16.1 Structure and function 3241 16.1.1 Blood vess

16.1 Structure and function 3241 16.1.1 Blood vessels and the endothelium 3241 Keith Channon and Patrick Vallance

CONTENTS 16.1.1 Blood vessels and the endothelium 3241 Keith Channon and Patrick Vallance 16.1.2 Cardiac physiology 3253 Rhys D. Evans, Kenneth T. MacLeod, Steven B. Marston, Nicholas J. Severs, and Peter H. Sugden 16.1.1 Blood vessels and the endothelium Keith Channon and Patrick Vallance ESSENTIALS Anatomy of blood vessels The blood vessel wall consists of three layers: the intima, media, and adventitia. Not all vessels have each layer, and the layers vary in size and structure between vessels. (1) The intima is made up of a single layer of endothelial cells on a basement membrane, beneath which—depending on vessel size—there may be a layer of fibroelastic connective tissue and an internal elastic lamina that provides both structure and flexibility. Embedded in the intima are pericytes. (2) The media is made up of smooth muscle cells, elastic laminae, and extra- cellular matrix. (3) The adventitia is the outermost part of the vessel, composed mainly of fibroelastic tissue but also containing nerves, small feeding blood vessels (the vasa vasorum), and lymph vessels. The adventitia is directly related to the surrounding perivascular adi- pose tissue. Function of particular constituents of blood vessels Endothelial cells are metabolically very active and exert a profound influence on vascular reactivity, thrombogenesis and coagulation, and the behaviour of circulating cells. Endothelial cells sense blood flow through transduction of shear stress (viscous drag), and align with the direction of blood flow through cytoskeletal functions. They produce key vasodilator mediators: nitric oxide,

prostanoids, and hyperpolarizing factors. Although the predominant influence of the healthy endothelium is as dilator, it also produces important vaso- constrictor factors, including endothelin, angiotensin-converting enzyme, certain prostanoids, and reactive oxygen species such as superoxide anion. The endothelium synthesizes and releases prothrombotic and antithrombotic factors, with antithrombotic factors predominating under basal conditions. The healthy endothelium allows leucocytes to roll along its surface, but prevents cells from adhering fully to the vessel wall. Vascular smooth muscle cells provide the contractile function of the vessel wall, but may adopt a range of other phenotypes: they can enter a replicative state, undergo cell death through apoptosis, migrate into the intima, adopt a secretory phenotype that results in matrix deposition (including developing bone-like features and cal- cification), and can contribute to inflammation within the vessel wall. The vessel is surrounded by adventitia and perivascular adipose tissue, which contain adipocytes, inflammatory cells, and fibro- blasts. Evidence suggests that there is continuous cross-talk between the vascular wall and perivascular tissues. Perivascular adipose tissue secretes a wide range of adipocytokines, which have paracrine ef- fects on the vessel wall. The vessel and its perivascular adipose tissue are now considered to be closely interrelated, with perivascular adipose tissue playing important roles in vascular homeostasis and pathophysiology. Integrated responses of blood vessels Blood flow elicits an endothelium-dependent dilator tone due to the production of nitric oxide, which provides a physiological counter- balance to the constrictor tone of the sympathetic nervous system. Veins differ from arteries and arterioles, and do not seem to be ac- tively dilated by continuous release of nitric oxide. Flow-mediated dilatation is an autoregulatory property of blood vessels that tends to oppose classical myogenic autoregulation—the process by which a blood vessel constricts in response to an in- crease in intraluminal pressure. There is a fourth-power relationship between resistance to flow and the radius of a blood vessel, which means that relatively small changes in the thickness or contractile state of smooth muscle in small arteries and arterioles have big ef- fects on systemic vascular resistance. There are important interactions between the sympathetic ner- vous, renin–angiotensin, and endothelin systems, with these acting in concert to control constrictor tone, and with the endothelin system providing a slowly modulating background constrictor tone. 16.1 Structure and function

 Additional endocrine signals that modulate vascular tone and func- tion include circulating cortisol and oestrogens. Pathophysiology Several clinical conditions associated with increased cardiovascular risk—including atherosclerosis, hypertension, hypercholesterol- aemia, and diabetes—are associated with reduced nitric oxide- mediated effects. Overproduction of nitric oxide may also contribute to disease, with induction of inducible nitric oxide synthase (e.g. in sepsis), leading to production of large amounts of nitric oxide and contributing to vascular paresis. Expression of adhesion molecules by the vascular endothelium is an important mechanism of cellular adhesion during inflammation and is also important in recruitment of monocytes in atherosclerosis. Impaired production and/or func- tion of endothelial progenitor cells, particularly with ageing, may contribute to the pathogenesis of endothelial dysfunction in disease, particularly in atherosclerosis and vascular injury, where endothelial cell turnover is increased. Introduction Blood vessels range in size from microscopic capillaries to large ves- sels such as the aorta and vena cava, and vary in specialized func- tion from tissue to tissue. They deliver oxygen and nutrients, remove waste, control the passage of cells and macromolecules from the blood into the tissues, and are equipped to sense and respond to physical and chemical signals. There are three basic layers to blood vessels—the intima, the media, and the adventitia

(Fig. 16.1.1.1). The intima comprises a single layer of endothelial cells on a base- ment membrane, beneath which—depending on vessel size—there may be a layer of fibroelastic connective tissue and an internal elastic lamina that provides both structure and flexibility. Embedded in the intima are pericytes—intriguing cells of smooth muscle cell lineage that make contact with multiple endothelial cells. The media is made up predominantly of smooth muscle cells and concentric elastic fibres making up the elastic laminae. The outermost part of the vessel is the adventitia, a less well-defined layer composed mainly of fibroelastic tissue that provides structural integrity to the vessel, but also contains nerves, small feeding blood vessels (the vasa vasorum), and lymph vessels. However, the adventitia is also in continuity with perivascular adipose tissue (PVAT) that has paracrine relationships with the vascular wall. In simple terms, the intima may be con- sidered as the layer that transduces signals from the lumen of the vessel to the rest of the vessel wall and controls the interface with the blood; the media is the mechanical workhorse of the vessel, and the adventitia links the vessel wall to the local and wider environment. Not all vessels have each layer, and the layers vary in size and struc- ture between vessels. For example, capillaries are essentially endo- thelial cell tubes surrounded by pericytes, resistance vessels have a relatively thick media, and the large conduit arteries have a high pro- portion of elastic tissue and a rich vasa vasorum. In disease states, particularly atherosclerosis (see Chapter 16.13.1), the vessel wall may have a high content of inflammatory cells in the intima, media, and adventitia. All three layers coordinate to regulate the function of the blood vessel, and all three are involved in the pathogenesis of vascular disease. Large ‘conduit’ arteries perform the function of mass transport; smaller arteries and arterioles provide the predominate resistance to flow, and are therefore key determinants of blood pressure; capil- laries are thin-walled and contribute most to passage of nutrients, gases, and cells through to tissues; venules provide postcapillary re- sistance and help determine capillary pressure; and larger venules and veins dynamically regulate the total capacitance of the circula- tory system. Cellular constituents of blood vessels Endothelium A monolayer of endothelial cells lines the intimal surface of the entire vascular tree (Fig. 16.1.1.2) to form the largest endocrine/ paracrine organ in the body. Endothelial cells are metabolically very active and exert a profound influence on vascular reactivity, thrombogenesis and coagulation, and the behaviour of circulating cells. Abnormalities of endothelial function have been implicated in a wide variety of diseases ranging from atheroma and hypertension to acute inflammation and septic shock. During early development, the endothelium forms the first layer of the circulatory system and extends to produce a network of interconnecting tubes. This ability of endothelial cells to form tube-like structures is retained even when they are grown in vitro. In vivo the endothelial tubes differentiate into arteries, arterioles, capillaries, veins, and lymph vessels, and regional differences in function and structure evolve such that the properties of endothe- lial cells vary between arterial and venous beds, between micro- and macrovasculature, between organs, and between different parts of individual organs—perhaps the most striking example being the specialized layer of endothelial cells and pericytes that forms the blood–brain barrier. Although heterogeneity of vascular

Fig. 16.1.1.1 Image of a human coronary artery, imaged in vivo using optical coherence tomography during coronary angiography. The asterisk denotes the circular cross-section of the imaging catheter, and the dotted lines show the optical shadow cast by the coronary guide wire, adjacent to the imaging catheter. The vessel lumen appears black, with the vessel wall highlighted in yellow pseudocolour. The layers of the vessel wall—intima (I), media (M), and adventitia (A)—are shown in the magnified inset box.

16.1.1 Blood vessels and the endothelium 3243 endothelium has long been recognized at the histological and im- munocytochemical level, recent studies using microarray analysis of global gene expression have begun to define these differences at the molecular level and promise to have important implications for understanding physiology, pathophysiology, and therapeutics. Heterogeneity of endothelial cell function undoubtedly has such im- plications. For example, endothelial cell heterogeneity may provide strategies to target therapeutic agents or imaging markers to specific organs by coupling them with antibodies or ligands to vascular bed- specific endothelial proteins. However, endothelial cells also have many features in common and several pathologies, including those causing premature vascular disease, are associated with widespread changes in the behaviour of endothelial cells. Anatomy of the endothelium Each endothelial cell is between 25 and 50 μm long, 10 to 15 μm wide, and up to 5 μm deep, and lies with its long axis aligned in the direction of the blood flow (Fig. 16.1.1.2). The underlying smooth muscle cells lie radially, are about 5 to 10 μm wide, and taper at either end so that a single endothelial cell can communicate with many smooth muscle cells, and vice versa. The endothelium also comes into intimate contact with circulating cells, and the total area of the luminal surface of the endothelium is in excess of 500 m2. This thin layer of cells is particularly susceptible to injury, and changes in endothelial cell morphology and turnover occur in experimental hypertension, diabetes, and atherosclerosis. Signal detection by endothelial cells The endothelial cell membrane expresses many receptors for circu- lating hormones, local mediators, and vasoactive factors released from blood cells. Endothelial cells sense pressure, stretch, and blood flow via a number of different sensors that vary in different vessels, reflecting the differences in pressure and blood flow across different vascular beds. For example, shear stress (viscous blood flow force) varies from c.10 dyn/cm2 in large conduit arteries such as the aorta, to c.50 dyn/cm2 in resistance arterioles, c.20 dyn/cm2 in post-capillary venules, and c.1 dyn/cm2 in large veins such as the vena cava. Endothelial cell mechanosensors on the luminal surface include G- protein-coupled receptors (such as the sphingosine 1-phosphate re- ceptor 1 and the bradykinin B2 receptor), heterotrimeric G-proteins, ion channels (such as TRPV4, TRPP2, TRPC1, Piezo1, and Piezo2), and the glycocalyx, a c.500 nm thick layer of glycosaminoglycans on the endothelial surface containing syndecan-1 and −4. Shear stress responses are also localized in microtubule-based primary cilia (con- taining with the ion channels PKD1 and PKD1) and protein-coated membrane ‘pits’ called caveolae, containing the proteins Caveolin 1–3 and Cavin 1–3. In addition, endothelial mechanosensors are present at cellular junctions, including PECAM-1, VE-cadherin, and VEGFR2, forming mechanosensory complexes that respond to shear stress. On the basal (outer) part of the endothelial cell, other mechanosensors such as the integrins interact with the intimal extra- cellular matrix (ECM). Rapid endothelial cell responses to shear stress include K+ and Ca2+ influx, activation of MAP kinases, Akt and eNOS, leading to nitric oxide (NO) production that causes vasodilatation and S-nitrosylation of endothelial cell proteins such heat shock proteins, as well as cytoskeletal components such as tropomyosin and vimentin. Small GTPases such as RhoA and Rac are highly sensitive to shear stress. RhoA is transiently downregulated within 5 min of onset of shear stress, allowing rearrangement of the actin cytoskeleton. Endothelial cell integrins modulate the response to shear stress, for example, by sensing the nature of connections with ECM compo- nents, and ensure spatial organization of focal adhesion complexes, microtubules, and intracellular signalling pathways such as p38 MAP kinase, JNK, and p21-activated kinase. Downstream consequences of shear stress transduction in- clude initial transcriptional activation of NF-kB target genes, such as ICAM-1, but in areas of sustained laminar flow there is downregulation of NF-kB and increased ‘atheroprotective’ gene expression, mediated by the transcription factor KLF2. In areas of disturbed

flow (i.e. loss of laminar shear stress), NF-kB and other inflammatory signalling are sustained, leading to ICAM-1 and VCAM-1 expression and enabling monocyte recruitment. The endothelial cells in vascular damage and repair Vascular endothelial cells move in response to specific chemical sig- nals and can migrate to recover areas of endothelial damage or de- nudation (Fig. 16.1.1.3). The basic mechanisms of movement share similarities with those required to form vessels during development Fig. 16.1.1.2 Left panel: Immunostaining of an en face preparation of artery with CD31 (cell bodies red, nuclei blue), showing endothelial cells. Note that the endothelial cells are aligned in the direction of blood flow. Right panel shows a section of human internal mammary artery immunostained for endothelial nitric oxide synthase (red staining), demonstrating the endothelial cell layer on the luminal surface.

 (vasculogenesis) or during the process of formation of new vessels in adults (e.g. in tumour angiogenesis). Circulating endothelial progenitor cells (EPCs), derived from bone marrow, have been identified and are involved in the pro- cesses of vascular repair and the response to tissue injury. These progenitor cells are characterized by the expression of specific cell surface markers (CD34 and CD133) and can form colonies when cultured in vitro. There is also evidence that resident stem cells lo- cated in the vessel wall with properties of clonality, self-renewal, and multipotentiality can replace local damaged or denuded endothelial cells. The relationship between the number of circulating endothelial cells and cardiovascular disease is complex. The number of circu- lating EPCs, typically estimated by colony growth in vitro from peripheral blood mononuclear cells, is thought to represent the restorative capacity of the vessel wall, with low numbers being in- dicative of disease progression and increased cardiovascular risk. Importantly, the number of circulating EPCs appears to decrease with age, and with known cardiovascular risk conditions such as dia- betes, hence it is likely that the ability to increase EPCs in response to vascular damage is a key feature of a healthy cardiovascular system able to repair itself. Acute adverse events such as myocardial infarc- tion are associated with a temporary increase in circulating EPC numbers. There is growing interest in the potential therapeutic delivery of EPCs—or bone marrow-derived cells capable of differentiating into EPCs and/or endothelial cells—for the treatment of cardiovascular disorders. For example, clinical studies have already been initiated in which autologous bone-marrow-derived cells have been admin- istered to patients for the treatment of acute myocardial infarction and peripheral vascular disease. In general, the results of these early clinical experiments have been mixed, and there remain important unanswered questions regarding the optimum cell type, the timing and route of delivery (e.g. intracoronary vs. intravenous), and the precise mechanism of potential beneficial effects. Other therapeutic strategies have been aimed at increasing endogenous EPC number and/or EPC function, either (e.g. using statin drugs) or acutely (by administration of erythropoietin, cytokines, or growth factors) in ischaemic events such as myocardial infarction. Pericytes Pericytes are long cells (approximately 70 μm) with extending cyto- plasmic processes around endothelial cells that make multiple cel- lular contacts (Fig. 16.1.1.4). In small capillaries, it also seems that pericytes may extend connections to more than one vessel, possibly exerting some sort of coordinating influence. The overall coverage of the endothelium by pericytes varies between vascular beds, from 10% to 50%. The junctions between pericytes and endothelial cells appear to be rich in growth factors (particularly epidermal growth factor) that are important in regulating endothelial cell growth and may be vital for angiogenesis and inflammation. Pericytes can also differentiate in to other cells, such as fibroblasts. The nature of the junction between pericytes and endothelial cells may be important for regulating permeability at specialized sites such as the blood–brain

barrier, and in the response to ischaemic injury. In other areas, the contractile function of pericytes may pre- dominate. In the retina, where pericytes are particularly prevalent, their loss is associated with impaired hierarchical organization of vessels or even vessel regression, and this might contribute to dia- betic retinopathy. In inflammation, neutrophil transmigration from venules is regulated by adhesion to pericytes. The only genetic disease Fig. 16.1.1.3 An endothelial cell moving. The front end of the cell with leading lamella is on the right, stress fibres of contractile elements are seen in the centre and these end in focal adhesions. The retracting rear end of the cell is on the left. Courtesy of Dr B. Wojciak-Stothard. Endothelial cell RBC Pericyte Fig. 16.1.1.4 Pericytes are observed outside small blood vessels in close association with endothelial cells. Reproduced with kind permission from the Department of Pathology and Laboratory Medicine, University of Pennsylvania.

16.1.1 Blood vessels and the endothelium 3245 to date in which pericyte loss has been implicated is Adams–Oliver syndrome, a rare developmental disorder characterized by scalp and limb malformations, telangiectasia, and vascular problems. The potential roles of pericytes are listed in Box 16.1.1.1. These rather underinvestigated cells seem to retain a plasticity that enables them to differentiate into smooth muscle cells. Vascular smooth muscle cells Smooth muscle cells lie mainly circumferentially in the vessel media to provide contractile function, which is influenced by hormonal, endothelial, neuronal, and intrinsic influences (‘myogenic tone’), with contraction being triggered by a wave of calcium release. The regulation of vascular smooth muscle cell (VSMC) Ca2+ signalling is complex and heterogeneous. Extracellular Ca2+ entry is regulated by activation of the plasma membrane voltage-dependent L-type Ca2+ channels (LTCC) and TRP channels. Intracellular Ca2+ release from the sarcoplasmic reticulum (SR) is regulated by agonist activation of SR-bound inositol trisphosphate (IP3) or ryanodine receptors (RyR). Calcium signalling is highly compartmentalized such that large changes in intracellular Ca2+ may lead to VSMC con- traction without activating other Ca2+-dependent pathways, and vice-versa. VSMC contraction is highly complex, but is effected by phos- phorylation of smooth muscle actin at Ser 19 by myosin light chain kinase (MLCK). MLCK is a Ca2+-calmodulin dependent kinase that is activated by VSMC Ca2+ signalling. Other important aspects of VSMC contractility, via the cytoskeleton and focal adhesion com- plexes, are regulated by complex networks of Ca2+ -dependent path- ways including the Ca/CaM-dependent kinase II (CaMKinase II) isoforms, protein kinase C (PKC), and MAP kinases. VSMC contraction is the key regulator of resistance to blood flow and hence blood pressure. There is a fourth-power relationship be- tween resistance to flow and the radius of the vessel, which means that relatively small changes in the contractile state of smooth muscle can produce large changes in the resistance offered by the vessel. This is particularly important for small arteries and arterioles, which are the major determinants of systemic vascular resistance. The rela- tive thickness of the vessel wall compared to the size of the lumen is also an important determinant of resistance. As the wall:lumen ratio increases, there is a comparatively larger reduction in lumen size for every incremental shortening of the smooth muscle. In this way, smooth muscle hypertrophy or hyperplasia can lead to a func- tional hyperreactivity of the vessel wall, exemplifying the intimate connection between structure and function. Vascular smooth muscle cells are remarkably plastic and may adopt a range of phenotypes in response to local environmental changes. They may leave the quiescent contractile state and enter a replicative state, migrate into the intima, adopt a secretory phenotype that results in matrix deposition (including the develop- ment of bone-like features and calcification), and may, under cer- tain conditions, contribute to inflammation within the vessel wall. Smooth muscle cells that replicate

and secrete matrix contribute to the process of thickening of the vessel wall in vasculoproliferative syndromes including atherosclerosis, transplant vasculopathy, and the neointimal hyperplasia that characterizes vascular restenosis fol- lowing arterial stent implantation. Phenotypic modulation of vascular smooth muscle cells is under coordinated transcriptional regulation. In the normal vessel wall, the contractile smooth muscle phenotype is maintained by a tran- scriptional pathway involving signalling from the actin cytoskel- eton to SRF, a ubiquitous transcription factor that functions in a smooth muscle cell-specific fashion by interacting with smooth muscle cell-restricted cofactors of the myocardin family. This actin–SRF–myocardin pathway directly regulates genes encoding contractile proteins such as smooth muscle myosin and SM22. However, in response to inflammatory and other pathological stimuli, the contractile transcriptional pathway is repressed, and alternate transcriptional pathways are activated that promote pro- liferation, production of inflammatory mediators, and synthesis of matrix proteins. Key mediators of the synthetic smooth muscle cell phenotype include the platelet-derived growth factor-BB (PDGF-BB) and Notch signalling pathways, and many of the Ca2+- dependent pathways that also regulate VSMC contractility, cyto- skeletal function, and interactions with the ECM. Recent evidence suggests that these transcriptional pathways are also regulated in an epigenetic fashion by smooth muscle cell-specific programmes for modification of histones within the chromatin structure of smooth muscle-restricted genes. As in the case of endothelial cells, there is clear heterogeneity in vascular smooth muscle cell phenotype in various vascular beds. Indeed, subsets of vascular smooth muscle cells are derived from distinct embryological precursors; vascular smooth muscle cells of the proximal aortic arch and great vessels are derived from neural crest (i.e. ectoderm), whereas vascular smooth muscle cells in the rest of the circulation are derived from somatic mesoderm. In the adult, another important example of functional heterogeneity is that the pulmonary and systemic vasculature differ markedly in their response to hypoxia. Hypoxia produces modest vasodilatation in the systemic vasculature, but marked vasoconstriction in the pul- monary circulation. This is likely an adaptive mechanism to prevent ventilation–perfusion mismatch in the presence of alveolar disease (e.g. pneumonia). However, chronic hypoxia (e.g. in the presence of chronic respiratory disease) can result in pulmonary hyperten- sion and lead to right heart hypertrophy and failure. The precise molecular mechanisms regulating hypoxic pulmonary vasocon- striction are incompletely understood, but oxygen sensing mechan- isms in the mitochondria and voltage-gated potassium channels on the plasma membrane of pulmonary vascular smooth muscle cells appear to play important roles. Control of vascular tone Endothelium extracts and inactivates circulating hormones, converts inactive precursors to active products, and synthesizes and releases a variety of vasoactive mediators (Fig. 16.1.1.5). Box 16.1.1.1 Roles of pericytes • Contractility • Barrier function and regulation of permeability • Neutrophil transmigration in inflammation • Signalling to control endothelial growth and angiogenesis • Vascular stabilization • Sensors of hypoxia and hypoglycaemia • Transdifferentiation into fibroblasts in wound healing, cancer metastasis

 Vasoconstrictor and vasodilator mediators allow the vessel to respond to changes in the local milieu, but the predominant background influence of the endothelium is dilator, with the re- moval of the endothelium leading to vasoconstriction. A basal endothelium-dependent dilator tone seems to provide a physio- logical counterbalance to the continuous constrictor tone of the sympathetic nervous system. Vasodilators The endothelium produces at least three key vasodilation mediators (Fig. 16.1.1.5): nitric oxide (NO), prostanoids, and hyperpolarizing factors. Nitric oxide Physiology The production of NO is responsible for endothelium-dependent dilator tone that is generated by blood flow. NO is synthesized from the

amino acid l-arginine by the nitric oxide synthase (NOS) enzymes (Fig. 16.1.1.6; see also Fig 16.1.1.8). The vasodilator ac- tions of NO are mediated through the second messenger cGMP, generated when NO activates soluble guanylate cyclase (sGC) by binding to the haem group in the enzyme. A similar mechanism mediates NO signalling by inhibition of cytochrome c oxidase, ini- tially in a reversible manner, but irreversibly under certain condi- tions. Inhibition of this enzyme decreases oxygen utilization, and the release of NO by endothelial cells appears to be an important determinant of oxygen consumption in the vasculature. However, the signalling actions of NO are much broader than modification of enzyme function by haem binding. NO modifies protein functions through numerous chemical reactions involving nitrosylation of cysteine residues and nitration of tyrosines, including ion chan- nels, enzymes, and transcription factors, leading to change such as reduced adhesiveness of the endothelial cell for circulating white cells. A key role for endothelium-derived NO is the nitrosylation of haemoglobin, leading to changes in oxygen affinity, which appear to play a fundamental role in oxygen delivery in the microvasculature. The arterial circulation of animals and humans is vasodilated continuously and actively by endothelium-derived NO, and inhibition of the synthesis of NO with certain guanidino- substituted analogues of l-arginine, including N-G-monomethyl- l-arginine, leads to vasoconstriction, hypertension, and sodium retention. Shear stress—the force caused by the viscous drag of flowing blood—is an important physiological stimulus for the continuous production of NO. Shear stress increases NO produc- tion so the blood vessel relaxes and dilates, thereby reducing the shear stress and increasing flow and/or reducing blood pressure. Noradrenaline ATP Neuropeptide Y Nitric oxide Blood cells Nerves Shear stress Hormones Autocoids Endothelial layer Fig. 16.1.1.5 Vascular endothelial cells lie at the interface between blood and the smooth muscle cells. They detect chemical and physical signals in the lumen of the blood vessel and adjust their output of biologically active mediators accordingly. This provides a mechanism of local regulation of vascular function. Rapid adjustment of vascular tone is probably achieved through a balance of endothelium-derived nitric oxide and neuronally derived noradrenaline. Endothelin provides a slowly modulating constrictor tone and angiotensin II has the capacity to fine-tune neuronal, endothelial, and smooth muscle function. ACE, angiotensin-converting enzyme. Fig. 16.1.1.6 (a) Nitric oxide synthases (NOS) catalyse the conversion of l-arginine and molecular oxygen to citrulline and NO. NOS enzymes are catalytically active as homodimers and require the binding of cofactors (flavin adenine dinucleotide, flavin mononucleotide (FMN), haem (Fe), and tetrahydrobiopterin (BH4)) and calmodulin (CaM) for optimal activity. Each NOS dimer coordinates a single atom of zinc. (b) Under conditions where NOS are ‘uncoupled’, the enzyme does not catalyse the conversion of l-arginine to citrulline and NO, but instead generates superoxide or other reactive oxygen species (ROS) by reduction of molecular oxygen, driven by electron flow from NADPH via the flavin domain. Factors that cause NOS uncoupling include low levels of the cofactor tetrahydrobiopterin (BH4), inadequate levels of the substrate l-arginine, or oxidative modification of the eNOS protein by glutathionylation (G) of specific cysteine residues.

 This process of flow-mediated dilatation appears is a homeostatic mechanism to regulate blood flow and coordinate tissue perfu- sion. The autoregulatory action of flow-mediated dilatation op- poses classical myogenic autoregulation—the process by which a blood vessel constricts in response to an increase in intraluminal pressure. Synthesis of NO is stimulated by acetylcholine, bradykinin, and substance P, and in many vessels the release of NO accounts for the vasodilator actions of these mediators, which are known as ‘endothelium-dependent vasodilators’. Circulating hormones, including insulin and oestrogens, may

also act on receptors on or within the endothelial cell to stimulate the release of NO acutely or to alter the expression of endothelial NO synthase chronically. Endothelial NO synthase (NOS) is activated either by increases in intracellular calcium, which causes binding of calmodulin, or by phosphorylation of specific serine or threonine residues in the protein, for example, by the kinases Akt or PKC (Fig. 16.1.1.8). Phosphorylation can either activate or inhibit the enzyme, for ex- ample at serine 1179 or threonine 495, respectively. Phosphorylation of eNOS mediates the physiological effects of shear stress, and hor- mones such as insulin, oestrogen, and vascular endothelial growth factor (VEGF). Veins differ from arteries and arterioles in that they do not seem to be actively dilated by the continuous release of NO, al- though the venous endothelium releases NO when it is stimulated by acetylcholine or bradykinin, and veins are highly sensitive to NO-mediated vasodilatation. Furthermore, human veins do not release much NO in response to platelet-derived mediators. Indeed, aggregating platelets constrict veins, due to the un- opposed action of vasoconstricting platelet-derived mediators on the vascular smooth muscle. The reasons for the arteriovenous difference in NO production are not fully understood, but one consequence is that the guanylyl cyclase in venous smooth muscle is relatively upregulated and veins respond to smaller amounts of NO than do arteries or arterioles. This is of therapeutic rele- vance; NO is the active moiety of glyceryl trinitrate and other nitrovasodilators, and the low basal synthesis of endogenous NO by venous endothelium accounts, in part, for the venoselective action of these drugs. Pathophysiology Loss of NO leads to arterial vasoconstriction, has the potential to enhance platelet and white cell adhesion, and, in experimental models, may enhance atherogenesis. Several clinical conditions— including atherosclerosis, hypertension, hypercholesterolaemia, and diabetes—are associated with a functional loss of NO- mediated effects. In the coronary vasculature, loss of NO predisposes to vasospasm and may contribute to the onset of anginal symptoms. Atherosclerotic coronary arteries constrict in response to the platelet-derived me- diator serotonin (5-hydroxytryptamine), whereas healthy vessels are stimulated to produce more NO and dilate. Flow-dependent dilatation is also lost in such vessels, and the response to sympa- thetic stimulation is converted from dilatation to unopposed con- striction. Endothelial dysfunction precedes the development of overt atheroma, and there is a relationship between risk factors for ischaemic heart disease and impaired responsiveness of coronary arteries to endothelium-dependent vasodilators. Furthermore, hypercholesterolaemia, even in the absence of angiographic evi- dence of atheroma in large vessels, is associated with abnormal endothelium-dependent vasodilatation in coronary and peripheral arterioles. Modified low-density lipoproteins appear to inhibit NO synthesis or accelerate its destruction, possibly by enhancing pro- duction of the superoxide anion. Basal endothelium-dependent dilatation is also impaired in patients with essential hypertension and the degree of impair- ment increases with increasing blood pressure. It is not known whether the defect is a consequence or a cause of the raised pres- sure, but the fact that endothelial function appears to be restored by antihypertensive therapy argues in favour of such dysfunction being a response to raised pressure. Patients with diabetes show diminished endothelium-dependent dilatation, and this defect does not reverse with treatment. Thus, patients with uncontrolled hypertension, diabetes, and hypercholesterolaemia all display defects of NO-mediated vasodilatation and this could provide a common mechanism of vascular dysfunction in these diseases. Overproduction of NO may also contribute to disease. Bacterial endotoxin and some cytokines, including interleukin (IL)-1 and interferon-γ, induce expression of another NOS enzyme (inducible NOS, iNOS, or NOS2) in the endothelium, vascular smooth muscle, and inflammatory cells invading the vessel wall. Unlike the consti- tutive eNOS enzyme present in healthy endothelium, iNOS is not dependent upon agonist activation and

produces large amounts of NO. In these quantities NO, either alone or in combination with superoxide, may contribute to tissue damage in addition to causing profound vasodilatation and hypotension such as that seen in septic shock. The NO pathway has been the basis for several important thera- peutic approaches. Administration of glyceryl trinitrate, a NO donor that directly activates soluble guanylate cyclase, has been a longstanding therapy (notably since the time of Alfred Nobel) for coronary ischaemia and heart failure because of its ability to produce systemic venous and coronary arterial vasodilatation, respectively. Inhibitors of phosphodiesterase-5 (e.g. sildenafil, vardenafil, and tadalafil), the enzyme that inactivates cGMP in VSMC, which is the key downstream signalling molecule for NO, were initially developed for hypertension, but have been much more widely used for erectile dysfunction because of their effects on augmenting blood flow into the corpus cavernosum. PDE-5 inhibitors are also used for the treatment of pulmonary hyper- tension, and ongoing studies are exploring their efficacy in pa- tients with heart failure related to primarily systolic or primarily diastolic dysfunction. Activators of soluble guanylate cyclase, the enzyme that produces cGMP, have also been developed as poten- tial therapies for systemic hypertension, pulmonary hyperten- sion, and peripheral vascular disease (see Box 16.1.1.2). Other commonly used drugs, such as statins, may also exert some of their beneficial effects through ‘pleiotropic’ mechanisms that are not primarily dependent upon cholesterol lowering but act to in- crease NO bioactivity. Prostanoids NO appears to be the dominant vasoactive factor released from endo- thelial cells under basal conditions, but it is by no means the only mediator produced. The endothelium is a rich source of prostanoids, including the vasodilators prostacyclin and prostaglandins E2 and

 D2 (PGE2 and PGD2). However, whereas inhibition of NO leads to profound and widespread changes in vascular tone, inhibition of prostanoid synthesis with aspirin (or other nonsteroidal anti- inflammatory drugs, NSAIDs) does not, excepting in the renal vas- culature where dilator prostanoids do appear to be important in the regulation of basal renal blood flow: aspirin and other NSAIDs lead to vasoconstriction in the kidney, indicating tonic release of vasodilator prostanoids in this vascular bed. Furthermore, in the fetus and newborn, indometacin leads to the closure of the ductus arteriosus and a fall in cerebral blood flow suggesting a significant contribution of endothelium-derived prostanoids to tonic vaso- dilatation in these beds, at least during development. The cerebral blood flow in adults also falls in response to indometacin, but not to aspirin and other cyclooxygenase (COX) inhibitors, and so the role of prostanoids is unclear. Vasodilator prostanoids are important in the vascular changes of inflammation, although whether these prostanoids derive exclusively from the endothelium is not known. The finding that the inhibition of COX-II appears to be associated with increased cardiovascular risk is important and suggests that the balance of prostanoids in the vessel wall, and between endothelium and platelets, is a key determinant of the ‘stickiness’ of the endothe- lium to platelets and other circulating cells. Hyperpolarizing factors An endothelium-derived hyperpolarizing factor has been identi- fied in some animal and human blood vessels. Hyperpolarization of vascular smooth muscle cells leads to a fall in calcium entry and vascular relaxation. Increasing evidence suggests that endothelium- dependent hyperpolarization may be particularly important in small arteries and arterioles. The chemical identity of endothelium-derived hyperpolarizing factor has not been clearly established, but products of activity of cytochrome P450, the cannabinoid anandamide, and the potassium ion have all been suggested as possible candidates. Recent data also suggests that the C-type natri- uretic peptide accounts for this activity in some vessels. A picture is emerging that endothelium-derived hyperpolarizing factor

is not a single entity, but rather that hyperpolarization is a mechanism utilized by different mediators that vary between vessels. In add- ition, direct contact through gap junctions also provides a means for endothelial cells to hyperpolarize smooth muscle cells. Without specific inhibitors, it is not yet clear what role the variations in endo- thelial cell hyperpolarization of smooth muscle cells plays in human disease. Vasoconstrictors Although the predominant background influence of the endothe- lium is dilator, important vasoconstrictor factors are also synthesized and released. Endothelin The endothelins are a family of potent vasoconstrictor peptides of 21 amino acids, which are closely related to the snake-venom toxin of the Israeli burrowing asp (Atractaspis engaddensis). Three types of endothelin have been described—endothelin 1, 2, and 3—and there are at least two endothelin receptors in human blood ves- sels, the endothelin A and endothelin B receptors. Endothelins vasoconstrict and can promote the growth of vascular smooth muscle cells. Effects are mediated in part through the stimulation of increases in calcium and in part through calcium-independent mechanisms, including activation of protein kinases. Endothelin 1 is synthesized from ‘big endothelin’ within human endothelial cells (Fig. 16.1.1.7). It is a potent and long- lasting constrictor of human blood vessels, and causes widespread vasoconstriction, hypertension, and sodium retention when in- fused into healthy volunteers. Antagonists of the endothelin A receptor cause vasodilatation and can lower blood pressure, indicating that there is a tonic synthesis and release of endothelin A. Several studies suggest that there may be important inter- actions between the sympathetic nervous system, the renin– angiotensin system, and the endothelin system, and that these may act in concert to control constrictor tone, with the endothelin system providing a slowly modulating background constrictor tone. Endothelins also exert an important influence on sodium reabsorption in the kidney. Although activation of endothelin B receptors on vascular smooth muscle causes constriction, activation of endothelial endothelin B receptors leads to the generation of vasodilator prostanoids and/ or NO, hence endothelin can also produce transient vasodilatation in some circumstances. Binding of endothelin to endothelin B re- ceptors also seems to be important to clear the peptide from the circulation. Stimuli for endothelin production include thrombin, insulin, ciclosporin, adrenaline, angiotensin II, cortisol, various proinflammatory cytokines, hypoxia, and shear stress. The concentrations of endothelins circulating in plasma are low and may not reflect local concentrations achieved within the vessel wall, making it difficult to interpret the elevated values reported in many conditions. Nonetheless, activation of the endothelin system has been implicated in the pathogenesis of certain cardiovascular conditions. For example, a role for endothelin in the pathogenesis of vasospasm associated with subarachnoid haemorrhage and some types of renal ischaemia is suggested by experiments in animals. In addition, the increased production of endothelin has also been clearly implicated in the pathogenesis of a very rare form of secondary sys- temic hypertension caused by malignant haemangioendothelioma, a vascular tumour characterized by intravascular proliferation of Box 16.1.1.2 PDE5 inhibitors, cGC activators, and ADMA The pulmonary vasculature seems to be particularly sensitive to NO and the inhibition of NO synthesis causes pulmonary hypertension. These observations have been utilized therapeut- ically in the form of inhaled NO treatment, and amplification of NO signalling by inhibition of cGMP phosphodiesterase with sildenafil (see Chapter 16.15.2). Levels of VSMC cGMP are also increased by drugs that increasing the activity of soluble guanylate cyclase (sGC), either by activating the enzyme independently of the haem group (which can be oxidized or lost in pathophysio- logical states), or by stimulating the activity of the intact enzyme (e.g. riociguat). A naturally occurring amino acid, asymmetric dimethylarginine (ADMA), acts as an important endogenous inhibitor of NO synthesis, and the concentration of ADMA in blood is a predictor of cardiovascular risk.

Accumulation of ADMA may be important in renal failure, providing a possible mechanism to link failing renal function with increased risk of atherothrombotic complications.

16.1.1 Blood vessels and the endothelium 3249 atypical endothelial cells. In this condition, the degree of hyperten- sion correlates with plasma levels of endothelin, and when the tu- mour is removed blood pressure and plasma endothelin levels fall. The role of endothelin in the pathogenesis of pulmonary hyper- tension and congestive heart failure has been studied most intensely. In pulmonary hypertension, selective ETA antagonists lower pul- monary vascular pressure in patients with advanced disease and have been approved for clinical use. However, the role of endothelin receptor antagonists in treating congestive heart failure is less clear.
A substantial body of preclinical evidence indicates that selective ETA or nonselective ETA/ETB antagonists prevent ventricular re- modelling and prolong survival in models of myocardial injury. However, although short-term studies with endothelin antagonists produced beneficial hemodynamic effects in heart failure patients, long-term studies failed to show significant effects on morbidity or mortality, and endothelin antagonists are not presently approved for heart failure.
Angiotensin-converting enzyme Angiotensin-converting enzyme (ACE) is located primarily on the luminal surface of the endothelium (see Fig. 16.1.1.5). This en- zyme converts angiotensin I to angiotensin II and also metabolizes bradykinin to inactive products. The pulmonary vasculature pro- vides the largest area of endothelium and is important in the regulation of circulating levels of angiotensin II, but the activity of endothelial ACE in systemic vessels may be more important in determining the final concentrations of angiotensin II and brady- kinin that reach the blood vessel wall. Furthermore, endothelial cells also have the ability to synthesize renin and its substrate. It seems, therefore, as though the enzymatic machinery for a complete renin– angiotensin system is present within the vessel wall. The activity of the renin–angiotensin system is clearly important in cardiovascular diseases including hypertension and heart failure, but the relative importance of local, compared with systemic, regu- lation of angiotensin II production is not yet clear. Furthermore, the full clinical significance of bradykinin metabolism by endothelial ACE has yet to be determined. It has been demonstrated that at least part of the vasodilator action of ACE inhibitors in certain isolated blood vessels is due to accumulation of bradykinin, which stimu- lates NO synthesis. Bradykinin and many other vasoactive pep- tides (e.g. substance P and natriuretic peptides) are broken down by the metalloendopeptidase, neprilysin (particularly in the lung and kidney). Neprilysin is the target of neprilysin inhibitor drugs such as sacubitril, which has been combined with valsartan (an angiotensin II receptor antagonist) for the treatment of heart failure.
Prostanoids The endothelium synthesizes thromboxane and the unstable pros- taglandin endoperoxides PGG2 and PGH2. Overproduction of constrictor prostanoids by the endothelium has been implicated in animal models of diabetes and hypertension, but the significance of these findings for human disease remains uncertain. Reactive oxygen species Production of reactive oxygen species (ROS) influences blood vessel physiology by direct interactions with nitric oxide, and by modu- lating redox-sensitive pathways such as gene expression and activity of key proteins by post-translational oxidative modification. ROS such as the superoxide anion ($O^{-}2$) are synthesized within the vas- cular wall by multiple enzyme systems within endothelial, vascular smooth muscle, and inflammatory cells (e.g. macrophages and neu- trophils). A major source are the NADPH oxidases that can be de- fined by at least five classes of a catalytic subunit termed Nox1–5. The Nox2 enzyme typified by the neutrophil NADPH oxidase (but also expressed in endothelial cells and other inflammatory cells) is a major source of vascular superoxide. Vascular smooth muscle cells, where Nox1 and Nox4 predominate, also contribute. Stimulation by

angiotensin II increases superoxide generation by Nox2 NADPH oxidases, and is a key feature of vascular pathophysiology. Other im- portant sources of ROS are mitochondria, xanthine oxidoreductase, and NOS. In addition to its important vasodilator properties, NO acts as a free radical scavenger. As is characteristic for such agents, NO itself is also a free radical (it has an unpaired electron in its outer orbit), and as such, reacts readily with other free radicals and ROS, re- sulting in the formation of the reactive nitrogen species peroxynitrite (ONOO−), and with other ROS to generate inorganic nitrite and nitrate (NO3−) in biological systems. Under certain physiological conditions (e.g. hypoxia), NO can be regenerated from nitrite by ni- trite reductases such as xanthine oxidoreductase, or haemoglobin. Lys-Arg Lys-Arg C N Trp-Val Big endothelin Endothelin-1 Activation of endothelin A and endothelin B receptors Dibasic-pair-specific endopeptidase Endothelin-converting enzyme Fig. 16.1.1.7 Endothelin-1 (ET-1), a cyclic (Cys1–Cys15 and Cys3–Cys11) 21-amino acid peptide, is synthesized within the vascular endothelium as the product of an ‘inactive’ 39-amino acid precursor known as ‘big ET-1’, a conversion catalysed by a specific membrane-bound zinc metalloproteinase endothelin converting enzyme (ECE). Big ET-1, in turn, is the catalytic product of a larger (203 amino acids) precursor polypeptide termed ‘preproET-1’ (a conversion that is believed to be mediated by a ‘furin-like’ protease). The ECE-mediated conversion of big ET-1 to mature ET-1 is an essential step in the expression of full biological activity. Upon release from the vascular endothelium, ET-1 interacts with the underlying smooth muscle cells resulting in vasoconstriction. This action is mediated by two distinct G-protein-coupled receptors, ETA and ETB. Although the predominant action of ET-1 is that of a vasoconstrictor, this effect is regulated by the concomitant release of vasodilatory factors (e.g. PGI2, NO) by the action of ET-1 on endothelial ETB-receptors. Although such an action tempers the contractile actions of ET-1, it is postulated that endothelial dysfunction (e.g. diminished ability to synthesize and/or release NO such as is seen in hypertension, atherosclerosis) results in aberrant ET-mediated vasoconstrictor tone due to a loss in concomitant endothelial regulation.

 eNOS can also generate ROS (see Fig. 16.1.1.6b). This aspect of eNOS function, termed ‘eNOS uncoupling’, occurs when levels of the cofactor for NOS, tetrahydrobiopterin (BH4), are lowered due to oxidation or reduced biosynthesis. Uncoupling is also caused by conformational changes in eNOS due to oxidative modification of cysteine residues on the enzyme by glutathionylation. The posi- tive feedback loop created for generation of ROS is an important pathway resulting in endothelial dysfunction and other alterations in vascular redox signalling. Regulation of platelet function and haemostasis The endothelium synthesizes and releases prothrombotic and antithrombotic factors. However, healthy endothelium presents a thromboresistant surface, indicating that the antithrombotic factors predominate under basal conditions. Platelets Endothelial cells inhibit the aggregation and adhesion of plate- lets, and disaggregate aggregating platelets. Two mediators are of particular importance: NO and prostacyclin (or PGE2 in the microvascular endothelium). These act synergistically through different second messenger systems: cGMP for NO and cAMP for prostacyclin. Thiols and sulphydryl-containing molecules react with NO to produce more stable adducts, including nitrosocysteine, nitrosoglutathione, nitrosoalbumin, and even nitrosohaemoglobin. Some of these compounds are formed in vivo and may enhance the antiplatelet effects of endothelium-derived NO. Furthermore, interaction between NO and tissue plasminogen activator leads to the formation of nitroso-tissue plasminogen activator, a molecule with fibrinolytic, antiplatelet, and vasorelaxant properties. It is not yet clear how important these NO adducts are in human physiology or pathophysiology.

Deficient production of NO has been implicated in a wide variety of cardiovascular diseases (see ‘Nitric oxide’ section earlier), and ab- normalities of prostanoid synthesis occur in experimental models of atherosclerosis and diabetes. In the presence of a quiescent healthy endothelium, loss of basal NO alone does not lead to significant sys- temic platelet activation. However, loss of NO and prostacyclin at sites of endothelial damage, dysfunction, or activation promotes the formation of platelet aggregates and may contribute to thrombosis and vessel occlusion. In animals, stenosed endothelium-denuded vessels lead to cyclical variations in flow as platelets stick to the vessel wall and release vasoactive and proaggregant mediators. If this also occurs in human vessels in vivo, it might be an important mech- anism of vasospasm and thrombosis. Under basal conditions the endothelium inhibits platelet activa- tion, but in response to certain stimuli, proaggregant, proadhesive mediators may be synthesized and released. Unstable prostaglandin endoperoxides activate platelets, platelet activating factor may be produced, and von Willebrand factor—which is synthesized and stored within endothelial cells—increases platelet adhesion. These changes occur in response to inflammatory mediators and may also result from endothelial ‘injury’, such as those occurring during cor- onary artery angioplasty or stent implantation. Coagulation Heparan sulphate is a glycosaminoglycan closely related to heparin, but less potent, which is found on the surface of endothelial cells. Antithrombin III is also expressed on the endothelial cell surface and, together with heparan sulphate, provides a mechanism for binding and inactivating thrombin. In addition, endothelial cells participate in the activation of the anticoagulant protein C, and se- cretion of protein S and thrombomodulin that is found on the cell surface. In the quiescent state, expression of anticoagulant factors pre- dominates, but when activated the endothelium may promote co- agulation. Receptors for clotting factors appear on the endothelial surface, von Willebrand factor is secreted, and tissue factor—the principal cellular initiator of coagulation—is expressed. Bacterial endotoxin, inflammatory cytokines, and glycosylated proteins acti- vate the endothelium and shift the balance in favour of coagulation. This may occur in response to infection, inflammation, or endothe- lial injury. Circulating levels of von Willebrand factor are increased in some patients with diabetes or hypertension. Fibrinolysis The endothelial cell surface has a fibrinolytic pathway. Urokinase and tissue plasminogen activator are secreted and there are spe- cific binding sites for plasminogen activators and plasminogen. Thrombin, adrenaline, vasopressin, and stasis of blood may be physiological stimuli for the release of tissue plasminogen activator from human endothelium. Plasminogen activator inhibitor 1 is also synthesized and bound by endothelium, providing a pathway for local inhibition of the fi- brinolytic system. Under basal conditions fibrinolysis is dominant, but the balance may be altered by a variety of local and circulating factors, including inflammatory cytokines and the atherogenic par- ticle lipoprotein(a), which inhibits plasminogen binding and hence plasmin generation. In the presence of atherosclerosis, the fibrino- lytic properties of the endothelium are diminished. Other important aspects of vascular and endothelial biology Cellular adhesion The resting endothelium prevents cells from adhering fully to the vessel wall, but allows leucocytes to ‘roll’ along its surface. The regulation of rolling, adhesion, and migration is governed largely by specialized glycoproteins known as cell adhesion molecules, which are expressed in varying amounts on the endothelial cell surface and interact with complementary adhesion molecules on circulating cells. Endothelial-leucocyte adhesion molecule 1 (ELAM-1, also known as E-selectin), vascular adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), and P-selectin (also known as GMP-140) are all expressed on cytokine-activated endothelium. The degree of expression and the type of adhesion molecules expressed determines the ‘stickiness’ of the endothelium for different cell types. Expression of adhesion molecules is an important mechanism of cellular adhesion during inflammation and is also

important in

16.1.1 Blood vessels and the endothelium 3251 recruitment of T cells and monocytes in atherosclerosis. Increased expression of E-selectin is seen in the coronary arteries of trans- planted hearts, and has been implicated in the rapid development of atherosclerosis in these vessels. NO and prostacyclin inhibit the adhesion of white cells to the endothelium and this effect may be mediated by changes in the expression or configuration of adhesion molecules. Certain endothelial cell adhesion molecules are shed into the plasma: changes in their concentration have been detected in a variety of cardiovascular diseases, but the significance of this is uncertain. Proinflammatory cytokines Cytokines are released from activated leucocytes in response to in- fection and immunological stimulation and are also produced by the vessel wall itself; IL-1, IL-6, and IL-8, and colony stimulating factors are synthesized by endotoxin-stimulated endothelial cells, and tumour necrosis factor (TNF) by human smooth muscle cells. A large number of cytokines and chemokines alter endothelial functions, upsetting the balance of vasoactive mediators, altering thrombotic activity and the expression of adhesion molecules, or initiating apoptosis (programmed cell death). IL-1 and some other proinflammatory cytokines alter the synthesis of NO (see ‘Nitric oxide’ section earlier) and a variety of prostaglandins; enhance the generation of thrombin, platelet activating factor, von Willebrand factor, and plasminogen activator inhibitor; alter endothelial per- meability; increase expression of ICAM-1 and VCAM-1; and may also cause endothelial cell damage and death. These findings are of direct relevance to the vascular changes occurring in inflammation and sepsis, and might also provide a link between acute or chronic immunological stimulation (e.g. infection) and the development of cardiovascular disease, including atherosclerosis or acute cardiovas- cular events. More recently it has been recognized that components of the innate immune pathway, such as Toll-like receptors (TLRs), are expressed by cells in the vascular wall and play a role in the patho- genesis of cardiovascular disease. These receptors recognize spe- cific, highly conserved structural motifs in nonhost pathogens, resulting in rapid activation of a coordinated innate immune re- sponse. However, TLRs may also be activated by damage-associated molecular pattern molecules such as proteins released by injured or necrotic cells (e.g. heat shock proteins, HMBG1), and/or modified by oxidation (e.g. oxidized LDL), by DNA released from the nucleus, or proteins that have been glycated in diabetes (advanced glycation end products, AGE), that are recognized by RAGE, the specific re- ceptor for AGE. These innate immune mechanisms are important in the vascular wall in atherosclerotic plaques or in the myocardium following ischaemic injury, by initiation and amplifying the patho- logic inflammatory response. Cell growth and angiogenesis The endothelium of healthy differentiated vessels inhibits prolif- eration of the underlying smooth muscle. Endothelium-derived vasodilator, antiplatelet, and antithrombotic mediators (e.g. NO, prostacyclin) tend to inhibit the growth of vascular smooth muscle cells, whereas vasoconstrictor and prothrombotic mediators (e.g. endothelin, angiotensin) tend to promote it. Thus, the basal state of the endothelium, in which dilatation and thromboresistance pre- dominates, also prevents the growth of smooth muscle. The heparin- like molecules prevent cell growth and molecules similar or identical to platelet-derived growth factor (PDGF) and fibroblast growth factor are endothelium-derived growth promoters. Others such as transforming growth factor β (TGFβ), produced by endothelial cells, may either inhibit or promote cell growth, and the precise role of this molecule in vivo is unclear. The basal antiproliferative effects of the endothelium may retard the development of atherosclerosis and intimal proliferation. In addition to affecting the growth of underlying smooth muscle, endothelial cells are essential for the formation of new blood ves- sels. The ability of endothelial cells to initiate the formation of new

vessels (angiogenesis and vasculogenesis; Fig. 16.1.1.8) is retained in adults, but the only place this occurs physiologically to any great extent is in the female reproductive tract. However, angiogenesis occurs in a wide range of disease states including atherosclerosis, rheumatoid arthritis, and tumour growth, and during wound Fig. 16.1.1.8 Formation of new blood vessels. Endothelial cells grown in a matrix (Matrigel) form tube-like structures. The right-hand panel shows the effect of inhibiting angiogenic signals such as vascular endothelial growth factor (VEGF). Reprinted from Biochemical and Biophysical Research Communications, Vol 308, Issue 4, Smith CL et al., Dimethylarginine dimethylaminohydrolase activity modulates ADMA levels, VEGF expression, and cell phenotype, pp. 984–89. Copyright (2003), with permission from Elsevier.

 healing or in response to ischaemia. Positive and negative regu- lators of angiogenesis have been identified and a wide variety of cytokines, growth factors, and local autacoids can act alone or in concert to promote endothelial cell growth, migration, and tube formation. Of particular interest is VEGF, a growth factor produced by smooth muscle cells in response to hypoxia, inflammatory cyto- kines, and certain other growth factors. There is good evidence that VEGF can promote angiogenesis in a variety of animal models and in humans. Therapeutics that inhibit angiogenesis by targeting the VEGF pathway have shown clinical benefit in diabetic retinopathy and certain cancers. Intriguingly, it appears as though VEGF can increase the production of NO by endothelial cells, and this may be one of the effector molecules mediating some of the actions of this growth factor. In order to form endothelial tubes through tis- sues (i.e. angiogenesis), endothelial cells must degrade matrix and they are capable of synthesizing and releasing a variety of matrix metalloproteinases. Some of these matrix metalloproteinases may, in turn, affect endothelial function by regulating cell attachment, proliferation, and migration. Failure of endothelial cells to initiate appropriate angiogenesis in response to ischaemia may lead to tissue hypoxia, while excessive or inappropriate angiogenesis may contribute to a sustained inflammatory response in the vessel wall, disrupt vessel wall architecture, or lead to haemorrhage into athero- sclerotic plaques. Transport and metabolism The endothelium presents a permeability barrier for molecules in the bloodstream. Transfer of molecules from the bloodstream into the vessel wall across the endothelium can occur by transport through the endothelial cells or between them. The junctions between endo- thelial cells are maintained by specialized molecules, including cadherins, and are actively regulated. Transport between cells occurs when endothelial cells contract to leave intercellular gaps. This is an important mechanism for formation of localized oedema. Transport through cells occurs by transcytosis and is an important mech- anism for the passage of some macromolecules, including insulin. In addition, specialized channels for transport of water have been identified—the aquaporins. The endothelium is intimately involved in lipid metabolism. Lipoprotein lipase is bound to proteoglycans on the endothelial cell surface, and receptors for low-density lipoproteins are present in varying amounts. In quiescent endothelium, lipoprotein lipase is ac- tive, but there are few low-density lipoprotein receptors, indicating that healthy endothelium provides a barrier for the entry of low- density lipoproteins into the vessel wall. However, under conditions in which a low-density lipoprotein is taken into the endothelium, modification by oxidation occurs and this step may stimulate atherogenesis. Endothelial-derived microvesicles Endothelial cell-derived particles or cell fragments were first de- tected as presumed evidence of damaged or dead endothelial cell fragments (exosomes, microparticles, or apoptotic bodies). However, endothelium-derived microvesicles have now been iden- tified as a potential biomarker of endothelial function and cardio- vascular disease, reflecting biological processes. Microvesicles are submicron-sized particles shed from the plasma membrane of cells in response to cell activation, cell damage, or

apoptosis. The number of circulating microparticles appears to be increased in patients with cardiovascular disease such that it is possible that they play a role in pathogenesis, and it has been proposed that they are a biomarker of endothelial function and vascular health. Microvesicles contain proteins, active lipids, and nucleic acids that may provide additional biological and pathophysiological in- formation, since these components are dependent on the nature and cause of microvesicle shedding. For example, microRNAs (e.g. miR- 126), which are endogenously expressed small noncoding RNAs that regulate gene expression at the post-transcriptional level, have been implicated in regulating endothelial cell function and angio- genesis. Microvesicles are also a vehicle for cell-to-cell transfer of these signalling molecules, for example, between endothelial cells, platelets, and inflammatory cells such as monocytes. Specificity of cell-to-cell communication is mediated by cell surface proteins on microvesicles that are ligands for receptors on target cells. The adventitia and perivascular adipose tissue Nerves supplying the vessel wall enter through the adventitia into the media to provide a key influence on the contraction of vas- cular smooth muscle cells. The sympathetic nervous system is, of course, of prime importance in determining the contractile state of the vessel. In addition, cholinergic innervation influences some vas- cular beds, as do purinergic nerves. Pharmacological observation suggests that not all vessels are equally affected by denervation or interruption of specific neuronal influences. Resistance vessels and capacitance veins seem to be particularly regulated by sympathetic tone, and blockade of the sympathetic system causes not only a fall in arterial pressure but also major venous dilatation that leads to postural hypotension. In the brain, local neuronal projections have been implicated in providing a link between cerebral activation and the consequent increase in blood flow. Lymph vessels also permeate the adventitia of large vessels and are important to remove fluid. A network of small blood vessels, the vasa vasorum, is found in the adventitia of larger blood vessels. Vasa vasorum are found mainly in vessels that have relatively thick walls with many layers of vascular smooth muscle cells. An increase in vasa vasorum may be taken as an indication of vessel wall hyp- oxia. Stripping the vasa vasorum in large veins may contribute to both smooth muscle and endothelial dysfunction and damage, and, in the arterial system, can stimulate smooth muscle cell replication and promote an atherogenic type of lesion. The vasa vasorum re- sponds to vasoactive agents, but the pharmacology of these vessels is relatively poorly understood. Infiltration of the adventitia with in- flammatory cells may be an important feature of atherogenesis (see Chapter 16.13.1), and perivascular fat may interfere with vascular function through the generation of adipokines and inflammatory cytokines; this process has been implicated in the pathogenesis of cardiovascular disease in obese individuals. The adventitia surrounding the vascular wall also contains large numbers of adipocytes, forming the perivascular adipose tissue (PVAT). Although PVAT is typically in continuity with other sur- rounding adipose tissue, PVAT has particular cellular composition and pathophysiological roles that are distinct from other adipose tissue depots such as subcutaneous and visceral adipose tissue me- diated by adipocytokines. PVAT has anticontractile properties on small vessels due to a variety of vasoactive molecules produced in this tissue, such as

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