PART I

The basis of endothelial involvement in vascular diseases

CHAPTER 1

Endothelial functions and dysfunctions

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Introduction

Key elements in the maintenance of tissue homeostasis, blood vessels serve as the conduits of circulation, transporting nutrients and oxygen to organs and tissues, and removing destructive catabolites and xenobiotics from the blood flow.

William Harvey first described the ceaseless and circular motion of the vascular system in 1628 [1]. Using primitive microscopes, Marcello Malpighi later elucidated a network of vessels throughout the body (Table 1.1). In 1661, Malpighi described the physical separation between blood and tissues and also identified the capillaries that connect small arteries with small veins [2]. During the 1880s, von Recklinghausen determined that tunnel-like blood vessels, lined by cells, bore deeply in the tissues [3,4]. After Starling proposed the law of capillary exchanges in 1896, scientists understood the endothelium as a selective physical barrier. By the mid-20th century, Palade's electron microscopy studies of the vessel wall [5] and Gowans' description of the interaction between lymphocytes and the endothelium of postcapillary venules [6] determined an active role of the endothelium in the circulation. Since then, numerous studies have elucidated our current view of the endothelium as a dynamic, heterogeneous, and widespread organ with vital synthetic, secretory, metabolic, and immunologic functions [1,7].

We have long understood two specialized endothelial functions: gas exchange in pulmonary circulation and fenestration in hepatic and splenic circulation. Under normal homeostatic conditions, the endothelium resists vasospasm, prevents leukocyte and platelet adhesion to the vessel wall, favors fibrinolysis, combats coagulation of blood, and inhibits the proliferation of vascular smooth muscle cells (SMC) (Figure 1.1). For these reasons, we now appreciate the vascular endothelium as a dynamic and heterogeneous autocrine/paracrine organ capable of synthesizing, secreting, and metabolizing a variety of substances as well as performing vital immunologic functions. This chapter will briefly review endothelial physiology.

Dysfunctional and activated endothelium: definitions

During the last two decades, accumulating evidence has described the vascular endothelium as an active endocrine, paracrine, and autocrine organ, indispensable for the maintenance of vascular homeostasis. Such maintenance occurs by continuous monitoring by the vascular endothelium of blood-borne and locally generated stimuli and also through subsequent immediate- or long-term responses to changes in its environment [8–10]. Altered homeostasis induced by various stimuli may cause localized alterations, or "endothelial dysfunctions," of the antihemostatic properties, vascular tone, heightened leukocyte adhesion, and increased production of cytokines and growth factors [9]. The term "endothelial activation" designates a subset of endothelial dysfunction whereby changes produced by various stimuli (e.g., inflammatory cytokines) elicit new functional and molecular properties. The dramatic change of endothelial interactions with blood leukocytes occurring in

Table 1.1 This brief history of vessel anatomy and biology as well as the discovery of the endothelium outline the principal theories and discoveries leading to the current understanding of blood circulation and the endothelium as a key regulator of vascular wall functions.

Who	*When*	*What*
Greek colony in Egypt	About 250 BC	Taught that the heart was connected with two separated sets of vessel: arteries full of air and veins full of blood. It was supposed that the two trees were entirely separated [4,223].
Erasistratos	304–250 BC	First hypothesized the existence of "synastomoses" between veins and arteries [4,223].
Galen	131–201 AD	Taught that blood passed from the right to the left ventricle through invisible pores in the interventricular septum [224].
Galilei	1609	Developed an *occhiolino* or compound microscope with a convex and a concave lens.
Cesalpino	1509–1603	Maintained that the blood circulated through the whole body [4].
Harvey	1628	Discovered the circulation of the blood without the use of the microscope [225].
Malpighi	1661	With the use of a primitive microscope, described the physical separation between blood and tissues and the existence of capillaries connecting small arteries with small veins [2].
Van Leuwenhoek	1674	Improved the microscope and finely described where a small artery ends and a vein begins [226].
von Recklinghausen	1881	Showed that vessels were lined by cells [3].
Starling	1896	Described the law of "capillary exchanges" [227].
Ernest Ruska	1931	Built the first electron microscope.
Pappenheimer	1953	For the first time proposes a physical theory on capillary permeability [228].
Palade	1953	Described the ultrastructure of the blood capillaries [5].
Gowans	1959	First described the interaction between lymphocytes and the endothelium in post-capillary venules [6].
Moncada and Vane	1976	Discovered prostacyclin [229].
Furchgott and Zawadzki	1980	Postulated the existence of an endothelium-derived vascular relaxing factor (EDRF) [8].
Ignarro and Palmer	1987	Demonstrated that endothelium-derived relaxing factor is nitric oxide (NO) [38,39].

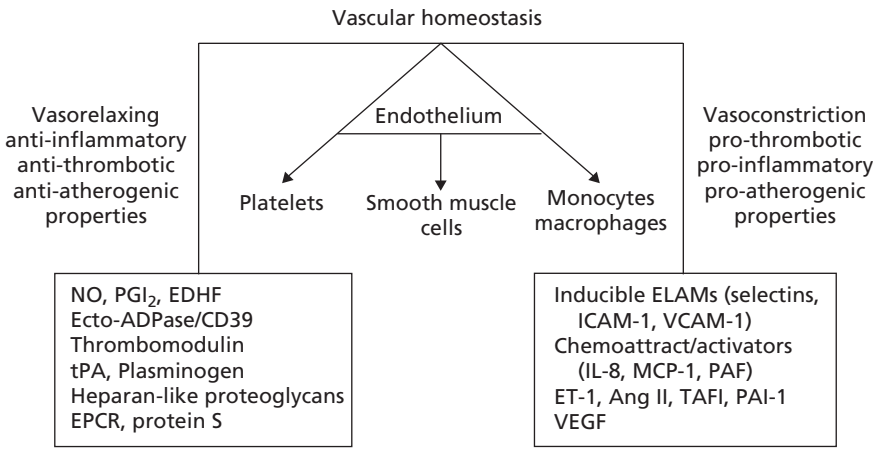

Figure 1.1 A schematic representation of the role of the endothelium in the maintenance of vascularhomeostasis. Endothelium-derived factors act in concert to appropriately control vasoconstriction, smooth muscle proliferation, thrombosis, inflammation, coagulation, and fibrinolysis. Endothelial dysfunction is defined as the loss of any of these balanced functions, with resulting pathologic consequences. NO: nitric oxide; PGI_2: prostacyclin; EDHF: endothelium-derived hyperpolarizing factor; EPCR: endothelial protein C receptor; ELAMs: endothelial-leukocyte adhesion molecules; ICAM-1: intercellular adhesion molecule-1; VCAM-1: Vascular cell adhesion molecule-1; IL-8: interleukin-8; MCP-1: monocyte chemoattractant protein-1; PAF: platelet-activating factor; ET-1: endothelin-1; Ang II: angiotensin II; TAFI: thrombin-activatable fibrinolysis inhibitor; PAI-1: plasminogen activator inhibitor-1; VEGF: vascular endothelial growth factor.

inflammation provides an example of endothelial activation [11].

Endothelial cell morphology

Generally of squamous morphology and usually elongated in the direction of blood flow, endothelial cells (EC) form a 0.2–4 μm-thick monolayer that lines the lumina of the entire surface of the vascular tree. Often considered the body's largest "organ," the 6 trillion cells of the endothelium cover an area of ≈5000 m^2 in a vessel network that develops beyond 100,000 km and weighs about 1 kg, representing about 1% of body mass [12]

The cytoskeleton determines EC shape and orientation. Flow variations result in the rearrangement of stress fibers (dense bundles of actin fibers) to localize predominantly in the periphery of the cell [13]. Stress fibers also associate with intermediate filaments rich in vimentin and tubulin, this last protein predominant in microtubules. Together, these three protein families largely maintain cell shape and orientation [13].

EC markers include angiotensin-converting enzyme (ACE); von Willebrand factor (vWF, stored in the Weibel–Palade bodies); vascular endothelial growth factor receptors (VEGFR)-1 and -2; the vascular endothelial (VE)-cadherin, platelet–EC adhesion molecule-1 (PECAM-1; CD31); P-selectin; the mucin-like molecule CD34; and E-selectin. While VE-cadherin, E-selectin, and VEGFR are entirely endothelium-specific, ACE, vWF, CD31, P-selectin, and CD34 also reside in megakaryocytes, platelets, and other predominantly hematopoietic cell types (Figure 1.2) [14].

Barrier function of the endothelium

The normal endothelium features a compact monolayer characterized by scant intercellular spaces, thus forming an active barrier between blood and the underlying tissues. Several elements, including intercellular junctions, cell-surface-binding proteins, electrostatic charges of endothelial membranes, and basement membrane composition, regulate endothelial integrity and permeability.

Transmembrane proteins linked to cytoplasmic and cytoskeletal proteins form intercellular junctions with close physical attachments between two contiguous cell membranes [15,16]. Within minutes, this highly dynamic and reversible system allows the passage of blood components into tissues. Three major intercellular junctions occur in EC: tight junctions, gap junctions, and *adherens* junctions (Figure 1.3).

Tight junction (*zonula occludens*)

Tight junctions allow very close contacts between adjacent cells. The number of tight junctions varies according to EC type, e.g., brain and large arteries contain many tight junctions, while post-capillary EC contain few or none [17]. Occludin, a transmembrane protein associated with intracellular proteins, i.e., *zonula occludens* (ZO)-1 and -2, cingulin, and RAB, member of RAS oncogene family-like 3, form tight junctions. ZO-1 likely localizes at initial cell-to-cell contacts, whereas cingulin and ZO-2 link to actin microfilaments [18,19]. Some evidence suggests that tight junctions may protect the endothelium against hemodynamic forces. Indeed, the number of tight junctions increases in cultured

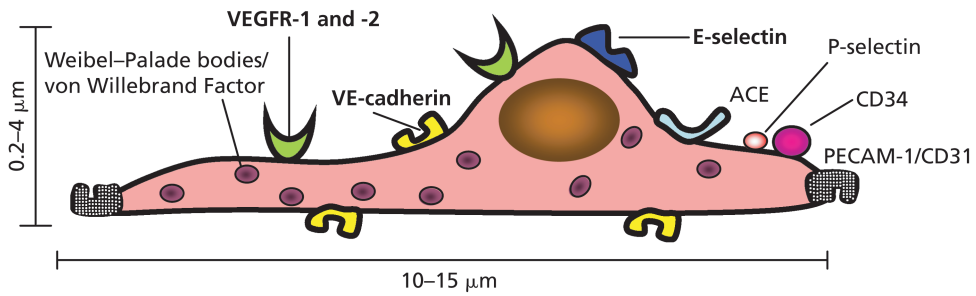

Figure 1.2 EC markers. VEGFR-1 and -2, VE-cadherin, and E-selectin (in bold) are specific for the endothelium. ACE, von Willebrand Factor (stored in the Weibel–Palade bodies), PECAM-1 (CD31), P-selectin, and the mucin-like molecule CD34 are also present in megakaryocytes, platelets, and some other predominantly hematopoietic cell types. VEGFR-1 and -2: vascular endothelial growth factors receptors-1 and -2; ACE: angiotensin-converting enzyme; vWF: von Willebrand factor; PECAM-1: platelet-EC adhesion molecule-1.

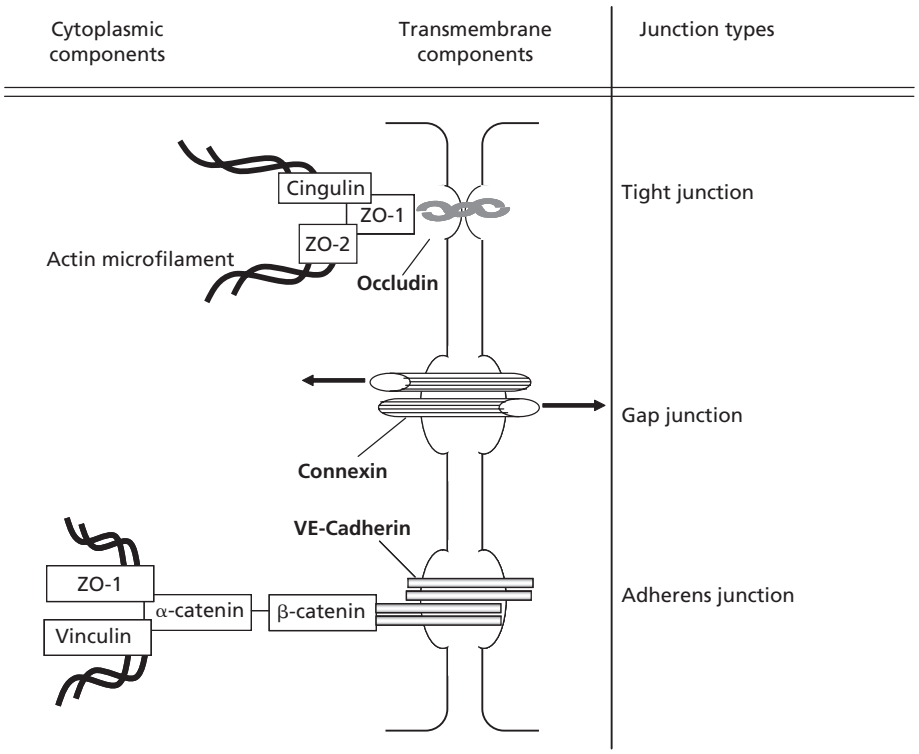

Figure 1.3 A schematic representation of the molecular organization of endothelial cell-to-cell junctions. ZO-1 and -2: zonula occludens-1 and -2; VE-cadherin: vascular endothelial-cadherin.

EC exposed to laminar shear stress [20,21], and tight junctions occur only rarely in aortic regions exposed to higher levels of shear stress [22], sites relatively spared by lipid deposition and atherosclerotic lesion formation.

Gap junction

Clusters of transmembrane channels, or gap junctions, link the cytoplasmic compartments of neighboring cells, allowing a direct exchange of ions and second messengers [23]. A gap junction channel consists of a pair of hemi-channels or connexons; each connexon contains six connexin molecules. In mammals, connexins form a multigene family comprising at least 15 members [24]. Each connexin forms channels with different properties of permeability and selective interaction with other connexin molecules. Gap junctions likely facilitate communication between EC (homotypic cell communication) and also between EC and other cell types, i.e., SMC or leukocytes (heterotypic cell communication). Some have suggested that gap junction signaling permits and coordinates EC migration and replication during angiogenesis or injury repair [15]. Recent findings indicate differentially regulated expression of EC and SMC connexins during atherogenesis [25]. The distribution pattern of individual connexins in disparate areas of the atherosclerotic plaque suggests that these molecules may differentially regulate the cell-to-cell transfer of factors important for lesion development, a hypothesis that raises the intriguing possibility that direct cell-to-cell communication via gap junctions may contribute to the pathogenesis of atherosclerosis in humans [25,26].

Adherens junction (zonula adherens)

Transmembrane proteins termed cadherins form *adherens* junctions that allow calcium-dependent homophilic recognition, i.e., recognition between EC [13]. Adherens junctions appear essential for the organization of EC contacts [27]. *Adherens* junctions likely participate importantly in the control of cell migration, growth, and differentiation

[13,15,18]. EC express specific and non-specific cadherins [28]. Non-specific cadherins, present in disparate cell types, include N-, P-, and E-cadherin. Their role in the determination of endothelial structure remains controversial. EC specifically possess a cadherin termed VE-cadherin [13,29], the most common component of *adherens* junctions [13]. Recent studies suggest that EC associated with neovascularization express VE-cadherin in atherosclerotic lesions [30]. In particular, reduced expression of VE-cadherin within intimal neovessels coincides with an increased entry of immunocompetent cells into the intimal matrix surrounding areas of neovascularization, thus suggesting that disorganized endothelial cell-to-cell interactions within neovessels may represent a significant event in atherogenesis [30].

Regulation of endothelial permeability

An altered rate of macromolecular diffusion or transport through endothelial junctions (paracellular transport) provides the major mechanism for increased endothelial permeability in response to selected inflammatory mediators such as thrombin or histamine [16]. These inflammatory substances can increase endothelial permeability within minutes by modulating the phosphorylation of proteins involved in the organization of endothelial junctions, followed by actin–myosin contraction, a centripetal retraction of EC, and increased formation of inter-endothelial gaps [16]. Thrombin alters the normal distribution of VE-cadherin, which disappears from areas of inter-EC pore formation and sequestration at the remaining sites of cell-to-cell contact, anchored to the cytoskeleton [31]. Signal transduction mechanisms involved in EC retraction and increased endothelial permeability can vary according to stimuli, and remain largely undetermined. Recent studies show that thrombin may increase intracellular calcium levels and, by the subsequent activation of protein kinase C (PKC), mediate the phosphorylation of junctional and actin-binding proteins [32,33]. Several important EC-derived factors can also modulate endothelial permeability. For example, the inhibition of basally produced endothelial nitric oxide (NO) increases permeability across vascular endothelium [34,35]. On the other hand, higher levels of NO induced by cytokines such as tumor necrosis factor alpha (TNF-α) also seem to mediate increased permeability [36]. Finally, mildly oxidized low-density lipoproteins (LDL), but not native LDL, may act in early atherosclerosis to increase endothelial permeability through the formation of actin stress fibers and intercellular gaps [37].

Endothelial regulation of vascular tone

We now recognize that the endothelium secretes mediators that influence vascular hemodynamics (Table 1.2). Moreover, the endothelium contributes to the regulation of blood pressure and blood flow by releasing vasodilators such as NO and prostacyclin (PGI_2) as well as vasoconstrictors, including endothelin (ET) and platelet-activating factor (PAF). These chemically diverse compounds usually do not reside preformed in intracellular granules; rather, their major biologic effects depend on their rapid synthesis.

Nitric oxide

NO is the main vasorelaxing factor produced by EC. In the early 1980s, Furchgott and Zawadzki demonstrated experimentally a role of EC in the obligatory vascular relaxation in response to factors like acetylcholine, thus postulating the existence of an endothelium-derived vascular relaxing factor (EDRF) [8]. In 1987, two research groups independently demonstrated that the relaxing factor was NO, an odorless gas previously known as an atmospheric pollutant [38–40].

NO arises from the conversion of L-arginine to L-citrulline in the presence of nicotinamide adenine dinucleotide phosphate (NADPH) derived electrons, a reaction catalyzed by NO synthase (NOS), which exists as three isoforms [41]. NOS III, or endothelial NOS (eNOS), is a constitutive EC enzyme that continuously produces small amounts of NO. The high-capacity inducible form (type II) and the constitutive neuronal form (type I) comprise the two other forms of NOS. Exposure of macrophages, SMC, and EC to cytokines such as interleukin (IL)-1 and TNF-α can induce type II NOS, which produces large amounts of NO during inflammatory processes [41].

The three NOS isoforms share structural similarities and have nearly identical catalytic mechanisms

Table 1.2 Vasoregulatory substances synthesized by the endothelium.

Substance	*Properties*	*Secretion/expression*	*Compound chemistry*	*Precursor compound*
NO	Vasorelaxing agent; inhibits leukocyte adhesion, exerts antiplatelet properties, inhibits SMC migration and proliferation.	Constitutive and inducible expression. Production is increased by thrombin, ADP, shear stress, cyclic strain, and cytokines	Heterodiatomic free radical	L-arginine
PGI_2	Antiplatelet and vasorelaxing agent	Constitutive and inducible expression at sites of vascular perturbation by proinflammatory agents	Eicosanoid	AA
EDHF	Vasorelaxing agent	Induced by acetylcholine, bradykinin, and shear stress	5,6 EET or Potassium, or Myoendothelial gap junction, or ?	AA
ACE	Catalyzes the conversion of Ang I into Ang II, which causes vasoconstriction. Also catalyzes the degradation of bradykinin	Endothelial surface	Enzyme	Ang I
ET-1	Cause vasoconstriction and proliferation of SMC	Induced by hypoxia, shear stress, and ischemia	21-amino acid peptide	Preproendothelin-1 (203-amino acids)

NO: nitric oxide; PGI_2: prostacyclin; EDHF: endothelium-derived hyperpolarizing factor; ACE: angiotensin-converting enzyme; Ang I and II: angiotensin I and II; ET-1: endothelin-1; SMC: smooth muscle cells; ADP: adenosine diphosphate; EET: epoxyeicosatrienoic acid; AA: arachidonic acid.

[42]. They all require a number of cofactors and prosthetic groups for their activity, including flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), heme, calmodulin (CaM), and tetrahydrobiopterin (BH_4). The catalytic activity of NOS isoforms requires three distinct domains. The C-terminus requires a reductase domain, a CaM-binding domain, and an oxygenase domain. The reductase domain contains the FAD and FMN moieties, and also transfers electrons from the NADPH to the oxygenase domain. The oxygenase domain catalyzes the conversion of arginine into citrulline and NO, and contains the binding sites for heme, BH_4, and arginine. The obligatory CaM cofactor functions as a sensor of intracellular calcium concentrations, exerting its activity only in response to elevated levels of intracellular calcium.

In EC, eNOS localizes mainly in *caveolae*, small cholesterol-rich invaginations of the plasma membrane [43] that contain the transmembrane protein caveolin and a number of signaling molecules such as G-protein-coupled receptors (including the muscarinic and the bradykinin receptors) and protein kinases [44]. eNOS binds caveolin or CaM in a mutually exclusive manner. In the resting condition, the caveolin binding suppresses eNOS activity; during activation, increased cytosolic calcium promotes a reversible dissociation of eNOS from caveolin and induces CaM binding, which augments eNOS activity [44]. Although eNOS depends primarily on calcium and CaM, recent evidence indicates the possibility of calcium-independent activation of this enzyme. The response to shear stress involves this type of eNOS activation. Signal transduction processes that lead to calcium-independent activation of eNOS involve tyrosine phosphorylation and the activation of phospholipase C-γ [43,45].

Although originally considered constitutive, current evidence suggests that different stimuli regulate eNOS at several levels. The best-known physiologic activators of endothelial NO formation include physical stimuli, such as shear stress [46] and cyclic

circumferential stretch [47]. Physical exercise, likely through such mechanisms, can stimulate NO production in human vascular beds [48], and also can induce eNOS gene expression in experimental animals [49]. Moreover, endothelial NO production rises in response to hormones such as estrogens [50,51], autacoids such as bradykinin [52] and histamine [53], and platelet-derived factors such as serotonin and adenosine diphosphate (ADP), as well as products of blood coagulation such as thrombin [54]. Moreover, transforming growth factor beta (TGF-β) [55] and high glucose concentrations [56] can enhance eNOS expression, and inflammatory cytokines such as TNF-α [57] and LDL [58] can limit eNOS activity.

NO has a half-life of ≈3–5 s [59]; when produced by EC, it can diffuse into the underlying tissues. By permeating the SMC plasma membrane, NO interacts with the iron atom of the heme prosthetic group of guanylate cyclase, causing its activation, which leads to increased cyclic guanosine monophosphate (cGMP) formation [60]. The ensuing prevention of calcium entry into SMC produces vascular relaxation [61]. Furthermore, EC release NO into the bloodstream where, before inactivation by oxyhemoglobin, NO can remain biologically active in close proximity to the endothelial surface, thus inhibiting leukocyte adhesion [11], augmenting fibrinolysis, and inhibiting platelet adhesion and activation synergistically with PGI_2 [62].

The loss of endothelium-dependent NO-mediated vasodilation occurs early during endothelial dysfunction [63]. Indeed, dysregulation of NO bioavailability (production or stabilization or disposal) accompanies numerous vascular diseases, including atherosclerosis [64]. NO bioavailability decreases in patients with established coronary artery disease [65,66]. Correspondingly, pharmacologic inhibition of NO synthesis in animal models of hypercholesterolemia accelerates atherosclerosis [67]; conversely, increased availability of NO decreases formation and may cause regression of atherosclerotic lesions [68–74]. However, although results of oral L-arginine supplementation in hypercholesterolemic animals have generally shown beneficial effects, the data in humans vary [75–78]. The inactivation of NO by reactive oxygen species can contribute to the development of endothelial dysfunction. Indeed, experimental studies show that antioxidants may re-establish NO-dependent endothelial function [79,80]. Finally, an increase in endogenous inhibitors of NO synthesis may also participate in the genesis of endothelial dysfunction. For example, levels of asymmetric dimethylarginine, which may compete with L-arginine in the synthesis of NO, increase in young individuals with hypercholesterolemia; this increase associates with endothelium-dependent vasomotor dysfunction [81].

Prostacyclin, thromboxane, and other eicosanoids

The term "eicosanoids" encompasses prostaglandins (PGs), thromboxanes (TX), leukotrienes, and hydroxyl-eicosatetraenoic acids formed from polyunsaturated 20-carbon fatty acids, including the most abundant and the most biologically prominent precursor, arachidonic acid (AA). AA, a normal component of cell membrane phospholipids, becomes available for eicosanoid synthesis only after its release from phospholipids through the action of several phospholipases, principally phospholipase A_2. Released AA serves as a substrate for PG H synthases-1 and -2, also known as cyclooxygenases (COX)-1 and -2; lipoxygenases (5-, 12-, or 15-lipoxygenase); or cytochrome P450 enzymes [82]. Both COX-1 and -2 catalyze the conversion of AA to PGH_2. While most tissues express COX-1 constitutively, COX-2 expression rises markedly only upon cell activation, such as in inflammation [82]. The direct product of COX catalysis, PGH_2, has a half-life of ≈5 min in aqueous media, causes vasoconstriction, and undergoes further enzymatic conversion into several PGs, such as PGD_2, PGE_2, PGF_2-α or PGI_2, and TX, depending on the local tissue distribution of specific PG or TX synthases. Thus, the synthesis of specific PGs varies among tissues or cell types. For example, EC primarily contain PGI synthase (PGIS), which catalyzes PGH_2 conversion into PGI_2. Prostanoids signal through a series of seven-membrane spanning G-protein-coupled receptors. Through activation of the IP receptor present on SMC and platelets, PGI_2 causes vasodilation and inhibits platelet aggregation. Activation of adenylyl cyclase mediates most biologic effects of PGI_2, with the consequent increase in intracellular levels of cyclic adenosine monophosphate (cAMP) [83]. COX-1 and PGIS constitutive actions produce PGI_2 in resting EC [84]. However, although IL-1β stimulation of human umbilical vein EC leads to increased accumulation of PGI_2, PGD_2 and PGE_2 also arise largely via COX-2

through both PGH_2 non-enzymatic transformation [85] and, particularly for PGE_2, through the additive effect of an inducible PG E synthase (PGES) activity [86]. Additionally, EC can synthesize TX, a vasoconstrictor primarily produced by platelets, as recently proven by the cloning of an endothelial TX synthase [82,87]. An interesting interaction between platelets and EC can occur in this regard. EC stimulated by thrombin can restore TX production of aspirin-treated platelets by supplying them with PGH_2 in a juxtacrine manner [84,88], one of the many known examples of eicosanoid transcellular metabolism. Other potential COX products include a variety of compounds termed isoprostanes, e.g., 8-iso-$PGF_{2\alpha}$. The isoprostanes, a family of oxygenated arachidonate products, share structural similarities with eicosanoids. Under conditions of oxidative stress, the production of isoprostanes from arachidonate requires reactive oxygen species but not COX activity [89]. Conditions that induce increased generation or impaired disposal of free radicals, however, also involve parallel COX-2 expression and the production of 8-isoprostanes, a process inhibitable by indomethacin [89]. One interpretation for endothelial COX-2-dependent production of isoprostanes involves reactive oxygen species, produced by COX-2 activity (having an oxygenase and a peroxidase activity), that lead themselves to the production of isoprostanes. Therefore, COX-2 activity may also affect vascular function by enhancing oxidative stress within the cell.

Despite extensive study, the specific roles of vascular COX-1 vs COX-2 and their contribution to the pathophysiology of atherosclerosis remain undetermined. We have demonstrated the presence of COX-1 and -2 mRNA and protein in atherosclerotic plaques, macrophages, and SMC; COX-2 resides in the endothelium of atherosclerotic vessels [90]. Overexpression of functionally coupled COX-2, PGES, and some matrix metalloproteinases (MMP) occurs simultaneously in symptomatic atherosclerotic lesions. Since MMP may participate in the digestion of the fibrous cap, the functionally coupled overexpression of COX-2 and inducible PGES may contribute to a destabilization of the atherosclerotic plaque [91]. Additionally, COX-2, MMP-9, and membrane type 1 MMP colocalize in the EC lining of *vasa vasorum* in human atherosclerotic aortas [92]. Moreover, several lines of evidence now indicate a proangiogenic role of COX-2 activity [93], a process that might further contribute to plaque destabilization [94]. Therefore, COX-2 inhibition emerged as a potential therapy to mitigate complications of atherosclerosis. Clinical trials, however, have proven controversial [95], and overall suggest a detrimental effect [96–99] on cardiovascular outcomes in recipients of COX-2 inhibitors, in common with many other non-steroidal anti-inflammatory agents. One explanation involves reduced production of COX-2-derived PGI_2 [100]. Slight but sustained increases in blood pressure also may tend to worsen cardiovascular outcomes in individuals treated with COX-2 inhibitors and other non-steroidal anti-inflammatory agents. Fuller understanding of the specific roles of endothelial COX-1 and -2 in the modulation of vascular function and in the formation, progression, and destabilization of atherosclerosis requires further study.

Endothelium-derived hyperpolarizing factor

In the early 1980s, several lines of evidence began to indicate that the release of either NO or PGI_2 did not fully explain endothelium-dependent relaxation. Indeed, pharmacologic inhibition or genetic inactivation of NOS and inhibition of PGI_2 production do not greatly affect endothelium-dependent relaxation in response to either chemical (acetylcholine, bradykinin) or mechanical (shear stress) stimulations, especially in small resistance arteries. The observation that this process involves hyperpolarization of the target vascular SMC (and of the endothelium itself), causing relaxation without increased intracellular levels of cyclic nucleotides, led to the concept of an "endothelium-derived hyperpolarizing factor" (EDHF) [101,102]. EDHF would transduce its cellular effects by either directly or indirectly opening K^+ channels on vascular SMC, or through a hyperpolarization of EC, facilitating the electrical coupling between EC and vascular SMC [103].

The chemical identity of EDHF has received considerable attention, but thus far has yielded no consensus. Tissue and species heterogeneity implies the existence of multiple such factors. Four major candidates for EDHF exist. Substantial evidence supports the hypothesis that AA metabolites may share some EDHF properties. Epoxy-eicosatrienoic acids (EET), metabolites of AA through the P450 epoxygenase

pathway, exert EDHF activity in at least some vascular beds upon stimulation of endothelial receptors. EET would be either synthesized or released from stored pools in the endothelium. Once released, EET would diffuse to the vascular SMC, where they would increase the probability of opening big conductance calcium-activated potassium channels (BK_{Ca}), and hyperpolarize vascular SMC [104]. Alternatively or additionally, another AA product, the endogenous cannabinoid anandamide, may act as EDHF: anandamide activates cannabinoid receptors in both EC and vascular SMC, and can hyperpolarize SMC [105]. A third hypothesis implicates potassium ions (K^+) as EDHF: stimulated endothelial receptors activate small and intermediate conductance calcium-activated potassium channels (SK_{Ca} and IK_{Ca}) and increase the probability that they will open in EC, leading to the efflux of K^+ from EC and increased levels of extracellular K^+. Moderately increased concentrations of endothelial K^+, such as that occurring upon stimulation of EC in the rat hepatic artery, can induce hyperpolarization and relaxation of vascular SMC by activating the inward rectifying K^+ channels (K_{IR}) and the Na^+-K^+-ATPase between EC and SMC gap junctions [103], which provide a low-resistance electrical pathway between these two cell layers [106]. The number of these "heterocellular" gap junctions increases as artery diameter decreases, paralleling the predominance of EDHF relevance in microvessels. Cell activation by an agonist facilitates myoendothelial gap junctional communication. Thus, gap junctions would transmit EC stimulation and subsequent hyperpolarization to the underlying vascular SMC. If cations (or a current) move through the gap junction to hyperpolarize vascular SMC, "EDHF" does not involve a factor *per se* but rather a process of current movement between cells. Although gap junctions also would allow movement of low molecular weight water-soluble molecules such as cAMP or cGMP from the endothelium to vascular SMC, thus accounting for EDHF properties, it is more likely that no "factor" transfer participates in the dynamic regulation of gap junctional communication. Other mechanisms remain unsubstantiated or await fuller scrutiny. However, several mechanisms for EDHF likely operate simultaneously or sequentially and act additively or synergistically in a given site of the circulation. The relative proportions of each mechanism likely depend on numerous variables including the activation state of the vascular smooth muscle, the density of myoendothelial gap junctions, expression level of cytochrome P450, and the appropriate isoforms of Na^+-K^+-ATPase and/or K_{IR} channels.

Pulsatile stretch [107] or receptor-dependent calcium-elevating agents result in EDHF release [104]. Biologic effects of EDHF decrease in vascular abnormalities, including those associated with aging and hypercholesterolemia [108]. Therefore, decreased EDHF may account, at least in part, for the alterations of vascular responses that occur in atherosclerosis [109].

Endothelins

In sharp contrast with the less well-defined substances described above, ET are well-characterized and potent vasoconstrictors [110]. ET, 21-amino acid peptides closely related to the snake venom safaratoxin, arise from different tissues as three different isoforms: ET-1, -2 and -3. ET-1, first discovered in 1988, was isolated from the conditioned medium of porcine aortic EC [111]. EC can only synthesize ET-1, a 203-amino acid precursor named preproendothelin, which cleaves to big ET (39-amino acids) and then, in a reaction catalyzed by an ET-converting enzyme (ECE), further converts to the 21-amino acid active peptide [112]. ECE exists as several different isoforms, e.g., ECE-1a, -1b, and -2. EC only appear to express the ECE-1a isoform [113,114].

EC release 75% of their ET abluminally, toward SMC; the remainder enters the vessel lumen, so that the plasma of normal healthy subjects has low levels of ET-1. ET-1 exerts its biologic effects by stimulating specific G-protein-coupled receptors, termed A (ET_A) and B (ET_B) receptors. SMC express ET_A and, at lower levels, ET_B receptors. Stimulation of SMC ET receptors induces vasoconstriction by two different mechanisms: increased intracellular calcium influx and activation of phospholipase C and A_2 [115]. EC also have ET_B receptors, whose stimulation promotes the formation of NO and PGI_2, a reaction that could serve as a feedback mechanism to restore normal vascular tone. Increased production and/or activity of ET may participate in several pathologic states related to a dysfunctional endothelium, such as pulmonary and systemic hypertension, heart failure, and atherosclerosis [116,117]. Factors implicated in atherogenesis, e.g., inflammatory cytokines [118,119]

and oxidized LDL [120–123], also induce ET-1 production by EC. Jones et al. observed increased expression of ET-1 in human atherosclerotic vessels at sites directly overlying atherosclerotic plaques [124]. The pathologic role of ET-1 in the development of atherosclerosis may involve not only local perturbation of blood flow but also mitogenic effects on SMC [115], stimulation of adhesion molecule expression [125], and leukocyte chemoattraction [126]. In early atherosclerosis or even in the presence of the sole risk factors, abnormal vasoconstriction arises in part from enhanced local production of ET [127].

The endothelial renin–angiotensin system

The renin–angiotensin–aldosterone system contributes to the regulation of electrolyte balance, fluid volume, and blood pressure by angiotensin II (Ang II) and, subsequently, aldosterone production. The elements of this system comprise a cascade of enzymatic reaction in which a first enzyme, renin, converts angiotensinogen to angiotensin I (Ang I), and a second enzyme termed ACE further cleaves Ang I into Ang II [128]. Ang II, a potent vasoconstrictor, increases blood pressure and stimulates aldosterone secretion, in turn promoting sodium retention. ACE arises in large part from EC [129]. In normal vessels, ACE localizes on the surface of EC, where it is easily available for the cleavage of plasma Ang I [129]. In addition to converting Ang I to Ang II, ACE degrades and inactivates bradykinin, a potent indirect vasodilator, by inducing endothelial NO, EDHF, and PGI_2.

Specific receptors termed angiotensin II receptor type 1 (with subclasses AT_{1A} and AT_{1B}) and angiotensin II receptor type 2 mediate the biologic effects of Ang II. AT_1 stimulation on SMC mediates the main physiologic effects of Ang II, vasoconstriction, and SMC proliferation [130]. However, EC contain AT_1 and, in smaller amounts, AT_2 [131]. Recent experimental evidence suggests an important role of Ang II in the development of atherosclerosis, through direct action on the endothelium. Ang II exerts proinflammatory effects on vascular endothelium by inducing the most important inflammatory genes implicated in the inception of atherosclerotic lesion, i.e., intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 (see below). The stimulation of AT_1 receptors appears to mediate this effect, since AT_1 receptor blockers prevent the inflammatory effect of angiotensin [132,133].

The endothelial control of hemostasis

The endothelium plays a key role in the control of hemostasis, exerting effects on platelets, the coagulation system, and fibrinolysis. In normal physiologic conditions, the endothelium displays antiplatelet, anticoagulant, and fibrinolytic properties, but dysfunctional EC promote a prothrombotic, antifibrinolytic state.

Control of platelet function

Normally, circulating platelets do not interact with the EC surface, due in part to the release of NO and PGI_2, and in part to catabolism of the proaggregatory mediator ADP that results from the constitutive expression of an ecto-ADPase (CD39). Endothelium-derived NO exerts a strong vasorelaxing action and also potently inhibits platelet adhesion, activation, secretion, and aggregation through a cyclic guanosine monophosphate (cGMP)-dependent mechanism [134].

Similarly, endothelial PGI_2 contributes to endothelial vasorelaxing properties even more efficiently than NO, and inhibits platelet aggregation through the activation of the IP receptor present on platelets [135]. NO also inhibits P-selectin expression on the platelet surface and, by inhibiting agonist-dependent increase in intraplatelet calcium [136], suppresses the calcium-sensitive conformational change in the heterodimeric integrin glycoprotein(GP) IIb–IIIa required for fibrinogen binding [137]. Additionally, NO appears to promote platelet disaggregation indirectly by impairing the activity of phosphoinositide 3-kinase, which normally supports conformational changes in GP IIb–IIIa, rendering its association with fibrinogen irreversible [138].

Another long-recognized antiplatelet property of normal EC involves ecto-ADPase/CD39, a membrane-associated ectonucleotidase. Released by platelets and other cells, ADP strongly activates platelets by interacting with the platelet $P2Y_{12}$ receptor [139,140]. The constitutive expression of ecto-ADPase, an adenosine triphosphate (ATP)-diphosphohydrolase, by EC allows efficient metabolism of this platelet agonist to AMP, thus maintaining platelets in their resting state [141].

Anticoagulant properties

Hemostatic clots form due to the polymerization of fibrinogen into fibrin, a conversion catalyzed by thrombin, a serine protease that cleaves fibrinogen, activates several other coagulation enzymes and cofactors and, in conjunction, activates platelets and EC [142]. Therefore, it is not surprising that several highly regulated pathways have evolved to constrain the generation and activity of thrombin [143]. The endothelium employs at least three anticoagulant strategies, i.e., heparin–antithrombin; tissue factor (TF) pathway inhibitor (TFPI); and thrombomodulin–protein C anticoagulant.

The matrix surrounding the endothelium contains cell surface heparan sulfate and related glycosaminoglycans that promote antithrombin III (AT-III) activity [144,145]. This complex inactivates thrombin and factors Xa, IXa, and XIa. The expression of heparan sulfate and proteoglycans likely responds to inflammatory conditions, as indicated by suppression during sepsis [146,147]. The endothelium also prevents thrombin formation by expressing TFPI, which binds to factor Xa within the TF/FVIIa/FXa complex [148]. TFPI and AT-III both contribute to physiologic hemostasis, and both show impairment in acquired thrombotic states [149,150].

Of these three anticoagulant systems, the thrombomodulin–protein C system has the greatest complexity. EC synthesize thrombomodulin present on the endothelial luminal surface of most vessels, in capillaries, arteries, veins, and lymphatics. Human umbilical vein EC contain 40,000–50,000 thrombomodulin molecules per cell [151]. Thrombin bound to thrombomodulin loses its procoagulant activity, and the complex instead becomes a potent activator of protein C, an anticoagulant protein synthesized by the liver [152], which circulates in plasma at a concentration of $\approx 4\,\mu g/mL$ [153]. The activation rate of protein C increases when it binds to the endothelial protein C receptor (EPCR), a specific receptor on EC [154]. Once activated (activated protein C, APC), protein C retains its binding affinity for EPCR, but appears to lose anticoagulant activity [155]. Indeed, APC reversibly binds to EPCR ($K_d \approx 30\,nM$); when APC dissociates from EPCR, it binds to protein S, a molecule synthesized both in the liver and EC [156]. This complex proteolytically inactivates factors Va and VIIIa [157]. Inflammatory cytokines such as TNF-α or IL-1β can reduce thrombomodulin expression. Indeed, TNF-α both inhibits transcription of thrombomodulin mRNA and augments the lysosomal degradation of the mature protein [158]. Recent data showing a lower degree of expression in patients with unstable angina also suggests a role for regulation of the anticoagulant protein S in vascular disease [159].

Procoagulant properties

The pivotal step in transforming the inner vessel surface from an anticoagulant to a procoagulant surface involves the induction of TF, a 263-amino acid residue membrane-bound GP comprised of a 219-amino acid residue extracellular domain, a single transmembrane sequence, and a short cytoplasmic domain [160]. TF dramatically accelerates factor VIIa-dependent activation of factors X and IX. Therefore, it is not surprising that the unperturbed endothelium in physiologic conditions does not express TF, at least in the adult [161]. TF participates importantly in various pathologic states, including atherosclerosis. TF accumulates in experimentally injured vessels and in atherosclerotic plaques [162]. The accumulation of TF likely accounts for the high thrombogenicity of some plaques [163]. Diverse agonists including thrombin, endotoxin [164], several cytokines such as IL-1α and β [165] and TNF-α [166], shear stress [167], and oxidized lipoproteins [168] induce endothelial synthesis of TF *in vitro*. The ligation of CD40, the expression of which rises after exposure to interferon-γ, by CD40 ligand (CD154) on T-cells or activated platelets also increases TF expression [169,170]. TF mRNA and protein levels decline despite continued exposure to agonists, a mechanism that may help to limit the extent of fibrin formation. Cells in culture also shed microvesicles containing TF, which may propagate the activation of thrombosis distal to the initial site of TF exposure [171]. Patients with disseminated intravascular coagulation have elevated plasma levels of TF [172].

EC also express several receptors for fibrin and specific fibrin degradation products, including a 130-kDa GP [173], a tissue transglutaminase [174], and the integrin $\alpha_v\beta_3$ (vitronectin receptor) [175]. The endothelial expression of this receptor is important, since the binding of fibrin promotes endothelial adhesion, spreading, proliferation, migration, retraction, and leukocyte adhesion and also inhibits PGI_2 synthesis [107].

Control of fibrinolysis

Fibrinolysis depends primarily on the action of plasmin, an active protease formed from its precursor, plasminogen, upon stimulation by tissue-type plasminogen activator (tPA). The contribution of EC to fibrinolysis varies with their metabolic status, i.e., quiescent vs activated, their site in the vasculature, and the concentration of other hemostatically active molecules in the local plasma milieu. Although several studies with EC cultured from various tissues originally indicated that all EC can produce and secrete tPA [176], more recent studies using *in situ* hybridization and immunohistochemistry have demonstrated tPA antigen and mRNA only in a distinct subset of quiescent microvascular EC of both primates and mice [177]. Hence, contrary to assumptions based on work with cultured EC, tPA production *in vivo* likely associates only with a distinct subpopulation of EC in the microvasculature. The urokinase-type plasminogen activator (uPA) appears absent in quiescent endothelium [178]. Consistent with the hypothesized importance of uPA in cell migration and tissue remodeling, EC involved in wound repair or angiogenesis express uPA as a fibrin-specific precursor termed prourokinase [179]. However, uPA has obvious importance for vascular homeostasis, since mice genetically deficient in uPA develop thrombosis and show thrombotic tissue injury in response to lipopolysaccharide (LPS) [180].

The endothelium also can produce large amounts of plasminogen activator inhibitor (PAI)-1 [181]. Experiments in mice have identified the liver at the major source of plasma PAI-1, and shown that quiescent EC express little or no PAI-1 [182]. However, the endothelium overexpresses this inhibitor of fibrinolysis in virtually all tissues after exposure to inflammatory stimuli [182].

With regard to the endothelial regulation of fibrinolysis, it is also important to consider the dual role exhibited by thrombomodulin. Binding of thrombin to thrombomodulin accelerates its capacity to activate a protein known as thrombin-activatable fibrinolysis inhibitor (TAFI) [183]. TAFI is a procarboxypeptidase-B-like molecule that, when activated, cleaves basic carboxyterminal residues within fibrin and other proteins, resulting in the loss of plasminogen/plasmin and tPA binding sites on fibrin and thus retarded fibrinolysis [183]. In this manner, through the regulated expression of thrombomodulin, EC could decrease the rate of intravascular fibrinolysis.

Control of leukocyte traffic during inflammatory reactions (see also Chapter 2)

The development of inflammatory reactions by the vascular endothelium participates integrally in normal host defense mechanisms initiated by injury or infection. Physiologically, such reactions maintain and/or repair normal structure and function of the vessel wall and of the entire organism, defending it from external insults or foreign agents. Mice lacking the selectin family of adhesion molecules suffer recurrent infections [184]. On the other hand, the initiation of abundant inflammatory reactions can lead to severe tissue damage, possibly including the development of atherosclerosis [185].

The interaction between EC and leukocytes depends on the expression of adhesion molecules and production of inflammatory cytokines, both critical factors in initiating and sustaining inflammatory processes. Among different chemokines, IL-8 [186] and monocyte chemoattractant protein-1 (MCP-1) [187] likely play key roles in the recruitment of polymorphonuclear leukocytes, lymphocytes, and monocytes into the vessel wall. Among several other cell types, EC produce IL-8 and MCP-1 through mechanisms mediated by nuclear factor kappa (NF-κB) [187,188].

During inflammation, loosely tethered leukocytes first roll over the EC surface, then arrest and spread, and finally emigrate between EC to reach the underlying tissues. The interaction between EC and leukocytes initially involves a relatively loose adhesion of leukocytes to EC, so that leukocytes "roll" over the endothelium [189]. Firmer adhesion and transmigration of leukocytes across EC follow this process. Leukocyte rolling involves molecules belonging to the selectin family of adhesion molecules, e.g., P-, E-, and L-selectin, typically characterized by an amino-terminal Ca^{2+}-dependent lectin domain, an epidermal growth factor (EGF) domain, a series of short consensus repeats, a transmembrane domain, and a cytoplasmic tail [190,191]. While E-selectin expression occurs only on activated EC, L-selectin

expresses on most leukocytes and binds to ligands constitutively expressed on high endothelial venules of lymphoid tissues; ligands induced on the endothelium at sites of inflammation; and ligands exposed on other leukocytes. P-selectin rapidly redistributes from secretory granules to the surface of platelets and EC stimulated with thrombin or histamine. In general, selectins bind to sialylated and fucosylated oligosaccharides that attach to proteins and lipids on most leukocytes and some EC. In particular, E-selectin preferentially binds to the GP E-selectin ligand (ESL)-1 [192,193]. L- and P-selectin bind preferentially to sialomucins. Involvement of individual types of selectins in the initial leukocyte rolling depends primarily on the type of stimuli. For example, immediate stimulation of leukocyte rolling induced by histamine or thrombin depends primarily on P-selectin stored in Weibel–Palade bodies [194]. EC activation can induce a rapid translocation and fusion of these organelles with plasma membranes and, therefore, the swift appearance, within minutes, of P-selectin on the EC surface. However, activated EC express P-selectin only temporarily; 30 min following the initial stimulus, surface levels of this adhesion molecule already wane [195]. In contrast to histamine or thrombin, TNF or IL-1 can stimulate delayed leukocyte rolling and adhesion to EC primarily through the induction of E-selectin. In contrast to P-selectin, EC do not store preformed E-selectin. Following stimulation with TNF or IL-1, the expression of E-selectin typically rises after ≈4 h, peaks at ≈12 h, and declines after 24 h [189]. Studies demonstrating the presence of both E- and P-selectin on the surface of EC overlying atherosclerotic plaques affirm the importance of the selectin family of adhesion molecules in the development of atherosclerosis [196]. Moreover, the absence of P-selectin delays fatty streak formation in mice predisposed to the development of atherosclerosis [197].

Firmer adhesion of leukocytes to the vascular endothelium involves adhesion molecules of the immunoglobulin superfamily, i.e., ICAM-1, -2, -3, VCAM-1, and PECAM-1 [198]. ICAM-1, -2 and -3 are closely related, and bind to the same leukocyte integrin receptors, i.e., CD11/CD18 [199]. Unstimulated, resting EC constitutively express low levels of ICAM-1, but exposure of EC to stimuli such as IL-1, TNF and interferon-γ [200] markedly increases ICAM-1 expression. After stimulation, ICAM-1 peaks at 6 h, and its levels remain sustained for at least 72 h [201]. ICAM-1 expression depends predominantly on transcriptional regulation. The ICAM-1 promoter region contains several enhancer sequences. Although several other transcription factors such as specificity protein (SP)1; activator protein (AP)-1, retinoic acid responsive element (RARE), and CCAAT/enhancer binding protein (C/EBP) participate in the activation of the ICAM-1 gene, particularly in EC, NF-κB likely is the most important regulatory element [202].

VCAM-1 principally mediates the adhesion of monocytes, lymphocyte, eosinophils, and basophils, but not neutrophils, to the surface of the vascular endothelium [203,204]. The best-known inducers of VCAM-1 include cytokines such as TNF-α, IL-1 [205,206], modified LDL [207], and advanced glycation end products (AGEs) [208,209]. Similar to ICAM-1 and E-selectin, the transcriptional regulation of VCAM-1 also requires NF-κB and AP-1 [210]. Recent findings in genetically engineered mice that express a form of VCAM-1 with reduced function have established the causal role of VCAM-1 in early atherogenesis [211].

The final phase of leukocyte emigration through the endothelium involves PECAM-1, another member of the immunoglobulin superfamily. PECAM-1, which likely participates in the regulation of endothelial permeability and leukocyte adhesion [18], localizes preferentially in the intercellular junctions of EC, where it forms homodimers linking two EC. Additionally, the leukocyte surface contains PECAM-1. The dissociation of homotypic PECAM-1 dimers between EC to form heterotypic dimers between emigrating leukocytes and EC appears critical for leukocyte diapedesis [212], through a mechanism that apparently involves a reactive oxygen species-induced phosphorylation of this adhesion molecule [213]. However, the role of PECAM-1 in the stimulation of leukocyte adhesion and migration during atherogenesis remains uncertain, since levels of PECAM-1 expression do not occur in human atherosclerotic coronary arteries [214] or atheromata in ApoE$^{-/-}$ mice [215]. Figure 1.4 provides a summary of endothelial functions and illustrates how the loss of homeostatic balance leads to the development of the atherosclerotic plaque.

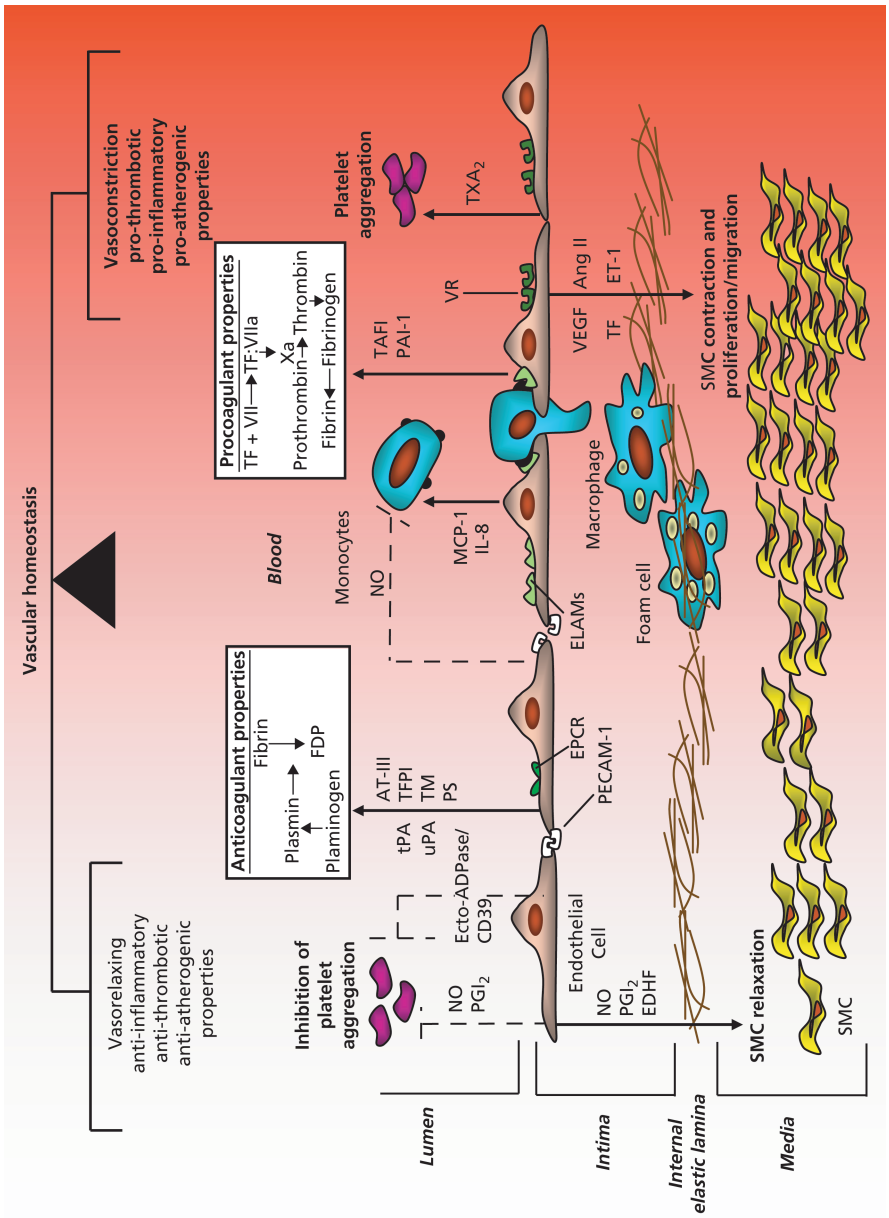

Figure 1.4 Molecular and cellular mediators of the antithrombotic and antiatherogenic properties of the endothelium. The normally functioning endothelium (left and white) produces several vasodilatory substances, e.g., prostacyclin (PGI_2), nitric oxide (NO), and endothelium-derived hyperpolarizing factor (EDHF), that maintain normal vascular tone, counteract platelet aggregation, exert anticoagulant properties that control fibrin production (thrombomodulin (TM), endothelial cell protein C receptor (EPCR), protein S (PS), tissue factor pathway inhibitor (TFPI)), inhibit tissue factor (TF) activity, and enhance fibrinolysis (tissue- and urokinase-type plasminogen activators (tPA, uPA)).

A dysfunctional endothelium (right and red) favors atherothrombosis, enhancing the production of pro-coagulants such as TF, anti-fibrinolytic factors such as plasminogen activator inhibitor (PAI)-1, and vasoconstrictors such as endothelin (ET)-1 and angiotensin (Ang)-II) and promoting leukocyte adhesion and migration through the production of endothelial-leukocyte adhesion molecules (ELAMs) and chemoattractants such as monocyte chemotactic protein (MCP-1) and interleukin (IL)-8, thus overall favoring inflammation, plaque formation, and growth as well as vasoconstriction and thrombosis. → induces; ----- inhibits; FDP: = fibrin-degrading products; TXA_2 = thromboxane A_2; VEGF = vascular endothelial growth factor; TAFI = thrombin-activatable fibrinolysis inhibitor; AT-III = antithrombin-III; VR: = vitronectin receptor.

Role of transcriptional factor NF-κB in endothelial dysfunction

The activation of NF-κB likely plays a key role in the development of endothelial dysfunction [216]. NF-κB comprises a family of transcription factors originally identified in B cells, expressed ubiquitously and highly phylogenetically conserved. Family members include Re1A (p65), Re1B, c-Re1, NF-κB1 (p50), NF-κB2 (p52), and their inhibitory subunits IκBα, IκBβ , and IκBε. NF-κB subunits form homo- and heterodimers, the most prominent being the p65/p50 heterodimer, and bind to the decameric consensus sequence GGG*RNNTYCC* (*R* = G or A, *Y* = C or T, *N* = nucleotide), thus inducing the expression of target genes. Interaction with IκB to form a heterotrimer retains NF-κB in the cytoplasm in an inactive state under basal conditions. NF-κB activates rapidly in response to a variety of stimuli that always lead to IκB degradation. Important stimuli include TNF-α, IL-1, bacterial LPS, hyperglycemia, PAF, shear stress, oxidized lipids, oxidant stress, and hypoxia/reperfusion [217]. NF-κB exhibits very rapid and transient activation, making it well suited for the expression of many immune- and "stress"-response genes that require action on demand for only a limited period of time. Independent of the type of stimuli, co-treatment with antioxidants or metal chelators can inhibit NF-κB activation. Therefore, changes in the cellular redox balance may alter NF-κB activation [210,218,219].

The promoter regions of most transcriptionally regulated genes expressed in EC in response to inflammatory mediators such as LPS, IL-1, or TNF-α contain functional NF-κB binding sites. Genes regulated by NF-κB include VCAM-1, E-selectin, IL-1, -6, -8, TF, PAI-1, COX-2, and iNOS, all strategically involved in endothelial activation and dysfunction. In addition, atherosclerotic lesions contain activated NF-κB [220,221]. Interestingly, the expression of inflammatory cytokines depends on activated NF-κB and, in turn, these cytokines can stimulate its activation. Thus, inflammatory cytokines may use NF-κB to amplify their own signals [216]. On the other hand, inhibition of basal endothelial NO production can activate NF-κB and augment the expression of NF-κB-regulated genes, i.e., genes encoding for VCAM-1 and ICAM-1 [11,222], and treating EC with NO donors decreases TNF-induced NF-κB activation and attenuates cytokine-induced expression of VCAM-1 and ICAM-1 [11].

General conclusions

We can no longer view the endothelium as a static physical barrier that simply separates blood from tissue. Healthy EC are vital for the correct maintenance of vascular homeostasis. By secretion or surface expression of a series of specific molecules, EC ensure appropriately regulated blood flow under normal conditions, and avoid intravascular activation of platelets and coagulation. In response to pathophysiologic mediators, EC properties modulate dynamically to support vessel growth or repair and guide the resolution of inflammatory or infectious processes. In most instances, this transient alteration of EC phenotype contributes to the successful restoration of vascular homeostasis. Certain disease states such as atherosclerosis, however, may involve a chronically perturbed EC behavior critical for disease progression. Increased understanding of EC biology has defined many endothelial functions at the molecular level and several mechanisms that cause acute and chronic changes in EC functions. Efforts to understand the physiologic features of the endothelium and mechanisms underlying long-term changes in endothelial properties as well as strategies to alleviate endothelial dysfunction provide a promising pathway for the treatment of diseases characterized by altered endothelial functions.

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