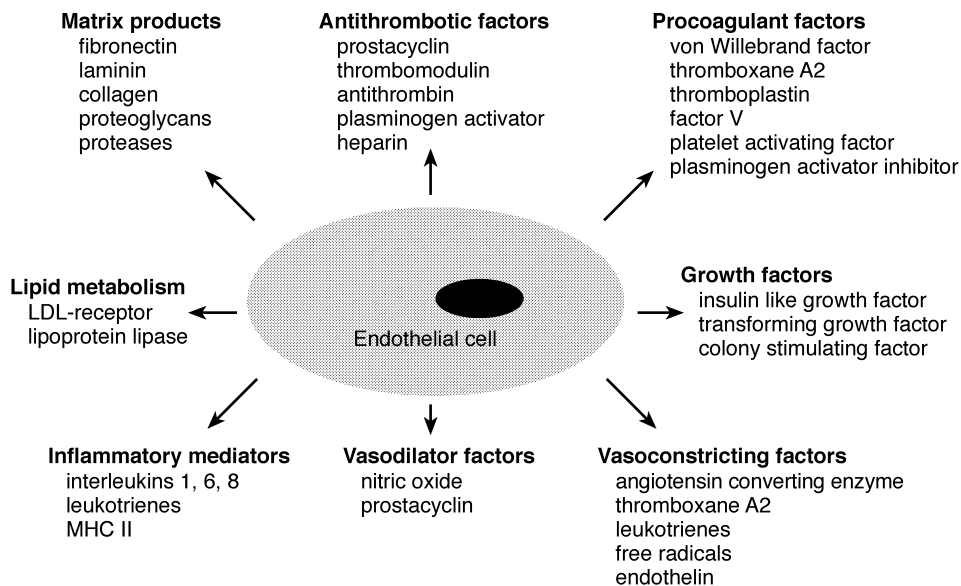


Fig 1 Endothelial cells have both metabolic and synthetic functions. Through the secretion of a large variety of mediators they are able to influence cellular function throughout the body. LDL, low-density lipoprotein.

endothelial cells to the subendothelial space to meet the metabolic needs of the surrounding tissue cells (reviewed in detail by Mann and colleagues).³⁶ In addition, the junctions between endothelial cells (the ‘tight’ junctions) act as a selective barrier to the egress of molecules from the circulation.

Glucose transport

There are seven facilitative glucose transporters of the GLUT supergene family but only *GLUT-1* and *GLUT-4* are expressed in endothelial cells. Regulation of *GLUT-4* expression is an essential process in the modulation of glucose transport and is particularly important in diabetes and hypoxaemia. The blood–brain barrier is the major endothelial tissue expressing GLUT transporters; however, glucose transporters have also been detected in endothelial cells throughout the body, including umbilical vein, adrenal capillaries, aorta, retina, heart, placenta, the eye, and testis.³⁶ *GLUT-1* is the most abundant isoform in endothelial cells.

Amino acid transport

There are multiple transport systems for amino acids in endothelial cells, but the system y^+ cationic amino acid transporter is perhaps most relevant, since this is how L-arginine, the substrate for nitric oxide, is transported. In view of the importance that nitric oxide plays in modulating vascular tone, it is surprising that only limited information is available on the effects of nitric oxide on amino acid transport in endothelial cells. Several studies have shown that cytokines such as tumour necrosis factor α (TNF α) are able to stimulate L-arginine transport in endothelial cells, resulting in increased nitric oxide production.³

Caveolae

Caveolae are invaginations in the cell membrane and are important vesicle carriers responsible for transcellular transport (transcytosis) in endothelial cells.⁴¹ For example, transcytosis via caveolae is the primary means of albumin transport across endothelium (Fig. 2). Caveolin-1 is a scaffolding protein that becomes inserted into the cytoplasmic face of the plasma membrane to regulate caveolar internalization. In the endothelium, caveolin-1 regulates nitric oxide signalling by binding to and maintaining type III (endothelial) nitric oxide synthase (NOS) in an inactive state.⁶ As calcium influx channels and pumps are localized in caveolae, caveolin-1 is also an important determinant of calcium signalling in endothelial cells.¹⁹

Tight junctions

Tight junctions are intercellular junctions important for paracellular transport. Although vascular permeability depends on both the paracellular pathway (tight junctions) and transcellular pathway (caveolae) of the endothelium, oedema develops mainly as a result of dysfunction of tight junctions. Within multicellular organisms, several organs are relatively independent of whole body homeostasis and are wrapped by endothelial cell sheets. A clear example is the blood–brain barrier, made of highly specialized endothelial cells whose tight junctions protect the central nervous system. Tight junctions can function as either a ‘gate’ (selected passage of molecules) or a ‘fence’ (no passage) (reviewed by Sawada and colleagues).⁴⁹ The gate function regulates the passage of ions, water and various macromolecules, even of cancer cells, through paracellular spaces. The gate function is important in oedema, jaundice, diarrhoea and blood-borne metastasis. The fence function

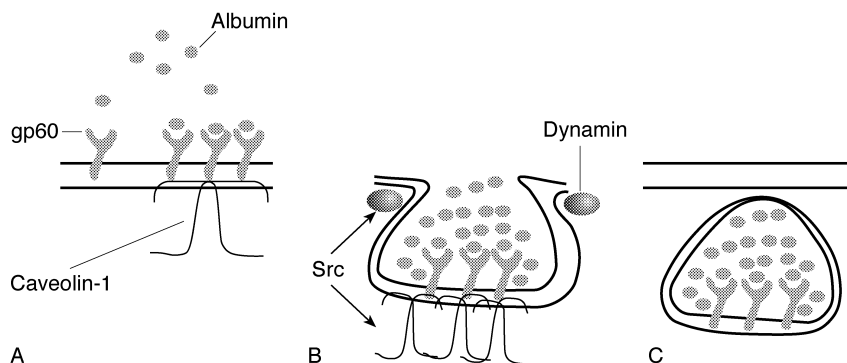


Fig 2 Caveolae are responsible for transcellular transport in endothelial cells. Caveolae are carriers of albumin. Albumin binding proteins (albumin binding glycoprotein, gp60) initiate endocytosis of albumin by associating with the scaffolding protein caveolin-1 (A) and activating the kinase Src. The Src enzyme phosphorylates caveolin-1 and a second protein dynamin (B). This results in the fission of caveolae and internalization of albumin (C).

maintains cell polarity (and hence an electrochemical barrier to the egress of charged molecules) by preventing mixing of molecules in the apical (in contact with blood) endothelial cell membrane with those in the lateral membrane. Some pathogenetic bacteria and viruses target and affect the tight junction function, leading to diseases affecting the vascular system (e.g. oedema), the gastrointestinal tract (e.g. bacterial enteritides) and the respiratory tract (e.g. acute respiratory distress syndrome).

Vascular tone

The endothelium produces a number of vasodilator and vasoconstrictor substances which regulate vasomotor tone and the recruitment and activity of inflammatory cells, and regulate thrombosis.⁵⁵

Nitric oxide

Endothelial cells have a major role in the regulation of vascular tone, through production of several vasoactive mediators. Nitric oxide, prostacyclin, endothelin (ET) and endothelial-derived hyperpolarizing factor are powerful vasoactive substances released from the endothelium in response to both humoral and mechanical stimuli, and can profoundly affect both the function and structure of the underlying vascular smooth muscle. Nitric oxide is a profound vasodilator. Constitutive production of nitric oxide by the endothelium maintains the vasculature in a state of vasodilatation. Both type II (cytokine-inducible) and type III (endothelial constitutive) NOS, which catalyse the conversion of L-arginine to nitric oxide (Fig. 3), have been found in endothelial cells. Caveolae play an integral part in regulating the activity of endothelial NOS. In addition, pro-inflammatory cytokines also increase the activity of GTP-cyclohydrolase, the rate-limiting enzyme for tetrahydrobiopterin production, which is a cofactor for NOS.²⁷ The y^+ amino acid transporter channels are co-located with NOS on caveolae, and re-circulation of L-arginine from L-citrulline mediated by cytokines has also been described in vascular endothelial cells.³⁴

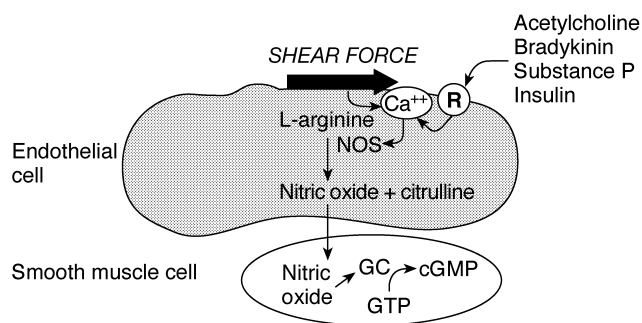


Fig 3 Nitric oxide synthase (NOS) type III catalyses the production of nitric oxide from the cationic amino acid L-arginine. The enzyme is activated via changes in intracellular calcium in response to changes in shear forces or via a receptor-mediated process. Released nitric oxide activates soluble guanylate cyclase (GC) in smooth muscle cells, converting GTP to cGMP. This activates a protein kinase which leads to the inhibition of calcium influx into the smooth muscle cell, and decreased calcium-calmodulin stimulation of myosin light chain kinase. This in turn decreases the phosphorylation of myosin light chains, decreasing smooth muscle tension development and causing vasodilatation.

Nuclear factor kappa B (NF κ B) is a redox-sensitive transcription factor which regulates, in part, gene expression of many cytokines, growth factors, adhesion molecules and enzymes involved in immune and inflammatory responses.⁵² It is maintained in a non-activated state in the cytoplasm by association with an inhibitor subunit, I κ B (Fig. 4). Proteolysis of I κ B in response to activation stimuli, including lipopolysaccharide (LPS, endotoxin) and cytokines with a common redox-sensitive step, reveals a nuclear recognition site. This then prompts the NF κ B to move into the nucleus, where it binds to target DNA and results in mRNA expression. Constitutive nitric oxide production by endothelial cells inhibits adhesion molecule expression through stabilization of I κ B, thus attenuating pro-inflammatory responses.

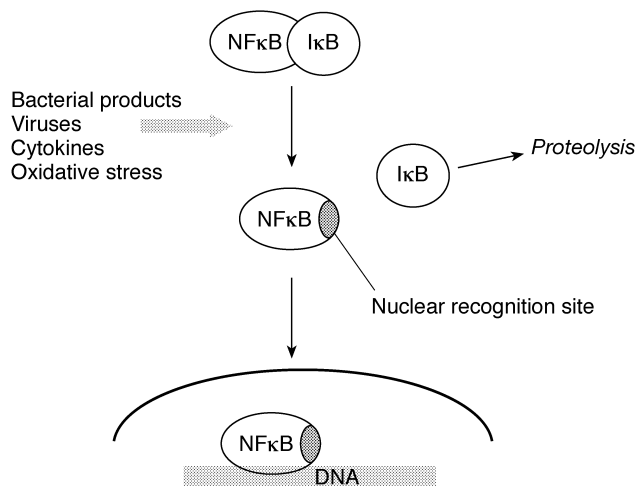


Fig 4 Nuclear factor kappa B (NFκB) is maintained in a non-activated state in the cytoplasm by association with an inhibitor subunit, IκB. Proteolysis of IκB in response to activation stimuli including bacterial products, viruses and cytokines, with a common redox-sensitive step, reveals a nuclear recognition site. This then prompts the NFκB to move into the nucleus where it binds onto target DNA and results in mRNA expression.

Endothelin

The vasoconstrictor ET is also produced by endothelial cells, with marked effects on vascular tone. There are three types of ET, but vascular endothelial cells produce only ET-1.²⁹ However, the distribution of ET receptors extends throughout the body and, in addition to causing vasoconstriction, ET has pleiotropic effects on non-vascular tissue. ET-1 exerts vasoconstrictor actions through stimulation of ET_A receptors in vascular smooth muscle and vasodilator actions through stimulation of ET_B receptors in endothelial cells. However, ET_B receptors also contribute to vasoconstriction in some blood vessels.^{23,54} ET-1 stimulates cell proliferation, increasing the expression of several genes, including collagenase, prostaglandin endoperoxidase synthase and platelet-derived growth factor. There is considerable cross-talk between endothelin, nitric oxide and prostacyclin in the control of vascular tone.³²

Leukotrienes

Leukotrienes regulate smooth muscle tone and have effects on permeability, adherence and chemotaxis. Endothelial cells do not contain 5-lipoxygenase, an essential enzyme in the arachidonic acid pathway, and therefore cannot generate leukotrienes from arachidonic acid, but cooperate with neutrophils to metabolize leukotrienes produced from activated neutrophils.¹⁶ Prostacyclin is also synthesized from arachidonic acid by endothelial cells in response to inflammatory mediators, including interleukin (IL) 1 and platelet-derived and epidermal growth factors. Like nitric oxide, prostacyclin is a potent vasodilator, and inhibits platelet aggregation and thrombosis and may synergize with nitric oxide in this respect.⁴⁶

Host defence

Endothelial cells are in a unique strategic position as key players in host defence and inflammation. Orchestration of immune and inflammatory responses depends on communication between cells by soluble molecules given the generic terms cytokines; these include chemokines, colony stimulating factors (CSF), IL, growth factors and interferons (IFN). They are low-molecular-weight proteins that regulate both the amplitude and duration of the immune and inflammatory responses. Endothelial cells produce and react to a variety of cytokines and other mediators.

Chemokines

The chemokine repertoire of endothelial cells includes α and β chemokines and fractalkine, with effects on neutrophils, eosinophils, T lymphocytes, natural killer cells and monocytes.²² Although the spectrum of action of chemokines is generally restricted to effects on leucocytes, some *in vitro* studies have also shown effects on endothelial cell function. IL-8, growth-related oncogene- α and some other α chemokines stimulate proliferation and migration of endothelial cells and are angiogenic *in vivo*. Endothelial cells are strategically located at the tissue–blood interface and present several chemokines to circulating leucocytes. When chemokines are produced in large amounts, such as in cancer or chronic inflammation, they contribute to systemic anti-inflammation by inducing the release of so-called decoy receptors for TNF α and IL-1 into the circulation.^{10,35}

Adhesion molecules

Endothelial cells also regulate leucocyte movement into tissues via a carefully regulated process involving adhesion molecules that mediate the adhesion of leucocytes to the endothelium by binding to specific ligands on the leucocytes.⁴² Endothelial cells express E-selectin, P-selectin, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule (VCAM). ICAM-2 is constitutively expressed on resting endothelial cells. ICAM-1 and VCAM are only minimally expressed on resting endothelial cells, but their expression can be increased by cytokines and LPS (endotoxin) activation. Lymphocytes, unlike platelets and other leucocytes, can interact with endothelial cells under basal conditions through the L-selectin receptor. Activated lymphocytes express integrins, such as leucocyte function-associated antigen-1 (LFA-1) or very late antigen-4 (VLA-4), which interact with ICAM and VCAM. Adhesion molecules of the L-selectin and β_2 integrin class, such as LFA-1 and Mac-1 (integrin alpha M, complement receptor 3) are involved in the transient adherence of leucocytes to endothelial cells. Activated endothelial cells secrete platelet activating factor (PAF) which upregulates LFA-1 and Mac-1 on the leucocytes and the expression of P-selectin and E-selectin. The mechanisms involve interaction of the platelet glycoprotein IIb/IIIa with fibrinogen and endothelial vitronectin receptors. Once adherent, platelets potentiate the adherence of neutrophils

to endothelium by expression of CD154 on activated platelets, which binds to CD40 on endothelial cells. This induces expression of leucocyte adhesion molecules and tissue factor on the endothelial surface. Thrombin or histamine stimulation selectively induces endothelial cell P-selectin while cytokines and LPS stimulation leads to E-selectin expression. Movement of the adherent cells between tightly adjacent endothelial cells and into the tissues to the site of infection or injury occurs via complex and tightly regulated sequential activation steps, allowing the integrity of the endothelium itself to be maintained whilst at the same time allowing the movement of activated inflammatory cells out of the circulation to the site in infection or injury.

Cytokines and growth factors

Endothelial cells produce a variety of cytokines and growth factors in response to stimulation with cytokines, bacterial products, hypoxaemia and other mediators.³⁷ These include granulocyte macrophage CSF, granulocyte CSF, macrophage CSF, the stem cell factors, and IL-1 and IL-6. Although endothelial cells do not produce the anti-inflammatory IL-1 receptor antagonist, they do express the TNF receptors p55 and p75.⁵ Endothelial cells also react to a vast array of cytokines, leading to responses involved in immunity, inflammation, thrombosis and angiogenesis. Many of the manifestations of these responses are implicated in pathogenetic processes.

Haemostasis and coagulation

Coagulation-related receptors on the surface of vascular cells and circulating coagulation proteins are tightly controlled, to regulate coagulation and initiate a coagulation response to vascular injury. Endothelial and smooth muscle cells express a variety of proteins directly participating in haemostasis. Engagement of activated coagulation proteins by their specific receptors on the vascular cell surface in turn activates these cells and leads to expression of genes involved in coagulation, angiogenesis, leucocyte adhesion, regulation of the vascular wall tone, etc. The signals inducing the expression of target genes are mediated by protease-activated receptors, which are shared among coagulation proteases. However, differences in mechanisms of activation of these receptors, as well as the presence of specific receptors for each coagulation protein and structures of promoters of target genes may provide specificity in the responses of vascular cell types to different coagulation factors.

Tissue factor is the receptor for factor VII and is pro-coagulant. It is inhibited by tissue factor pathway inhibitor, which is synthesized mainly by endothelial cells under basal conditions and is bound to the endothelial cell surface. Tissue factor expression leads to the activation of factor X, which then combines with factor Va to convert prothrombin to thrombin. Thrombin is a multifunctional protein with

some important homeostatic anticoagulant effects as well as pro-coagulant activity. It binds to thrombomodulin expressed on the endothelial cell surface, which is the major physiological buffer for the pro-coagulant effects of thrombin in normal vessels. Because thrombomodulin binds to the same site on thrombin that would normally bind to fibrinogen, platelets or factor V, all of these functions are blocked. Instead, the thrombin–thrombomodulin complex activates protein C (through a different site on the thrombin molecule), resulting in initiation of the activated protein C pathway.¹⁴ This process is augmented by the endothelial protein C receptor (EPCR). Activated protein C must dissociate from EPCR before it can bind to protein S and function as an effective anticoagulant through the inactivation of factor Va.

In addition to its pro-coagulant and anticoagulant effects, thrombin is also involved in the process of inflammation and can up-regulate endothelial cell P-selectin expression through von Willebrand factor (vWF).⁴⁸ Thrombin is also chemotactic for polymorphonuclear leucocytes and is a potent inducer of PAF expression in endothelial cells. The majority of vWF is derived from endothelial cells, which synthesize two forms: vWF dimers that are secreted into the plasma and sub-endothelial matrix, and granular vWF multimers that are stored in Weibel–Palade bodies in the endothelial cells for rapid mobilization in response to activating molecules such as thrombin. The vWF binds and stabilizes factor VIII and is a cofactor for platelet binding to exposed extracellular matrix in injured vessel walls. Both infection and inflammatory processes can lead to elevation of plasma vWF.³⁰

Endothelial cells also produce ectonucleotidases, which are enzymes that dephosphorylate ADP to AMP and then to adenosine and inhibit platelet aggregation.³⁸

Angiogenesis

Vascular endothelial growth factor (VEGF) is an angiogenic factor produced by a variety of cells, including endothelial cells, with specific receptors on the endothelium.¹¹ Angiogenesis – the formation of new blood vessels from pre-existing endothelium – is mediated by VEGF. VEGF contributes to the inflammatory response through stimulation of the release of adhesion molecules, metalloproteinases and nitric oxide, via the transcription factor activator protein-1 (AP-1).

The role of the endothelial cell in disease

Atherosclerosis

Disturbed endothelial function may play a large role in cardiovascular disease. Atherosclerosis results from excessive inflammatory and fibroproliferative responses to vascular insults and the earliest alterations in the vessel wall are formation of the fatty streak and monocyte adhesion.²⁴

Cytokines, growth factors, lipids and enzymes modulate cell function, leading to lipid accumulation, vasoconstriction and promotion of thrombosis. Active endothelial participation is required for monocyte adhesion and migration to the sub-endothelium. In addition, endothelin is a profound chemoattractant for monocytes. The endothelium oxidizes native low-density lipoprotein (LDL); the accumulation of monocytes could represent an attempt to remove the sub-endothelial oxidized LDL. The activated monocytes secrete TNF α and IL-1, with secondary up-regulation of growth factors by endothelial and smooth muscle cells. Oxidised LDL stimulates both endothelin and E-selectin production.

Lipids (particularly LDL) and oxidant stress play a major role in impairing endothelial function by reducing the bioavailability of nitric oxide and activating pro-inflammatory signalling pathways such as NF κ B. LDL-cholesterol and oxidant stress also impair caveolae structure and function. Biomechanical forces on the endothelium, including shear stress from disturbed turbulent blood flow, also activate the endothelium, increasing vasomotor dysfunction and promoting inflammation by up-regulating pro-atherogenic genes. In contrast, normal laminar shear stress promotes the expression of genes that may protect against atherosclerosis.

Nitric oxide

Many studies demonstrate that endothelial dysfunction in terms of reduced nitric oxide activity is one of the earliest markers in patients with atherogenic risk factors (male gender,⁵⁷ ageing,⁶¹ hypertension,^{13 61} diabetes,⁴⁴ smoking,⁶² family history⁵⁰) in the absence of angiographic evidence of atherosclerosis. Improved endothelial function is a clinical marker of atherogenic risk factor modification, for example nitric oxide responses are improved by cholesterol-lowering therapy. Even 3 days' treatment with statins increased nitric oxide activity and decreased soluble VCAM-1 levels in patients with coronary heart disease or diabetes, without affecting serum lipids.³⁹ Mice lacking the gene for type-III NOS have hypertension and heightened responses to injury.²⁸ Antioxidant therapy or direct inhibition of NF κ B activation prevents vascular injury.³¹

Endothelin

In addition to acute vasoconstrictor effects, ET-1 appears to be implicated in proliferative responses associated with vascular disease. Oxidized LDL induces ET-1 expression in human endothelial cells.⁴ ET-1 stimulates the expression of *c-fos*, *c-jun* and *c-myc*, so-called immediate early genes, expression of which can be used as an indication of gene activation, and induces proliferation of vascular smooth muscle cells and fibroblasts.⁴⁵ ET-1 has a synergistic action with growth factors, including VEGF, and may function as a co-mitogen.¹² It promotes synthesis and secretion of glycoproteins, thrombospondin and fibronectin, modifying extracellular constituents in cardiovascular tissues, and increases neutrophil and platelet adhesion.²⁶ Endothelial dysfunction characterized by loss of nitric-oxide-dependent

vasodilatation may simultaneously involve augmented ET activity in disease states, including atherosclerosis. In a mouse model of atherosclerosis, chronic ET_A receptor blockade restored nitric-oxide-mediated endothelial function and inhibited atherosclerotic plaque development.²

Infection and inflammation

Severe infection with Gram-negative organisms leads to the appearance of endotoxin or LPS in the bloodstream, which interacts with LPS-binding protein (LBP) and binds to CD14 receptors, transducing signals via Toll-like receptors (TLR), which culminate in the activation of NF κ B. NF κ B activation leads to increased gene expression of several mediators, including chemokines, cytokines, adhesion molecules, tissue factor, metalloenzymes and NOS. Although endothelial cells do not themselves express CD14, LPS can activate these cells via interaction with soluble CD14 and LBP present in the circulation.

TLRs are pathogen-associated molecular pattern receptors for a variety of diverse molecules derived from bacteria, viruses and fungi. To date, ten TLR family members have been identified. TLR2 is crucial for the propagation of the inflammatory response to components of Gram-positive and Gram-negative bacteria and mycobacteria such as peptidoglycan, lipoteichoic acid, bacterial lipoproteins, lipopeptides and lipoarabinomannan. TLR2 is predominantly expressed in the cells involved in first-line host defence, including monocytes, macrophages, dendritic cells and neutrophils, but some expression is also seen in endothelial cells. TLR4 has been identified as the receptor for LPS and lipoteichoic acid.^{40 53}

Endothelial cells express predominantly TLR4 and little TLR2, and respond vigorously to LPS via TLR4, but not to *Mycobacterium tuberculosis* lipoprotein, a TLR2 ligand. Several microbial antigens such as Gram-positive cell wall fragments, bacterial, spirochetal and mycobacterial lipoproteins, and fungi require TLR2 to activate cells, such that the regulation of TLR2 expression in endothelial cells could influence immune responses. Faure and colleagues¹⁵ showed that TLR4 was up-regulated by LPS in vascular endothelial cells but that in addition, LPS, TNF α and IFN γ were also able to up-regulate TLR2 expression in a mechanism involving NF κ B. Induction and up-regulation of TLR2 in response to inflammatory stimuli may help explain the well-known synergy between LPS and lipoproteins.

During infection and inflammation, for example in sepsis, many cytokines, growth factors such as VEGF, adhesion molecules, chemokines and enzymes such as matrix metalloproteinases and NOS are upregulated in response to a variety of microbial mediators and cytokines and contribute to the broad pathophysiological manifestations of sepsis and its sequelae.⁵⁹ The cytokines released during sepsis, including TNF α , IL-1 and IL-6, result in increased endothelial permeability, induction of tissue factor synthesis and up-regulation of adhesion molecules. Endotoxin and

cytokines, including TNF α and IFN γ , induce increased permeability of some cells through effects on tight junction proteins and increased VEGF expression.⁴⁹ When T cells are activated as part of the host defence process, they express their own tight junction proteins to make the transendothelium process smooth.¹

Anatomical damage to the endothelium occurs during septic shock, and a single injection of LPS in animals denudes endothelium.⁴⁷ Endothelial cells become detached and sub-endothelial oedema occurs. Cellular damage is apparent as early as 15 min after LPS injection, with nuclear vacuolization, cytoplasmic swelling and protrusion, cytoplasmic fragmentation and detachment of the endothelium from its underlying layer.³³ In a caecal ligation and puncture rat sepsis model, similar events are seen after 10 h.⁵⁸ Circulating shed endothelial cells have also been identified in human sepsis using antibodies to vWF and the VEGF receptor.⁴³ The number of circulating endothelial cells was higher in non-surviving patients than survivors.

Endothelial injury exacerbates sepsis-induced coagulation abnormalities. Release of nitric oxide and prostacyclin is impaired, facilitating leucocyte and platelet aggregation and aggravating coagulopathy.

Hyperglycaemia, diabetes and hypertension

Hyperglycaemia has been implicated in the pathogenesis of micro- and macrovascular complications in diabetes yet little is known concerning the regulation of glucose transporters in endothelial cells. Chronic hyperglycaemia in diabetes promotes endothelial cell dysfunction and is a major factor in the development of micro- and macrovascular disease. Hyperglycaemia associated with insulin resistance is common in critically ill patients, even those who have not previously had diabetes. Adaptive responses in system γ^+ activity, increased nitric oxide synthesis and increased type-III NOS mRNA have been identified in human umbilical vein endothelial cells exposed to elevated glucose. In human endothelial cells, exposure to glucose 25 mM results in up-regulation of several genes, including IL-8 and ICAM-1.⁷ Increased IL-8 secretion was also seen in human endothelial cells cultured for 7 days in glucose 25 mM compared with cells cultured in glucose 5.5 mM and this was accompanied by up-regulation of AP-1 and glucose-response element promoter.⁵¹

Hypertension is one of the clinical conditions where endothelial damage has been confirmed, although it is not clear whether such damage is the cause or the consequence of the hypertension. There is certainly an imbalance between decreased production or receptor function of vasodilatory factors and an increased formation of, or sensitivity to, vasoconstrictive agents. Endothelium damage may also play a key role in the development of organ damage in hypertension. It has been suggested that ET-1 is involved in the pathogenesis of hypertension, although plasma levels and severity of hypertension conflict.²⁵ In

hypertensive rats however, ET-1 in vascular tissue did correlate with systemic arterial pressure which was reduced by endothelin antagonists.⁵⁶ The involvement of endogenous ET-1 in mild-to-moderate hypertension remains controversial but it may be implicated in some more severe types of hypertension.

Pulmonary hypertension however, has been shown to be associated with increased ET-1 production both in animal models and in patients.⁶⁰

Thrombosis

There are a number of inherited or acquired thrombotic disorders of endothelium-derived and regulated proteins.¹⁸ These include deficiency of protein C and protein S, defective synthesis or release of tissue plasminogen activator, enhanced plasminogen activator inhibitor secretion and mutations of factor V synthesis, such as factor V Leiden mutation (also known as activated protein C resistance). There is also a group of inherited or acquired diseases that result in the accumulation of components that perturb endothelial haemostatic properties, including homocysteinaemia and hypercholesterolaemia.

Metastatic disease

Endothelial cells within tumours are a target for angiogenic signals, a pathway for dissemination and implantation leading to metastasis.¹⁷ During metastasis, tumours often use the same molecular pathways as leucocytes. Cytokines produced constitutively by cancer cells activate expression of adhesion molecules on endothelial cells which are required for recognition by tumour cells. For instance, melanoma cells express the receptor VLA-4, which recognises VCAM-1.⁸ Chemokines and VEGF produced by endothelial cells may influence metastasis of cancer cells to specific sites. Cancer cells also secrete VEGF to induce angiogenesis and to enable forcible passage through the tight junctions to facilitate metastasis.

Summary

Endothelial cells have finally emerged as key immunoreactive cells involved in host defence and inflammation. These cells both produce and react to a wide variety of mediators including cytokines, growth factors, adhesion molecules, vasoactive substances and chemokines, with effects on many different cells. Endothelial cells are also intimately involved in the manifestations of infection, atherogenesis, hypertension and cancer.

References

- 1 Alexander JS, Dayton T, Davis C, et al. Activated T-lymphocytes express occludin, a component of tight junctions. *Inflammation* 1998; **22**: 573–82
- 2 Barton M, Haudenschild CC, d'Uscio LV, Shaw S, Munter K,

- Luscher TF. Endothelin ETA receptor blockade restores NO-mediated endothelial function and inhibits atherosclerosis in apolipoprotein E-deficient mice. *Proc Natl Acad Sci USA* 1998; **95**: 14367–72
- 3 Bogle RG, Macallister RJ, Whitley GSJ, Vallance P. Induction of N^G-monomethyl-L-arginine uptake: a mechanism for differential inhibition of NO synthases? *Am J Physiol Cell Physiol* 1995; **269**: C750–6
 - 4 Boulanger CM, Tanner FC, Bea ML, Hahn AW, Werner A, Luscher TF. Oxidized low density lipoproteins induce mRNA expression and release of endothelin from human and porcine endothelium. *Circ Res* 1992; **70**: 1191–7
 - 5 Bradley JR, Thiru S, Pober JS. Disparate localization of 55-kd and 75-kd tumor necrosis factor receptors in human endothelial cells. *Am J Pathol* 1995; **146**: 27–32
 - 6 Bucci M, Gratton JP, Rudic RD, et al. In vivo delivery of the caveolin-1 scaffolding domain inhibits nitric oxide synthesis and reduces inflammation. *Nature Med* 2000; **6**: 1362–7
 - 7 Chettab K, Zibara K, Belaiba SR, McGregor JL. Acute hyperglycaemia induces changes in the transcription levels of major genes in human endothelial cells: macroarrays-based expression analysis. *Thromb Haemost* 2002; **87**: 141–8
 - 8 Chirivi RG, Nicoletti MI, Remuzzi A, Giavazzi R. Cytokines and cell adhesion molecules in tumor-endothelial cell interaction and metastasis. *Cell Adhes Commun* 1994; **2**: 219–24
 - 9 Choi K, Kennedy M, Kazarov A, Papadimitriou JC, Keller G. A common precursor for hematopoietic and endothelial cells. *Development* 1998; **125**: 725–32
 - 10 Colotta F, Orlando S, Fadlon EJ, Sozzani S, Matteucci C, Mantovani A. Chemoattractants induce rapid release of the interleukin 1 type II decoy receptor in human polymorphonuclear cells. *J Exp Med* 1995; **181**: 2181–6
 - 11 Cross MJ, Dixelius J, Matsumoto T, Claesson-Welsh L. VEGF-receptor signal transduction. *Trends Biochem Sci* 2003; **28**: 488–94
 - 12 Douglas SA, Loudon C, Vickery-Clark LM, et al. A role for endogenous endothelin-1 in neointimal formation after rat carotid artery balloon angioplasty. Protective effects of the novel nonpeptide endothelin receptor antagonist SB 209670. *Circ Res* 1994; **75**: 190–7
 - 13 Egashira K, Suzuki S, Hirooka Y, et al. Impaired endothelium-dependent vasodilation of large epicardial and resistance coronary arteries in patients with essential hypertension. Different responses to acetylcholine and substance P. *Hypertension* 1995; **25**: 201–6
 - 14 Esmon CT. The protein C anticoagulant pathway. *Arterioscler Thromb* 1992; **12**: 135–45
 - 15 Faure E, Thomas L, Xu H, Medvedev A, Equils O, Arditi M. Bacterial lipopolysaccharide and IFN-gamma induce Toll-like receptor 2 and Toll-like receptor 4 expression in human endothelial cells: role of NF-kappa B activation. *J Immunol* 2001; **166**: 2018–24
 - 16 Feinmark SJ. The role of the endothelial cell in leukotriene biosynthesis. *Am Rev Respir Dis* 1992; **146**: S51–5
 - 17 Folkman J. Tumor angiogenesis: therapeutic implications. *N Engl J Med* 1971; **285**: 1182–6
 - 18 Fourrier F, Chopin C, Goudemand J, et al. Septic shock, multiple organ failure, and disseminated intravascular coagulation. Compared patterns of antithrombin III, protein C, and protein S deficiencies. *Chest* 1992; **101**: 816–23
 - 19 Fujimoto T. Calcium pump of the plasma membrane is localized in caveolae. *J Cell Biol* 1993; **120**: 1147–57
 - 20 Gallagher G, Sumpio BE. Vascular endothelial cells. In: Sumpio BE, Sidawy AS, eds. *Basic Science of Vascular Disease*. Mt. Kisco: Futura Publishing Co., 1997; 151–86
 - 21 Galley HF, Blaylock MG, Dubbels AM, Webster NR. Variability in E-selectin expression, mRNA levels and sE-selectin release between endothelial cell lines and primary endothelial cells. *Cell Biol Int* 2000; **24**: 91–9
 - 22 Galley HF. Endothelial cell-cytokine interactions. *Br J Intensive Care* 1999; **9**: 147–51
 - 23 Gardiner SM, Kemp PA, March JE, Bennett T, Davenport AP, Edvinsson L. Effects of an ET1-receptor antagonist, FR139317, on regional haemodynamic responses to endothelin-1 and [Ala11,15] Ac-endothelin-1 (6–21) in conscious rats. *Br J Pharmacol* 1994; **112**: 477–86
 - 24 Gerrity RG. The role of the monocyte in atherogenesis: II. Migration of foam cells from atherosclerotic lesions. *Am J Pathol* 1981; **103**: 191–200
 - 25 Goto K. Basic and therapeutic relevance of endothelin-mediated regulation. *Biol Pharm Bull* 2001; **24**: 1219–30
 - 26 Hahn AW, Resink TJ, Kern F, Buhler FR. Peptide vasoconstrictors, vessel structure, and vascular smooth-muscle proliferation. *J Cardiovasc Pharmacol* 1993; **22**: S37–43
 - 27 Harrison DG. Cellular and molecular mechanisms of endothelial cell dysfunction. *J Clin Invest* 1997; **100**: 2153–7
 - 28 Huang PL, Huang Z, Mashimo H, et al. Hypertension in mice lacking the gene for endothelial nitric oxide synthase. *Nature* 1995; **377**: 239–42
 - 29 Inoue A, Yanagisawa M, Kimura S, et al. The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three separate genes. *Proc Natl Acad Sci USA* 1989; **86**: 2863–7
 - 30 Kayal S, Jais JP, Aguin N, Chaudiere J, Labrousse J. Circulating E-selectin, intercellular adhesion molecule 1, and von Willebrand factor in patients with severe infection. *Am J Respir Crit Care Med* 1998; **157**: 776–84
 - 31 Kitamoto S, Egashira K, Kataoka C, et al. Increased activity of nuclear factor-kappa B participates in cardiovascular remodeling induced by chronic inhibition of nitric oxide synthesis in rats. *Circulation* 2000; **102**: 806–12
 - 32 Lavallee M, Takamura M, Parent R, Thorin E. Crosstalk between endothelin and nitric oxide in the control of vascular tone. *Heart Fail Rev* 2001; **6**: 265–76
 - 33 Lee MM, Schuessler GB, Chien S. Time-dependent effects of endotoxin on the ultrastructure of aortic endothelium. *Artery* 1988; **15**: 71–89
 - 34 Lee TJ, Yu JG. L-Citrulline recycle for synthesis of NO in cerebral perivascular nerves and endothelial cells. *Ann NY Acad Sci* 2002; **962**: 73–80
 - 35 Ley K, Baker JB, Cybulsky MI, Gimbrone MA Jr, Luscinskas FW. Intravenous interleukin-8 inhibits granulocyte emigration from rabbit mesenteric venules without altering L-selectin expression or leukocyte rolling. *J Immunol* 1993; **151**: 6347–57
 - 36 Mann GE, Yudilevich DL, Sobrevia L. Regulation of amino acid and glucose transporters in endothelial and smooth muscle cells. *Physiol Rev* 2003; **83**: 183–252
 - 37 Mantovani A, Bussolino F, Introna M. Cytokine regulation of endothelial cell function: from molecular level to the bedside. *Immunol Today* 1997; **18**: 231–40
 - 38 Marcus AJ, Broekman MJ, Drosopoulos JH. The endothelial cell ecto-ADPase responsible for inhibition of platelet function is CD39. *J Clin Invest* 1997; **99**: 1351–60
 - 39 Masumoto A, Hirooka Y, Hironaga K, et al. Effect of pravastatin on endothelial function in patients with coronary artery disease (cholesterol-independent effect of pravastatin). *Am J Cardiol* 2001; **88**: 1291–4

- 40 Medzhitov R. Toll-like receptors and innate immunity. *Nature Rev Immunol* 2001; **1**: 135–45
- 41 Minshall RD, Sessa WC, Stan RV, Anderson RG, Malik AB. Caveolin regulation of endothelial function. *Am J Physiol Lung Cell Mol Physiol* 2003; **285**: L1179–83
- 42 Muller WA. Leukocyte-endothelial-cell interactions in leukocyte transmigration and the inflammatory response. *Trends Immunol* 2003; **24**: 327–34
- 43 Mutunga M, Fulton B, Bullock R, et al. Circulating endothelial cells in patients with septic shock. *Am J Respir Crit Care Med* 2001; **163**: 195–200
- 44 Nitenberg A, Valensi P, Sachs R, Dali M, Aptekar E, Attali JR. Impairment of coronary vascular reserve and ACh-induced coronary vasodilation in diabetic patients with angiographically normal coronary arteries and normal left ventricular systolic function. *Diabetes* 1993; **42**: 1017–25
- 45 Pribnow D, Muldoon LL, Fajardo M, Theodor L, Chen LY, Magun BE. Endothelin induces transcription of fos/jun family genes: a prominent role for calcium ion. *Mol Endocrinol* 1992; **6**: 1003–12
- 46 Radomski MW, Palmer RM, Moncada S. The anti-aggregating properties of vascular endothelium: interactions between prostacyclin and nitric oxide. *Br J Pharmacol* 1987; **92**: 639–46
- 47 Reidy MA, Bowyer DE. Scanning electron microscopy: morphology of aortic endothelium following injury by endotoxin and during subsequent repair. *Atherosclerosis* 1977; **26**: 319–28
- 48 Royo T, Martinez-Gonzalez J, Vilahur G, Badimon L. Differential intracellular trafficking of von Willebrand factor (vWF) and vWF propeptide in porcine endothelial cells lacking Weibel-Palade bodies and in human endothelial cells. *Atherosclerosis* 2003; **167**: 55–63
- 49 Sawada N, Murata M, Kikuchi K, et al. Tight junctions and human diseases. *Med Electron Microsc* 2003; **36**: 147–56
- 50 Schachinger V, Britten MB, Elsner M, Walter DH, Scharrer I, Zeiher AM. A positive family history of premature coronary artery disease is associated with impaired endothelium-dependent coronary blood flow regulation. *Circulation* 1999; **100**: 1502–8
- 51 Srinivasan S, Yeh M, Danziger EC, et al. Glucose regulates monocyte adhesion through endothelial production of interleukin-8. *Circ Res* 2003; **92**: 371–7
- 52 Sun Y, Oberley LW. Redox regulation of transcriptional activators. *Free Rad Biol Med* 1996; **21**: 335–48
- 53 Takeuchi O, Hoshino K, Kawai T, et al. Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components. *Immunity* 1999; **11**: 443–51
- 54 Teerlink JR, Breu V, Sprecher U, Clozel M, Clozel JP. Potent vasoconstriction mediated by endothelin ETB receptors in canine coronary arteries. *Circ Res* 1994; **74**: 105–14
- 55 Vane JR, Anggard EE, Botting RM. Regulatory functions of the vascular endothelium. *N Engl J Med* 1990; **323**: 27–36
- 56 Verma S, Bhanot S, McNeill JH. Effect of chronic endothelin blockade in hyperinsulinemic hypertensive rats. *Am J Physiol* 1995; **269**: H2017–21
- 57 Vita JA, Treasure CB, Nabel EG, et al. Coronary vasomotor response to acetylcholine relates to risk factors for coronary artery disease. *Circulation* 1990; **81**: 491–7
- 58 Wang P, Wood TJ, Zhou M, Ba ZF, Chaudry IH. Inhibition of the biologic activity of tumor necrosis factor maintains vascular endothelial cell function during hyperdynamic sepsis. *J Trauma* 1996; **40**: 694–700
- 59 Webster NR, Galley HF. Sepsis. In: Hemmings H Jr, Hopkins P, eds. *Foundations of Anesthesia: Basic and Clinical Sciences*. London: Mosby International, 2000; 721–8
- 60 Yoshibayashi M, Nishioka K, Nakao K, et al. Plasma endothelin concentrations in patients with pulmonary hypertension associated with congenital heart defects. Evidence for increased production of endothelin in pulmonary circulation. *Circulation* 1991; **84**: 2280–5
- 61 Zeiher AM, Drexler H, Saubier B, Just H. Endothelium-mediated coronary blood flow modulation in humans. Effects of age, atherosclerosis, hypercholesterolemia, and hypertension. *J Clin Invest* 1993; **92**: 652–62
- 62 Zeiher AM, Schachinger V, Minners J. Long-term cigarette smoking impairs endothelium-dependent coronary arterial vasodilator function. *Circulation* 1995; **92**: 1094–100