Endothelium—the axis of vascular health and disease

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Blood vessels are lined by endothelial cells which are responsible for maintaining thromboresistance. Over the past decade it has become clear that endothelial cells have many additional functions, while endothelial cell dysfunction has been implicated in several diseases. Although in 1987 there were more than 1,400 publications on endothelial cell biology, relatively little has yet been communicated to a general readership. It is thus the objective of this review to introduce the endothelial cell and to outline its role in health and disease.

Table 1. Functions of the vascular endothelium.

Maintenance of thromboresistance
Maintenance of selective permeability
Integration and transduction of blood-borne signals
Modulation of leukocyte interactions with tissues
Regulation of inflammatory and immune reactions
Regulation of vascular tone
Regulation of vascular growth
Synthesis and secretion of peptides

The vascular endothelium in health

Despite its apparently simple structure, the vascular endothelium is a highly specialised, metabolically active organ. It has central roles not only in the maintenance of vascular tone and permeability, but also in the regulation of leukocyte traffic, and in the modulation of haemostasis and thrombosis (Table 1). Although only one cell thick, it constitutes a dynamic interface between the blood and the rest of the body. It has been well described as a 'container for blood' [1]. It is now becoming clear that endothelial cells are heterogeneous, with significant variations in their functional specialisation at different anatomical sites. Endothelium can make important contributions to the differentiated functions of the various organs in which it resides. As with any metabolically active and physiologically responsive tissue, the endothelium is susceptible to injury.

Insights into the functional organisation of vascular endothelium were derived initially from ultrastructural [2] and physiological [3] studies *in vivo,* and more recently from experiments using endothelial cell culture *in vitro* [4,5]. More than 50 different types of endothelial cell have now been isolated and cultured from a variety of human and animal sources, both from large vessels and from the microvasculature.

Properties of endothelial cells

1. Procoagulant and prothrombotic properties

Endothelial cells rest on a basement membrane. This serves to anchor them within the vessel wall and provides a secondary barrier to the exchange of fluid, protein and the formed elements of the blood with extravascular tissue. The basement membrane is a complex structure containing collagens, elastin, laminin, fibronectin, thrombospondin, microfilaments, and at least three types of proteoglycan. Not only do endothelial cells synthesise these membrane components [6], but the matrix so formed modulates the growth, proliferation and phenotypic expression of the endothelium [7]. Endothelial cells can also remodel basement membranes, and the stimulated release of collagenases is believed to be an important early stage in angiogenesis [8].

Unstimulated platelets do not normally adhere to intact endothelial cells. However, when the basement membrane is exposed. for instance by removal or contraction of endothelial cells, platelets rapidly adhere to it. They may aggregate further and degranulate if they also come in contact with collagen fibrils in the membrane. Adhesion of platelets to the subendothelium, particularly at high shear rates, is critically dependent on von Willebrand factor (vWF) which acts as a cofactor in binding platelets to the subendothelium. Endothelial cells synthesise and store this glycoprotein and secrete it into plasma and the extracellular matrix. In the circulation its role is to act as a carrier for factor VIII [9].

Endothelial cells do not initiate coagulation by the intrinsic pathway, but it has recently become clear that, once the coagulation cascade has been triggered, endothelial cells (like blood platelets) localise and greatly promote prothrombin activation by providing an organised surface with receptors for factors Xa, IXa, Va and thrombin [10].

In common with other tissue cells, endothelium has the capacity to express tissue factor (thromboplastin) and hence to initiate the extrinsic pathway of coagulation.

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Unlike other tissue cells, however, factor VII binding activity is present only when endothelial cells are perturbed by a variety of stimuli, of which the best characterised are thrombin, bacterial lipopolysaccharides and cytokines [11,12]. This change towards procoagulant status is only one of a series of alterations of endothelial cell surface properties in response to these agents, and is of pathophysiological importance in a number of diseases.

2. *Anticoagulant properties*

Endothelium is the major site for two anticoagulant reactions involving thrombin. One binds thrombin and catalyses its inactivation by antithrombin which is also expressed at the endothelial cell luminal surface [13] and is present in the circulation. This reaction, which occurs only slowly *in vitro* unless enhanced by heparin, is probably accelerated *in vivo* by the presence of heparin-like glycosaminoglycans on the surface of the endothelium.

The second reaction is due to the binding of thrombin by a specific endothelial cell surface protein, thrombomodulin, which dramatically increases the ability of thrombin to activate proteolytically the circulating anticoagulant protein C [14]. Endothelial cells further contribute to this process by synthesising and secreting protein S, the cofactor for protein C activity [15].

3. *Fibrinolytic properties*

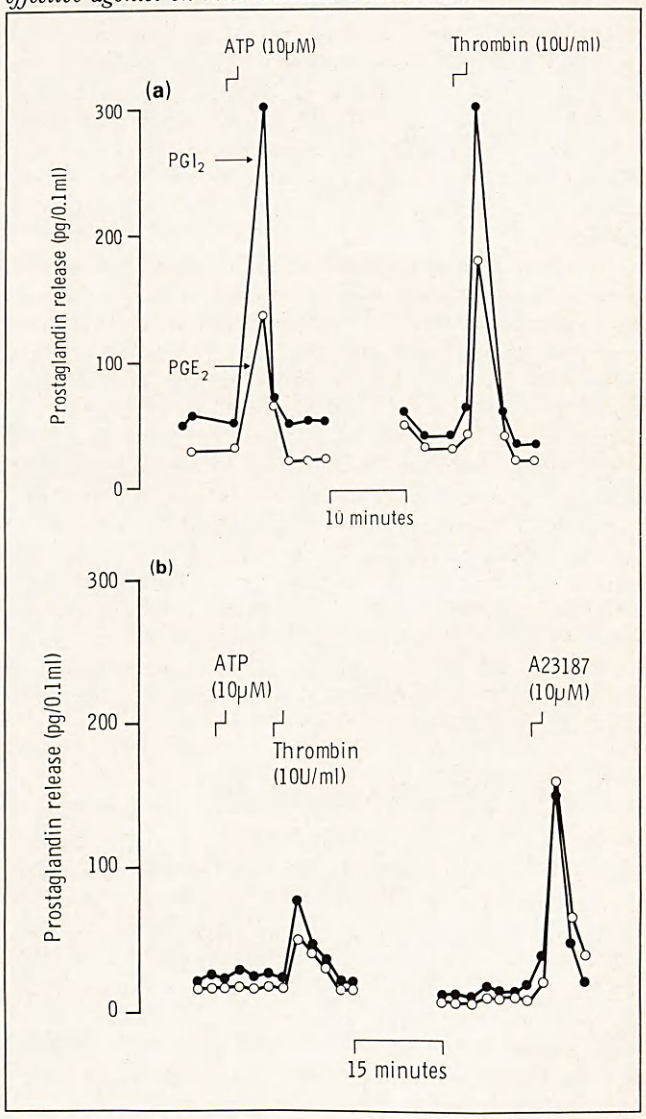
Resolution of a fibrin clot is due to two types of plasminogen activators—urokinase which activates plasminogen in the fluid phase, and tissue type plasminogen activator (t-PA) which is active only when bound to fibrin. Although endothelial cells in culture can secrete both forms it is only endothelial cell-derived t-PA [16] that is released into the blood stream stimulated *in vivo* by venostasis or by agonists such as vasopressin or noradrenaline. The regulation of fibrinolytic activity secreted by endothelium is complex and not yet fully understood. Studies *in vitro* have shown that thrombin directly induces t-PA release, but they have as yet failed to demonstrate vasopressin- or noradrenaline-induced release. In addition, the major circulating fast-acting t-PA inhibitor, plasminogen activator inhibitor 1 (PAI-1), is concurrently synthesised and released by endothelium. Interestingly, cytokines upregulate PAI-1 but not t-PA secretion by endothelial cells, thus providing an additional element in the shift towards procoagulant activity induced by these agents [16].

4. *Antiplatelet properties*

Endothelial cells are intrinsically non-thrombogenic—unstimulated platelets will not adhere to them. The inhibition of the adhesion of stimulated platelets to endothelial cells is powerfully inhibited by prostacyclin (PGI_2). This labile arachidonic acid metabolite is transiently synthesised and secreted by endothelium in response to a variety of stimuli including thrombin, bradykinin, adenosine diphosphate (ADP) and adenosine triphosphate (ATP) [17]. Thus mediators generated in plasma during coagulation and platelet aggregation induce a response from undamaged endothelium to limit the size of a forming thrombus. PGI_2 inhibits platelet stimulation by activating adenylate cyclase. It is also a powerful vasodilator in several vascular beds, acting on vascular smooth muscle through the same adenylate cyclase mechanism [18]. It is therefore of considerable interest that the recently characterised vasodilator, endothelium-derived relaxing factor (EDRF)—another highly labile endothelial cell product released in response to similar stimuli (see Section 9 below)—has now been shown to interact synergistically with PGI_2 to inhibit platelet function [19].

Although PGI_2 is the major prostanoid produced by large-vessel endothelium in response to stimuli, microvascular endothelial cells in culture often release more PGE_2

Fig. 1. *Stimulated production of prostacyclin (PGI_2; closed circles) and prostaglandin E_2 (PGE_2; open circles) by* **(a)** *human umbilical vein endothelial cells and* **(b)** *human microvascular endothelial cells from omental fat, in response to ATP, thrombin and ionophore A23187. Note that ATP was not an effective agonist on microvascular endothelium.*



than PGI_2 [20]. Figure 1 shows examples of agonist-stimulated PGI_2 and PGE_2 release from human microvascular (MVEC) and umbilical vein endothelial cells (HUVEC), illustrating this difference.

Aggregating platelets release ATP and ADP, which both induce EDRF and PGI_2 release from endothelium. ADP is pro-aggregatory, recruiting platelets into the developing platelet plug. Enzymes on the outer surface of endothelial cells inactivate these vasoactive metabolites. They produce adenosine (an inhibitor of platelet aggregation) which is then cleared from the circulation by uptake into endothelial cells and erythrocytes. Thus a series of interactions between adenosine metabolites and endothelium regulates vascular tone and platelet function [21]. These reactions can occur in the endothelium of large vessels and of capillary beds, but not equally at all sites. For example, microvascular cells cultured from human omentum, unlike cells from umbilical vein, do not dephosphorylate extracellular ATP or ADP (Fig. 2).

5. *Lipoprotein metabolism*

Lipoprotein lipase, the enzyme which hydrolyses the triacylglycerols and diacylglycerols of chylomicrons and of very low density lipoproteins (VLDL) is located at the luminal surface of capillary endothelial cells, bound to heparan sulphate [22,23]. Lipoprotein lipase is synthesised by parenchymal cells, particularly adipocytes and macrophages, from which it is secreted and transferred to endothelial cells to perform its role in the assimilation of dietary fat.

Receptor-mediated uptake of low density lipoproteins (LDL) by endothelial cells in culture occurs readily in growing, non-confluent monolayers, but is markedly down-regulated in confluent monolayers [24], indicating that endothelial cells act as a metabolic as well as a physical barrier to LDL uptake by the vessel wall. Alterations in this control of endothelial cell LDL uptake can occur at sites of vessel injury and may be important in atherogenesis (see below). In addition, endothelial cells can modify LDL molecules to a form which is recognised by the macrophage receptor for acetylated LDL (the 'scavenger' receptor) and thus is accumulated and degraded more rapidly by these cells [25]. Similarly, LDL preincubated with monocytes is more rapidly taken up than untreated LDL by aortic endothelial cells *in vivo* [26]. These observations raise the possibility that LDL modification is involved at an early stage of atherosclerosis.

6. *Endothelial cell-derived growth factors*

Endothelial cells in culture produce a vascular smooth muscle cell mitogen indistinguishable from platelet-derived growth factor (PDGF) [27], as well as a separate factor which supports the growth of other endothelial cells [28]. *In vivo* observations that intact endothelium inhibits intimal smooth muscle cell proliferation, however, have led to the demonstration that heparin-like oligosaccharides secreted by endothelial cells powerfully inhibit smooth muscle cell growth [29]. Thus there is an interplay of growth factors within the vessel wall.

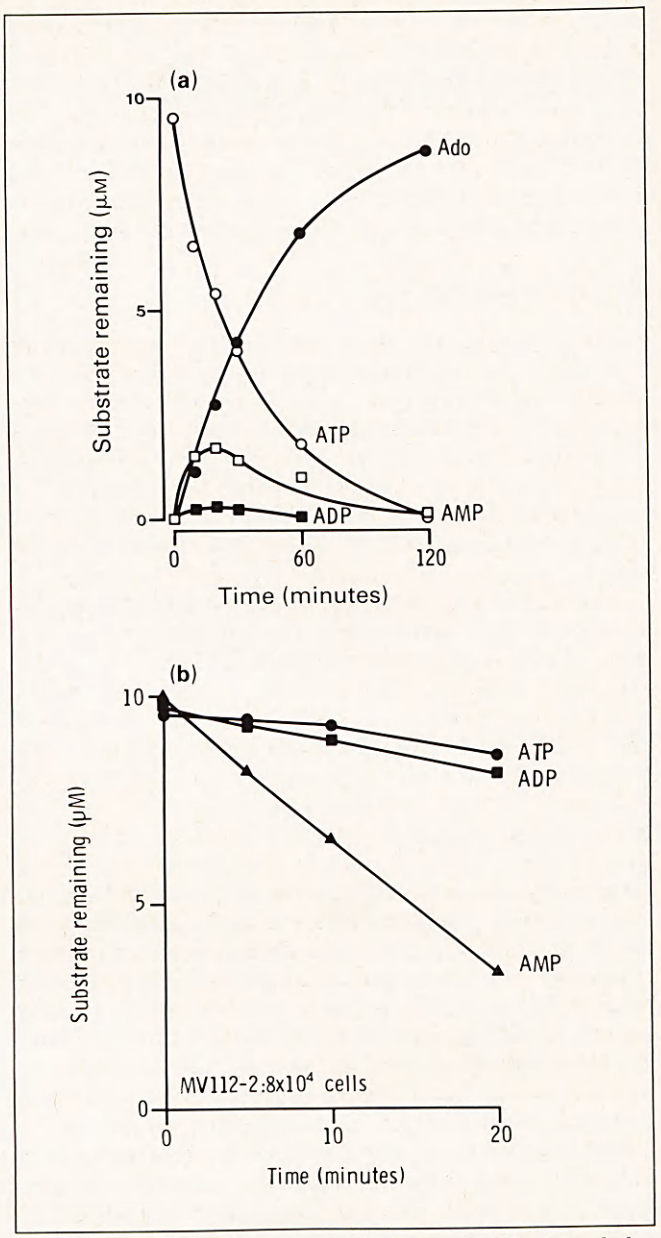


Fig. 2. *Hydrolysis of exogenous adenine nucleotides by endothelial ectonucleotidase enzymes.* **(a)** *The pattern of breakdown of added ATP by human umbilical vein endothelial cells, indicating sequential dephosphorylation to adenosine (Ado).* **(b)** *Added AMP, but not ADP or ATP, is hydrolysed by ectonucleotidases on microvascular endothelial cells from omental fat.*

7. *Interactions of endothelial cells with neutrophil leukocytes*

Endothelial cells are a favoured cellular substratum for neutrophil adhesion, and the blood pool of neutrophils is in dynamic equilibrium between freely circulating cells and a high proportion which is temporarily adherent to endothelium. An increase of this adhesive interaction and of subsequent neutrophil emigration is the mechanism by which the inflammatory response is initiated. This can be achieved both by stimulation of the neutrophil, particularly by chemotactic agents, and by alteration of endothe-

lial cell properties [30]. The most dramatic example of the latter is the development of greatly enhanced adhesiveness for neutrophils by cytokine- or lipopolysaccharide-treated endothelium. This is due to the novel surface expression of at least one specific endothelium–leukocyte adhesion molecule (ELAM-1) [12,31]. In addition, soluble mediators including platelet activating factor (PAF) and thrombin can rapidly and transiently enhance neutrophil adhesion by acting on the endothelium [32].

It has also been suggested that endothelial cells may regulate granulopoiesis, since they synthesise and release colony-stimulating activity which acts on granulocyte-macrophage colonies [33].

8. Immunological properties

Endothelium at specialised sites (high endothelial venules) is responsible for the directed emigration of lymphocytes from the bloodstream to lymphoid tissue. These morphologically distinct endothelial cells have specific properties, as yet not fully characterised at the molecular level, recognised by surface markers on lymphocytes [34]. The maintenance of lymphocyte traffic seems to depend on the action of lymphocyte secretory products—again not yet fully defined—on the endothelium. Analogous changes in endothelial cell morphology and function occur *in vivo* at sites of chronic inflammation associated with lymphocyte emigration [34]. *In vitro* studies have demonstrated that increased activity of at least one specific molecule, ICAM-1, on endothelial cells in response to gamma-interferon (γIFN) contributes to increased lymphocyte adhesion [35].

Endothelial cells are unusual amongst non-haemopoietic cells in that, in addition to the expression of blood group and major histocompatibility (MHC) class I antigens, class II MHC (Ia) antigens are also expressed to a variable degree, depending on anatomical site and local mediators. Ia antigens are strongly induced on endothelial cells by co-cultivation with T lymphocytes or by γIFN [36]. In association with this Ia expression, endothelium can present antigen to T lymphocytes and trigger lymphocyte proliferation [37], perhaps due to its recently described ability to secrete interleukin 6 (IL-6; B cell stimulating factor 2) [38]. Endothelial cells are therefore capable of replacing classical antigen presenting cells in the generation of the immune response.

9. Vasoactive properties

Endothelium has a central role in modulating the tone of underlying smooth muscle in response to physiological stimuli and pharmacological agents. In addition to producing powerful vasodilator and vasoconstrictor agonists, it is responsible for inactivating many circulating vasoactive mediators: thrombin, by binding and catalysing its interaction with antithrombin; adenine nucleotides, inactivated by specific ectoenzymes; bradykinin, inactivated by angiotensin converting enzyme [39].

Endothelium-dependent vasodilatation in many vascular beds is predominantly due to the release of EDRF in response to soluble mediators [40], although PGI_2 release from endothelium (Section 4 above) may contribute to it. EDRF acts by stimulating smooth muscle guanylate cyclase, which gives a rise in intracellular cyclic guanosine monophosphate, which in turn inhibits smooth muscle contractility and induces relaxation. Chemically, EDRF is nitric oxide. It is produced in endothelial cells by the metabolism of L-arginine [41,42] and is very unstable. It is possible that there may be more than one endothelium-dependent relaxing factor, but the term EDRF should be reserved for nitric oxide. Recently another dilator, endothelium-derived hyperpolarising factor, has been reported and its significance is being evaluated [43]. EDRF release occurs in response to a wide range of soluble mediators; its release is also triggered by changing shear forces on the endothelium, and this mechanism is thought to contribute to blood flow related control of vascular tone [44].

EDRF is also a potent inhibitor of platelet stimulation, acting in synergy with PGI_2 [19]. Since venous endothelium releases less EDRF than arterial endothelium [45], this relative deficiency in platelet inhibitory and vasodilator capacity may contribute to the lower patency rate of venous rather than of arterial coronary artery bypass grafts [46].

In certain vessels endothelium-dependent constriction can occur. This was first shown in veins but it also occurs in coronary and cerebral arteries, particularly in response to hypoxia. Thromboxane A2 and superoxide anions have been suggested as candidate mediators, but current interest is focused on the recently isolated endothelial cell-derived peptide endothelin, which is one of the most potent vasoconstrictors known [47,48].

10. Maintenance of permeability

Normal vascular permeability is maintained by a combination of endothelial cell integrity and basement membrane characteristics. The degree of hydraulic conductivity is clearly related to functional specialisation, ranging from the very low permeability of cerebral endothelium to the high permeability of fenestrated endothelium in exocrine glands and liver, and is also affected by the structural composition of the endothelial cell glycocalyx. In different vascular beds the movement of macromolecules may either be through the endothelial cell or between individual cells at cell–cell junctions. Thus situations which alter the physical characteristics of endothelial cells will alter permeability. The possibilities for modulation of permeability may be increased in cells where there is significant transcellular transport [49]. Permeability to macromolecules such as LDL may normally be due to a significant transcellular rather than intercellular route across endothelium. However, in localised oedema (the hallmark of an acute inflammatory response) the exudate is protein rich and reaches the tissue between endothelial cells, particularly in venules, whose intercellular junctions have been disrupted either by direct action of soluble inflammatory mediators (eg histamine or bradykinin) on endothelium, or as a consequence of increased neutrophil emigration [50]. Uncontrolled increases in permeability may be crucial in

conditions such as septic shock and in the adult respiratory distress syndrome.

Endothelial cells contain contractile proteins. In contrast to the view that transvascular flux of molecules is a passive process determined by diffusion barriers and Starling forces, there is increasing evidence that vascular permeability is regulated by physiological and pharmacological stimuli. This has led to the interesting suggestion that it may be possible to prevent increases in vascular permeability and oedema formation due to the local release of mediators, as in inflammation, with endothelial cell 'stabilisers' as potential novel anti-inflammatory agents [51].

Endothelial cell heterogeneity

A constellation of properties can be used to distinguish endothelial cells from other cell types, but there is considerable structural and functional heterogeneity between endothelial cells from different vascular sites. Some of the more obvious structural differences and their functional consequences are: the presence of tight junctional complexes in brain and retinal capillaries; the fenestrated endothelium of exocrine glands, liver, kidney and bone marrow; the high endothelial venules of lymphoid tissue. Moreover, arterial, venous and microvascular endothelial cells differ quantitatively in properties such as the pattern of prostanoid and EDRF release, or ectoenzyme complement. Thus cells from different sites cannot necessarily be identified by the same criteria. Two further examples are illustrated in Fig. 3. One is structural—the ability of microvascular endothelial cells *in vitro* spontaneously to produce capillary-like structures, which is not commonly observed in cultured large-vessel endothelial cells. The second shows that these microvascular cells take up modified LDL, in the same way as umbilical vein cells, but differ significantly in the manner of processing vWF, which is concentrated in granular stores in the large-vessel cells only. Endothelial cell properties are therefore closely interrelated with, and often modulated by, their local environment, and care must be taken before assuming that properties measured at one site will be shared with cells from another vascular location.

The vascular endothelium and disease

Endothelium may be damaged mechanically, chemically or immunologically. The damage may be sufficiently severe to cause endothelial desquamation with resulting loss of the thromboresistant lining and of the barrier function which regulates macromolecular transport into the arterial wall. Alternatively it may result in temporary or persistent specific alterations in endothelial cell function. For example, certain leukocyte products reversibly alter endothelial cell thrombotic and coagulant properties as well as modulating leukocyte adherence and migration. Some of these changes can also be induced by bacterial lipopolysaccharides. Further instances of the influence of infectious agents on endothelium include the recognition that several viruses can non-lytically infect endothelial cells and alter their properties [52], and that certain bacteria attach to endothelium, suggesting a mechanism for penetration of tissues by blood-borne organisms [53].

It is now certain that endothelial cells are involved in a wide spectrum of disease processes—ranging from inflammatory reactions and the various forms of vasculitis, to graft rejection and tumour metastasis (Table 2).

Table 2. Diseases with an endothelial cell component.

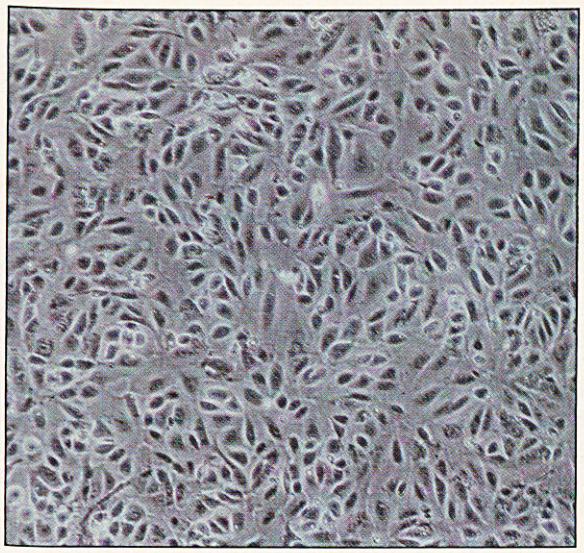
Definite	**Presumed**
Atherosclerosis	Thrombosis
Diabetic vasculopathy	Hypertension
Tumour angiogenesis	Cerebral vasospasm
Vasculitis	Coronary artery spasm
Inflammation	Migraine
Graft rejection	Raynaud's phenomenon
Haemolytic uraemic syndrome	Adult respiratory distress syndrome
Kawasaki syndrome	Septic shock
Thrombotic thrombocytopaenic purpura	
Idiopathic thrombocytopaenic purpura	

Atherosclerosis

Atherogenesis is a highly complex multifactorial process but endothelial cell damage or dysfunction is widely regarded as a critical initiating factor [54,55] (Fig. 4). Frank endothelial cell damage may promote at least two atherogenic processes. First, it reduces the significant barrier to lipoprotein accumulation within the vessel wall. Second, it initiates smooth muscle cell migration and proliferation, either by loss of endothelium-derived negative growth regulators [29] or by allowing platelet adhesion and secretion of the potent smooth muscle cell mitogen, PDGF [56].

In experimental models, mechanical damage to endothelium can lead to lipid-rich atherosclerotic lesions in normolipidaemic animals [57]. Homocystinaemia, which causes endothelial cell injury, is associated with aggressive premature atherosclerosis [58,59], and accelerated peripheral vascular and cerebral occlusive disease even occurs in heterozygotes [60]. Hypertension, strongly im-

Fig. 3. **(a)** *Confluent human adult microvascular endothelial cells derived from omental fat showing characteristic cobblestone morphology in culture.* **(b)** *Confluent microvascular endothelial cells showing spontaneous tube formation.* **(c)** *Immunofluorescent localisation of vWF in human umbilical vein endothelial cells showing characteristic punctate staining.* **(d)** *Immunofluorescent localisation of vWF in human microvascular endothelial cells showing perinuclear haze and absence of punctate staining.* **(e)** *Intracellular uptake of fluorescein-labelled acetylated LDL by umbilical vein endothelial cells.* **(f)** *Intracellular uptake of fluorescein-labelled acetylated LDL by microvascular endothelial cells.*



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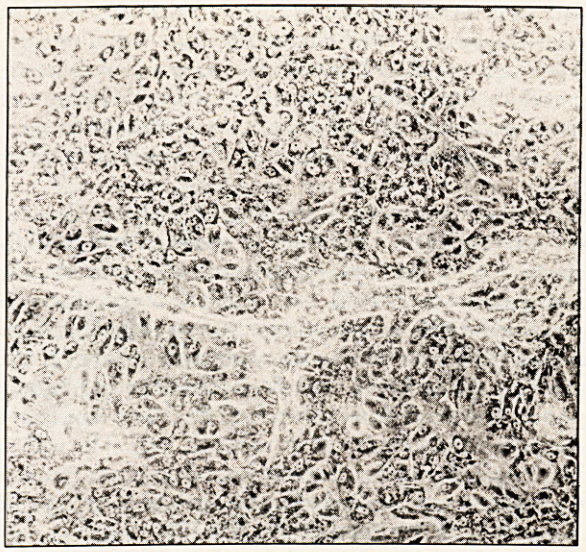

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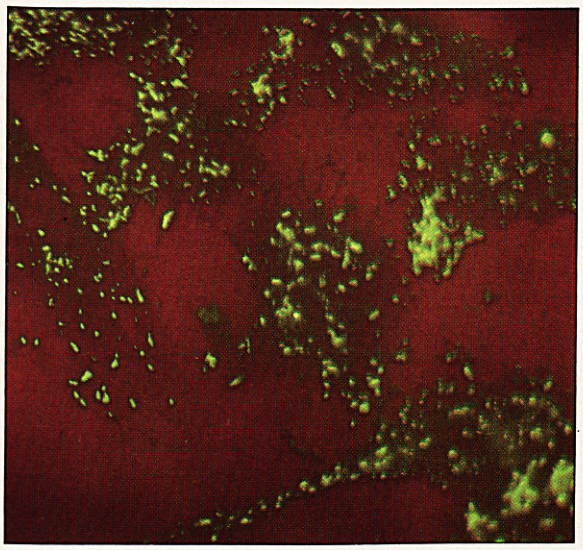

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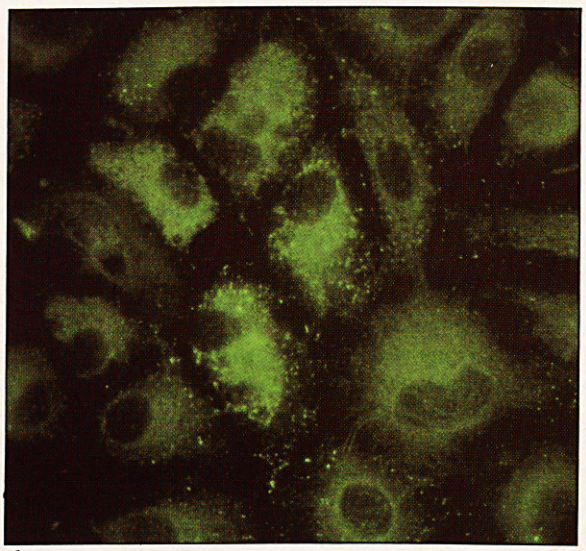

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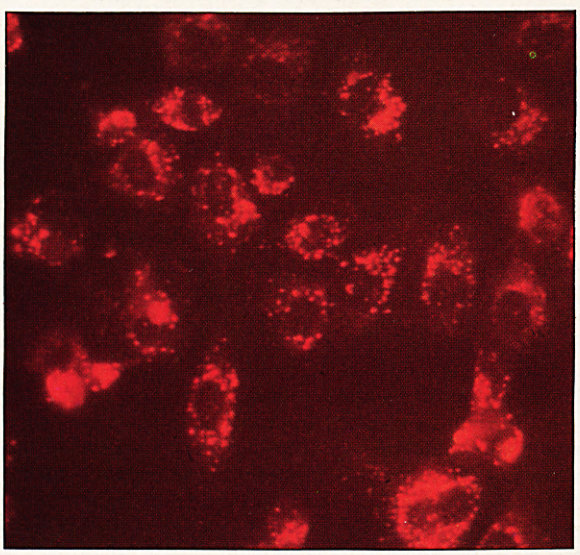

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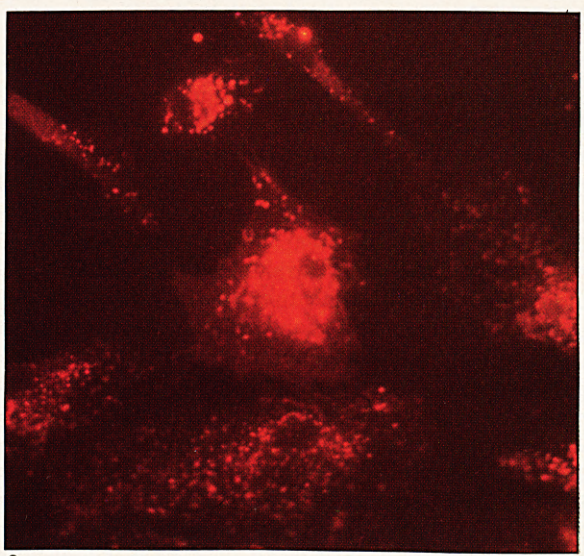

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plicated as a risk factor for ischaemic heart disease, has been shown experimentally to disrupt endothelial integrity [61]. There is also experimental evidence that regenerated endothelium has properties, related either to increased permeability to lipoproteins or to altered lipoprotein metabolism, that enhance intimal lipid accumulation [62].

Infection of human and animal endothelial cells with herpes virus has been implicated in atherogenesis [63,64]. The possibility of immunological involvement in accelerated atherosclerosis derives from clinical observations in certain patients with auto-immune diseases including rheumatic fever [65], systemic lupus erythematosus [66,67], and rheumatoid arthritis [68], and the realisation that rapidly progressive atherosclerosis is one of the most important long-term complications of graft rejection [69]. The role of immunological injury has been substantiated in a number of experimental models, including one in which endothelial cells were used as the immunogen [70–72].

It is now agreed that macrophages, derived from blood monocytes, are a significant feature of human atherosclerotic lesions [54,73] (Fig. 4). These cells can contribute to lipid modification and accumulation [25], and also secrete PDGF-like mitogens [74]. In view of the rapidly increasing knowledge of how leukocyte adhesion and emigration can be modulated by alterations of endothelial cell properties, it seems highly likely that the endothelium plays an active role in recruiting monocytes. Once present, macrophage-derived IL-1 could serve to maintain and amplify such alterations in endothelial cell function [12].

Finally, endothelial cell dysfunction, particularly in relation to the maintenance of thromboresistance or vascular tone, may play a critical role in the precipitating events of myocardial ischaemia, namely thrombus formation or vasospasm. A convincing example of this in man is the recent demonstration that atherosclerotic segments of coronary artery fail to dilate *in vivo* during infusion of acetylcholine (a potent stimulus for EDRF release) and respond with paradoxical vasospasm [75].

Diabetic vascular disease and endothelial injury

Several lines of evidence suggest that endothelial damage occurs in diabetes: there are increases in circulating levels of vWF [76], alterations in PGI_2 metabolism [77], abnormalities of coagulation pathways [78] and vascular permeability [79–81], all of which imply endothelial cell pathology. In addition, the increased incidence of atherosclerosis in diabetic patients can be interpreted as a reflection of endothelial cell dysfunction. Endothelial cell proliferation is a critical feature of the progression of diabetic retinopathy. This may be due to the presence of altered systemic or local growth influences. It has recently been shown that serum from patients with proliferative diabetic retinopathy contains a specific promoter of endothelial cell proliferation, which is not present in patients with non-proliferative background retinopathy [82]. In addition, loss of pericytes is an initial feature in diabetic retinopathy, and it has been convincingly demonstrated by *in vitro* co-culture methods that pericytes inhibit endothelial replication [83].

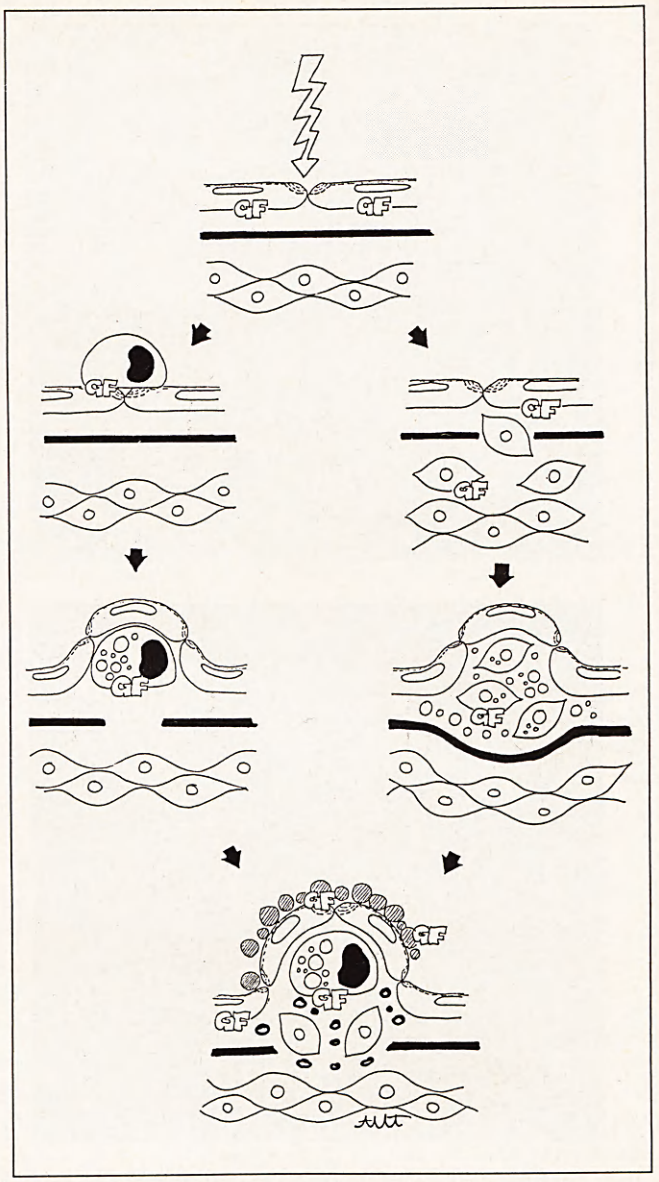

Fig. 4. *The revised response to injury hypothesis of atherogenesis* [based on Ross, 1986 (Ref 54)]
Intimal proliferation may occur by at least two separate pathways. Injury to endothelium is the primary event and may lead to the release of smooth muscle cell mitogens (GF) by endothelial cells.

In the left-hand path, endothelial damage is followed by monocyte adhesion and persistent growth factor release. Subsequently, monocytes migrate through the endothelial monolayer and lead to the formation of the fatty streak. Further growth factor release leads to the formation of fibrous plaque. Monocytes may also injure or stimulate the overlying endothelium, which can cause platelet attachment. Finally endothelial cells, monocytes and platelets continue to secrete growth factors, as may neo-intimal smooth muscle cells.

In the right-hand path, injured but intact endothelium increases its production and turnover of smooth muscle cell mitogens. These growth factors lead to smooth muscle cell migration from the media to the intima. The migrating smooth muscle cells also produce growth factor—probably PDGF. The interaction of endothelial and smooth muscle cell growth factors leads to fibrous plaque formation.

Angiogenesis and tumour growth

Capillary endothelial cells are normally in a resting state, and their rate of proliferation is so low that turnover times are measured in years [84]. However, under certain special conditions, particularly when new capillaries are being formed, these same cells can proliferate rapidly. Since the original suggestion that the growth of solid tumours may be dependent upon the formation of new capillaries, ie angiogenesis, there has been intense interest in determining whether tumours secrete angiogenic factors and whether anti-angiogenic agents can block tumour growth [85,86]. Angiogenesis occurs normally in embryogenesis, wound healing and ovulation, probably during osteogenesis (particularly marked in the regenerating deer antler [87]), and perhaps in skeletal muscle undergoing exercise hypertrophy [88]. However, uncontrolled angiogenesis may support diabetic retinopathy, rheumatoid arthritis and psoriasis.

A tumour-derived angiogenesis factor (TAF) was first isolated by demonstrating that it induced the formation of new vessels in the chorioallantoic membrane assay [89]. A second tumour-derived factor—angiogenin—has been isolated, characterised as a single peptide and cloned [90]. TAF is clearly not the same as angiogenin; it binds to heparin, and it induces proliferation or migration of tissue-cultured endothelial cells, which angiogenin does not. Since angiogenesis is a multistage process, involving disorganisation of basement membrane, cell migration and proliferation, it is likely that a range of angiogenic factors exists. An emerging concept is that angiogenesis can be regarded as a response to injury, triggered for example by local release of growth factors or basement membrane degrading enzymes. Alternatively, angiogenesis may occur as the result of loss of inhibitors of endothelial cell proliferation, such as pericytes.

One class of angiogenic inhibitors has already been shown to induce regression of tumour growth [91]—a fascinating new approach to the treatment of cancer, which may also prove to be valuable in the treatment of diabetic retinopathy, psoriasis and rheumatoid arthritis.

Tumour metastasis

Tumour metastases have distinct organ preferences, related to the nature of the primary tumour. Extravasation of tumour cells is initially dependent on adhesion of these cells to vascular endothelium. Experimental studies indicate a good correlation between metastatic pattern and affinity of adhesion to endothelial cells from different sites [92], possibly because of organ-specific heterogeneity between endothelial cells. Interference with this response has been suggested as a rationale for reducing tumour spread.

Haemolytic uraemic syndrome

Haemolytic uraemic syndrome (HUS) is a severe childhood disease characterised by microangiopathic haemolytic anaemia, thrombocytopaenia and acute renal failure. It is associated with platelet occlusion of arterioles and capillaries. There is some overlap between the syndrome and the acute non-relapsing form of thrombotic thrombocytopaenic purpura (TTP) in adults. Endothelial cell disturbance is evident from the presence of abnormally large vWF multimers in plasma. These may contribute to the pathogenesis of the disease by inducing or potentiating platelet stimulation; alternatively they may be a marker of endothelial damage (albeit unusual in that the altered vWF pattern is specific to HUS), which of itself may lead to loss of thromboresistance [93,94]. Although the aetiology of HUS and TTP, as with most autoimmune diseases, is far from clear, it is known that some TTP patients have circulating cytotoxic anti-endothelial antibodies [95]. Additionally, 13/14 HUS patients had complement-fixing antibodies that lysed cultured HUVEC [96]. The likelihood that these antibodies may be pathogenic is strengthened by the finding that they appear to be directed against a gamma-interferon sensitive antigen [96].

Kawasaki disease (mucocutaneous lymphnode syndrome)

This is another rare, life-threatening disease of children, in which arterial endothelial cell damage and proliferation occur and coronary aneurysms develop [97]. Cytotoxic autoantibodies directed against endothelial cells, in this case reactive with lymphokine- or monokine-induced antigens, are found in a high proportion of cases studied [98,99].

Idiopathic thrombocytopaenic purpura (ITP)

There is an intimate association between endothelial cells and the platelet. Platelets contribute to the maintenance of vascular permeability, while perturbations of the endothelium lead to platelet activation. In one recent study, sera from patients with ITP were reported to be deficient in a platelet-derived endothelial cell growth stimulator of low molecular weight, but the relevance of this factor in normal control of permeability has not been studied [100].

Systemic sclerosis (scleroderma)

There is increasing evidence to suggest that organ damage in scleroderma occurs as a result of a widespread disorder of the microvasculature [101]. Several studies have shown increased levels of vWF, and disturbances of coagulation and platelet function in scleroderma [102]. Endothelial cell-binding antibodies are common and distinct from the other antibodies (eg anti-collagen, anti-DNA) found in the disease [103]. Some sera have also been reported to contain non-antibody direct cytotoxic factors [104] and, while this may be an artefact related to oxidised lipoproteins [105], it is clear that sera from certain patients can induce antibody-dependent lymphocyte mediated killing of endothelium [106].

Other vasculitic disorders

Disorders of endothelial cell function, indicated by raised levels of vWF or the presence of autoantibodies directed

against endothelium have been recorded in a variety of other vasculitic disorders, such as giant cell arteritis, rheumatoid arthritis, and systemic lupus erythematosus [103,105,107–109]. Whether this merely monitors an association of endothelial cell dysfunction with the disease, or whether, as is suggested increasingly, it reflects a causal relationship with the pathogenesis of vasculitis [110], remains to be determined.

Generalised racemose livedo (Ehrmann's disease)

This is a rare illness frequently associated with cerebral infarctions, when it is known as Sneddon's syndrome. It typically occurs in women between 20 and 40, and is marked by occlusive lesions of small dermal arteries, associated with intimal proliferation [111]. A striking incidence of endothelial cell-directed cytotoxic antibodies has been found in this condition, suggesting that endothelial cell dysfunction plays some role [112].

Transplant rejection

The rejection of transplanted organs is usually associated with damage to vascular endothelium mediated by immunological mechanisms. Endothelial cells express both ABH and MHC class I antigens and can also express MHC class II antigens. Nonetheless, acute rejection in HLA-identical grafts has been shown to be due to pre-existent antibodies recognising a separate antigen system (EM) restricted to endothelial cells, monocytes and occasionally granulocytes [113]. It is of interest that these antigens are enhanced on lymphokine-treated endothelium, and are apparently present in patients with vasculitic disease [114,115], raising the possibility that they are related to the autoantibodies discussed above.

Conclusions

Endothelial cell health is crucial for normal vascular homeostasis, and endothelial cell dysfunction is implicated in a variety of diseases. The apparently simple monolayer of endothelium lining our blood vessels has emerged as a multifunctional cell type whose responses are carefully modulated by physiological stimuli. It can thus contribute to the pathogenesis of diseases where any one of these responses is inappropriately altered or lost, initiating the incorrect regulation of vessel growth, vessel tone, blood coagulation, platelet function or leukocyte traffic.

In addition to plans for new therapeutic strategies specifically aimed at the endothelium in, for example, tumour management, the consequences of infectious or inflammatory diseases, or the alleviation of diabetic vascular complications, novel approaches to therapy involving endothelium are being developed. These include the positive control of neovascularisation in wound healing, and the seeding of vascular prostheses with pre-cultured endothelial cells to provide the appropriate thromboresistant surface. An appreciation of endothelial cell biology will become an increasingly important element of our understanding of many diseases and how to manage and cure them.

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