

REVIEW ARTICLE

Endothelial Cells in Physiology and in the Pathophysiology of Vascular Disorders

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PART I

THE ENDOTHELIUM has long been viewed as an inert cellophane-like membrane that lines the circulatory system with its primary essential function being the maintenance of vessel wall permeability. Shortly after the first description of circulating blood by William Harvey in 1628, the existence of a network of vessels arose from studies of Malpighi, who described the physical separation between blood and tissue.¹ In the 1800s, von Reckinghausen established that vessels were not merely tunnels bored through tissues but were lined by cells. The strength of Starling's experiments and his law of capillary exchange proposed in 1896 served to solidify the belief that the endothelium was principally a selective but static physical barrier, not withstanding Heidenhahn's description in 1891 of the endothelium as an active secretory cell system. However, electron microscopic studies of the vessel wall by Palade in 1953 and physiological studies by Gowan in 1959 describing the interaction between lymphocytes and endothelium of post-capillary venules stimulated numerous subsequent studies that led to the current view of the endothelium as a dynamic, heterogeneous, disseminated organ that possesses vital secretory, synthetic, metabolic, and immunologic functions.¹

The endothelial cell (EC) surface in an adult human is composed of approximately 1 to 6×10^{13} cells, weighs approximately 1 kg, and covers a surface area of approximately 1 to 7 m².² ECs line vessels in every organ system and regulate the flow of nutrient substances, diverse biologically active molecules, and the blood cells themselves. This gate-keeping role of endothelium is effected through the presence of membrane-bound receptors for numerous molecules including proteins (eg, growth factors, coagulant, and anticoagulant proteins), lipid transporting particle (eg, low-density lipoprotein [LDL]), metabolites (eg, nitrous oxide and serotonin), and hormones (eg, endothelin-1), as well as through specific junctional proteins and receptors that govern cell-cell and cell-matrix interactions.

The endothelium also plays a pivotal role in regulating blood flow. In part, this results from the capacity of quiescent ECs to generate an active antithrombotic surface that facilitates transit of plasma and cellular constituents throughout the vasculature. Perturbations, such as those that may occur at sites of inflamma-

tion or high hydrodynamic shear stress, disrupt these activities and induce ECs to create a prothrombotic and antifibrinolytic microenvironment. Blood flow is also regulated, in part, through secretion and uptake of vasoactive substances by the endothelium that act in a paracrine manner to constrict and dilate specific vascular beds in response to stimuli such as endotoxin.

Detailed study of endothelial function first became feasible with the development in the 1970s of techniques to culture ECs in vitro.³⁻⁵ Limitations of this approach have become apparent recently with the realization that cell culture perturbs ECs from their quiescent in vivo state (0.1% replications per day) to an activated phenotype (1% to 10% replications per day) with loss of specialized functions associated with diverse vessels and organ systems. More complex analytic systems now exist that incorporate changes in EC properties imparted by plasma and cellular blood elements, by rheologic factors, and by cell-cell

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interactions that occur within the vessel wall. Genetic recombination studies in mice are likely to advance understanding of ECs in both their physiologic and pathologic roles in thrombosis, atherosclerosis, tumor metastasis, and organ rejection.

The purpose of this review is to provide a broad overview of EC participation in several biological processes judged to be relevant to clinical hematologists and investigators of vascular biology. Part I will principally examine the known physiologic roles of the endothelium, whereas Part II will discuss the interactions between ECs and blood cells and emphasize the contribution of the EC to the pathogenesis of specific diseases. Because of the introductory nature of this review, many topics and important contributions have been omitted, including the involvement of ECs in hematopoiesis, neuroendocrinology, cell aging, cellular integrins and matrix interactions, vascular permeability, lipid metabolism, the lymphatic vasculature, and the endothelium as a target for gene therapy. We hope that the abbreviated bibliographies will serve as an introduction to readers who wish to further investigate EC biology.

VASCULOGENESIS AND ANGIOGENESIS

Overview of early vascular development. Recently developed techniques that permit alteration of genomic sequences and manipulation of developing embryonic tissues have provided important insights into molecular and genetic elements that regulate vascular development.⁶ These studies show that the cardiovascular system is the first system to form in the gastrulating embryo. The *de novo* organization of ECs into vessels in the absence of any pre-existing vascular system is referred to as vasculogenesis and only occurs in the early embryo. Angiogenesis, the continued expansion of the vascular tree as a result of ECs sprouting from existing vessels, occurs in avascular regions of the embryo and is repeated many times in the mature animal, most commonly during wound healing and tumor metastasis⁷ (Fig 1). It remains uncertain how the pattern of the vascular tree is established or which factors govern the site of sprouting or the route taken by migrating ECs during angiogenic expansion.

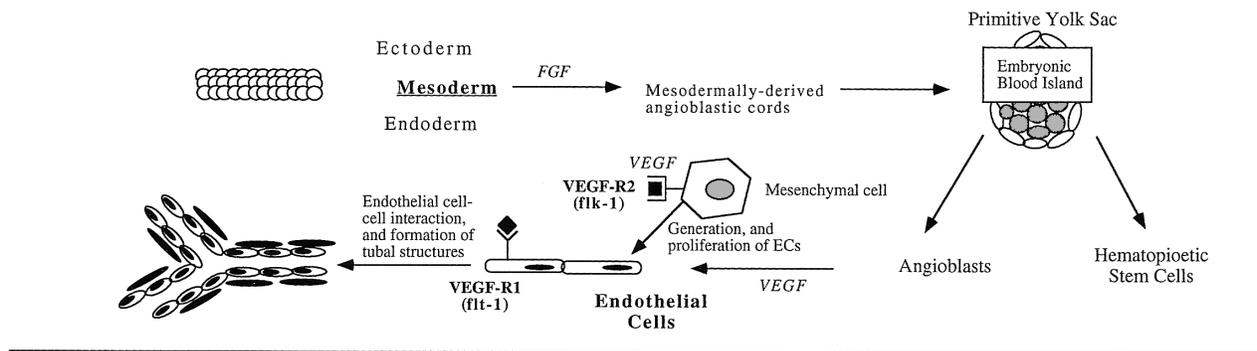
Origin of the vascular endothelium. Molecular events involved in EC differentiation from the early mesoderm remain uncertain. Vascular and hematopoietic tissues develop together, beginning shortly after implantation with the formation of blood islands within the primitive yolk sac⁸ (Fig 1) composed of two cell types: (1) angioblasts that form the outer layer of ECs encasing the blood island; and (2) hematopoietic stem cells, in the inner cluster, from which the first embryonic blood cells develop. Angioblasts committed to EC differentiation are found primarily in embryonic mesoderm,⁹ whereas the early epiblast also contains a subpopulation of hematopoietic stem cells.¹⁰ Recently, angioblasts have been identified in the adult as well.¹¹ The endoderm is the initial stimulus for angioblast formation.^{8,9} Within the embryo proper, the first angioblasts arise from the lateral mesodermal plate and cardiac crescent^{8,9}; some cells migrate into the forming brain, whereas others assemble into the endocardium of the early heart tube. Other angioblasts form a plexus of ECs at the base of the primitive heart tube that assemble into the vitelline vessels, allowing blood cells from the yolk sac to circulate within the body of the embryo.⁸ The vasculature of the viscera is formed from ECs that differentiate

directly from the surrounding mesenchyme incorporated into the angiogenic extensions of invading vessels.¹² For example, as the airway of the developing lung expands, endodermally derived cells of the branching airway provide directional queues for advancing branchial arteries and induce formation of angioblasts that become part of the pulmonary vasculature.¹³ Vasculogenic activity of early organ rudiments was shown through engraftment experiments using pieces of early quail lung and chick embryos. These experiments lead to the hypothesis that endoderm, but not ectoderm, induces vasculogenesis but that both endoderm and ectoderm can support angiogenesis¹⁴; recent experiments suggest additional sources of endothelial growth factors may exist.¹⁵

Genetic programs regulating EC differentiation and early vascular development. The best insight into molecular events required to initiate and maintain vascular development has come from detailed analyses of mouse embryos in which the genes for specific polypeptide growth factors or their transmembrane receptor tyrosine kinases (RTKs) have been inactivated. Such experiments show that initiation of vascular development requires both basic fibroblast growth factor (β BFGF) and vascular EC growth factors (VEGF; see Beck and D'Amore¹⁶ for a detailed discussion of growth factors and vascular development). Three alternatively spliced isoforms of VEGFs, members of the platelet-derived growth factor family (VEGF, VEGF-B, and VEGF-C¹⁷), interact with specific tyrosine kinase receptors. The growth factor-receptor interactions include VEGFR-1 (also known as flt-1 or fms-like tyrosine kinase-1) with VEGF and a related placenta growth factor (PlGF); VEGFR-2 (known alternatively as flk-1, fetal liver kinase-1; or Kdr, kinase-inserted domain containing receptor) with both VEGF and VEGF-C; and VEGFR-3 (originally designated flk-4) with VEGF-C. All VEGFs stimulate receptor autophosphorylation and EC replication and migration. The crucial role of this ligand in early vasculogenesis is demonstrated by the fact that loss of the VEGF gene results in embryonic death. Subsequent assembly of ECs into vessels requires activation of VEGFR-1 on the surface of the newly differentiated cells.^{18,19} The decision for a vessel to become a vein or an artery appears to be under the control of yet another growth factor, VEGFR-3,²⁰ that is expressed later in development only on ECs that will become veins or lymphatic vessels.

Expansion of the vascular tree, continued endocardial and ventricular development, and formation of the vascular wall is controlled by two members of a second family of RTKs and their ligands,¹⁹ tie-1 (tyrosine kinase with Ig and epidermal growth factor homology domains) and tie-2 or tek (tunica interna EC kinase). Two ligands, termed angiopoietin-1 and angiopoietin-2,^{21,22} are specific for tie-2 and are synthesized by cells surrounding the developing vessels. Ligand binding results in autophosphorylation of tyrosine residues in the intracellular domain of tie-2, but does not lead to EC replication or tube formation, as is the case for other endothelium-associated receptor-ligand interactions. Interestingly, angiopoietin-2 appears to function as an antagonist for angiopoietin-1, blocking its binding to tie-2. Targeted mutations of the genes for either tie-2 or angiopoietin-1 result in embryos with abnormal hearts

Vasculogenesis



Angiogenesis

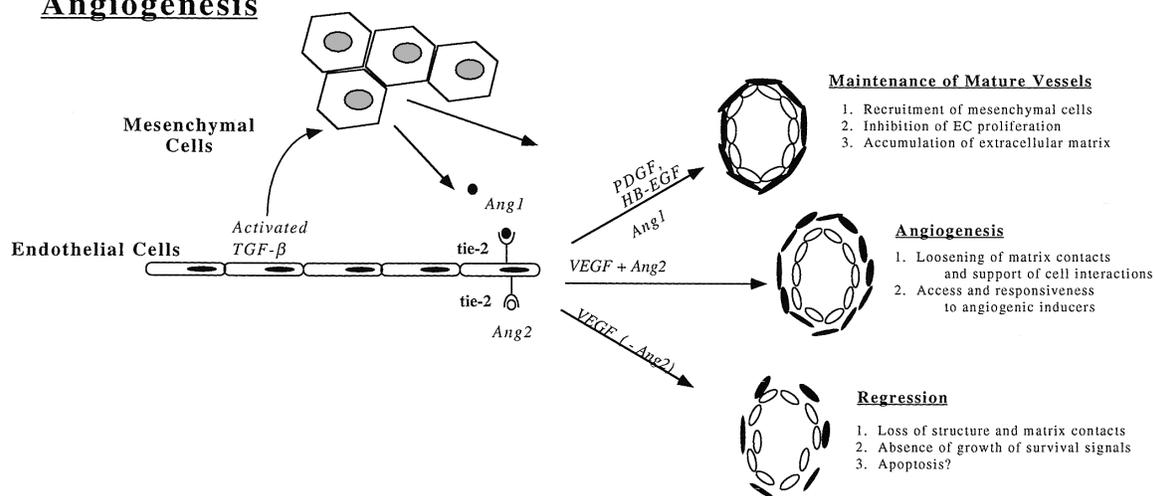


Fig 1. The formation of new vessels during vasculogenesis and angiogenesis. Vasculogenesis, the de novo organization of ECs into vessels in the absence of preexisting vascular structures, takes place during embryogenesis in the blood islands of the yolk sac (pictured) and in the embryo through expression of growth factors, in particular fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF). The tyrosine receptor kinases, VEGFR-1 (flk-1) and VEGFR-2 (flt-1), are expressed on mesenchymal cells and newly formed ECs, respectively, and are essential for the generation and proliferation of new ECs and the formation of tubal EC structures. Angiogenesis, the continued expansion of the vascular tree, is mediated through the expression of additional tyrosine kinase receptors, tie-2 (tek), which binds to Ang1 and Ang2 (angiopoietins), resulting in the maintenance of mature vessels, the development of new vessels, and the regression of formed vessels in processes dependent on a combination of factors, most notably the presence or absence of growth factors.

and vessels with poorly formed walls.²³ This has led to the suggestion that angiopoietin-1 acts via its receptor on ECs to stimulate the production of growth factors that, in turn, stimulate the differentiation of surrounding mesenchyme into pericytes or smooth muscle cells required for vessel wall formation.²⁴ This is consistent with the phenotype of a Tie-2 mutation in humans that leads to smooth muscle deficiencies around small vessels and microaneurysms.²⁵ These observations suggest that carefully regulated activity of tie-1 and 2 is required for continued vascular branching and vessel remodeling. Thus, the assembly of the early vascular tree depends on the programmed expression of at least two sets of RTKs and their ligands, one set for EC differentiation and initiation of vessel formation and the other for subsequent branching, establishment of capillary beds, and vessel wall formation.

Although genetic manipulations now possible in the mouse have provided important insights, there is much that we do not

know about vascular development. Random genetic mutations introduced into the zebrafish have also generated many surprising and fascinating cardiovascular anomalies.^{6,26} For example, zebrafish can be induced to develop with hearts that do not contain an endocardium although the remainder of the vascular system appears functional. Thus, it is likely that a combination of genetics and developmental biology, unencumbered by previous assumptions, will continue to show new genes and suggest new paradigms that will advance our understanding of vascular development.

Extracellular matrix and matrix adhesion receptors in vascular development. The ability of ECs to form capillary-like tubes is regulated not only by specific cytokine/receptor combinations, but also by the extracellular matrix. For example, human umbilical vein ECs (HUVECs) exposed to transforming growth factor-β (TGF-β) grow as a rapidly dividing monolayer if cultured on a flat surface coated with type I collagen.²⁷

However, under similar TGF- β exposure, but within a type I collagen gel, ECs spontaneously organize into capillary-like tubules and continue to divide. Several kinds of molecules on the EC surface act together to mediate cell-extracellular matrix (ECM) interactions, including proteoglycans as well as proteins. The best studied family of receptors that mediate cell-matrix interactions is the integrins,²⁸ which serve both a tethering and an information transfer function. Integrin-ligand binding triggers cytoskeletal organization at specific sites on the surface membrane to facilitate cell movement or maintain tissue stability. Binding also activates intercellular pathways that can result in either cell replication or programmed cell death.²⁹

Cells express more than one integrin and the combination of integrins expressed during embryonic development is constantly changing, suggesting that specific combinations are required as development proceeds. Experimental results in the developing mouse embryo suggest that functional compensation by integrins can occur during embryogenesis. It is also possible that receptors required for angiogenesis in early development may differ from those required for collateral vessel formation or tumor angiogenesis or that both gene inactivation and the introduction of inhibitory agents have unknown secondary effects. Similarly, mouse embryos continue to develop normally when genes for certain ECM components have been inactivated, whereas inactivation of other genes, such as the abundant fibronectin, results in early embryonic lethality.³⁰ Similarly, knocking out the gene for a fibronectin receptor subunit ($\alpha_5\beta_1$) also results in a poorly developed heart and vascular system and early embryonic death.³⁰ Again, other integrins, most likely $\alpha_v\beta_3$, appear to compensate for the absence of $\alpha_5\beta_1$ during preimplantation development and early gastrulation, but are unable to do so as development proceeds.

Endothelial cell-cell interactions and vessel formation. Angioblasts and ECs must contact like cells for vessels to sprout and lengthen. Such cell-cell adhesion is mediated by a distinct series of cell surface receptors that includes platelet EC adhesion molecule (PECAM-1),³¹ a member of the Ig superfamily, and vascular endothelial (VE)-cadherin.⁸ ECs express two isoforms of PECAM that mediate cell adhesion that differ in their requirement for divalent cations and sulfated proteoglycans. VE-cadherin, also known as cadherin-5, found almost exclusively on ECs, promotes cell-cell adhesion by a calcium-dependent homotypic mechanism,³² ie, in the presence of calcium, VE cadherin from one cell binds to the VE-cadherin expressed on an adjacent cell. As the vessel matures, more classic junctional complexes, such as tight junctions and gap junctions, form depending on the function of the particular vascular bed. Thus, during vascular development, junction formation initially involves rather weak adhesion complexes, likely required for cell-cell recognition, that facilitate the assembly of additional junctional complexes. However, the factors that determine the organ-specific nature of junction formation remain unknown.

Proteinases and vascular development. Vascular development may be regulated by some of the same factors that are involved in the control of blood clotting and capillary formation. EC movement through the ECM is tightly regulated and requires integrin mediated cell-matrix adhesion complex formation and subsequent disassembly. This involves repetitive cycles

of reversible integrin/matrix binding, assembly, and disassembly of cytoskeletal elements as well as matrix degradation restricted to the advancing edge of the moving cell.³³ This is accomplished through the organization of active molecular complexes that approximate integrins and integrin ligands with matrix metalloproteinases and plasminogen activators with their respective substrates and inhibitors at sites of cell-matrix interaction.³⁴ It has long been hypothesized that, when ECs are exposed to angiogenic stimuli, plasminogen activation is initiated through the binding of plasminogen and urokinase to their receptors. This leads to the formation of plasmin that activates prometalloproteinases, degrades noncollagenous components of the matrix, provides a path for cell migration, and releases peptides that can promote or inhibit continued angiogenesis.³⁵ Two peptides, angiostatin and endostatin,^{36,37} are currently being evaluated for clinical use in reversing tumor angiogenesis.³⁶ However, much of the dogma regarding the role of plasmin in angiogenesis may have to be revisited in light of recent data indicating that transgenic animals with targeted disruption in the genes for tissue-type plasminogen activator (t-PA), urokinase (u-PA), the urokinase receptor (u-PAR), and plasminogen appear to develop a normal vasculature in the absence of trauma or other stressors (see below).

Perspective. The induction of embryonic angioblasts to differentiate into ECs, organize into a vascular network, and subsequently populate the specialized vascular bed of an organ results from a complex genetic program, the details of which are only now emerging. This program is not only sensitive to the composition and structure of the ECM but is influenced by cell-cell contact as well as angiogenic and angiostatic growth factors and peptides generated by vascular expansion itself. Many of these same events are recapitulated after injury or as part of an inflammatory response and, if allowed to proceed unchecked (eg, tumor angiogenesis and diabetic retinopathy), can have serious consequences for the organism. Insight into the molecular and genetic programs involved in vascular differentiation may suggest better approaches to minimize ischemic tissue damage, avoid tissue rejection, stimulate wound healing, and inhibit tumor growth. This section has focused on the development of the vascular endothelium; however, the formation of the basement membrane, the induction and differentiation of vascular smooth muscle cells, and the complex process of assembling the elastic lamina are all essential to vessel formation and are under separate regulation. Ultimately, prevention of devastating effects from congenital abnormalities and facilitation of normal vascular function will, in a large part, be influenced by our ability to manipulate molecular mechanisms involved in vascular development.

EC HETEROGENEITY

Many human vascular diseases are exquisitely restricted to specific types of vessels. For example, the contribution of platelets to the pathogenesis of arterial and venous thrombosis differs as does the susceptibility of these two types of vessels to atherosclerosis. It is also common for vasculitis to show marked predilection for specific arteries, veins, or capillaries or for certain organs. Tumor cells may show similar predilection to metastasize through particular vascular beds.³⁸ Even when systemic risk factors are clearly evident, such as is the case with

inherited disorders of lipoprotein metabolism or proteins that control coagulation, there is marked regional variation in disease expression. Furthermore, clinical events such as thromboses are generally episodic and often localized to single vessels. The basis for variation is poorly understood, but may lie, in part, in the heterogeneity of ECs themselves (see Augustin et al² and McCarthy et al³⁹ for reviews).

To date, appreciation of EC function has been largely based on the behavior of cultured umbilical vein ECs (HUVECs). Indeed, it is remarkable that so many concepts in vascular biology have been predicated on the repertoire of umbilical ECs studied under such potentially unphysiologic in vitro conditions; this is especially true considering their derivation from a type of vessel that rarely, if ever, is affected by the most common human vascular disorders. More recently, there has been greater appreciation that EC heterogeneity may contribute both to the maintenance of adaptive processes and to the development of disorders restricted to specific vascular beds.

EC heterogeneity among and within tissues. Variation in the appearance of capillary endothelium from different vascular sites has long been recognized and appears well suited to postulated differences in function (Fig 2). For example, the brain and retina are lined by continuous ECs connected by tight junctions that help to maintain the blood-brain barrier; the liver, spleen, and bone marrow sinusoids are lined by discontinuous ECs that allow cellular trafficking between intercellular gaps; while the intestinal villi, endocrine glands, and kidneys are lined by fenestrated ECs that facilitate selective permeability required for efficient absorption, secretion, and filtering (see

Dejana³² for review). ECs from diverse tissues are also heterogeneous with respect to their surface phenotype and protein expression. For example, von Willebrand factor (vWF), used commonly as a marker for ECs, is not expressed uniformly on cells from all types of vessels,^{40,41} the expression of tissue type plasminogen activator is limited in vivo to approximately 3% of vascular ECs,⁴² and the constitutive expression of u-PA is reportedly confined to renal ECs,^{43,44} which are also uniquely susceptible to injury by verotoxin.⁴⁵ Microvascular ECs also differ in their susceptibility to undergo apoptosis induced by plasma from patients with thrombotic thrombocytopenic purpura.⁴⁶ The induction of tissue factor after infusion of cytokines or endotoxin is similarly restricted to specific vessels,⁴⁷ among many other examples of heterogeneity at the level of protein expression.

One of the clearest examples of EC heterogeneity lies in the expression of homing receptors involved in cell trafficking. In the mouse, Lu-ECAM-1 (lung-specific EC adhesion molecule) is exclusively expressed by pulmonary postcapillary ECs and some splenic venules,⁴⁸ whereas Mad-CAM-1 (mucosal addressin cell adhesion molecule-1) is expressed primarily on high endothelial venules in Peyer's patches of the small intestine.⁴⁹ Microvascular ECs derived from the bone marrow show an affinity for binding megakaryocytes and CD34⁺ progenitor cells and constitutively secrete hematopoietic stimulating factors such as Kit-ligand, granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, and interleukin-6 (IL-6), which help control trafficking, proliferation, and hematopoietic lineage-specific differentiation.⁵⁰

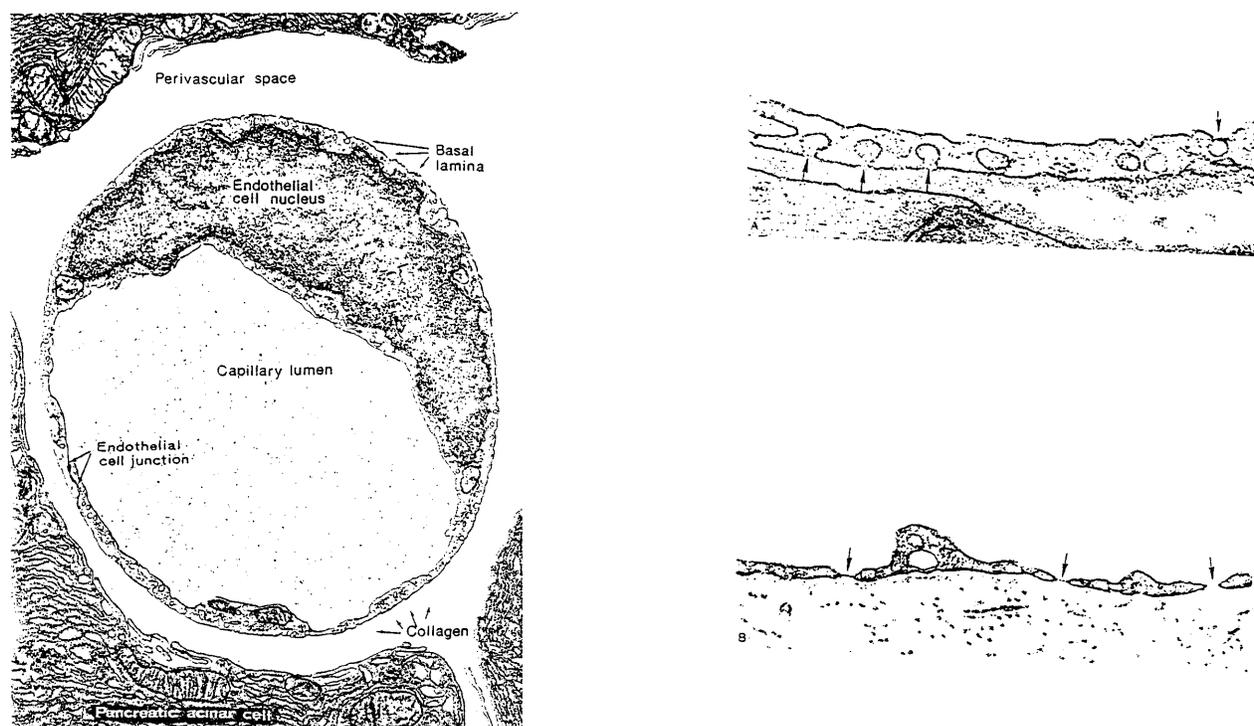


Fig 2. EC heterogeneity. (A) Electron micrograph showing the junction between two capillary ECs in a guinea pig pancreas (micrographs reprinted with permission from R.F. Bolender, *The Journal of Cell Biology*, 1974, vol. 61, p. 269). (B) Electron micrograph demonstrating the diversity of ECs from two types of capillaries: (1) vesicular invaginations (arrow) on both luminal and abluminal plasma membrane of a muscle capillary EC; (2) fenestrated capillary from the lamina propria of the colon with thin diaphragms (arrow) covering the plasma membrane pores (micrographs reprinted with permission from E. Weihe, *Textbook of Histology*, (ed 12), 1994, p. 391, courtesy of Chapman and Hall).

Tumor cells may show clear preferential adhesion to the endothelium of specific organs paralleling their *in vivo* metastatic propensities (see McCarthy et al³⁹ for review).

Microvascular ECs cultured from the brain, liver, and other organs each express distinct patterns of cell surface markers, protein transporters, and intracellular enzymes.⁵¹ These tissue-specific phenotypic differences can be maintained for some time under identical tissue culture conditions (eg, Grau et al⁵²). Distinct subsets of ECs often exist within a single organ. *In situ* studies of adult human liver show two distinct sinusoidal EC phenotypes: hepatic periportal vessels express PECAM-1 and CD34, whereas sinusoidal intrahepatic ECs do not.⁵³ During the development of the human liver, ECs progress from a phenotype closely resembling adult hematopoietic sinusoidal bone marrow ECs, which supports fetal intrahepatic hematopoiesis, to one resembling adult hepatic sinusoidal ECs, including the expression of the T-lymphocyte marker CD4.⁵⁴

Environmental and genetic regulation of EC phenotype. There is extensive evidence to indicate that heterogeneity develops in part as a result of variation in exposure of EC to environmental stimuli, some of which act only over short distances or even require cell-cell contact to effect change. Numerous exogenous factors affect EC phenotype, including mechanical forces, soluble growth promoters and inhibitors, cytokines, plasma lipids and proteins [eg, thrombin, plasmin, antibodies, Lp(a), etc], and contact with circulating and tissue-based cells (eg, smooth muscle cells and pericytes) and with the ECM, microbes, and their soluble products.

There are numerous examples of how the microenvironment can regulate the endothelial phenotype, a phenomenon that has been referred to as transdifferentiation.² For example, aortic ECs cultured on extracellular matrix derived from the lung are induced to express Lu-ECAM-1,⁴⁸ whereas the cells develop fenestrae when cultured on matrix derived from kidney-derived MDCK cells.⁵⁵ Transplantation studies in the chick-quail system illustrate that ECs can take on the characteristics of the tissue into which they are transplanted *in vivo*,⁵⁶ whereas other examples show that ECs acquire a different phenotype *ex vivo*.⁵⁷ Studies in transgenic mice expressing the Lac Z reporter gene under control of 2,182 bp of the 5' flanking sequence and the first exon and intron of the vWF gene suggest that expression is regulated by signals derived from the local microenvironment that influence pathways specific for particular vascular beds.⁵⁸

EC heterogeneity can thus arise as a consequence of local concentrations of exogenous effectors or due to intrinsic variations in responsiveness (reviewed in McCarthy et al³⁹). ECs grown on extracts of basement membrane from different organs have been observed to develop preferential adhesivity for tumor cells prone to metastasize to that organ.⁵⁹ ECs derived from saphenous vein have been reported to synthesize less prostaglandin I₂ (PGI₂) than those from the internal mammary artery, a finding that may contribute to the rapidity with which pathogenic changes may develop in venous bypass grafts placed under arterial pressure.⁶⁰ Possible genetic bases for EC diversity have only recently been considered and have not been studied in depth. Microvascular and macrovascular ECs differ in the fastidiousness of their growth, propensity to form capillary-like structures, synthesis of PGI₂, and expression of adhesion

receptors for lymphocytes, among other properties (see Ades et al⁶¹ for review). Tissue-specific transcription factors or signal transduction molecules responsible for activating and/or depressing transcription apparatus in a tissue-specific manner are only beginning to be understood. Identification of these control factors will be important in the design of vectors that will enable expression of EC proteins in a tissue-specific manner.

The effects of cell culture. Only in the past few years has the technology become available that permits *in situ* study of EC behavior. These studies indicate that the constitutive phenotype of ECs is unstable and their behavior can change rapidly once explanted. Commonly used culture conditions may activate or otherwise alter the endothelial phenotype (eg, Grant et al⁶²). There is, as yet, no model for generating the resting EC *in vitro*. Thus, all the information described in subsequent sections should be considered in the context of the cell source as well as the *ex vivo* culture conditions, including passaging, the presence/omission of shear forces, and factors released into blood that alter the behavior of the endothelium from that which occurs in healthy blood vessels *in vivo*. Thus, much may be gained in the future by a more critical consideration of EC heterogeneity both in terms of understanding homeostasis and vascular pathology, as well as in targeting the delivery of gene therapy, antithrombotic agents, and antitumor agents to an anatomically or functionally distinct endothelial region.⁶³

VASOREGULATION

The endothelium not only provides a structural barrier between the circulation and surrounding tissue, but ECs also secrete mediators that influence vascular hemodynamics in the physiologic state (Table 1). ECs contribute to the regulation of blood pressure and blood flow by releasing vasodilators such as nitric oxide (NO) and prostacyclin (PGI₂), as well as vasoconstrictors, including endothelin (ET) and platelet-activating factor (PAF). These chemically diverse compounds are not stored in intracellular granules. Rather, their major biologic effects are regulated by localization of specific receptors on vascular cells, through their rapid metabolism, or at the level of gene transcription. NO is constitutively secreted by ECs, but its production is modulated by a number of exogenous chemical and physical stimuli, whereas the other known mediators (PGI₂, ET, and PAF) are synthesized primarily in response to changes in the external environment.

NO. ECs elaborate NO, a heterodiatomic free radical product generated through the oxidation of L-arginine to L-citrulline by NO synthases.⁶⁴ One isoform, eNOS or the Nos3 gene product, is constitutively active in ECs but is stimulated further by receptor-dependent agonists that increase intracellular calcium and perturb plasma membrane phospholipid asymmetry.⁶⁵ Receptor-dependent agonists that stimulate eNOS include thrombin, adenosine 5'-diphosphate, bradykinin, substance P, and muscarinic agonists, in addition to shear stress⁶⁶ and cyclic strain.⁶⁷ The increase in eNOS activity evoked by shear stress contributes to the phenomenon of flow-mediated vasodilatation, an important autoregulatory mechanism by which blood flow increases in response to exercise.⁶⁸ This is in part a result of shear-induced transcriptional activation due to the presence of a shear response consensus sequence, GAGACC, in the promoter of the Nos3 gene.⁶⁹ In addition to eNOS,

Table 1. Vasoregulatory Substances Synthesized by the Endothelium

Substance	Principal Effect	Other Effects	Secretion	Compound	Precursor Compound
NO (nitric oxide)	Vasodilatation	Maintains basal tone of vessels; inhibits leukocyte adhesion; inhibits platelet adhesion, activation, secretion, and aggregation; promotes platelet disaggregation; inhibits smooth muscle cell migration and proliferation	Paracrine/Constitutive and induced by thrombin, ADP, bradykinin, substance P, muscarinic agonists, shear stress, cyclic strain, cytokines	Heterodiatomic free radical	L-arginine
PGI ₂ (prostacyclin)	Vasodilatation	Retards platelet aggregation and deposition	Paracrine/Induced at sites of vascular perturbation	Eicosanoid	Arachidonic acid
PAF (platelet-activating factor)	Vasoconstriction	Promotes leukocyte adhesion at cell surface	Juxtacrine/Induced	Phospholipid	Arachidonic acid
ET-1 (endothelin-1)	Vasoconstriction	Mitogen for smooth muscle cells; modulates effect of numerous compounds	Paracrine/Induced by hypoxia, shear stress, and ischemia	21 Amino acid peptide	Preproendothelin-1 (203 amino acids)

Principal regulatory compounds synthesized by the endothelium, their effects on the vasculature and other processes, their mode of secretion, and the nature of their chemical composition and precursor compound.

cytokines have been shown to stimulate bovine microvascular endothelium in culture to express an inducible isoform of NO synthase, iNOS, or the Nos2 gene product.⁷⁰ EC-derived NO has several important effects on the vasculature. First, NO maintains basal tone by relaxing vascular smooth muscle cells⁷¹ through the binding of NO to the heme prosthetic group of guanylyl cyclase. Endothelial-derived NO also inhibits platelet adhesion, activation, secretion, and aggregation and promotes platelet disaggregation, in part through a cyclic GMP-dependent mechanism.⁷² PGI₂, which does not affect platelet adhesion,⁷³ acts synergistically with NO to inhibit other steps in the platelet activation cascade.⁷⁴ NO also inhibits expression of P-selectin on platelets and, by inhibiting the agonist-dependent increase in intraplatelet calcium,⁷² suppresses the calcium-sensitive conformational change in the heterodimeric integrin glycoprotein $\alpha_{IIb}\beta_3$ (GP IIb-IIIa) required for fibrinogen binding.⁷⁵ Additionally, NO appears to promote platelet disaggregation indirectly by impairing the activity of phosphoinositide 3-kinase, which normally supports conformational changes in $\alpha_{IIb}\beta_{IIIa}$, rendering its association with fibrinogen effectively irreversible.⁷⁶

In addition to these effects on the vasculature, endothelial-derived NO inhibits leukocyte adhesion to the endothelium^{77,78} and inhibits smooth muscle cell migration⁷⁹ and proliferation.⁸⁰ These latter effects serve to limit neointimal proliferation that occurs after vascular injury and, combined with its stimulatory effect on EC migration and proliferation,⁸¹ suggest that NO helps to sustain vascular reparative mechanisms.

ECs also produce a less well-characterized compound known as endothelium-derived hyperpolarizing factor (EDHF) that promotes vascular smooth muscle relaxation (see Garland et al⁸² for review). Muscarinic agonists stimulate ECs to release

EDHF, causing a transient hyperpolarization of the cell membrane. It has been proposed that EDHF exerts its vascular effects by activating ATP-sensitive potassium channels, smooth muscle sodium-potassium ATPase, or both,⁸³ but its role in vascular (patho)physiology requires further study.

ET. Remarkably, ECs produce not only the potent vasodilator NO, but also synthesize endothelin-1 (ET),⁸⁴ the most potent vasoconstrictor identified to date. Endothelins comprise a family of 21-amino acid peptides produced by many cell types.⁸⁴ ET-1 is not stored in granules⁸⁵ but is formed after transcription of the gene encoding preproendothelin-1, the inactive precursor of ET-1, after stimulation by hypoxia, shear stress, and ischemia. ET-1 released from ECs binds to the abundant G-protein-coupled ET-A receptor expressed on vascular smooth muscle cells, which results in an increased intracellular calcium concentration and, in turn, increases vascular smooth muscle cell tone.⁸⁶ Of interest, this effect of ET-1 persists after the hormone dissociates from its receptor through longer-lived effects on intracellular calcium. NO shortens the duration of these effects by accelerating the restoration of intracellular calcium to basal levels.⁸⁷ The interplay between ET-1 and ET-A receptors likely contributes to basal vascular tone as well. ET-1 potentiates the vasoconstrictor actions of catecholamines, which, in turn, potentiate the actions of ET-1. In states of endothelial dysfunction, such as atherosclerosis, in which concentrations of bioactive NO are reduced, the relatively unopposed actions of ET-1 promote vasoconstriction and smooth muscle proliferation.⁸⁸

Prostacyclin (PGI₂) and PAF. The contribution of ECs to the regulation of vasomotor tone is even more finely regulated as evidenced by the production of additional vasoactive compounds such as prostacyclin (PGI₂) and PAF. Prostacyclin and

PAF factor provide an interesting contrast. Both are intercellular signaling molecules synthesized by stimulated ECs in vitro and in vivo.⁸⁹ Both are lipids: PGI₂ being an eicosanoid and PAF being a phospholipid.^{90,91} Neither is constitutively present in resting human ECs nor stored within the cell. The synthesis of each is induced rapidly by humoral and mechanical stimuli via discrete, regulated pathways.^{90,91} Once formed, PGI₂ and PAF have relatively short half lives, one of several features that limits the magnitude of their signals and exerts control over their biologic activities.^{92,93}

However, a major difference between the two factors lies in the range over which they exert their effects: PAF acts in a juxtacrine fashion, whereas PGI₂ acts as a paracrine signaling molecule. PAF, expressed on the surface of the endothelium, remains cell-associated even in the presence of physiologic concentrations of albumin or other acceptor molecules⁹¹ and binds to and activates its receptor on leukocytes,⁹⁴ fulfilling critical criteria of a juxtacrine signaling molecule. Consistent with this notion, PAF synthesized by cultured human ECs acts in concert with P-selectin (see below) to promote leukocyte adhesion.⁹⁴

In contrast, PGI₂ is rapidly released from ECs,⁹⁵ although the export mechanism has not been precisely defined. Thus, PAF and PGI₂ have spatially differentiated realms of signaling, even though both derive from a common precursor and are synthesized concurrently.^{91,96} This feature may contribute to differences in their actions at the endothelial interface with the blood: PAF is specialized to signal leukocytes at the cell surface, whereas PGI₂ acts primarily in solution to retard platelet aggregation and deposition. Both PGI₂ and PAF also elicit autocrine effects on ECs,^{91,92} which may be important in modulating angiogenesis and controlling the synthesis of EC-derived mediators.

PGI₂ was the first endothelial-derived vascular smooth muscle relaxing factor to be identified. PGI₂, which was generated locally, and PGI₂ or its analogs, which were infused systemically, caused vasodilatation and altered regional blood flow.⁹³ A receptor for PGI₂, the IP receptor, is present on vascular smooth muscle as well as on platelets,⁹⁷ consistent with early experimental observations, indicating that PGI₂ acts principally to modulate the function of these two cell types.⁹⁸ Although IP receptors are present in the arterial vascular wall, PGI₂ is not constitutively produced and does not appear to regulate basal systemic vascular tone.⁹⁹ Rather, PGI₂ synthesis is induced at sites of vascular perturbation, where it may regulate vasoconstriction and platelet deposition.⁶⁶ Because of its effects on blood flow and relevant cell-cell interactions, PGI₂ may influence local inflammatory responses as well. An important recent advance has been the identification of prostaglandin H synthase-II (PHS-II), an inducible form of a key enzyme in PGI₂ formation providing a mechanism by which the production of PGI₂ and other eicosanoids can be sustained in chronic states of inflammation and vascular injury.

The receptor for PAF, the first receptor characterized at a molecular level that recognizes a biologically-active lipid, is a member of the serpentine G-protein-linked family (reviewed in Whatley et al⁹¹). Intravascular infusion of PAF causes either vasodilatation or vasoconstriction, depending on the concentration administered, the time, and the specific vascular bed

studied.⁹³ Some hemodynamic effects of PAF in vivo are indirect and depend on the generation of eicosanoids or leukotrienes or mediators derived from activated leukocytes or platelets and on cardiac effects.⁹¹⁻⁹³ In shock and other in vivo pathologic states, PAF acts concomitantly or sequentially with other classes of mediators, including leukotrienes and tumor necrosis factor- α (TNF- α).^{90,91,100} As with PGI₂, it is unlikely that PAF is a circulating regulator of blood pressure under basal conditions, despite early studies suggesting that PAF-like activity is released from kidneys.⁹²

THE ROLE OF THE ENDOTHELIUM IN COAGULATION

A crucial physiologic function of the endothelium is to facilitate blood flow by providing an antithrombotic surface that inhibits platelet adhesion and clotting. However, when the endothelium is perturbed by physical forces or by specific chemical factors, the cells undergo programmatic biochemical changes that culminate in their transformation to a prothrombotic surface. A dynamic equilibrium exists between these two states, modulated both at the level of gene transcription and at the level of the intact cell, that often permits the injured endothelium to return to its unperturbed state once the procoagulant stimulus has dissipated (Table 2; see Bombeli et al¹⁰¹ for review). Although the fibrin clots formed as a consequence of procoagulant activity may serve a protective organ function by limiting vascular damage induced by trauma, infection, and inflammation, the loss of anticoagulant activity may predispose to several common thrombotic disorders discussed in the sections that follow.

Anticoagulant mechanisms. Control of thrombin generation is a pivotal step in the balance between the natural antithrombotic and the induced procoagulant activities of the endothelium. Thrombin, a serine protease, serves diverse functions in coagulation, including the activation of platelets, several coagulation enzymes, and cofactors. Thrombin also stimulates procoagulant pathways on the ECs themselves. Therefore, it is not surprising that several highly regulated pathways have evolved to constrain the generation and activity of thrombin (see Rosenberg and Rosenberg¹⁰² for review), such that little enzyme activity is found in the plasma of healthy individuals.¹⁰³ The matrix surrounding the endothelium contains heparan sulfate and related glycosaminoglycans (GAGs) that promote the activity of cell/matrix associated antithrombin III (AT-III)¹⁰⁴; the subendothelium contains dermatan sulfate, which promotes the antithrombin activity of heparin cofactor II.¹⁰⁵ ECs also prevent thrombin formation through the expression of tissue factor pathway inhibitor (TFPI), which binds to factor Xa within the tissue-factor/VIIa/Xa complex (see Broze¹⁰⁶ for review). TFPI is released from its EC stores by heparin. TFPI and AT-III both contribute to physiologic hemostasis and can be depleted in acquired thrombotic states.^{107,108}

The endothelium also helps to contain thrombin activity through the expression of thrombomodulin (see Esmon and Fukudome¹⁰⁹ for review). Binding of thrombin to TM facilitates the enzyme's ability to activate the anticoagulant protein C. In turn, the activity of activated protein C (APC) is enhanced by its cofactor protein S, which is synthesized by EC, among other cell types.¹¹⁰ ECs also express receptors for APC¹¹¹ that regulate the activity of this pathway. APC, in turn, promotes the

Table 2. Regulation of Hemostasis and Thrombosis by the Endothelium

	Antithrombotic	Prothrombotic
Coagulation protein binding sites	Glycosaminoglycans/ATIII TFPI Thrombomodulin	Binding sites for: fibrin, FIX, IXa, X, Xa, FXII, kallikrein Tissue factor Thrombin receptor Receptor for protein C/APC
Products produced and/or stored by platelets	PGI ₂ NO ADPase	vWF PAF Fibrinogen FV FXI
Fibrinolytic factors	t-PA production u-PA expression u-PAR Plasminogen binding sites Annexin II	PAI-1, PAI-2 PAI-3 (protein C Inhibitor) TAFI activation
Vasomotor factors	NO PGI ₂	TxA ₂ Endothelin-1

Outline of the antithrombotic and prothrombotic properties of ECs detailing the binding sites expressed by ECs, the factors stored and/or secreted by ECs that affect platelet function, factors produced by the EC that influence the fibrinolytic state of the vasculature, and vasomotor substances secreted by the EC.

Abbreviations: ATIII, antithrombin III; PGI₂, prostacyclin; TFPI, tissue factor pathway inhibitor; APC, activated protein C; PAF, platelet-activating factor; t-PA, tissue plasminogen activator; u-PA, urokinase plasminogen activator; u-PAR, urokinase plasminogen activator receptor; PAI, plasminogen activator inhibitor; TAFI, thrombin activatable fibrinolysis inhibitor; TxA₂, thromboxane A₂.

inactivation of activated factors V and VIII. Binding of thrombin to TM also dampens the enzyme's ability to activate platelets, factor V, factor XIII, and fibrinogen and promotes EC fibrinolytic activity (see below). TM also inhibits prothrombinase activity indirectly by binding factor Xa.¹¹² Thrombin bound to TM is rapidly endocytosed and degraded.¹¹³ Various inflammatory cytokines downregulate TM gene transcription and accelerate TM internalization^{114,115} while at the same time promote tissue factor expression (see below). Soluble TM is also shed into plasma and elevated plasma levels have been identified in various disorders associated with EC injury (see Cucurull and Gharavi¹¹⁶ and below).

Procoagulant mechanisms. The pivotal step in transforming the EC membrane from an anticoagulant to a procoagulant surface is the induction of tissue factor (TF). TF dramatically accelerates factor VIIa-dependent activation of factors X and IX, so it is not surprising that TF is not expressed by unperturbed endothelium, at least in the adult organism.¹¹⁷ Interruption of the gene for TF is associated with impaired vascular development and lethal embryonic bleeding,¹¹⁸⁻¹²⁰ but the source and function of TF during development have not been elucidated. Synthesis of TF is induced in vitro by diverse

agonists, including thrombin, endotoxin, several cytokines, shear, hypoxia, oxidized lipoproteins, and many other provocations (see Rapaport and Rao¹²¹ and Nemerson¹²² for reviews). Procoagulant activity is accelerated by exposure of anionic phospholipids that may occur as a consequence of apoptosis.¹⁰⁸ TF is localized primarily beneath and between cultured ECs,¹²³ although some evidence for expression on the cell surface has been presented.¹²⁴ TF mRNA and protein levels decline despite continued exposure to agonists, a mechanism that may help contain the extent of fibrin formation. Cells in culture also shed microvesicles containing TF,¹²⁵ and plasma levels of TF are elevated in patients with disseminated intravascular coagulation,¹²⁶ although the cellular source has not been established. TF expression is rapidly induced after vascular injury,¹²⁷ and TF is found associated with ECs within atherosclerotic plaque^{128,129} and in tumor-derived vessels.¹³⁰ TF may also contribute to the regulation of angiogenesis and tumor metastases through mechanisms independent of coagulation.^{131,132} Yet, it has been difficult to demonstrate expression of TF by ECs in vivo even in response to potent provocations where expression was expected, another example of the dissociation between the behavior of these cells in culture and that seen in the whole organism.⁴⁷

Once ECs expressing TF are exposed to plasma, prothrombinase activity is generated and fibrin is formed on the surface of the cells.¹³³ This implies that ECs express binding sites for factors IX, IXa, X, and Xa; thrombin; and fibrin.¹³⁴ Yet, the identity and location of most of these binding sites is unknown, as is their role in either physiologic hemostasis or in thrombosis. Factor IX has recently been shown to bind type IV collagen in the EC matrix,¹³⁵ although its cellular association site promoting assembly of the intrinsic FX activation complex has not been identified. Several candidate FX/Xa binding sites have been reported,^{136,137} whereas others may be induced as a result of exogenous stimuli.¹³⁸ ECs also express receptors for proteins of the contact factor pathway,¹³⁹ but their role in hemostasis is uncertain.

The most thoroughly characterized EC binding site for a coagulation protein is the thrombin receptor, also termed the protease-activated receptor-1 (PAR1). The thrombin receptor is a high-affinity G-protein-coupled protein¹⁴⁰ that is activated when a fragment derived from the amino terminus of the protein, formed as a result of cleavage by thrombin, binds to the remaining cell-associated receptor fragment. Binding of thrombin leads to a wide array of changes in expression of prothrombotic and antithrombotic molecules by cultured ECs, including TF, PAI-1, NO, PAF, ET, and PGI₂, among others (see Kanthou and Benzakour¹⁴¹ for review) and disruption of cell-cell contacts (see Garcia et al¹⁴² for review). Thrombin is also mitogenic for ECs, fibroblasts, and smooth muscle cells and is chemotactic for monocytes.

The seemingly normal phenotype of surviving adult mice with targeted disruptions in the thrombin receptor gene¹⁴³ was unanticipated and raised questions about the physiologic role of this protein as well as other proteinase activated receptors expressed on ECs.¹⁴⁴ The subsequent discovery of two additional protease-activated G-protein-coupled receptors, PAR-2 and PAR-3, helped to explain this observation.¹⁴⁵ Both PAR-1 and PAR-2 are present on some human ECs, whereas PAR-1,

but not PAR-2, is expressed on human platelets. PAR-3 is expressed by human bone marrow and mouse megakaryocytes, but its expression on ECs has not been established. Notable tissue- and species-specific differences in expression and cellular distribution of PARs have been described,^{145,146} making it difficult at present to relate the phenotype of the various murine knockouts to human physiology.

ECs also express several receptors for fibrin and specific fibrin degradation products,¹⁴⁷ including a 130-kD glycoprotein,¹⁴⁸ a tissue transglutaminase,¹⁴⁹ and the $\alpha_v\beta_3$ integrin, although evidence regarding their expression in vivo is only now emerging.^{150,151} Binding of fibrin promotes EC adhesion, spreading, proliferation, and migration; cell retraction; leukocyte adhesion; and inhibition of PGI₂ synthesis. Cultured ECs also express glycoprotein Ib, which binds vWF secreted constitutively by ECs and, presumably, the ultralarge vWF multimers released from Weibel-Palade bodies in response to a number of agonists (see Wagner and Bonfanti¹⁵² for review). Expression of GPIb α by ECs is enhanced by TNF- α ,¹⁵³ but whether this glycoprotein participates in the physiologic or pathophysiologic binding of vWF in vivo requires additional study. The $\alpha_v\beta_3$ integrin also binds vWF in the EC substratum. In vitro, conditions that decrease $\alpha_v\beta_3$ expression (TNF- α plus interferon- γ [IFN- γ] or arterial shear stress) increase GPIb α expression,¹⁵⁴ suggesting that the EC state may affect the availability of adhesion receptors, although these findings require confirmation in vivo.

Undoubtedly, additional receptors for coagulation proteins with distinct functions will be characterized in the future. Genetic and acquired alterations in the structure, expression, and function of these EC receptors may contribute to hitherto unexplained hemorrhagic and thrombotic disorders. However, despite rather extensive study of EC procoagulant function in culture, the extent to which platelet adherence or fibrin formation actually occurs on the surface of the intact endothelium in vivo (as opposed to subendothelial matrix exposed to blood) remains unclear. Perhaps the best indirect evidence comes from animal models of *Escherichia coli* sepsis and cytokine infusion in which TF- and contact factor-dependent intravascular coagulation and multiorgan ischemic injury occurs in the absence of overt EC disruption.¹⁵⁵ Nevertheless, the contribution of endothelium, platelets, monocytes, and other cell types in these models will require further study.

ECs AND FIBRINOLYSIS

Experiments using cultured ECs have yielded a concept that the endothelial surface is profibrinolytic and thus helps maintain blood in its fluid state.¹⁵⁶ However, experiments using animal models have shown this conceptually satisfying hypothesis does not accurately reflect the situation in vivo.^{42,157} It has also become clear that the contribution of ECs to fibrinolysis varies with their metabolic status (ie, quiescent or activated), their vascular derivation, and the concentration of other hemostatically active molecules in the local plasma milieu.

Plasminogen activators. Studies with ECs cultured from various tissues have led to the widely held inference that t-PA production and secretion is a property of all ECs.¹⁵⁶ However, a few studies have gone largely unnoticed in which PA activity, demonstrated by fibrin zymography, was observed only in

association with the adventitia and not with luminal ECs.¹⁵⁷ More recent studies using in situ hybridization and immunohistochemistry have demonstrated t-PA antigen and mRNA only in a distinct subset of quiescent microvascular ECs of both primates and mice.^{42,158} Hence, contrary to assumptions based on work with cultured ECs, t-PA is associated only with a distinct subpopulation of the microvasculature, even after provocation. In both murine brain and lung, the percentage of microvascular ECs producing t-PA increases markedly upon exposure to pertinent stimuli; however, in both cases, t-PA production remains an exclusive property of microvascular ECs.⁴² Hence, invoking local EC production of t-PA in large vessels as a mechanism of maintaining blood flow may not adequately describe the in vivo situation.

t-PA production by cultured ECs is regulated by a variety of external stimuli at the level of gene transcription and cellular release.¹⁵⁹ Measurements of plasma t-PA levels suggest such regulated production/secretion occurs in vivo as well.¹⁶⁰ Intracellular signaling pathways operative in stimulated t-PA release have been described in vitro.¹⁶¹ The mechanisms that control EC t-PA production in vivo are less well understood, but clearly such regulation occurs.¹⁶² Humans exhibit higher plasma levels of t-PA after exercise or venous compression, but the cellular source of this increase has not been established.¹⁶¹

The other mammalian PA, u-PA, appears not to be produced by most quiescent ECs.⁴³ Rather, it is expressed by ECs involved in wound repair or angiogenesis,¹⁶³ consistent with the hypothesized importance of u-PA in cell migration and tissue remodeling. Yet, u-PA is obviously important to vascular homeostasis, because mice genetically deficient in u-PA develop inflammation induced thrombi¹⁶⁴ and manifest thrombotic tissue injury in response to lipopolysaccharide (LPS).¹⁶⁵ However, the extrarenal source of u-PA in physiologic states has not been established.

PA receptors. The presence of EC receptors for t-PA has been reported by several groups.¹⁶⁶⁻¹⁷¹ Binding of t-PA to ECs has been reported to promote its fibrinolytic activity^{166,172} and to stimulate cell proliferation.¹⁷³ Recently, one such t-PA binding site has been identified as annexin II, which is expressed on ECs¹⁷⁴ and binds t-PA in a specific and saturable manner in vitro.¹⁷² However, the expression of annexin II on the endothelium in vivo has not yet been demonstrated.

The u-PA receptor (u-PAR) expressed by ECs appears identical to that expressed on other cell types.^{175,176} u-PAR is a three-domain protein linked to cell surfaces by a glycerophosphatidyl inositol anchor. Single-chain u-PA (the form found in plasma¹⁷⁷) bound to cells via u-PAR exhibits increased plasminogen activating efficiency^{175,178} and is relatively protected from inhibition by PAI-1 and PAI-2.^{179,180} u-PAR may be expressed primarily on the surface of migrating ECs participating in angiogenesis, rather than on quiescent ECs lining normal vessels.¹⁸¹ Mice genetically lacking u-PAR develop normally and do not exhibit spontaneous vascular occlusion.¹⁸² Hence, u-PAR has yet to be shown to participate in maintaining physiologic blood fluidity, although it may be important in vascular repair.

Cells express diverse binding sites for plasminogen, among which are proteins that exhibit a carboxyterminal lysine (see Plow et al³⁵ for review). Plasminogen binds to ECs in vitro with

an affinity that would predict receptor occupancy at physiologic plasma concentrations.¹⁸³ Cell-associated plasmin may be relatively protected from inhibition by α_2 -plasmin inhibitor.¹⁸⁴ However, the exact identity of these EC plasminogen binding sites remains uncertain and their expression in vivo has not been established.¹⁸⁵ Lp(a) competes for the binding of plasminogen to ECs,¹⁸⁶ which may contribute to the prothrombotic effects of this lipoprotein.¹⁸⁷

Plasminogen activator inhibitors (PAIs). ECs in culture produce abundant PAI-1 that is associated primarily with its extracellular matrix, resulting in stabilization of its activity.¹⁸⁸ PAI-1 synthesis is stimulated by numerous agents, including thrombin, endotoxin, various cytokines, Lp(a), and oxidized LDL, among others.¹⁸⁹ Yet, experiments in mice have shown that liver is the major source of plasma PAI-1 and that quiescent EC express little or no inhibitor.¹⁹⁰ However, after exposure to inflammatory stimuli, ECs in virtually every tissue express PAI-1.¹⁹⁰ PAI-2 is found normally in plasma only during pregnancy¹⁹¹ and is not synthesized by ECs to an appreciable extent. However, multiply passaged ECs express PAI-2 in response to some agonists that may point to a local effect in select settings.¹⁹² PAI-3 (also known as the protein C inhibitor) has a much lower affinity for u-PA and t-PA than does PAI-1, but it is present in plasma at much higher concentrations.¹⁹³ Production of PAI-3 by ECs has not been reported, but PAI-3 antigen can bind to heparan sulfate proteoglycan on the luminal surface of ECs, thereby increasing its activity.¹⁹⁴

Thrombomodulin. Binding of thrombin to thrombomodulin (see "The Role of the Endothelium in Coagulation" above) accelerates its capacity to activate a protein known as thrombin-activatable fibrinolysis inhibitor (TAFI).¹⁹⁵ TAFI is a procarboxypeptidase-B-like molecule that, when activated, cleaves basic carboxyterminal residues within fibrin and other proteins. This results in the loss of plasminogen/plasmin and t-PA binding sites on fibrin such that fibrinolysis is retarded.¹⁹⁵ Thus, through the regulated expression of thrombomodulin, ECs serve as potent templates to decrease the rate of intravascular fibrinolysis.

Although a simple balance between profibrinolytic (PAs) and antifibrinolytic (PAIs) pathways seemed an attractive mechanism to explain the clinical experience that unperturbed endothelium helps maintain blood fluidity, more recent in vivo data have shown that the mechanism may not be quite so straightforward. Indeed, ECs seem to express more antifibrinolytic than profibrinolytic activity in many settings studied to date. Clearly, more work will be required to clarify the contribution of quiescent and activated ECs to fibrinolysis.

Summary. The first part of this two part series has focused on the development of the vasculature and the physiological functions of the endothelium as a gate-keeper regulating blood flow and hemostasis. Current insights into the generally unappreciated heterogeneity of endothelium from different vascular sites have been noted as potential discrepancies between the quiescent state of the endothelium in vivo and the behavior of these cells in culture. The second part of this review will concentrate on the mechanism by which the endothelium contributes to cell trafficking and the impact of endothelial injury on the development of several common human vascular disorders.

PART II

The endothelium, positioned at the interface between blood and tissue, is equipped to respond quickly to local changes in biological needs caused by trauma or inflammation. In the first part of this review, the capacity of the endothelium to move rapidly between an antithrombotic and prothrombotic state was discussed. In this second part, the mechanisms by which the endothelium regulates the trafficking of the cellular elements of the blood will be considered first, after which the impact of EC dysfunction on the pathogenesis of several common vascular disorders will be reviewed.

INTERACTION BETWEEN ECs AND BLOOD CELLS

In addition to the above-mentioned contribution of the endothelium to regulating blood coagulation, ECs also express cell surface-molecules that orchestrate the trafficking of circulating blood cells. These cell-associated molecules help direct the migration of leukocytes into specific organs under physiologic conditions and accelerate migration towards sites of inflammation, eg, in response to IL-6¹⁹⁶ or IL-8,¹⁹⁷ among many others. Recently, these pathways have also been implicated in the adhesion of platelets and erythrocytes in several common disorders associated with vascular occlusion.

Interactions of platelets and leukocytes with the vessel wall. Flowing leukocytes and platelets may adhere to specific regions of the endothelium, to exposed subendothelial components, or to each other during the process of immune surveillance as well as in response to tissue injury or infection. These multicellular interactions are essential precursors of physiologic inflammation and hemostasis. Conversely, uncontrolled adhesion of leukocytes and platelets contributes to inflammatory and thrombotic disorders. Under shear forces, both platelets and leukocytes interact with vessel surfaces through a multistep process that includes (1) initial formation of usually reversible attachments; (2) activation of the attached cells; (3) development of stronger, shear-resistant adhesion; and (4) spreading, emigration, and other sequelae (Fig 3).

Platelet adhesion during hemostasis. Circulating platelets normally do not interact with the EC surface (see Schafer¹⁹⁸ for review), in part due to the release of PGI₂, the release of NO, and the recently described expression of an Ecto-ADPase (CD39).¹⁹⁹ However, during hemorrhage, platelets adhere avidly to exposed subendothelial components, where they are rapidly activated. Circulating platelets interact with the adherent platelets, producing a hemostatic plug that promotes thrombin generation and development of a stable fibrin clot. Platelets adhere particularly efficiently to the subendothelium under high shear stress, accounting for the greater number of platelets in fibrin clots within arteries compared with those within veins.²⁰⁰

Under the influence of arterial shear stress, unactivated platelets attach first to the subendothelium through interactions of the platelet glycoprotein (GP) Ib-IX-V complex with immobilized vWF, a large, multimeric protein with binding sites for several other molecules, including subendothelial collagen.²⁰¹ The GPIb-IX-V complex consists of four proteins, each with one or more leucine-rich repeats: the disulfide-linked GPIb α and GPIb β and the noncovalently associated GPIX and GPV. The binding site for vWF is located on GPIb α , between the amino-terminal leucine-rich repeats and the membrane-

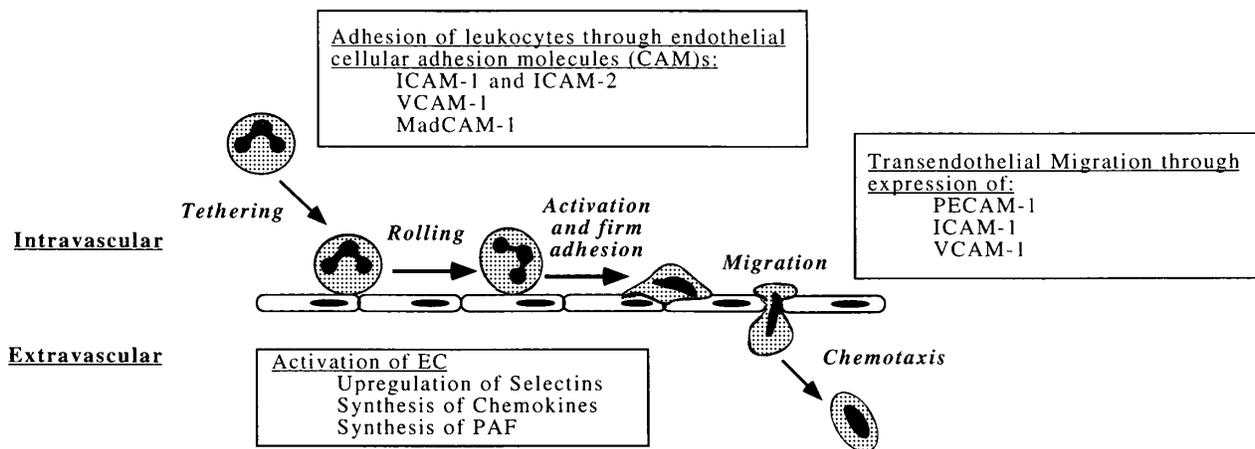


Fig 3. Physiologic interaction of leukocytes with the endothelium. Leukocyte adhesion and transmigration occurs during inflammation, usually at the postcapillary venules where shear stress is lowest.

proximal O-glycan-rich domain. The region includes clustered tyrosines that must be sulfated for GPIIb α to bind vWF.²⁰² GPIIb α binds weakly to plasma vWF, but with high activity to immobilized vWF under conditions of high shear stress, which may favor binding by altering the conformation of GPIIb α and/or vWF.^{200,203} Flowing platelets attach transiently to vWF, resulting in continuous movement of the cells along the surface.²⁰⁴ Such cellular interactions require very fast molecular rates for attachment and detachment; the fast dissociation rates are not significantly accelerated by shear stresses for detachment. Under the lower shear stresses found in veins, unactivated platelets use the integrin $\alpha_{IIb}\beta_3$ to attach to and immediately arrest on immobilized fibrinogen.²⁰⁴ Under low shear conditions, platelets may also use integrins or other molecules to attach to subendothelial matrix components, such as fibronectin, laminin, and thrombospondin.²⁰⁵

Once platelets adhere to either vWF or fibrinogen, they are activated by secreted products such as ADP or epinephrine or by surface molecules, such as collagen, that cross-link the integrin $\alpha_2\beta_1$ and other platelet receptors. The activated platelets spread and adhere more avidly to the subendothelial surface, principally through binding of activated $\alpha_{IIb}\beta_3$ to fibrinogen, which recruits additional platelets into aggregates²⁰⁶; platelet $\alpha_{IIb}\beta_3$ also binds to a distinct site on vWF.²⁰¹ Shear-resistant adhesion may be further enhanced by interactions of other integrins or receptors with laminin, fibronectin, and thrombospondin.²⁰⁰ As thrombin is generated, converting bound fibrinogen to fibrin, the aggregated platelets contract to strengthen the clot. Signaling through adhesion receptors, particularly integrins, may regulate the cytoskeletal-protein redistributions required for clot retraction.²⁰⁶ Increased bleeding is observed in patients with inherited defects in molecules that mediate platelet adhesion such as Bernard-Soulier disease (absence/dysfunction of GPIIb-IX-V) and Glanzmann's thrombasthenia (absence/dysfunction of $\alpha_{IIb}\beta_3$), confirming their physiological function.²⁰³

Leukocyte adhesion during inflammation. During inflammation, leukocytes tether to and roll on the EC surface. The cells then arrest, spread, and finally emigrate between ECs to reach the underlying tissues. Unlike platelets, which typically attach

to the subendothelium of arteries under high shear stresses, leukocytes usually attach to the ECs, where shear stresses are lowest, in the lining postcapillary venules.

In most circumstances, interactions with selectins, transmembrane glycoproteins that recognize cell-surface carbohydrate ligands found on leukocytes, initiate and mediate tethering and rolling of leukocytes on the EC surface.²⁰⁷ Selectins constitute a family of three known molecules, each of which has an amino-terminal Ca^{2+} -dependent lectin domain, an EGF domain, a series of short consensus repeats, a transmembrane domain, and a cytoplasmic tail. L-selectin is expressed on most leukocytes and binds to ligands constitutively expressed on high endothelial venules of lymphoid tissues, to ligands induced on endothelium at sites of inflammation, and to ligands exposed on other leukocytes. E-selectin is expressed on activated ECs and leukocytes. P-selectin is rapidly redistributed from secretory granules to the surface of platelets and ECs stimulated with thrombin or other secretagogues. Like E-selectin, P-selectin binds to ligands on leukocytes. Leukocytes adherent to the endothelium can make contact with flowing leukocytes through the L-selectin molecule, resulting in amplification of leukocyte recruitment to sites of inflammation.²⁰⁸ At sites of hemorrhage, leukocytes tether to and roll on adherent platelets.²⁰⁹ Monocytes recruited in this manner may augment fibrin generation, perhaps by elaborating tissue factor after their activation.²¹⁰ Selectin ligands expressed on high endothelial venules also mediate rolling of activated platelets and enhance accumulation of lymphocytes in lymph nodes.²¹¹ Thus, selectins initiate inflammatory, immune, and hemostatic responses by promoting transient multicellular interactions under conditions of shear stress.

The selectins bind weakly to sialylated and fucosylated oligosaccharides, such as sialyl Lewis x, a terminal component of glycans attached to many proteins and lipids on most leukocytes and some ECs. Strikingly, the selectins bind with higher affinity to only a few sialylated and fucosylated glycoproteins on target cells.²⁰⁷ E-selectin binds preferentially to ESL-1, a protein with at most five N-glycans and no described O-glycans. L-selectin and P-selectin bind preferentially to sialomucins whose recognition requires sulfation as well as sialylation

and fucosylation. The sulfate esters are attached to O-glycans on GlyCAM-1, a ligand for L-selectin secreted by high endothelial venules.²¹² In contrast, the sulfate esters are attached to tyrosines near the amino terminus of PSGL-1, a ligand for selectins on leukocytes.²¹³ Construction of some glycans may be restricted to specific sites on the polypeptide backbone of only a few proteins.²¹⁴ Of the described glycoprotein ligands for selectins, only PSGL-1, a ligand for selectins on leukocytes, has been shown to mediate cell-cell interactions under shear conditions (reviewed in McEver and Cummings²¹⁵). The $\alpha_4\beta_1$ and $\alpha_4\beta_7$ integrins, which are expressed on mononuclear cells and eosinophils, but not on neutrophils, also mediate tethering and rolling and occasionally arrest the flow of leukocytes on ECs by binding to the Ig-like ligands VCAM-1 and MAdCAM-1.^{216,217} Some lymphocytes use CD44 to roll on hyaluronate-bearing surfaces.²¹⁸

Under hydrodynamic flow, cell tethering and rolling requires bonds with sufficient mechanical strength between adhesion molecules and rapid rates of association and dissociation.²¹⁹ Interestingly, attachment of leukocytes through selectins requires a threshold hydrodynamic shear force that may prevent leukocyte aggregation in regions of low flow.²²⁰ A higher shear threshold for L-selectin may reflect faster dissociation rates of L-selectin ligand bonds²²⁰ and/or adhesion-induced shedding of L-selectin from the cell surface.²²¹ Because L-selectin, PSGL-1, and α_4 integrins are concentrated on the tips of the leukocyte microvilli, the probability of rapid contact with PSGL-1 is increased and the repulsion is minimized between the charged glycocalyxes of apposing cells.²²²

The slow velocities of rolling leukocytes favor encounters with chemokines or lipid autacoids presented at or near the apical surface of the endothelium. These mediators transduce signals that cooperate with those produced by engagement of L-selectin or PSGL-1 to activate the leukocytes.²²³ This crucial activation event, coupled with the slow rolling velocities, enables the β_2 integrins on leukocytes to bind to Ig-like ligands such as ICAM-1 and ICAM-2 on the EC surfaces.²²⁴ Plasma fibrinogen also links leukocytes to the endothelium by binding simultaneously to $\alpha_M\beta_2$ and ICAM-1,²²⁵ two integrins on the vessel wall that provide shear-resistant attachments. Subsequently, leukocytes migrate between ECs into tissues by mechanisms that are not completely understood but are affected by gradients of chemokines with restricted specificities,⁴⁹ β_1 and β_2 integrin activation states, and homotypic interactions with the Ig-like receptor, PECAM-1.²²⁶ This may require disruption of homotypic interaction of cadherins at endothelial tight junctions.²²⁷

Leukocyte recruitment to lymphoid tissues or inflammatory sites requires the coordinated expression of specific combinations of adhesion and signaling molecules. Diversity at each step of the multistep cascade ensures that the appropriate leukocytes accumulate for a restricted period in response to a specific challenge.^{49,224} Absence of P-selectin delays fatty streak formation in mice predisposed to developing atherosclerotic lesions.²²⁸ On the other hand, increased numbers of infections are observed in patients who are congenitally deficient in β_2 integrins²²⁹ or in fucosylated ligands for selectins,²³⁰ confirming the physiologic significance of these molecules in immune and inflammatory responses. Increased susceptibility to infection

combined with impaired leukocyte accumulation in mice rendered genetically deficient in selectins,²³¹⁻²³⁴ in fucosyltransferases,²³⁵ in ICAM-1,²³⁶⁻²³⁹ or in α_4 integrins²⁴⁰ further support the overlapping functions of these molecules.

Endothelium in cell-mediated immunity (CMI). CMI is defined as the protective set of immune reactions that can be adoptively transferred from a sensitized individual to an unimmunized host by a subset of T lymphocytes but not by antibodies. Vascular ECs may play two important roles in the evolution of CMI reactions: (1) antigen presentation to T cells (reviewed in Pober et al²⁴¹) and (2) recruitment of inflammatory cells.⁴⁹ Recall responses such as CMI reactions develop directly in peripheral tissues in which circulating memory T cells are activated by antigen presented on the surface of a resident cell population. This is in contrast to primary immunity that begins in the secondary lymphoid organs such as lymph node or spleen, where naive T cells encounter antigen on the surface of a specialized antigen-presenting cell. Once effector and memory cells develop in a secondary lymphoid organ, they may emigrate to the peripheral site via the blood stream, where reactivation by the antigenic stimulus is possible.

The two cell types that may present antigen to specific T cells in peripheral tissues are macrophages, resident in the tissues, or local microvascular ECs. In vitro, cultured human ECs from a variety of vascular beds constitutively express class I MHC molecules (used to present peptides derived from foreign proteins to CD8⁺ T cells). IFN- γ can induce ECs to express class II MHC molecules (used to present peptides derived from foreign proteins to CD4⁺ T cells). There is only limited information on antigen processing by cultured ECs, but indirect evidence (ie, the formation of functional peptide-MHC molecule complexes that can be recognized by cultured T-cell lines) suggests that ECs are fully competent to perform this function. In vivo, microvascular ECs constitutively express both class I and class II MHC molecules, although the levels of both molecules can be increased further by cytokines (eg, IFN- γ).

How do ECs compare with tissue macrophages as antigen-presenting cells? This has been a difficult question to address experimentally in humans because the relevant cell populations (ie, ECs, macrophages, and T cells) are not readily isolated from one immunized individual. A commonly used indirect approach is to examine the response of T cells isolated from one donor to cultured ECs and monocytes isolated from a second donor. This allogeneic response of T cells is directed against complexes formed between peptides associated with allogeneic MHC molecules and results from a cross-reaction of T cells that are specific for a foreign peptide associated with a self MHC molecule; it is an excellent model of normal immunity that avoids the requirement for isolating ECs from immunized donors. ECs activate about one fifth as many allogeneic T cells (either CD8⁺ or CD4⁺) as monocytes, determined in limiting dilution analyses for production of IL-2. Some of this difference may arise from a larger number of different peptide-MHC molecule complexes displayed on freshly isolated monocytes compared with serially cultured ECs, but a critical contribution to this difference is that monocytes can activate both naive and memory T cells, whereas ECs can only activate memory T cells.²⁴² It has recently been reported that ECs actually make naive T cells unresponsive to stimulation (ie, induce clonal

nergy).²⁴³ The ability of ECs to activate memory but not naive T cells in vitro is consistent with a role in presentation of antigen as an initiating event in CMI, which is a memory T-cell response.

The differences in ability among cell types to activate resting naive or memory T cells are best explained by differences in expression of cell surface ligands, called costimulators, that provide antigen-independent signals that complement those provided by T-cell antigen-dependent recognition.²⁴¹ Human ECs primarily provide costimulation to T cells through LFA-3 (CD58), which interacts with T-cell CD2. Monocytes and other professional antigen-presenting cells additionally provide costimulation to T cells through B7.1 (CD80) or B7.2 (CD86), which interact with T-cell CD28. Surprisingly, pig ECs express B7.2 and, more remarkably, pig B7.2 can functionally costimulate human T cells through CD28, a potential problem facing those who wish to use pigs as organ donors in human transplantation.²⁴⁴

The recruitment of inflammatory cells is the second role played by ECs in CMI. Once memory T cells become activated by antigens, they, in turn, can activate a variety of EC functions that contribute to recruitment of inflammatory cells. The signals provided by T cells to activate ECs may involve contact-dependent signals (eg, T-cell CD40 ligand may engage EC CD40)²⁴⁵ or cytokines (eg, T-cell–secreted TNF/LT, IFN- γ , or IL-4 may act on the EC; reviewed in Pober and Cotran²⁴⁶). Several different responses of ECs to these signals contribute to inflammation, including production of vasodilators to increase delivery of leukocytes to the tissue, expression of adhesion molecules that tether and bind circulating leukocytes, synthesis of chemokines that contribute to transendothelial migration, and leakage of plasma proteins that form a provisional matrix in tissues for migration of extravasated leukocytes (reviewed in Pober and Cotran²⁴⁷).

The expression of various adhesion molecules on the endothelial surface changes over time, favoring neutrophil recruitment initially (eg, dependent on E-selectin expression) and recruitment of other leukocytes at later times (eg, dependent on VCAM-1 expression). The identity of chemokines may also change over time from synthesis of neutrophil-activating C-X-C chemokines early on to the subsequent synthesis of C-C chemokines that act on other leukocytes. The net result is that the composition of infiltrates change over time from neutrophil-rich to T-cell–rich and monocyte-rich delayed hypersensitivity (DTH) reactions or to T-cell–rich, eosinophil-rich, and basophil-rich late-phase reactions. The differences between DTH and late-phase inflammation appear to be attributable to local production of IFN- γ versus IL-4 and IL-5, respectively. The T cells recruited in DTH reactions are predominantly Th1-like cells (that mediate DTH), whereas those in late-phase reactions are predominantly Th2-like cells (that mediate late-phase reactions). The selective recruitment of Th1 cells into DTH reactions may be mediated by E-selectins and P-selectins.²⁴⁸ Interestingly, although Th1 and Th2 cells appear to express equivalent levels of PSGL-1, only Th1 cells are able to bind P-selectin.²⁴⁹

Once infiltrates develop, a cytokine-rich milieu is generated that is sustained until the antigen is eliminated. Such chronic cytokine exposure has effects on ECs not seen at early times.

For example, over the first few days, adhesion molecules that are initially expressed diffusely on the luminal surface redistribute to inter-EC junctions.²⁵⁰ The basement membrane becomes enriched in sulfated glycosaminoglycans,²⁵¹ and the cells assume an altered morphology characteristic of endothelium at sites of high lymphocyte extravasation, such as the high endothelial venules of lymph nodes.²⁵² These features may promote leukocyte extravasation in acute settings. More chronic CMI reactions result in angiogenesis and tissue remodeling.

In general, CMI reactions do not produce endothelial injury, perhaps because T cells efficiently focus the response on the source of antigen, microbe-infected cells. An exception may be instances in which ECs are themselves infected by intracellular microbes (eg, viruses), so that cytolytic T lymphocytes (CTLs) kill the infected endothelium. Endothelial injury may also develop in transplantation, in which the immune system may perceive engrafted cells as self-cells that have been infected by virus. Some of the peptides recognized by graft-rejecting CTLs in association with allogeneic MHC molecules may be EC-specific and not found on leukocytes.²⁵³ In these instances, the CTL response may be directed at the endothelium. In addition, endothelium may be killed when CTL or natural killer (NK) cells are overstimulated by cytokines and lose their specificity. The actions of such lymphokine-activated killer (LAK) cells may contribute to the vascular leak syndrome associated with IL-2 or LAK therapy in cancer patients.²⁵⁴ It is increasingly appreciated that ECs may actively resist immune-mediated injury and that several of the resistance mechanisms involve cytokine-inducible genes.²⁵⁵ Thus, the CMI response itself may protect ECs if the onset is sufficiently gradual (or if it is delayed by immunosuppression), so that the cells have had adequate time to acquire the resistant phenotype.

Erythrocyte-endothelial adherence. Interactions between the endothelium and erythrocytes may contribute to the vascular complications of sickle cell anemia (SSA),²⁵⁶ infection with *Plasmodium falciparum* malaria,²⁵⁷ and diabetes.²⁵⁸ Red blood cell (RBC) adherence may initiate or promote intravascular sludging and occlusion leading to ischemic tissue and organ damage, retinopathy, dermal ulcers, strokes, and other infarctive pathologies. RBC adherence is dependent on EC surface molecules and is modulated by local hemodynamic factors. Recently, some of the RBC receptors, EC adhesion molecules, cytokines, and other vaso-active substances involved in adherence have been identified.

Sickle cell anemia. Although the tendency for hemoglobin SS to polymerize at low oxygen tension is assumed to be the dominant factor in the pathogenesis of occlusive pain episodes, morphologic evidence of sickling is not seen immediately after hemoglobin is deoxygenated.²⁵⁹ Rather, adherence of SS-RBCs to vascular endothelium, which retards transit through the microvasculature, may be an important initiating event in this cascade.^{260,261} Adherence of SS-RBCs in vitro is sufficiently strong to withstand fluid shear forces typical of those seen in postcapillary venules.²⁶² The resultant delay in capillary transit may allow time for sickle cells containing deoxygenated hemoglobin to deform leading to stable vascular obstruction²⁶³ and the resultant development of painful crises.

Adherence of SS-RBCs appears to result not only from the intrinsic membrane abnormalities induced in the erythrocytes,

but also as a result of specific plasma factors and the state of EC activation (reviewed in Wick and Eckman²⁶⁴). For example, plasma from patients with SSA promotes RBC adherence in excess of that seen with normal plasma,^{265,266} and plasma collected during painful crises promotes adherence to an even greater extent.²⁶⁵ Fibrinogen,²⁶⁵ fibronectin,²⁶² vWF,²⁶² and thrombospondin^{267,268} have all been identified as factors in plasma that modulate SS-RBC adhesion.

Several adherence pathways have been described in vitro, including (1) bridging of GPIIb-like molecules on sickle cells with their cognate receptors on ECs by unusually large vWF multimers released from activated platelets or by the stimulated endothelium itself²⁶²; (2) bridging by thrombospondin via CD36 on sickle reticulocytes²⁶⁹ and the $\alpha_v\beta_3$ integrin on large vessel ECs²⁶⁷ or $\alpha_v\beta_3$ and CD36 on microvascular endothelium²⁶⁸; (3) binding of sickle reticulocytes via $\alpha_4\beta_1$ receptors^{269,270} to VCAM-1 expressed on ECs stimulated by cytokines^{270,271} or by double-stranded RNA²⁷²; (4) binding of sickle reticulocyte via $\alpha_4\beta_1$ activated by phorbol ester or IL-8 to EC-associated fibronectin²⁷³; and (5) binding of SS-RBCs to E-selectin expressed on ECs stimulated by IL-1 β .²⁷⁴ The expression of VLA-4 and CD36 on reticulocytes from sickle cell patients is reduced by treatment with hydroxyurea.²⁷⁵ However, the precise contribution of each of these or additional pathways to SS-RBC adherence in vivo requires further study.

In vitro, adherence of sickled RBCs to large venous vessels differs both qualitatively and quantitatively from adherence to microvascular endothelium.²⁷⁶ High molecular weight vWF multimers promote greater adherence to venous than to microvascular endothelium.²⁷⁶ Autologous plasma promotes greater adherence of sickle RBCs to microvascular endothelium than does plasma from individuals without SS disease.²⁷⁶ Sickle cell adherence is localized to postcapillary venules in ex vivo tissue perfusion studies, with no adherence observed in either capillaries or arterioles.²⁶³ These differences are likely due, at least in part, to variation in the expression of adhesion molecules and their receptors on the vasculature (see, eg, Swerlick et al²⁷⁷).

Sickle cell adherence is also dependent on local hemodynamic conditions (reviewed in Wick and Eckman²⁶⁴). Under static conditions, the dense SS-RBCs adhere most avidly, possibly due to intrinsic membrane alterations.²⁶¹ In contrast, the least-dense sickle cells and reticulocytes are most adherent to the endothelium in vitro^{270,273,274} and ex vivo under flow conditions.²³⁰ Sickle cell adherence under flow is also more tenacious than under static conditions.²⁷⁸ Thus, adherence of reticulocytes expressing adhesion receptors may dominate in vivo in situations in which flow is maintained.²⁶⁴ Adherence and trapping of membrane-damaged sickled erythrocytes may follow once flow has been further impeded, ultimately leading to complete vascular occlusion.²⁷⁹

Painful crises frequently accompany ischemia, infarction, infection, or inflammation, situations in which the coagulation cascade may be activated as well.²⁵⁶ Activation of leukocytes and/or platelets may result in the generation and release of cytokines, adhesive proteins, or other factors that modulate endothelial or sickle cell adhesivity. Observations that activation of sickle cells²⁷³ and ECs with cytokines,²⁷⁰ virus,²⁷² or thrombogenic plasma proteins^{262,265,267,268} promotes RBC adherence suggests a mechanism by which infection and inflamma-

tion may initiate or propagate vaso-occlusion and episodic pain. Presumably, sickle cell adherence in vivo is most extensive at sites where the relevant adherence molecules are expressed most highly and shear stresses are sufficiently low to permit binding.²⁶⁴ Adherence of sickle cells may alter their metabolism,²⁸⁰ promote leukocyte adhesion,²⁸¹ and contribute to their desquamation.²⁸¹

Malaria. *Plasmodium falciparum* causes cerebral manifestations, perhaps the most serious complication of malaria. Maturation of the parasite within host RBCs induces membrane changes which promote adherence to cerebral microvascular endothelium in vitro and may lead to vascular congestion and hypoxia in vivo.²⁵⁷ It has been proposed that cytoadherence provides selective advantage to the invading parasites by facilitating their growth under the conditions of reduced oxygen tension found in the cerebral microcirculation and by enabling parasitized RBCs to avoid splenic filtration.²⁵⁷

Parasitized RBCs bind to cell surface molecules on ECs, including CD36,²⁵⁷ ICAM-1,²⁵⁷ VCAM-1,²⁸² and E-selectin.²⁸² Additionally, thrombospondin allows bridging between parasitized RBCs and CD36 receptors on ECs.²⁵⁷ The various endothelial receptors act synergistically to slow and arrest parasitized RBCs under flow conditions and at shear stresses in the physiological range.^{283,284} Additional binding sites for parasitized RBCs may be induced as a consequence of the inflammatory response to *Plasmodium falciparum* infection²⁵⁷ due to leukocyte activation,²⁸⁵ cytokine release,²⁸⁶ and EC activation.²⁸⁵

Binding to the endothelium occurs through knobs on RBCs induced by the parasite. These knobs contain *Plasmodium falciparum* erythrocyte membrane protein 1 that appears to participate in this process.²⁵⁷ Cytoadherence is inhibited by peptide fragments of the erythrocyte band 3 protein²⁸⁷ and by monoclonal antibodies that recognize band 3 on RBCs infected with mature parasites, suggesting involvement of cryptic regions of the protein exposed or altered during the course of infection.²⁸⁸ It has also been reported that some field isolates of *Plasmodium falciparum* promote adherence to chondroitin sulfate A.²⁸⁹ These data provide additional opportunities for antimalarial therapy based on inhibition of interactions between parasitized RBCs and ECs.

Diabetes mellitus. Erythrocytes from patients with diabetes mellitus are more adherent to normal ECs than are RBCs from healthy donors.²⁹⁰ The extent of adhesion correlates with the severity of vascular complications²⁹⁰; greater adherence is observed in the absence of plasma,²⁶⁶ suggesting a defect intrinsic to the RBC. Adhesion is augmented further by plasma from diabetic patients as well as by fibrinogen,²⁵⁸ suggesting a mechanism by which acute-phase reactants may modulate vascular obstruction. Persistent exposure to hyperglycemia induces the formation of advanced glycation end products (AGEs) that modify structures on erythrocyte. AGE modification of erythrocytes allows them to engage a specific receptor, RAGE, (receptor for advanced glycation endproducts), which has been identified immunohistochemically and by in situ hybridization in the vasculature in vivo.²⁵⁸ Exposure of RBCs harvested from patients with diabetes to cultured endothelium results in increased adherence compared with those from euglycemic individuals due to erythrocyte-associated AGEs

binding to endothelial RAGE.²⁵⁸ Furthermore, it has been hypothesized that interaction of diabetic erythrocytes with endothelium through an AGE/RAGE linkage may promote oxidant stress leading to EC activation (see below).

EC PERTURBATION AND VASCULAR DISEASE

It is currently believed that endothelium must remain in a resting or unperturbed state to optimize expression of anticoagulant activities which prevent thrombus formation (see “The Role of the Endothelium in Coagulation” above). However, the endothelium is a dynamic organ that responds to an array of agonists and environmental challenges by undergoing an activation process not unlike that of platelets, which eventuates in the loss of anticoagulant properties and/or acquisition of procoagulant function. Although the role of the endothelium in the pathogenesis of thrombosis *in vivo* remains unproven, accumulating evidence points towards dysregulation of EC function as pivotal in the development of several important thrombotic disorders. Plasma factors such as antibodies or lipoproteins that perturb EC function *in vitro* have been identified. It is likely that genetic differences in EC responsiveness to environmental pressures will be uncovered as contributors to the development of other common vascular diseases.

The endothelium in atherosclerosis. Atherosclerosis is the most prevalent vascular disease in developed countries. The concept that atherosclerosis arises in response to endothelial injury was first proposed more than 20 years ago, when it was appreciated that irregularities in EC organization are often found overlying early fatty streaks, whereas overt endothelial denudation is seen only in the late stages of the disease (see Ross and Glomset²⁹¹ for review; Fig 4). There is now extensive evidence that this morphologically abnormal endothelium is also dysfunctional and actually contributes to the propagation of lesions (see Ross²⁹² and McGorisk and Treasure²⁹³ for reviews).

These findings not only provide insight into the pathogenesis of atherosclerosis, but also suggest means to monitor the progression of lesions and effectiveness of treatment.

Atherosclerosis is a multifactorial disease with numerous predisposing factors, including smoking, diabetes, hyperlipidemia, hypertension, mechanical stress, and inflammation. Such diverse and complex processes may perturb EC function through a common pathway. Alternatively, the endothelium may react to diverse stimuli with a limited repertoire of reparative, but ultimately dysfunctional, responses.

Oxidant stress. Oxidant stress has been proposed as a mechanism common to diverse injuries such as unsaturated lipids that can be converted to cytotoxic lipid peroxidation products, various chemicals, radiation, and reactive oxygen metabolites released by leukocytes that migrate into the vasculature in response to infection and autoimmune injury. The pathways involved in the initiation and control of oxidant injury are receiving considerable study. Oxidized LDL and its peroxide derivative lysophosphatidylcholine stimulate protein kinase C activity, phosphoinositide turnover, and release of internal calcium; impair EC replication and angiogenesis; and induce apoptosis (reviewed in Henry²⁹⁴). Cytokines, such as TNF- α , can both induce reactive oxygen species in ECs and stimulate the ubiquitous transcription factor NF- κ B, resulting in a transcriptional activation of other proatherogenic molecules such as VCAM-1 (reviewed in Larrick and Wright²⁹⁵). Oxidation reactions also promote the formation of AGEs that contribute to diabetic vasculopathy (reviewed in Schmidt et al²⁹⁶) and initiate transcriptional activation of VCAM-1²⁹⁷ and monocyte chemoattractant protein-1 (MCP-1), which promotes monocyte entry into the vessel wall (reviewed in Gimbrone²⁹⁸).

Shear stress. ECs are exposed continuously to fluid shear stresses that lead to a dynamic interaction between the cell and the substratum via focal contact sites. Shear-induced changes in

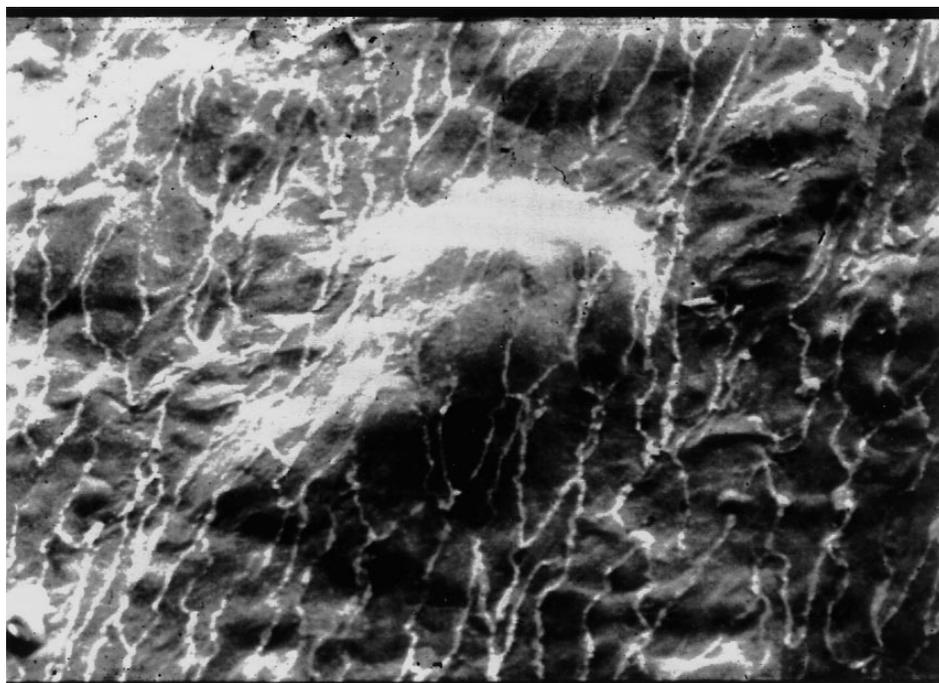


Fig 4. Very early development of atherosclerosis in a nonhuman primate. The focal origin of atherosclerosis is apparent beneath an intact endothelium. Scanning electron microscopy of ECs outlined by silver deposition in a thoracic aorta (photograph courtesy of Peter F. Davies, PhD, Institute for Medicine and Engineering, University of Pennsylvania, Philadelphia, PA).

transduced biomechanical forces can cause not only cytoskeletal rearrangement and altered morphology but changes in endothelial gene expression^{299,300} (Fig 5). Most studies have examined primarily changes that occur within hours of initiating flow, which may best reflect the situation in vascular beds exposed to newly flowing blood such as postangioplasty, but the adaptive response of endothelium to shear forces is less well characterized.

An effect of shear on vascular biology is suggested by the observation, eg, of decreased vasodilator function at coronary branch points that have a predilection for atherosclerosis.³⁰¹ Consistent with this notion, a number of genes relevant to the development of atherosclerosis expressed by ECs have shear stress response elements that coordinate their induction. Shear modulates EC production of products regulating vasoconstriction (NO, endothelin-1), vessel growth (β FGF, platelet derived growth factor [PDGF]-A and -B, and TGF- β), fibrinolysis (t-PA), and cell adhesion (MCP-1, VCAM-1, and ICAM-1) (see Malek and Izumo,³⁰⁰ Ando and Kamiya,³⁰² and Tsao et al³⁰³ for reviews). Shear has been reported to modulate the expression of thrombomodulin in a reversible manner²⁹⁹ and abrogate cytokine-induced EC tissue factor expression.³⁰⁴

The mechanism(s) responsible for the modulation of gene expression by shear is under study. At least part of these shear-induced effects are mediated through modulation of gene transcription. A number of genes, such as PDGF-B, contain one or more shear stress responsive elements (SSREs) that include an NF- κ B-responsive GAGACC promoter sequence³⁰⁵ in the 5' upstream region. However, the induction of TGF- β and MCP-1 appear to occur through alternative sites, eg, a TRE/AP-1-responsive element.³⁰⁶ Levels of transcription factors NF- κ B and AP-1 are increased in sheared EC.³⁰⁷ How shear-related transcription factors affect immediate and persistent gene transcription and how this inductive pathway differs from other injury-related responses remain to be elucidated.

Homocysteine. Homocysteine is a sulfhydryl amino acid formed during the conversion of methionine to cysteine. Elevated plasma levels of homocysteine may result from deficiencies of cystathionine- β -synthase, deficiencies of enzymes involved in the folate-dependent pathway of homocysteine remethylation, or deficiencies of folate or vitamin B12

themselves (see Guba et al³⁰⁸ and Rees and Rodgers³⁰⁹ for reviews). Homozygous deficiency of cystathionine β -synthase leads to markedly elevated plasma concentrations of homocysteine and is associated with premature atherosclerosis and arterial thrombosis. The results of several recent large prospective and case-controlled studies suggest that even modestly elevated levels of homocysteine may pose a risk factor for atherosclerosis as well as for arterial and venous thrombosis (reviewed in Mayer et al³¹⁰), although it is not clear that all pathways leading to hyperhomocysteinemia pose comparable risks. Several large prospective studies are underway to assess the magnitude of this risk.

These observations have led to the identification of several pathways by which homocysteine may affect the anticoagulant and procoagulant functions of cultured ECs (Table 2).³¹¹ It is important to consider that most studies to date have used cultured cells exposed for brief periods to concentrations of homocysteine that exceed that which is observed in vivo. Clearly, such concentrations of homocysteine induce formation of hydrogen peroxide³¹² and oxidized LDL³¹³ and may be directly cytotoxic for ECs; NO is at least partially protective.³¹⁴ However, induction of TF,³¹⁵ activation of factor V,³¹⁶ decreased binding of t-PA to annexin-II,³¹⁷ inhibition of thrombomodulin,³¹⁸ and reduced expression of heparan sulfate³¹⁹ and possibly PGI₂,³²⁰ among many other changes, have all been observed in ECs exposed to high concentrations of homocysteine. Homocysteine is also mitogenic for rat aortic smooth muscle cells.³²¹ That similar effects may occur in vivo is suggested by vascular dysfunction in monkeys with diet-induced hyperhomocysteinemia³²² and elevated levels of thrombomodulin and vWF in the plasma of homocysteinemic patients with peripheral arterial disease. Therapy with pyridoxine and folic acid led to rapid reductions in the levels of both markers.³²³ Studies in animal models in which moderately elevated levels of homocysteine is sustained may provide additional insight into the role of the endothelium and other pathogenic mechanisms of thrombosis and atherosclerosis.

Consequences of EC injury on endothelial-derived vasoactive factors. Healthy human epicardial coronary arteries dilate when acetylcholine is infused,³²⁴ whereas atherosclerotic arteries constrict³²⁵ due to impaired release of NO (see "Vasoregula-

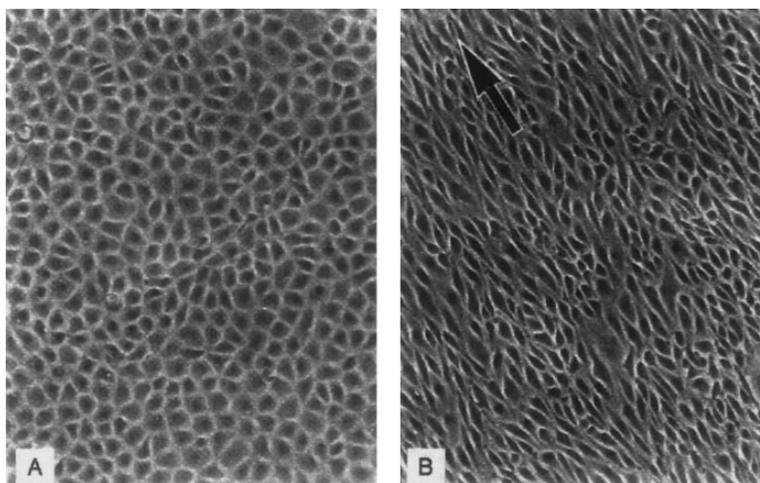


Fig 5. EC alignment by directional steady flow in vitro (photographs courtesy of Peter F. Davies, PhD, Institute for Medicine and Engineering, University of Pennsylvania, Philadelphia, PA). (A) Before exposure to shear stress (no flow). (B) Twenty-four hours after exposure to flow (shear stress, 10 dynes/cm²).

tion" above) and PGI₂ from the endothelium.³²⁶ Loss of vasodilatation in the absence of overt stenosis has been observed in patients with hypercholesterolemia,³²⁷ increased Lp(a),³²⁸ diabetes,³²⁹ homocystinuria,³³⁰ and possibly hypertension (reviewed in Woodman³³¹ and Heistad et al³³²). Similar effects have been observed with advancing age,³³³ exposure to cigarette smoke,^{330,334} and sedentary life style.³³⁵ Endothelial dysfunction is almost universal 12 to 24 months after cardiac transplantation.³³⁶ Indeed, abnormal vasodilator function may be a more sensitive marker of coronary artery disease in some settings than is angiography.³³⁷

The reduction in NO is attributable in part to lowered levels of eNOS in atherosclerotic vessels,³³⁸ but the reason for this is uncertain. One clue may be that impaired vasodilatation is especially marked at coronary branch points where flow and shear stress have been shown to stimulate NO production in healthy vessels.³⁰¹ In addition, free oxygen radicals and hydrogen peroxide generated by EC exposed to high levels of LDL may inactivate NO.³³⁹

Inhibition of NO activity accelerates atherosclerosis in animal models,³⁴⁰ whereas supplementation with L-arginine, the precursor of NO, diminishes lesion formation (reviewed in Cooke³⁴¹) and reverses endothelial dysfunction in otherwise healthy young humans with hypercholesterolemia.³⁴² The loss of NO may impact on multiple steps in the atherogenic process.³⁴¹ NO may also counteract the proatherogenic effects of endothelin (reviewed in Mathew et al³⁴³). Oxidized LDL binds to a newly described receptor on ECs³⁴⁴ and increases the production and secretion of ET in cultured ECs and in intact blood vessels.³⁴⁵ ET is also released by dysfunctional coronary arteries that constrict in response to acetylcholine.³⁴⁶ Plasma concentrations of ET are elevated in asymptomatic patients with hypercholesterolemia and increased plasma levels of both Lp(a)³⁴⁷ and ET have been reported to correlate with the severity of atherosclerosis.³⁴⁶

Monitoring the reversal of EC dysfunction. A significant reduction in cardiac events has been reported as a result of lipid lowering therapy, but the mechanism responsible for this benefit has not been elucidated. Little reduction in the cross-sectional area of preexisting lesions is seen, although progression of the lesions may be slowed³⁴⁸ and incidence of plaque rupture may be lessened. What is clear is that there is an improvement in various indirect measures of EC function in vivo possibly as a result of a more favorable profile of vasoactive agents that are produced at critical locations.³⁴⁹ Similar beneficial effects on surrogate markers of EC function have been seen with the use of antioxidants,³⁵⁰ whereas in other studies the acute administration of vitamin C has been associated with improved EC-dependent vasodilatation in chronic smokers³⁵¹ and patients with coronary artery disease.³⁵² Similar beneficial effects of angiotensin converting enzyme inhibitors have been confirmed in some³⁵³ but not in all³⁵⁴ animal models (reviewed in Lonn et al³⁵⁵).

Thus, with the advent of potential means to treat atherosclerosis, the need for reliable, noninvasive surrogate markers of risk and vascular function have become apparent. Several candidate molecules have emerged. Abnormalities in serotonin-induced arterial vasodilatation, a process dependent on NO, precede the development of clinical disease and resolve within 12 weeks of

the institution of cholesterol lowering therapy.³⁵⁶ Elevated plasma levels of PAI-1 and thrombomodulin revert towards normal as well.³⁵⁷ In contrast, little change has been seen in the elevated plasma levels of E-selectin, VCAM-1, and ICAM-1.³⁵⁸ There is also interest in measuring levels of 8-epi PGF_{2α}, an isoprostane with potent vasoconstrictor activity in the pulmonary and renal circulations generated through free radical catalyzed peroxidation of arachidonic acid by ECs and other vascular cells. Levels of 8-epi PGF_{2α} in the urine have been used as a marker of oxidant stress in vivo (reviewed in Morrow and Roberts³⁵⁹). Levels in smokers are elevated and fall with cessation of smoking or treatment with vitamin C but not with vitamin E or aspirin therapy.³⁶⁰

Perspective. Healthy ECs contribute to the prevention of atherosclerosis in medium to large arteries by inhibiting platelet activation, limiting the entry of cells and lipids into the vessel wall, maintaining a nonproliferative and biochemically quiescent intima,³⁶¹ and secreting products under appropriate stimuli that limit potentially injurious responses that occur as a byproduct of host response to injury. These self-protective mechanisms are impaired as a result of oxidant, chemical, and shear stress, while at the same time the biochemical profile of the endothelium changes in a way that promotes inflammatory and fibroproliferative responses. The role of ECs in preventing or limiting the effects of plaque rupture and terminal thrombosis is little understood. The injurious processes that initiate atherosclerosis appear to persist throughout life in most individuals. Studies are now being conducted to determine the extent to which the biochemical and functional changes in the vessel wall can be reversed at different stages of the disease.

Endothelial perturbation and vascular dysfunction in diabetes. Vascular dysfunction is a contributing factor in the etiology of several clinically important secondary complications of diabetes mellitus including retinopathy, accelerated atherosclerosis, microvascular disease, nephropathy, neuropathy, and impaired wound healing.^{362,363} The effects of hyperglycemia on EC function can be imparted through several pathways: (1) production of reactive oxygen intermediates; (2) direct activation of protein kinase C; (3) activation of the aldose reductase pathway resulting in an accumulation of sorbitol and diminished levels of myo-inositol; and (4) nonenzymatic glycoxylation of long-lived macromolecules.^{362,363} Because glycoxylation of proteins and lipids occurs ubiquitously in patients with diabetes and is irreversible, its consequences are especially relevant to long-term vascular dysfunction. Initially, exposure of free amino groups to reducing sugars, such as glucose, results in the formation of early glycation products, Schiff bases, and Amadori products. These are reversibly modified species, such as hemoglobin A1c, used for long-term monitoring of blood sugar in diabetic patients. Further molecular rearrangements occur, in part due to oxidation, resulting in irreversible AGEs (Fig 6). The latter have pathophysiologic relevance in that AGE-modified proteins may not function normally and/or may perturb cellular properties in a manner distinct from that of the native molecule. This occurs when the AGE form of the molecule binds to cellular receptors which recognize AGEs, including RAGE^{364,365} and the macrophage scavenger receptor.³⁶⁶ RAGE is expressed by endothelium, monocytes, and

Fig 6. A two-hit model of vascular perturbation. Stage 1 shows the interaction between AGEs, modified biochemical species and their receptors (RAGEs), transmembrane protein receptors of the Ig family present at low levels on a range of cells including ECs in addition to macrophages (Mfs), smooth muscle cells, and neurons. AGEs may perturb the normal physiologic function of the modified species and thus alter the EC's normal vascular functions. Stage 2 details subsequent perturbations resulting from the superimposed stimuli of accumulated lipoproteins as seen in atherosclerotic lesions, foreign materials that may be seen in wound repair, and bacterial infection that may be seen in periodontal disease.

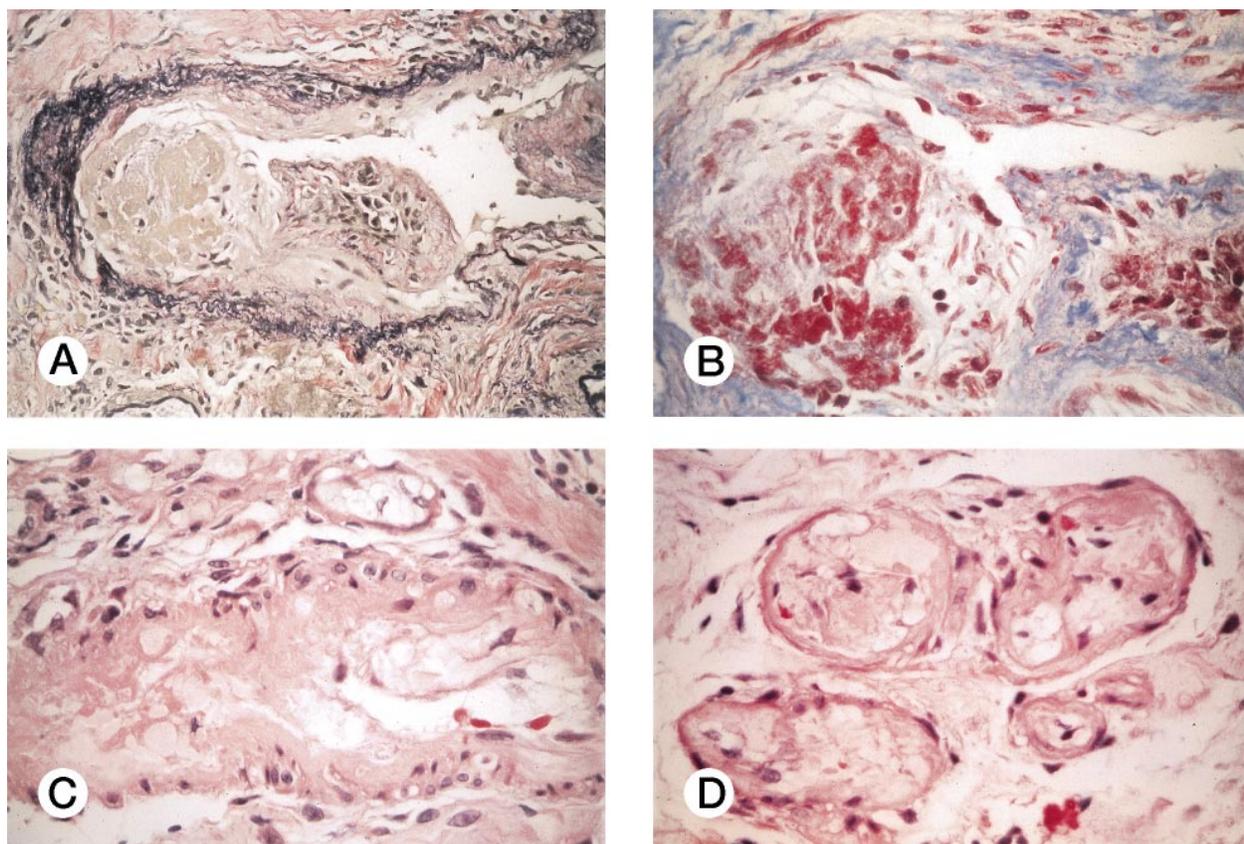
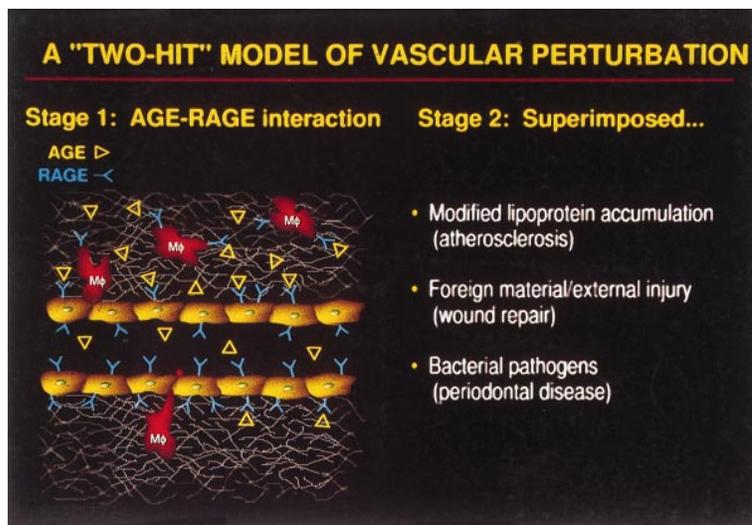


Fig 7. EC injury in a case of antiphospholipid antibody syndrome (photographs courtesy of Emma E. Furth, MD, Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA). EC injury seen in a duodenal biopsy from a 40-year-old woman who presented with profuse intestinal bleeding and was found to have a lupus anticoagulant and a markedly positive anticardiolipin antibody. (A) Elastic stain (original magnification $\times 200$) highlighting a fresh thrombus (right) with the beginning stages of organization and EC ingrowth, an older organized thrombus with fibroblast proliferation (center), within a vessel showing vacuolated, injured, and disrupted ECs. (B) A trichrome stain (original magnification $\times 400$) of the same field highlighting the thrombus material (red acellular material on the right) with early stages of organization. (C) A hematoxylin and eosin (H and E) stain (original magnification $\times 400$) showing fibrinoid intimal necrosis (right) in the absence of an inflammatory reaction within a small vessel in the same duodenal biopsy as shown in (A) and (B). The ECs show marked vacuolization (left). The surrounding eosinophilic vascular cells are smooth muscle cells surrounded by fibroblasts. (D) A higher power view (original magnification $\times 600$) of the same biopsy showing four capillaries with grossly vacuolated ECs and luminal effacement.

smooth muscle cells and is likely to play a major role in the development of vascular disease in diabetics.³⁶⁵

RAGE is a member of the Ig superfamily of cell surface molecules. It is composed of an extracellular domain with one V-type, followed by two C-type regions.^{364,365} There is a single transmembrane spanning domain and a short, highly charged cytosolic tail that likely transmits the signal of ligand occupancy by interacting cytosolic transduction molecules. The single RAGE gene is located on chromosome 6 in the major histocompatibility complex between genes for class II and class III molecules. This proximity of RAGE to genes contributing to the host response is in keeping with expression of the receptor. In mature animals, RAGE is present at low levels in a range of cells (endothelium, smooth muscle cells, mononuclear phagocytes, and neurons), but after perturbation, as in diabetes, immune/inflammatory disorders, or Alzheimer's disease, RAGE expression is dramatically upregulated. In diabetes, AGE-modified proteins appear to act as ligands for RAGE modulating a number of secondary messenger pathways. For example, AGE interaction with RAGE results in cellular oxidant stress eventuating in activation of p21ras, MAP kinases (erk's 1 and 2), and the transcription factor NF- κ B.³⁶⁷ Such activation of NF- κ B results from binding of p50/p65 heterodimers to DNA binding motifs, as in the gene for vascular cell adhesion molecule-1 (VCAM-1). RAGE-dependent enhanced VCAM-1 expression is observed both in cultured ECs and in vivo in mice infused with AGEs.³⁶⁸ Increased VCAM-1 levels are also observed in diabetic vasculature upon immunohistological analysis. In parallel with expression of VCAM-1 on the cell surface, ECs release a soluble form of VCAM-1 (sVCAM-1) into culture supernatants, potentially providing a means of monitoring cellular stress in vivo. Patients with diabetes and microalbuminuria, the latter considered a harbinger of impending future vascular complications, display higher plasma sVCAM-1 than those without microalbuminuria.³⁶⁹

AGE-RAGE interaction likely underlies vascular hyperpermeability, another salient feature of diabetic vasculopathy. Such hyperpermeability is blocked by anti-RAGE IgG or by preventing AGE binding by infusion of a soluble form of the extracellular domain of RAGE (the latter is termed sRAGE). Vascular leakage of solutes in diabetic animals can be largely blocked by infusion of sRAGE. These data identify a reversible component of diabetic vascular dysfunction and suggest that AGE-RAGE-induced cellular perturbation may be a contributor. The principal insights to be gained from analysis of RAGE binding to nonenzymatically glycosylated ligands is probably in the setting of chronic vascular perturbation.³⁶⁵ Because AGE modification of proteins is irreversible, AGEs accumulated in the vessel wall are present for extended periods of time. Thus, the diabetic vascular milieu has properties that distinguish it from that in euglycemic subjects. A two-hit model can be envisioned (Fig 6) in which tissue and blood AGEs interacting with RAGE provide a baseline state of vascular activation for the first-hit. The second stage comprises a superimposed stimulus, such as accumulated lipoproteins in atherosclerotic lesions, foreign material in wounds, and bacterial infection in periodontal disease. AGE-RAGE interaction provides a backdrop of chronic inflammation, with increased expression of proinflammatory cytokines, thrombogenic factors, cell adherence mol-

ecules, and vascular permeability, which aggravates and probably accelerates the development of vascular lesions.

Antibody-mediated EC injury: Solid organ transplantation. Transplantation of vascularized organs, such as kidney, heart, lung, and liver, has become the treatment of choice for end-stage organ failure. The key limitations on clinical transplantation today are rejection of allografts posttransplantation and the shortage of available donor organs. The EC lining of graft vessels plays a prominent role in both of these clinical problems.

Despite the enormous advances in clinical immunosuppression of transplant recipients that have been made in the last 50 years of practice, the principal cause of graft failure is still rejection, ie, immunological reactions of the host against graft cell alloantigens that injure and destroy the graft. ECs play three crucial roles in the process of graft rejection: (1) ECs stimulate the host immune system by presenting alloantigens in an immunogenic form to host lymphocytes, thereby helping to initiate graft rejection; (2) ECs respond to host stimuli, eg, inflammatory cytokines, to promote intragraft inflammation and thrombosis that contribute to graft injury; and (3) ECs lining graft vessels are primary cellular targets of the host antigraft response. In addition, graft ECs are sensors and mediators of antigen-independent injury to which the graft is subjected during harvest, transport, and implantation, a dramatic instance of ischemia reperfusion. The mechanisms by which ECs present antigens to lymphocytes, promote inflammation in response to cytokines, are injured by the immune response, and respond to oxidant injury have been discussed in previous sections of this review and will not be duplicated in this section. We will focus here on specific issues related to immunologic allograft rejection.

Rejection reactions are commonly classified according to the time when they occur after surgical transplantation and by their histopathologic features.³⁷⁰ Hyperacute rejection occurs within minutes to hours of perfusion of the graft by host blood, ie, in the perioperative period. It is characterized by extensive intravascular thrombosis of graft vessels and consequent graft ischemic infarction. Hyperacute rejection is mediated by host antigraft antibodies, usually IgM antibodies reactive with graft endothelial carbohydrate epitopes such as ABO blood groups, and by complement activation that is initiated by the antibodies bound to graft ECs. Thrombosis results from EC lysis and desquamation, exposing thrombogenic subendothelial basement membrane, or, in cases of sublytic quantities of complement deposition, by loss of endothelial antithrombotic mechanisms (eg, shedding of cell surface anticoagulant heparan sulfate) combined with activation of endothelial prothrombotic mechanisms (eg, release of stored high molecular weight vWF, release of lipid procoagulants, and possibly induction of TF).³⁷¹ Matching of donors and recipients for ABO blood groups has significantly reduced the incidence of hyperacute rejection in allografts.

Acute rejection reactions usually develop between the first and second week after transplantation. Although a variety of effector mechanisms may be involved, recent studies of human allograft biopsies have emphasized the primary role of cytolytic cells, principally CTLs.^{372,373} Some host CTLs recovered from rejecting grafts have shown specificity for graft ECs over graft

leukocytes.³⁷⁴ Because the specificity of CTL is determined by the antigens involved in their induction (ie, the same antigen receptor mediates initial differentiation and subsequent effector function of CTL), graft ECs must have played a role in stimulating host CTL development by presenting antigen to precursor cells, ie, to resting CD8⁺ T cells recruited from the circulation into the graft. Lysis of microvascular ECs is a prominent and early component of acute cell-mediated rejection.³⁷⁵ More severe rejection reactions typically involve injury of larger graft vessels as well. Such vascular rejection is believed to start as a host CTL reaction against graft arterial or arteriolar ECs (called endothelialitis or intimal arteritis at this early stage³⁷³) and may progress to severe necrotizing, transmural vasculitis. The most severe vascular rejection reactions appear to involve host antigraft antibodies (both IgG and IgM) as well as cytolytic lymphocytes. Presensitized hosts (eg, resulting from a prior transplant procedure) who have expanded numbers of memory T or B cells reactive with the donor may show accelerated acute rejection during the first few days after transplantation³⁷⁰). These accelerated rejection reactions are similar to experimental second set rejection seen in animal models of retransplantation. The use of tissue typing (for renal transplantation) and of improved immunosuppression (especially since the introduction of cyclosporin A) have reduced the incidence of graft loss due to acute and/or accelerated rejection to fewer than 10% of organs. ECs are considered prime targets for further improvements in immunosuppression, eg, by targeting adhesion molecules such as ICAM-1 to reduce posttransplant ischemia-reperfusion, inflammation, and CTL effector functions.

With current advances in controlling acute rejection, the major cause of graft loss has become chronic rejection.³⁷⁶ Chronic rejection appears in biopsies as replacement fibrosis of graft parenchyma, developing over months to years. These changes are widely thought, at least in cardiac and renal transplantation, to be secondary to graft ischemia caused by progressive occlusion of the lumen of graft arteries (called graft arteriosclerosis). Graft arteriosclerosis is characterized by concentric, diffuse intimal hyperplasia of large, medium, and small graft arteries. These changes are accelerated compared with atherosclerosis in that they can develop into clinically significant lesions as early as 6 months to a few years posttransplantation. Despite all of the advances in immunosuppression for acute rejection, the incidence of allograft failure to chronic rejection has remained at about 10% of grafts per year, with no evidence of improvement.

Graft arteriosclerosis is restricted to graft vessels, ie, it completely spares the host's vessels. Involved graft arteries contain increased numbers of intimal smooth muscle cells and deposition of extracellular matrix accompanied (or preceded) by a sparse subendothelial infiltrate of host T cells and macrophages.³⁷⁷ Cytolytic effector cells are markedly fewer than in acute vascular rejection,³⁷⁸ and the endothelium shows only rare apoptotic cells.³⁷⁹ The major theories of pathogenesis are that graft arteriosclerosis results from chronic, low-level endothelial injury (ie, persistent endothelialitis caused by CTLs and/or alloantibodies), followed by fibroproliferative repair, or that graft arteriosclerosis results from a conversion of an acute cytolytic immune response to a chronic delayed-type hypersen-

sitivity reaction in response to persistent immune stimulation by graft ECs. There is currently no therapy for graft arteriosclerosis except retransplantation. Future therapy may be targeted at preventing the intimal smooth muscle fibroplastic reaction rather than further increases in immunosuppression.

The next horizon in transplantation is to address the donor organ shortage by xenotransplantation of animal organs (eg, pigs) into human recipients.³⁸⁰ The use of pig organs has raised concerns about introducing new infectious agents into the human population, but the major practical problem is a very high incidence of hyperacute rejection. Two factors contribute to development of severe, uncontrollable hyperacute rejection of pig xenografts by human or old world monkey recipients. First, all mammals, except humans and old world monkeys, express a galactose α -1,3 galactose carbohydrate epitope instead of ABO blood groups on their ECs.³⁸¹ Moreover, virtually all humans have high levels of circulating natural IgM antibodies reactive with this alternative epitope, so that ABO matching cannot be used to evade hyperacute rejection in xenotransplantation. Second, the problem of abundant natural antibody is compounded by the fact that pig ECs express complement regulatory proteins, eg, DAF and CD59, that are unable to control the human complement system, ie, they are species specific for pig complement proteins.³⁸² The combination of high levels of complement-activating IgM antibodies and limited EC resistance to human (or primates) complement proteins invariably leads to rapid and overwhelming intravascular graft thrombosis.

A major current effort is underway to produce transgenic pigs that will have reduced levels of galactose α -1,3 galactose and/or express human complement regulatory proteins. If successful, transplanters will still need to address later phases of the human-antipig rejection reaction (eg, potentially strong acute cellular rejection due to expression B7.2 costimulator molecules on pig ECs³⁸³ or to strong NK reactions³⁸⁴ due to possibly antibody binding and absence of self class I MHC molecules on pig ECs). Some of these problems may be partly ameliorated by the failure of pigs to respond to certain human cytokines (eg, to IFN- γ ³⁸⁵), but additional studies will be needed to determine the significance of these differences.

Systemic lupus erythematosus (SLE) and the antiphospholipid antibody syndrome (aPS). Immune-mediated endothelial dysfunction may contribute to the development of thrombosis in patients with SLE and the aPS (see McCrae and Cines³⁸⁶ for review). One mechanism by which endothelial damage and/or activation may occur is through the effects of EC-reactive antibodies. Several groups have demonstrated anti-EC antibodies (AECA) in sera of patients with SLE³⁸⁷ and in patients with primary and secondary aPS (see McCrae et al,³⁸⁸ among others). Controversy remains as to whether antiphospholipid antibodies per se comprise the biologically important subpopulation of AECA (see Cines³⁸⁹ for review), although recent studies suggest that they may activate ECs through an effect on the plasma protein β_2 -glycoprotein I.³⁹⁰ AECA have been shown to alter the anticoagulant and procoagulant activities of cultured ECs in a number of ways. However, the in vivo importance of these antibodies in the pathogenesis of thrombosis remains unknown. Indeed, in only some studies has the presence of AECA correlated with thrombotic events or disease activity.³⁸⁸ Never-

theless, the fact that such antibodies induce the secretion of markers of EC injury/activation such as vWF from cultured ECs,³⁸⁸ when considered in light of reports demonstrating elevated levels of vWF in the plasma of patients with SLE,³⁹¹ suggests that at least some of these in vitro effects may reflect processes that occur in vivo.

Heparin-induced thrombocytopenia and thrombosis (HITT). Approximately 1% to 3% of patients who receive heparin develop severe thrombocytopenia, approximately 20% of whom also develop venous and/or arterial thrombi (see Arepally and Cines³⁹² and McCrae and Cines³⁹³ for reviews). Plasma from approximately 90% of patients with HITT contain antibodies that bind to complexes of heparin and platelet factor 4 (PF4).³⁹⁴ PF4 released from activated platelets may form complexes with heparin on the surface of activated platelets, targeting them for Fc_γRIIA-dependent activation by antiheparin-PF4 antibodies. However, the remarkable propensity of patients with HITT to develop thrombosis may also be promoted by the capacity of these antibodies to recognize PF4 bound to EC-heparan sulfate proteoglycans, which stimulate ECs to express TF and to bind platelets.^{395,396}

EC-reactive antibodies in other vasculitic disorders. AECAs have been described in several other disorders in which their role in pathogenesis is even less certain. PR-3, a common target of antineutrophil cytoplasmic antibodies (ANCA) is expressed on activated ECs in vitro³⁹⁷ and has been implicated in vascular injury in an animal model of vasculitis.³⁹⁸ However, EC-specific antibodies, apparently distinct from ANCA, have also been described in many patients with Wegener's granulomatosis.³⁹⁹ Plasma from children with the hemolytic uremic syndrome contain lytic AECA that recognize an unidentified EC surface protein that is suppressed by IFN- γ in vitro.⁴⁰⁰ AECA have also been implicated in mixed connective tissue disease, rheumatoid arthritis, and scleroderma,⁴⁰¹ in which EC apoptosis has been implicated,⁴⁰² as well as in atherosclerosis,⁴⁰³ Kawasaki's disease,⁴⁰⁴ Behcet's disease,⁴⁰⁵ and various forms of vasculitis.⁴⁰⁶ In each of these conditions, elevated levels of EC-derived proteins have also been described in the plasma of affected patients implying endothelial activation or injury.⁴⁰⁷ However, a pathogenic role of AECA or immune complexes in human vasculitis has not been proven. It is also pertinent to note that complex changes in antigen expression occur when ECs have been activated by cytokines or other agonists in vitro (see Favaloro⁴⁰⁸ for review) and in vivo.⁴⁰⁹ This activation promotes cell mediated immunity, promotes leukocyte/platelet adhesion (see "EC Perturbation and Vascular Disease" above) and exposes cryptic autoantigens. Fc_γ receptors capable of binding circulating immune complexes may exist⁴¹⁰ or can be induced on ECs⁴¹¹ at certain vascular sites.

Complement-mediated EC activation. The vascular endothelium is exposed to activated complement components as a consequence of antigen-antibody interactions that occur naturally in plasma and within the vessel wall in certain pathologic conditions.³⁸⁷ ECs also constitutively express several complement proteins⁴¹² and can be induced to synthesize others by various cytokines,⁴¹³ at least in vitro. Complement deposition on the vasculature is controlled, in part, by concerted actions of the regulatory proteins C1-esterase inhibitor, which is secreted by ECs,⁴¹⁴ and by decay-accelerating factor, membrane cofactor

protein of complement, and homologous restriction factor, which are expressed on the cell surface.⁴¹⁵⁻⁴¹⁹ Complement that deposits on the endothelium when these containment mechanisms are exceeded can increase vascular permeability, stimulate procoagulant pathways, and recruit ECs to become active participants in the inflammatory processes.

For example, multiple components of the complement cascade act in concert to augment the recruitment of leukocytes to sites of vascular inflammation. Binding of C1q to specific EC receptors⁴²⁰ augments expression of E-selectin and possibly ICAM-1 and VCAM-1,⁴²¹ C5a upregulates the expression of P-selectin,⁴²² and the terminal C5b-9 membrane attack complex (MAC) acts synergistically with TNF- α to stimulate expression of E-selectin and ICAM-1.⁴²³ Sublytic concentrations of MAC also activate the NF- κ B pathway leading to secretion of IL-8 and MCP-1.⁴²⁴

MAC also stimulates cultured ECs to express TF,^{425,426} to release large vWF multimers,⁴²⁷ and to release procoagulant microvesicles containing anionic phospholipids and binding sites for factor Va that accelerate prothrombinase activity.⁴²⁸ On the other hand, MAC also stimulates production of PGI₂⁴²⁹ and the complement components C7 and C9 provide sites for the binding and activation of plasminogen.⁴³⁰ However, as with other areas of research in vascular biology, studies have been confined largely to cultured cells and additional studies are required to elucidate the relative contribution of complement-dependent responses in vivo.

Thrombotic disorders of uncertain cause: Thrombotic thrombocytopenic purpura (TTP) and the hemolytic uremic syndrome (HUS). TTP and HUS are related-disorders characterized pathologically by the development of platelet microthrombi that occlude small arterioles and capillaries and clinically by microangiopathic hemolytic anemia and thrombocytopenia. Endothelial dysfunction plays a prominent role in the pathogenesis of both disorders (see Moake⁴³¹ and Heild⁴³²). Approximately 90% of cases of HUS occur in early childhood, often after an episode of bloody diarrhea caused by enteropathic strains of *Escherichia coli* that release an exotoxin, designated verotoxin-1 (VT-1), which is similar to the 70-kD Shiga toxin 18. VT-1 binds with high affinity to globotriosylceramide (Gb3) receptors expressed at the highest density on renal glomerular ECs.⁴³¹ VT-1 is directly cytotoxic to ECs. In addition, VT-1 promotes neutrophil-mediated EC injury^{433,434} and induces the production of TNF- α by monocytes⁴³⁵ and cells within the kidney.⁴³⁶ In turn, TNF- α , in concert with IL-1, increases Gb3 expression and exacerbates the sensitivity of the endothelium to toxin-mediated⁴³⁷ and antibody-mediated⁴⁰⁴ cytotoxicity, promotes vWF release,⁴³⁸ and impairs fibrinolytic activity.⁴⁴ In accord with this putative pathogenic mechanism, elevated plasma levels of PAI-1 have been reported to be a sign of a poor prognosis in childhood HUS.⁴³⁹ EC injury may also contribute to the pathogenesis of the microangiopathic syndromes that may follow the use of certain chemotherapeutic agents,⁴⁴⁰ cyclosporin,⁴⁴¹ quinine/quinidine (see Gottschall et al⁴⁴² for review), or after bone transplantation.⁴⁴⁰

There is considerable evidence to suggest that EC injury plays a role in the pathogenesis of TTP. The most exhaustively studied protein in this regard is plasma vWF, which circulates in plasma as oligomers that range in size from 1 to 15 \times 10⁶ kD.

The so-called unusually large vWF multimers (ULvWF) are normally found in subendothelial matrix, in the supernatant of cultured ECs,⁴³¹ and in platelet releasates, but are not normally detected in plasma.⁴³¹ Platelet microthrombi in TTP contain abundant vWF but little fibrinogen, in contrast to those seen in DIC. A subgroup of patients has been identified who suffer from chronic, relapsing TTP and whose plasma continues to contain elevated levels of ULvWF between relapses.⁴⁴³ Furthermore, plasma from patients with sporadic or isolated episodes of TTP often contains either ULvWF or decreased amounts of larger vWF multimers during periods of active disease. ULvWFs may exacerbate microvascular thrombosis through their ability to aggregate platelets at high levels of shear stress. The secretion of ULvWF by cultured ECs is stimulated by many agonists including Shiga toxin.⁴³¹ However, elevated levels of vWF occur in other thrombotic microangiopathies, and their exact role in TTP/HUS requires further study. Reports of elevated levels of thrombomodulin,⁴⁴⁴ tissue-type plasminogen activator (t-PA), plasminogen activator inhibitor type 1 (PAI-1),⁴⁴⁵ ELAM-1,⁴⁴⁶ and decreased levels of PGI₂⁴⁴⁷ and TFPI⁴⁴⁸ in plasma from patients with TTP provides additional support for the notion that endothelial damage plays a pivotal role in the pathogenesis of the disease.

The events that initiate TTP remain unknown. AECA have been described in TTP and HUS, but their role is uncertain. More recently, plasma from patients with TTP and HUS has been reported to induce apoptosis in microvascular ECs; it is of great interest that cells from dermal, renal, and cerebral origin were most susceptible, whereas pulmonary and coronary arterial cells were not.⁴⁶ The plasma factors responsible for these changes remain to be identified.

Pregnancy-induced hypertension. Pregnancy-induced hypertension (or preeclampsia) is the most common medical disorder of pregnancy, affecting 5% to 13% of all primaras. Although the clinical manifestations of preeclampsia are generally not evident until the third trimester, the pathogenesis of this disorder may involve a deficiency in placentation,⁴⁴⁹ the process in which fetal trophoblast cells remodel the maternal uterine spiral arteries early in pregnancy. Incomplete remodeling of the spiral arteries leads to compromised placental perfusion. Alterations in EC morphology occur within the placenta⁴⁵⁰ and in the glomerular capillaries (glomerular endotheliosis), the latter being characterized by EC swelling and lipid accumulation (see Ferris⁴⁵¹ for review). Fibrin deposition in microvasculature is common. Affected women show increased responsiveness to the pressor effects of angiotensin II,³¹⁴ increased amounts of thromboxane A₂ relative to PGI₂ in their urine,⁴⁵² elevated plasma levels of endothelin,⁴⁵³ and their umbilical vessels demonstrate less PGI₂ synthesis and decreased NO release in response to bradykinin (see Ferris⁴⁵¹ for review). Additional evidence suggesting endothelial damage is the reported findings of elevated plasma levels of EC-derived fibronectin,^{454,455} vWF,⁴⁵⁵ and PAI-1⁴⁵⁵ in affected women. Importantly, increased levels of vWF⁴⁵⁶ and cellular fibronectin⁴⁵⁴ may be detected before the onset of clinical manifestations.

The pathophysiology of EC damage in preeclampsia remains a mystery. Some,^{457,458} but not all groups,^{459,460} have reported that plasma from affected women is cytotoxic for cultured ECs.

Preeclamptic sera have also been reported to impair EC proliferation,⁴⁶¹ stimulate fibronectin release,⁴⁶² increase triglyceride accumulation,⁴⁶³ and increase PDGF synthesis⁴⁶⁴; variable effects on PGI₂ synthesis have been reported.^{463,465} A role for abnormalities of lipid peroxidation,⁴⁶⁶ for immunologic factors, and for underlying, but otherwise inapparent, maternal vascular disease (see Ness and Roberts⁴⁶⁷ for review) have also been advanced. Yet, it must be noted that all of these studies remain largely unconfirmed, the putative injurious plasma factor(s) has yet to be identified, and the pathophysiology and significance of EC injury outside of the terminal cases of eclampsia remains enigmatic.

CONCLUSION

The endothelium can no longer be viewed as a static physical barrier that simply separates blood from tissue. Rather, it is now clear that the endothelium helps to coordinate functions of differentiated tissues in a way that meets the requirements of the organism as a whole. In part, this is accomplished by the location of the endothelium at the interface with the blood and the capacity of these specialized cells to receive and transmit biochemical and physical information bidirectionally. Information sensed on the luminal surface of the endothelium can be transmitted either by direct permeation or active transport of soluble mediators through the capillaries to deeper tissues or indirectly through the capacity of ECs to modulate the behavior of smooth muscle cells and other components of the vessel wall. In turn, physiologic and pathophysiologic events in tissue alter EC interactions with soluble and cellular blood components.

The endothelium, as with all cell types, displays an immediate and prototypic response to diverse agonists that is modulated in complex ways by subsequent events. In the case of the endothelium, this first response appears designed to prevent physical disruption of the vessel wall by trauma, microbial organisms, toxins, or other threats to the maintenance of intravascular volume and oxygen delivery. This protective response is accomplished by the rapid transformation of the endothelium to a procoagulant, vasoconstrictive, and proinflammatory state that has multiple effects on of its structure and behavior.

Several ramifications of this reflexive, adaptive response of the endothelium have now become evident. First, it is clear that ECs rapidly undergo some of these same biochemical and phenotypic changes soon after being placed in culture, as a consequence of which the behavior of the unperturbed endothelium cannot be reliably inferred from currently available in vitro techniques.

Second, an extensive experimental literature has emerged supporting the notion that several common human vascular diseases are in part a consequence of the same responses of the endothelium to stress; ie, that prolonged or exaggerated endothelial activation leads to dysfunction that is an early, often preclinical component of vascular disease. Unfortunately, it is generally impossible to access vascular tissue directly and sequentially during these preclinical stages of disease development; without such tissue, the EC contribution to disease development can only be inferred. As a consequence, most research in vascular biology continues to (1) focus on the footprints of disease by analyzing damaged vessels, generally at

the endstage of the process; (2) link putative circulatory factors to disorders through their effect on cultured ECs, often derived from unaffected tissue; and (3) develop animal models that may simulate human diseases.

Third, it is now clear that the endothelium is not a homogeneous organ. ECs from different vascular beds show highly differentiated functions as a consequence of genetic diversity and the impact of specialized surroundings. These biochemical and phenotypic differences extend to their susceptibility to injury and effect function of the vasculature as a whole.

Fourth, there is remarkably little information on the potential contribution of genetic differences in EC behavior among individuals with respect to bleeding disorders, thrombosis, atherosclerosis, and vasculitis. Without such information, our current approach to studying these major vascular disorders can be compared with the study of anemia without appreciating the existence of hemoglobinopathies or the study of bleeding disorders without appreciating the contribution of genetic abnormalities in platelet function. It is hoped that future research will enable the direct study of EC behavior and thereby enhance our understanding of the contribution of the endothelium to vascular biology.

REFERENCES

1. Fishman AP: Endothelium: A distributed organ of diverse capabilities. *Ann NY Acad Sci* 401:1, 1982
2. Augustin HG, Kozian DH, Johnson RC: Differentiation of endothelial cells: Analysis of the constitutive and activated endothelial cell phenotypes. *Bioessays* 16:901, 1994
3. Jaffe EA, Nachman RL, Becker CG, Minick CR: Culture of human endothelial cells derived from umbilical veins: Identification by morphologic criteria. *J Clin Invest* 52:2745, 1973
4. Gimbrone MA, Cotran RS, Folkman J: Human vascular endothelial cells in culture. Growth and DNA synthesis. *J Cell Biol* 60:673, 1974
5. Lewis LJ, Hoak JC, Maca RD, Fry GL: Replication of human endothelial cells in culture. *Science* 181:453, 1973
6. Fishman MC, Stainier DYS: Cardiovascular development: Prospects for a genetic approach. *Circ Res* 74:757, 1994
7. Hanahan D, Folkman J: Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell* 86:353, 1996
8. Risau W, Flamme I: Vasculogenesis. *Annu Rev Cell Dev Biol* 11:73, 1995
9. Palis J, McGrath KE, Kingsley PD: Initiation of hematopoiesis and vasculogenesis in murine yolk sac explants. *Blood* 86:156, 1995
10. Flamme I, Baranowski A, Risau W: A new model of vasculogenesis and angiogenesis in vitro as compared with vascular growth in the avian area vasculosa. *Anat Rec* 237:49, 1993
11. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witzenbichler B, Schatteman G, Isner JM: Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 275:964, 1997
12. Risau W: Differentiation of endothelium. *Endothelium Differ* 9:926, 1995
13. Buck CA, Edelman JM, Buck CE, Kennedy G, Baldwin HS: Expression patterns of adhesion receptors in the developing mouse lung: Function implications. *Cell Adhesion Commun* 4:69, 1996
14. Pardanaud L, Yassine F, Dieterlen-Lievre F: Relationship between vasculogenesis, angiogenesis and haemopoiesis during avian ontogeny. *Development* 105:473, 1989
15. Bielinska M, Narita N, Heikinheimo M, Porter SB, Wilson DB: Erythropoiesis and vasculogenesis in embryoid bodies lacking visceral yolk sac endoderm. *Blood* 88:3720, 1996
16. Beck L Jr, D'Amore P: Vascular development: Cellular and molecular regulation. *FASEB J* 11:365, 1997
17. Breier G, Risau W: The role of vascular endothelial growth factor in blood vessel formation. *Trends Cell Biol* 6:454, 1996
18. Shalaby R, Rossant J, Yamaguchi TP, Gertsenstein M, Wu X-F, Breitman ML, Schuh AC: Failure of blood-island formation and vasculogenesis in FLK-1-deficient mice. *Nature* 376:62, 1995
19. Sato TN, Tozawa Y, Deutsch U, Wolburg-Buchholz K, Fujiwara Y, Gendron-Maguire M, Gridley T, Wolburg H, Risau W, Qin Y: Distinct roles of the receptor tyrosine kinases Tie-1 and Tie-2 in blood vessel formation. *Nature* 376:70, 1995
20. Mustonen T, Alitalo K: Endothelial receptor tyrosine kinases involved in angiogenesis. *J Cell Biol* 129:895, 1995
21. Davis S, Aldrich TH, Jones PF, Acheson A, Compton DL, Jain V, Ryan TE, Bruno J, Radziejewski C, Maisonpierre PC, Yancopoulos GD: Isolation of angiopoietin-1, a ligand for the TIE2 receptor, by secretion-trap expression cloning. *Cell* 87:1161, 1996
22. Maisonpierre P, Suri G, Jones P, Bartunkova S, Wiedgand S, Radziejewski C, Compton D, McClain J, Aldrich T, Papadonopoulou N, Daly T, Davis S, Sato T, Yancopoulos GD: Angiopoietin-2, a natural antagonist for Tie-2 that disrupts in vivo angiogenesis. *Science* 277:55, 1997
23. Suri C, Jones PF, Patan S, Bartunkova S, Maisonpierre PC, Davis S, Sato TN, Yancopoulos GD: Requisite role of angiopoietin-1, a ligand for the Tie-2 receptor during embryonic angiogenesis. *Cell* 87:1171, 1996
24. Folkman J, D'Amore PA: Blood vessel formation: What is the molecular basis? *Cell* 87:1153, 1996
25. Vikkula M, Boou L, Carraway K, Calvert J, D'Amore PA, Goomnerov B, Pasyk K, Marchuk D, Warman M, Cantley L, Mulliken J, Olsen BR: Vascular dysmorphogenesis caused by an activating mutation in the receptor tyrosine kinase. *Cell* 87:1181, 1996
26. Stainier DYS, Fishman MC: The zebrafish as a model system to study cardiovascular development. *Trends Cardiovasc Med* 4:207, 1994
27. Sankar S, Mahooti-Brooks N, Bensen L, McCarthy TL, Centrella M, Madri JA: Modulation of transforming growth factor β receptor levels on microvascular endothelial cells during in vitro angiogenesis. *J Clin Invest* 97:1436, 1996
28. Schwartz MA, Schaller MD, Ginsburg MH: Integrins: emerging paradigms of signal transduction. *Annu Rev Cell Develop Biol* 11:549, 1995
29. Varner JA, Brooks PC, Cheresh DA: Review: The integrin $\alpha_v\beta_3$: Angiogenesis and apoptosis. *Cell Adhesion Commun* 3:367, 1995
30. Hynes RO: Targeted mutations in cell adhesion genes: What have we learned from them? *Dev Biol* 180:402, 1996
31. Albelda SM, Muller WA, Buck CA, Newman PJ: Molecular and cellular properties of PECAM-1 (endoCAM/CD31): A novel vascular cell-cell adhesion molecule. *J Cell Biol* 114:1059, 1991
32. Dejana E: Endothelial adherens junctions: Implications in the control of vascular permeability and angiogenesis. *J Clin Invest* 98:1949, 1996
33. Bretscher MS: Moving membrane up to the front of migrating cells. *Cell* 85:465, 1996
34. Carmeliet P, Collen D: Gene targeting and gene transfer studies of the plasminogen/plasmin system: Implications in thrombosis, hemostasis, neointima formation, and atherosclerosis. *FASEB J* 9:934, 1995
35. Plow EF, Herren T, Redlitz A, Miles LA, Hoover-Plow JL: The cell biology of the plasminogen system. *FASEB J* 9:939, 1995
36. O'Reilly MS, Holmgren L, Shing Y, Chen C, Rosenthal RA, Moses M, Lane WS, Cao Y, Sage EH, Folkman J: Angiostatin: A novel angiogenesis inhibitor that mediates the suppression of metastases by a Lewis lung carcinoma. *Cell* 79:315, 1994
37. O'Reilly MS, Boehm T, Shing Y, Fukui N, Vasios G, Lane WS, Flynn E, Birkhead JR, Olsen BR, Folkman J: Endostatin: An endogenous inhibitor of angiogenesis and tumor growth. *Cell* 88:277, 1997

38. Zetter BR: On target with tumor blood vessels. *Nat Biotechnol* 15:1243, 1997
39. McCarthy SA, Kuzu I, Gatter KC, Bicknell R: Heterogeneity of the endothelial cell and its role in organ preference of tumour metastasis. *TIPS* 12:462, 1991
40. Kumar S, West DC, Ager A: Heterogeneity in endothelial cells from large vessels and microvessels. *Differentiation* 36:57, 1987
41. Turner RR, Beckstead JH, Warnke RA, Wood GA: Endothelial cell phenotypic diversity. *In situ* demonstration of immunologic and enzymatic heterogeneity that correlates with specific morphologic subtypes. *Am J Clin Pathol* 87:569, 1985
42. Levin EG, Osborn KG: The expression of endothelial cell tissue plasminogen activator *in vivo*: A function defined by vessel size and anatomic location. *J Cell Sci* 11:139, 1997
43. Wojta J, Hoover RL, Daniel TO: Vascular origin determines plasminogen activator expression in human endothelial cells. Renal endothelial cells produce large amounts of single chain urokinase type plasminogen activator. *J Biol Chem* 264:2846, 1989
44. Louise CB, Obrig TG: Human renal microvascular endothelial cells as a potential target in the development of the hemolytic uremic syndrome as related to fibrinolysis factor expression, *in vitro*. *Microvasc Res* 47:377, 1994
45. Obrig TG, Louise CB, Lingwood CA, Boyd B, Daniel TO: Endothelial cell heterogeneity in shiga toxin receptors and responses. *J Biol Chem* 268:15484, 1993
46. Mitra D, Jaffe EA, Weksler B, Hajjar KA, Doserland C, Laurence J: Thrombotic thrombocytopenic purpura and sporadic hemolytic-uremic syndrome plasmas induce apoptosis in restricted lineages of human microvascular endothelial cells. *Blood* 89:1224, 1997
47. Drake TA, Cheng J, Chang A, Taylor FB Jr: Expression of tissue factor, thrombomodulin, and E-selectin in baboons with lethal *Escherichia coli* sepsis. *Am J Pathol* 142:1458, 1993
48. Zhu D, Cheng C-F, Pauli BU: Mediation of lung metastasis of murine melanoma by a lung-specific endothelial cell adhesion molecule. *Proc Natl Acad Sci USA* 88:9568, 1991
49. Butcher EC, Picker LJ: Lymphocyte homing and homeostasis. *Science* 272:60, 1996
50. Rafii S, Shapiro F, Pettengall R, Ferris B, Nachman RL, Moore MA, Asch AS: Human bone marrow microvascular endothelial cells support long-term proliferation and differentiation of myeloid and megakaryocyte progenitors. *Blood* 86:3353, 1995
51. Owman C, Hardbeo JE: Functional heterogeneity of cerebrovascular endothelium. *Brain Behav Evol* 32:65, 1988
52. Grau GE, de Morelouse P, Bulla O, Lou J, Lei Z, Reber R, Mili N, Ricou B, Morel DR, Suter PM: Haemostatic properties of human pulmonary and cerebral microvascular endothelial cells. *Thromb Haemost* 77:585, 1997
53. Morin O, Patry P, Lafleur L: Heterogeneity of endothelial cells of adult rat liver as resolved by sedimentation velocity and flow cytometry. *J Cell Physiol* 119:327, 1984
54. Couveland A, Scoazec J-Y, Dauge M-C, Bringer A-F, Potet FGF: Structural and functional differentiation of sinusoidal endothelial cells during liver organogenesis in humans. *Blood* 87:4568, 1996
55. Milici AJ, Furie MB, Carley WW: The formation of fenestrations and channels by capillary endothelium *in vitro*. *Proc Natl Acad Sci USA* 82:6181, 1985
56. Pardanau L, Altmann C, Kitos P, Dieterlen-Lievre F, Buck CA: Vasculogenesis in the early quail blastodisc as studied with a monoclonal antibody recognizing endothelial cells. *Development* 100:339, 1987
57. Grau GE, Mili N, Lou JN, Morel DR, Ricou B, Lucas R, Suter PM: Phenotypic and functional analysis of pulmonary microvascular endothelial cells from patients with acute respiratory distress syndrome. *Lab Invest* 74:761, 1996
58. Aird WC, Edelberg JM, Weiler-Guettler H, Simmons WW, Smith TW, Rosenberg RD: Vascular bed-specific expression of an endothelial cell gene is programmed by the tissue microenvironment. *J Cell Biol* 138:1117, 1997
59. Pauli BU, Lee C-L: Organ preference of metastasis. The role of organ-specifically modulated endothelial cells. *Lab Invest* 58:379, 1988
60. Subramanian VA, Hernandez Y, Tack-Goldman K, Grabowski EF, Weksler BB: Prostacyclin production by internal mammary artery as a factor in coronary artery bypass grafts. *Surgery* 100:376, 1986
61. Ades EW, Candal FJ, Swerlick RA, George VG, Summers S, Bosse DC, Lawley TJ: HMEC-1: Establishment of an immortalized human microvascular endothelial cell line. *J Invest Dermatol* 99:683, 1992
62. Grant DS, Tashiro K-I, Segui-Real B, Yamada Y, Martin GR, Kleinman HK: Two different laminin domains mediate the differentiation of human endothelial cells into capillary-like structures *in vitro*. *Cell* 58:933, 1989
63. Gibbons GH, Dzau VJ: Molecular therapies for vascular diseases. *Science* 272:689, 1996
64. Stamler JS, Singel DJ, Loscalzo J: Biochemistry of nitric oxide and its redox-activated forms. *Science* 258:1898, 1992
65. Venema RC, Sayegh HS, Arnal JF, Harrison DG: Role of the enzyme calmodulin-binding domain in membrane association and phospholipid inhibition of endothelial nitric oxide synthase. *J Biol Chem* 270:14705, 1995
66. Topper JN, Cai J, Falb D, Gimbrone MA Jr: Identification of vascular endothelial genes differentially responsive to fluid mechanical stimuli: Cyclooxygenase-3, manganese superoxide dismutase, and endothelial cell nitric oxide synthase are selectively up-regulated by steady laminar shear stress. *Proc Natl Acad Sci USA* 93:10417, 1996
67. Awolesi MA, Widmann MD, Sessa WC, Sumpio BE: Cyclic strain increases endothelial nitric oxide synthase activity. *Surgery* 116:439, 1994
68. Loscalzo J, Vita JA: Ischemia, hyperemia, exercise, and NO: Complex physiology and complex molecular adaptations. *Circulation* 90:2556, 1994
69. Venema RC, Nishida K, Alexander RW, Harrison DG, Murphy TJ: Organization of the bovine gene encoding the endothelial nitric oxide synthase. *Biochem Biophys Acta* 1218:413, 1994
70. Balligand JL, Ungureanu-Longroid D, Simmons WW, Kobzik L, Lowenstein CJ, Lamas S, Kelly RA, Smith TW, Michel T: Induction of nitric oxide synthase in rat cardiac microvascular endothelial cells by IL-beta and IFN-gamma. *Am J Physiol* 268:H1293, 1995
71. Loscalzo J, Welch G: Nitric oxide and its role in the cardiovascular system. *Prog Cardiovasc Dis* 38:87, 1995
72. Mendelsohn ME, O'Neill S, George D, Loscalzo J: Inhibition of fibrinogen binding to human platelets by S-nitroso-N-acetylcysteine. *J Biol Chem* 265:19028, 1990
73. Radomski MW, Palmer RMJ, Moncada S: Comparative pharmacology of endothelium-derived relaxing factor, nitric oxide, and prostacyclin in platelets. *Br J Pharmacol* 92:181, 1987
74. Stamler J, Vaughan DE, Loscalzo J: Synergistic disaggregation of platelets by tissue plasminogen activator, prostaglandin E₁, and nitroglycerin. *Circ Res* 65:769, 1989
75. Michelson AD, Benoit SE, Furman MI, Breckwoldt WL, Rohrer MJ, Barnard MR, Loscalzo J: Effects of endothelium-derived relaxing factor/nitric oxide on platelet surface glycoproteins. *Am J Physiol* 39:H1640, 1996
76. Pigazzi A, Folli F, Heydrich S, Loscalzo J: Nitric oxide inhibits thrombin-induced phosphoinositide 3-kinase activity in human platelets. *Circulation* (in press)
77. Kubes P, Suzuki M, Granger DN: Nitric oxide: An endogenous modulator of leukocyte adhesion. *Proc Natl Acad Sci USA* 88:4651, 1991
78. De Caterina R, Libby P, Peng HB, Thannickal VJ, Rajavashisth TB, Gimbrone MA Jr, Shin WS, Liao JK: Nitric oxide decreases cytokine-induced endothelial activation. Nitric oxide selectively re-

duces endothelial expression of adhesion molecules and proinflammatory cytokines. *J Clin Invest* 96:60, 1995

79. Marks DS, Vita JA, Folts JD, Keaney JF Jr, Welch GN, Loscalzo J: Inhibition of neointimal proliferation in rabbits following vascular injury by a single treatment with a protein adduct of nitric oxide. *J Clin Invest* 96:2630, 1995
80. Garg UC, Hassid A: Nitric oxide (NO) and 8-bromo-cyclic GMP inhibit mitogenesis and proliferation of cultured rat vascular smooth muscle cells. *J Clin Invest* 83:1974, 1989
81. Fukuo K, Inoue T, Morimoto S, Nakahasi T, Yasuda O, Kitanno S, Sasanda A, Ogihara T: Nitric oxide mediates cytotoxicity and basic fibroblast growth factor release in cultured vascular smooth muscle cells. *J Clin Invest* 95:669, 1995
82. Garland CJ, Plane F, Kemp BK, Cocks TM: Endothelium-dependent hyperpolarization: A role in the control of vascular tone. *Trends Biol Sci* 16:23, 1995
83. Feletou M, Vanhoutte PM: Endothelium-dependent hyperpolarization of canine coronary smooth muscle. *Br J Pharmacol* 93:515, 1988
84. Levin ER: Endothelins. *N Engl J Med* 333:356, 1996
85. Nakamura S, Naruse M, Maruse K, Demura H, Uemura H: Immunocytochemical localization of endothelin in cultured bovine endothelial cell. *Histochemistry* 94:475, 1990
86. Simonson MS, Dunn MJ: Cellular signaling by peptides of the endothelin gene family. *FASEB J* 4:2989, 1990
87. Goligorsky MS, Tsukahara H, Magazine H, Andersen TT, Malik AB, Bahou WF: Termination of endothelin signaling: Role of nitric oxide. *J Cell Physiol* 158:485, 1994
88. Lopez JA, Armstrong ML, Piegors DJ, Heistad DD: Vascular responses to endothelin-1 in atherosclerotic primates. *Arteriosclerosis* 10:1113, 1990
89. Zimmerman GA, Whatley RE, Benson DE, McIntyre TM, Prescott SM: Endothelial cells for studies of platelet-activating factor and arachidonate metabolites. *Methods Enzymol* 187:520, 1990
90. Meade EA, Jones DA, Zimmerman GA, McIntyre TM, Prescott SM: Prostaglandins and related compounds. Lipid messengers with many actions. *Handbook Lipid Res* 8:285, 1996
91. Whatley RE, Zimmerman GA, Prescott SM, McIntyre TM: Platelet-activating factor and PAF-like mimetics. *Handbook Lipid Res* 8:239, 1996
92. Imaizumi T, Stafforini DM, Yamada Y, McIntyre TM, Prescott SM, Zimmerman GA: Platelet-activating factor: A mediator for clinicians. *J Intern Med* 238:5, 1995
93. Campbell WB, Halushka PV: Eicosanoids and platelet-activating factor, in Hardman JG, Limbird LE, Molinoff PM, Ruddon RW, Gilman AG (eds): Goodman and Gilman's The Pharmacologic Basis of Therapeutics. New York, NY, McGraw-Hill, 1996, p 601
94. Lorant DE, Zimmerman GA, McIntyre TM, Prescott SM: Platelet-activating factor mediates procoagulant activity on the surface of endothelial cells by promoting leukocyte adhesion. *Semin Cell Biol* 6:295, 1995
95. Weksler BB, Marcus AJ, Jaffe EA: Synthesis of prostaglandin I₂ (prostacyclin) by cultured human and bovine endothelial cells. *Proc Natl Acad Sci USA* 74:3922, 1977
96. McIntyre TM, Zimmerman GA, Satoh K, Prescott SM: Cultured endothelial cells synthesize both platelet-activating factor and prostacyclin in response to histamine, bradykinin and ATP. *J Clin Invest* 76:271, 1985
97. Coleman RA, Smith WL, Narumiya S: Classification of prostanoid receptors: Properties, distribution, and structure of the receptors and their subtypes. *Pharmacol Rev* 46:205, 1994
98. Majerus PW: Arachidonate metabolism in vascular disorders. *J Clin Invest* 72:1521, 1983
99. FitzGerald GA, Pedersen AK, Patrono C: Analysis of prostacyclin and thromboxane biosynthesis in cardiovascular disease. *Circulation* 67:1174, 1983
100. Chen X-S, Sheller JR, Johnson EN, Funk CD: Role of leukotrienes revealed by targeted disruption of the 5-lipoxygenase gene. *Nature* 372:179, 1992
101. Bombeli T, Meller M, Haeblerli A: Anticoagulant properties of the vascular endothelium. *Thromb Haemost* 77:408, 1997
102. Rosenberg RD, Rosenberg JS: Natural anticoagulant mechanisms. *J Clin Invest* 74:1, 1984
103. Bauer KA, Weiss LM, Sparrow D, Vakonas PS, Rosenberg RD: Aging-associated changes in indices of thrombin generation and protein C activation in human. Normative aging study. *J Clin Invest* 80:1527, 1987
104. Marcum JA, Rosenberg RD: Anticoagulant active heparin-like molecules from vascular tissue. *Biochemistry* 23:1730, 1984
105. Tollefsen DM, Pestka CA: Heparin cofactor II activity in patients with disseminated intravascular coagulation and hepatic failure. *Blood* 66:769, 1985
106. Broze GJ Jr: Tissue factor pathway inhibitor. *Thromb Haemost* 74:90, 1995
107. Jesty J, Lorenz A, Rodriguez J, Wun T-C: Initiation of the tissue factor pathway of coagulation in the presence of heparin: Control by antithrombin III and tissue factor pathway inhibitor. *Blood* 87:2301, 1996
108. Bombeli T, Karsan A, Tait JF, Harlan JM: Apoptotic vascular endothelial cells become procoagulant. *Blood* 90:2429, 1997
109. Esmon CT, Fukudome K: Cellular regulation of the protein C pathway. *Semin Cell Biol* 6:259, 1995
110. Fair DS, Marlar RA, Levin EG: Human endothelial cells synthesize protein S. *Blood* 67:1168, 1986
111. Fukudome K, Kurosawa S, Stearns-Kurosawa D-J, He X, Rezaie AR, Esmon CT: The endothelial cell protein C receptor. Cell surface expression and direct ligand binding by the soluble receptor. *J Biol Chem* 271:17491, 1996
112. Thompson EA, Salem HH: Inhibition by human thrombomodulin of factor Xa-mediated cleavage of prothrombin. *J Clin Invest* 78:13, 1986
113. Esmon CT: Molecular events that control the protein C anticoagulant pathway. *Thromb Haemost* 70:29, 1993
114. Nawroth PP, Stern DM: Modulation of endothelial cell hemostatic properties by tumor necrosis factor. *J Exp Med* 163:740, 1986
115. Mantovani A, Sozzani S, Vecchi A, Introna M, Allavena P: Cytokine activation of endothelial cells: new molecules for an old paradigm. *Thromb Haemost* 78:406, 1997
116. Cucurull E, Gharavi AE: Thrombomodulin: A new frontier in lupus research? *Clin Exp Rheumatol* 15:1, 1997
117. Drake TA, Morrissey JH, Edgington TS: Selective cellular expression of tissue factor in human tissues. Implications for disorders of hemostasis and thrombosis. *Am J Pathol* 134:1087, 1989
118. Carmeliet P, Mackman N, Moons L, Luther T, Gressens P, van Vlaenderen I, Demunck H, Kaspar M, Breier G, Evrard P, Muller M, Risau W, Edgington T, Collen D: Role of tissue factor in embryonic blood vessel development. *Nature* 383:73, 1996
119. Bugge TH, Xiao Q, Kormbrinck KW, Flick MJ, Holmback K, Danton MJS, Colbert MC, Witte DP, Fujikawa K, Davie E, Degen JL: Fatal embryonic bleeding events in mice lacking tissue factor, the cell-associated initiation of blood coagulation. *Proc Natl Acad Sci USA* 93:6258, 1996
120. Toomey JR, Kratzer KE, Lasky NM, Stanton JJ, Broze GJ Jr: Targeted disruption of the murine tissue factor gene results in embryonic lethality. *Blood* 88:1583, 1996
121. Rapaport SI, Rao VM: The tissue factor pathway: How it has become a "Prima Ballerina". *Thromb Haemost* 74:7, 1995
122. Nemerson Y: Tissue factor: Then and now. *Thromb Haemost* 74:180, 1995
123. Ryan J, Brett J, Tjburg P, Bach RR, Kisiel W, Stern D: Tumor necrosis factor-induced endothelial tissue factor is associated with

subendothelial matrix vesicles but is not expressed on the apical surface. *Blood* 80:966, 1992

124. Narahara N, Enden T, Wiiger M, Prydz H: Polar expression of tissue factor in human umbilical vein endothelial cells. *Arterioscler Thromb* 14:1815, 1994
125. Bona R, Lee E, Rickles F: Tissue factor apoprotein: Intracellular transport and expression in shed membrane vesicles. *Thromb Res* 48:487, 1987
126. Shimura M, Wada H, Waskita Y, Nakse T, Hiyoyama K, Nagaya S, Shiku H: Plasma tissue factor and tissue factor pathway inhibitor levels in patients with disseminated intravascular coagulation. *Am J Pathol* 52:165, 1996
127. Marmur JD, Rossikhina M, Guha A, Fyle B, Friedrich V, Mendlowitz M, Nemerson Y, Taubman MB: Tissue factor is rapidly induced in arterial smooth muscle after balloon injury. *J Clin Invest* 91:2253, 1993
128. Thiruvikraman SV, Guha A, Roboz J, Taubman MB, Nemerson Y, Fallon JT: In situ localization of tissue factor in human atherosclerotic plaques by binding of digoxigenin-labeled factors VIIa and X. *Lab Invest* 75:451, 1996
129. Hatakeyama K, Asada Y, Marutsuka K, Sato Y, Kamikubo Y, Sumiyoshi A: Localization and activity of tissue factor in human aortic atherosclerotic lesions. *Atherosclerosis* 133:213, 1997
130. Contrino J, Hair G, Kreutzer DL, Rickles FR: In situ detection of tissue factor in vascular endothelial cells: Correlation with the malignant phenotype of human breast disease. *Nat Med* 2:209, 1996
131. Sato Y, Asada Y, Marutsuka K, Hatakeyama K, Sumiyoshi A: Tissue factor induces migration of cultured aortic smooth muscle cells. *Thromb Haemost* 75:389, 1996
132. Zhang Y, Deng Y, Luther T, Muller M, Ziegler R, Waldherr R, Stern DM, Nawroth PP: Tissue factor controls the balance of angiogenic and antiangiogenic properties of tumor cells in mice. *J Clin Invest* 94:1320, 1994
133. Stern D, Nawroth P, Handley D, Kiesel W: An endothelial cell-dependent pathway of coagulation. *Proc Natl Acad Sci USA* 82:2523, 1985
134. Brinkman H-JM, Mertens K, Holthius J, Zwart-Huinink LA, Grijm K, van Mourik JA: The activation of blood coagulation factor X on the surface of endothelial cells: A comparison with various vascular cells, platelets and monocytes. *Br J Haematol* 87:332, 1994
135. Cheung WF, van den Born J, Kuhn K, Kjellen L, Hudson BG, Stafford DW: Identification of the endothelial cell binding site for factor IX. *Proc Natl Acad Sci USA* 80:7001, 1996
136. Altieri DC: Xa receptor EPR-1. *FASEB J* 9:860, 1995
137. Bono F, Herault J-P, Avril C, Schaeffer P, Lormeau J-C, Herbert J-M: Human umbilical vein endothelial cells express high affinity receptors for factor Xa. *J Cell Physiol* 172:36, 1997
138. Etingen OR, Silverstein RL, Hajjar DP: Identification of a monocyte receptor on herpes-infected endothelial cells. *Proc Natl Acad Sci USA* 88:7200, 1991
139. Schmaier AH, Kuo A, Cines DB: High molecular weight kininogen expression on human endothelial cells. *J Biol Chem* 31:16327, 1988
140. Woolkanis MJ, DeMelfi TM Jr, Blanchard N, Hoxie JA, Brass LF: Regulation of thrombin receptors on HUVECs. *J Biol Chem* 270:9686, 1995
141. Kanthou C, Benzakour O: Cellular effects of thrombin and their signalling pathways. *Cell Pharmacol* 2:293, 1995
142. Garcia JGN, Pavalko FM, Patterson CE: Vascular endothelial cell activation and permeability responses to thrombin. *Blood Coag Fibrinol* 6:609, 1995
143. Connolly AJ, Ishihara H, Kahn MI, Ferese RV Jr, Coughlin SR: Role of the thrombin receptor in development and evidence for a second receptor. *Nature* 381:516, 1996
144. Mirtza H, Yatsula V, Bahou WF: The proteinase activated receptor-2 (PAR-2) mediates mitogenic responses in human vascular endothelial cells. Molecular characterization and evidence for functional coupling to the thrombin receptor. *J Clin Invest* 97:1705, 1996
145. Ishihara H, Connolly AJ, Zeng D, Kahn ML, Zheng YW, Timmons C, Tram T, Coughlin SR: Protease-activated receptor 3 is a second thrombin receptor in humans. *Nature* 386:502, 1997
146. Brass LF, Molino M: Protease-activated G protein-coupled receptors on human platelets and endothelial cells. *Thromb Haemost* 78:234, 1997
147. Odrijn TM, Francis CW, Sporn LA, Bunce LA, Marder VJ, Simpson-Haidaris PJ: Heparin-binding domain of fibrin mediates its binding to endothelial cells. *Arterioscler Thromb Vasc Biol* 16:1544, 1996
148. Erban JK, Wagner DD: A 130-kDa protein on endothelial cells binds to amino acids 15-42 of the B β chain of fibrinogen. *J Biol Chem* 267:2451, 1992
149. Greenburg CS, Birckbichler PJ, Rice RH: Transglutaminases: Multifunctional cross-linking enzymes that stabilize tissues. *FASEB J* 5:3071, 1991
150. Okada Y, Copeland BR, Hamann GF, Koziol JA, Cheresch DA, Del Zoppo GJ: Integrin $\alpha_v\beta_3$ is expressed in selected microvessels after focal cerebral ischemia. *Am J Pathol* 149:37, 1996
151. Ruoslahti E, Engvall E: Integrins and vascular extracellular matrix assembly. *J Clin Invest* 99:1149, 1997
152. Wagner DD, Bonfanti R: Von Willebrand factor and the endothelium. *Mayo Clin Proc* 66:621, 1991
153. Rajagopalan V, Essex DW, Shapiro SS, Konkle BA: Tumor necrosis factor- α modulation of glycoprotein Ib α expression in human endothelial and erythroleukemia cells. *Blood* 80:153, 1992
154. Beacham DA, Tran LP, Ludlow LB, Huang R, Konkle BA, Shapiro SS: Endothelial glycoprotein Ib α expression is upregulated by arterial shear stress. *Blood* 86:2188a, 1995 (abstr, suppl)
155. Taylor FB Jr: Studies on the inflammatory-coagulant axis in the baboon response to *E. coli*: Regulatory role of proteins C, S, C4bBP and of inhibitors of tissue factor. *Prog Clin Biol Res* 388:175, 1994
156. Hirsh J, Salzman EW, Marder VJ, Colman RW: Overview of the thrombotic process and its therapy, in Colman RW, Hirsh J, Marder VJ, Salzman EW (eds): *Hemostasis and Thrombosis. Basic Principles and Clinical Practice*. Philadelphia, PA, Lippincott, 1994, p 1151
157. Todd AS: Histological localization of fibrinolytic activator. *J Pathol Bacteriol* 78:281, 1959
158. Pandolfi M: Histochemistry of tissue plasminogen activator. *Thromb Diath Haemorrh* 34:661, 1975
159. Levin EG, Marotti KR, Santell L: Protein kinase C and the stimulation of tissue plasminogen activator release from human endothelial cells. *J Biol Chem* 264:16030, 1989
160. Cugno M, Uziel L, Fabrezi I, Bottasso B, Maggiolini F, Agostoni A: Fibrinolytic response in normal subjects to venous occlusion and DDAVP infusion. *Thromb Res* 56:625, 1989
161. Levin EG, Santell L: Thrombin- and histamine-induced signal transduction in human endothelial cells. Stimulation and agonist-dependent desensitization of protein phosphorylation. *J Biol Chem* 266:174, 1991
162. McCrae KR, Aceto JF, Cines DB: Clinical aspects of the plasminogen activator system. *Adv Pathol Lab Med* 7:467, 1994
163. Bacharach E, Itin A, Keshet E: *In vivo* patterns of expression of urokinase and its inhibitor PAI-1 suggest a concerted role in regulating physiologic angiogenesis. *Proc Natl Acad Sci USA* 89:10686, 1992
164. Carmeliet P, Schoonjans L, Kieckens L, Ream B, Degan J, Bronson R, De Vos R, van den Oord JJ, Collen D, Mulligan RC: Physiological consequences of loss of plasminogen activator gene function in mice. *Nature* 369:419, 1994
165. Yamamoto K, Loskutoff DJ: Fibrin deposition in tissues from endotoxin-treated mice correlates with decreases in the expression of

- urokinase-type but not tissue type plasminogen activator. *J Clin Invest* 97:2440, 1996
166. Barnathan ES, Kuo A, van der Keyl H, McCrae KM, Cines DB: Binding of tissue type plasminogen activator to human endothelial cells: Evidence for two distinct binding sites. *J Biol Chem* 263:7792, 1988
167. Beebe DP, Miles LA, Plow EF: A linear amino acid sequence involved in the interaction of t-PA with its endothelial cell receptor. *Blood* 74:2034, 1989
168. Fukao H, Hagiya Y, Nonaka T, Okada K, Matsuo O: Analysis of binding protein for tissue-type plasminogen activator in human endothelial cells. *Biochem Biophys Res Commun* 187:956, 1992
169. Ramakrishnan V, Sinicropi DV, Dere R, Darbonne WC, Bechtol KB, Baker JB: Interaction of wild-type and catalytically inactive mutant forms of tissue-type plasminogen activator with human umbilical vein endothelial cell monolayers. *J Biol Chem* 265:2755, 1990
170. Cheng X-F, Pohl G, Back O, Wallen P: Characterization of receptors interacting specially with the B-chain of tissue plasminogen activator on endothelial cells. *Fibrinolysis* 10:167, 1996
171. Hajjar KA, Hamel NM, Harpel PC, Nachman RL: Binding of tissue plasminogen activator to cultured human endothelial cells. *J Clin Invest* 80:1712, 1987
172. Hajjar KA, Hamel NM: Identification and characterization of human endothelial cell membrane binding sites for tissue plasminogen activator and urokinase. *J Biol Chem* 265:2908, 1990
173. Weling TH, Huber TS, Messina LM, Stanley JC: Tissue plasminogen activator increases canine endothelial cell proliferation rate through a plasmin-independent, receptor-mediated mechanism. *J Surg Res* 66:36, 1996
174. Hajjar KA, Jacovina AT, Checko J: An endothelial cell receptor for plasminogen/tissue plasminogen activator. *J Biol Chem* 269:21191, 1994
175. Barnathan ES, Kuo A, Rosenfeld L, Kariko K, Leski M, Robbiati F, Nolli ML, Henkin J, Cines DB: Interaction of single chain urokinase type plasminogen activator with human endothelial cells. *J Biol Chem* 265:2865, 1990
176. Barnathan ES, Kuo A, Kariko K, Rosenfeld L, Murray SC, Behrendt N, Ronne E, Weiner D, Henkin J, Cines DB: Characterization of human endothelial cell urokinase-type plasminogen activator receptor protein and mRNA. *Blood* 76:1795, 1990
177. Wun TC, Schleuning W-D, Reich E: Isolation and characterization of urokinase from human plasma. *J Biol Chem* 257:3276, 1982
178. Manchanda N, Schwartz BS: Single chain urokinase. Augmentation of enzymatic activity upon binding to monocytes. *J Biol Chem* 266:14580, 1991
179. Schwartz BS: Differential inhibition of soluble and cell surface receptor bound scu-PA by plasminogen activator inhibitor. *J Biol Chem* 269:8319, 1994
180. Higazi AA-R, Mazar A, Wang J, Reilly R, Henkin J, Kniss D, Cines D: Single chain urokinase-type plasminogen activator bound to its receptor is relatively resistant to plasminogen activator inhibitor type 1. *Blood* 87:3545, 1996
181. Pepper MS, Sappino A-P, Stocklin R, Montesano R, Orci L, Vassalli J-D: Upregulation of urokinase receptor expression on migrating endothelial cells. *J Cell Biol* 122:673, 1993
182. Bugge TH, Flick MJ, Danton MJS, Daugherty CC, Romer J, Dano K, Carmeliet P, Collen D, Degen JL: Urokinase-type plasminogen activator is effective in fibrin clearance in the absence of its receptor or tissue-type plasminogen activator. *Proc Natl Acad Sci USA* 93:5899, 1996
183. Miles LA, Levin EG, Plescia J, Collen D, Plow EF: Plasminogen receptors, urokinase receptors, and their modulation on human endothelial cells. *Blood* 72:628, 1988
184. Plow EF, Freaney DE, Plescia J, Miles LA: The plasminogen system and cell surfaces: Evidence for plasminogen and urokinase receptors on the same cell type. *J Cell Biol* 103:2411, 1986
185. Ploplis VA, Carmeliet P, Vazirzadeh S, van Vlaenderen I, Moons L, Plow EF, Collen D: Effects of disruption of the plasminogen gene on thrombosis, growth, and health in mice. *Circulation* 92:2585, 1995
186. Miles LA, Fless GM, Levin EG, Scanu AM, Plow EF: A potential basis for the thrombotic risks associated with lipoprotein(a). *Nature* 339:301, 1989
187. Grainger DJ, Kemp PR, Metcalfe JC, Liu AC, Lawn RM, Williams NR, Grace AA, Schofield PM, Chauhan A: The serum concentration of active transforming growth factor- β is severely depressed in advanced atherosclerosis. *Nat Med* 1:74, 1995
188. Levin EG, Santell L: Association of a plasminogen activator inhibitor (PAI-1) with the growth substratum and membrane of human endothelial cells. *J Cell Biol* 105:2543, 1987
189. Loskutoff DJ, Sawdey M, Mimuro J: Type 1 plasminogen activator inhibitor, in Coller BS (ed): *Progress in Hemostasis and Thrombosis*. Philadelphia, PA, Saunders, 1989, p 87
190. Sawdey MS, Loskutoff DJ: Regulation of murine type 1 plasminogen activator inhibitor gene *in vivo*. Tissue specificity and induction by lipopolysaccharide, tumor necrosis factor- α , and transforming growth factor- β . *J Clin Invest* 88:1346, 1991
191. Astedt B, Hagerstrand I, Lecander I: Cellular localization in placenta of placental type plasminogen activator inhibitor. *Thromb Haemost* 56:63, 1986
192. Scarpati EM, Sadler JE: Regulation of endothelial cells coagulant properties. *J Biol Chem* 264:20705, 1989
193. Suzuki K, Nishioka J, Hashimoto S: Protein C inhibitor. Purification from human plasma and characterization. *J Biol Chem* 258:163, 1983
194. Geiger M, Prilinger U, Griffin JH, Binder BR: Urinary protein C inhibitor. Glycosaminoglycans synthesized by the epithelial cell line TC1-598 enhance its interaction with urokinase. *J Biol Chem* 266:11851, 1991
195. Bajzar L, Morser J, Nesheim M: TAFI, or plasma procarboxypeptidase B, couples the coagulation and fibrinolytic cascades through the thrombin-thrombomodulin complex. *J Biol Chem* 271:16603, 1996
196. Romano M, Sironi M, Toniatti C, Polentarutti N, Fruscella P, Ghezzi P, van Hinsbergh V, Sozzani S, Bussolino F, Poli V, Ciliberto G, Mantovani A: Role of IL-6 and its soluble receptor in induction of chemokines and leukocyte recruitment. *Immunity* 6:315, 1997
197. Middleton J, Neil S, Wintle J, Clark-Lewis I, Moore H, Lam C, Auer M, Hub E, Rot A: Transcytosis and surface presentation of IL-8 by venular endothelial cells. *Cell* 91:385, 1997
198. Schafer AI: Vascular endothelium: In defense of blood fluidity. *J Clin Invest* 99:1143, 1997
199. Marcus AJ, Broekman MJ, Drosopoulos JHF, Islam N, Alyoncheva TN, Safer LB, Hajjar KA, Posnett DN, Shoenborn MA, Schooflet KA, Gayle RB, Maliszewski CR: The endothelial cell ecto-ADPase responsible for inhibition of platelet function is CD39. *J Clin Invest* 99:1351, 1997
200. Roth GJ: Platelets and blood vessels: The adhesion event. *Immunol Today* 13:100, 1992
201. Ruggeri Z, Ware J: von Willebrand factor. *FASEB J* 7:308, 1993
202. Dong J-f, Hyun W, Lopez JA: Aggregation of mammalian cells expressing the platelet glycoprotein (GP) Ib-IX complex and the requirement for tyrosine sulfation of GPIb α . *Blood* 86:4175, 1995
203. Lopez JA: The platelet glycoprotein Ib-IX complex. *Blood Coagul Fibrinol* 5:97, 1994
204. Savage B, Saldivar E, Ruggeri ZM: Initiation of platelet adhesion by arrest onto fibrinogen or translocation on von Willebrand factor. *Cell* 84:289, 1996
205. Krall MH, Hellums JD, McIntire LV, Schafer AI, Moake JL: Platelets and shear stress. *Blood* 88:1525, 1996

206. Calvete JJ: Clues for understanding the structure and function of a prototypic human integrin: The platelet glycoprotein IIb/IIIa complex. *Thromb Haemostasis* 72:1, 1994
207. McEver RP, Moore KL, Cummings RD: Leukocyte trafficking mediated by selectin-carbohydrate interactions. *J Biol Chem* 270:11025, 1995
208. Walcheck B, Moore KL, McEver RP, Kishimoto TK: Neutrophil-neutrophil interactions under hydrodynamic shear stress involve L-selectin and PSGL-1: A mechanism that amplifies initial leukocyte accumulation on P-selectin *in vitro*. *J Clin Invest* 98:1081, 1996
209. Buttrum SM, Hatton R, Nash GB: Selectin-mediated rolling of neutrophils on immobilized platelets. *Blood* 82:1165, 1993
210. Palabrica T, Lobb R, Furie BC, Aronovitz M, Benjamin C, Hsu Y-M, Sajer SA, Furie B: Leukocyte accumulation promoting fibrin deposition is mediated *in vivo* by P-selectin on adherent platelets. *Nature* 359:848, 1992
211. Diacovo TG, Puri KD, Warnock RA, Springer TA, Von Andrian UH: Platelet-mediated lymphocyte delivery to high endothelial venules. *Science* 273:252, 1996
212. Hemmerich S, Leffler H, Rosen SD: Structure of the O-glycans in GlyCAM-1, an endothelial-derived ligand for L-selectin. *J Biol Chem* 270:12035, 1995
213. Wilkins PP, Moore KL, McEver RP, Cummings RD: Tyrosine sulfation of P-selectin glycoprotein ligand-1 is required for high affinity binding to P-selectin. *J Biol Chem* 270:22677, 1995
214. Wilkins PP, McEver RP, Cummings RD: Structures of the O-glycans on P-selectin glycoprotein ligand-1 from HL-60 cells. *J Biol Chem* 271:18732, 1996
215. McEver RP, Cummings RD: Role of PSGL-1 binding to selectins in leukocyte recruitment. *J Clin Invest* 100:485, 1997
216. Berlin C, Bargatz RF, Campbell JJ, Von Andrian UH, Szabo MC, Hasslen SR, Nelson RD, Berg EL, Erlandsen SL, Butcher EC: $\alpha 4$ integrins mediate lymphocyte attachment and rolling under physiologic flow. *Cell* 80:413, 1995
217. Alon R, Kassner PD, Carr MW, Finger EB, Hemler ME, Springer TA: The integrin VLA-4 supports tethering and rolling in flow on VCAM-1. *J Cell Biol* 128:1243, 1995
218. DeGrendele HC, Estess P, Picker LJ, Siegelman MH: CD44 and its ligand hyaluronate mediate rolling under physiologic flow: A novel lymphocyte-endothelial cell primary adhesion pathway. *J Exp Med* 183:1119, 1996
219. Alon R, Hammer DA, Springer TA: Lifetime of the P-selectin: Carbohydrate bond and its response to tensile force in hydrodynamic flow. *Nature* 374:539, 1995
220. Finger EB, Puri KD, Alon R, Lawrence MB, Von Andrian UH, Springer TA: Adhesion through L-selectin requires a threshold hydrodynamic shear. *Nature* 379:266, 1996
221. Walcheck B, Kahn J, Fisher JM, Wang BB, Fisk RS, Payan DG, Feehan C, Betageri R, Darlak K, Spatola AF, Kishimoto TK: Neutrophil rolling altered by inhibition of L-selectin shedding *in vitro*. *Nature* 380:720, 1996
222. Patel KD, Nollert MU, McEver RP: P-selectin must extend a sufficient length from the plasma membrane to mediate rolling of neutrophils. *J Cell Biol* 131:1893, 1995
223. Weyrich AS, McIntyre TM, McEver RP, Prescott SM, Zimmerman GA: Monocyte tethering by P-selectin regulates monocyte chemoattractant protein-1 and tumor necrosis factor- α secretion. *J Clin Invest* 95:2297, 1995
224. Springer TA: Traffic signals on endothelium for lymphocyte recirculation and leukocyte emigration. *Annu Rev Physiol* 57:827, 1995
225. Languino LR, Plescia J, Duperray A, Brian AA, Plow EF, Geltosky JE, Altieri DC: Fibrinogen mediates leukocyte adhesion to vascular endothelium through an ICAM-1-dependent pathway. *Cell* 73:1423, 1993
226. Muller WA, Weigl SA: Monocyte-selective transendothelial migration: Dissection of the binding and transmigration phases by an *in vitro* assay. *J Exp Med* 176:819, 1992
227. Dejana E, Corada M, Lampugnani MG: Endothelial cell-to-cell junctions. *FASEB J* 9:910, 1995
228. Johnson RC, Chapman SM, Dong ZM, Ordovas JM, Mayadas TN, Herz J, Hynes RO, Schaefer EJ, Wagner DD: Absence of P-selectin delays fatty streak formation in mice. *J Clin Invest* 99:1037, 1997
229. Anderson DC, Springer TA: Leukocyte adhesion deficiency: An inherited defect in the Mac-1, LFA-1, and p150,95 glycoproteins. *Annu Rev Med* 38:175, 1987
230. Etzioni A, Frydman M, Pollack S, Avidor I, Phillips ML, Paulson JC, Gershoni-Baruch R: Brief report: Recurrent severe infections caused by a novel leukocyte adhesion deficiency. *N Engl J Med* 327:1789, 1992
231. Ley K, Tedder TF: Leukocyte interactions with vascular endothelium: New insights into selectin-mediated attachment and rolling. *J Immunol* 155:525, 1995
232. Frenette PS, Mayadas TN, Rayburn H, Hynes RO, Wagner DD: Susceptibility to infection and altered hematopoiesis and mice deficient in both P- and E-selectin. *Cell* 84:563, 1996
233. Bullard DC, Kunkel EJ, Kubo H, Hicks MJ, Lorenzo I, Doyle NA, Doerschuk CM, Ley K, Beaudet AL: Infectious susceptibility and severe deficiency of leukocyte rolling and recruitment in E-selectin and P-selectin double mutant mice. *J Exp Med* 183:2329, 1996
234. Frenette PS, Mayadas TN, Rayburn H, Hynes RO, Wagner DD: Susceptibility to infection and altered hematopoiesis in mice deficient in both P- and E-selectins. *Cell* 84:563, 1996
235. Maly P, Thall AD, Petryniak B, Rogers CE, Smith PL, Marks RM, Kelly RJ, Gersten KM, Cheng G, Saunders TL, Camper SA, Camphausen RT, Sullivan FX, Isogai Y, Hindsgaul O, von Andrian UH, Lowe JB: The $\alpha(1,3)$ fucosyltransferase Fuc-TVII controls leukocyte trafficking through an essential role in L-, E-, and P-selectin ligand biosynthesis. *Cell* 86:643, 1996
236. Kunkel EJ, Jung U, Bullard DC, Norman KE, Wolitzky BA, Vestweber D, Beaudet AL, Ley K: Absence of trauma-induced leukocyte rolling in mice deficient in both P-selectin and intercellular adhesion molecule 1. *J Exp Med* 183:571, 1996
237. Schmits R, Kundig TM, Baker IM, Shumaker G, Simard JLL, Duncan G, Wakeham A, Shaninian A, van der Heiden A, Bachman MF, Ohashi PS, Mak TW, Hickstein DD: LFA-1-deficient mice show normal CTL responses to virus but fail to reject immunogenic tumor. *J Exp Med* 183:1415, 1996
238. Coxon A, Rieu P, Barkalow FJ, Askari S, Sharpe AH, von Andrian UH, Arnaut MA, Mayadas TN: A novel role for the beta 2 integrin CD11b/CD18 in neutrophil apoptosis: A homeostatic mechanism in inflammation. *Immunity* 5:653, 1996
239. Lu H, Smith CW, Perrard J, Bullard D, Tang L, Shappell SB, Entman ML, Beaudet AL, Ballantyne CM: LFA-1 is sufficient in mediating neutrophil emigration in MAC-1 deficient mice. *J Clin Invest* 99:1340, 1997
240. Arroyo AG, Yang JT, Rayburn H, Hynes RO: Differential requirements for $\alpha 4$ integrins during fetal and adult hematopoiesis. *Cell* 85:997, 1996
241. Pober JS, Orosz CG, Rose ML, Savage COS: Can graft endothelial cells initiate a host anti-graft immune response? *Transplant* 61:343, 1996
242. Murray AG, Libby P, Pober JS: Human vascular smooth muscle cells poorly costimulate and actively inhibit allogeneic CD4+ T cell proliferation *in vitro*. *J Immunol* 154:151, 1995
243. Marcelli-Berg FM, Hargreaves REG, Carmichael P, Dorling A, Lombardi G, Lechler RI: Major histocompatibility complex class II-expressing endothelial cells induce allospecific non-responsiveness in naive T cells. *J Exp Med* 183:1603, 1996
244. Murray AG, Khodadoust MM, Pober JS, Bothwell ALM: Porcine aortic endothelial cells strongly activate human T cells: Direct

presentation of swine MHC antigens and effective costimulation by swine ligands for human CD2 and CD28. *Immunity* 1:57, 1994

245. Karman K, Hughes CCW, Schechner J, Fnaslow WC, Pober JS: CD40 on human endothelial cells: Inducibility by cytokines and functional regulation of adhesion molecule expression. *Proc Natl Acad Sci USA* 92:4342, 1995

246. Pober JS, Cotran RS: Cytokines and endothelial cell biology. *Physiol Rev* 70:427, 1990

247. Pober JS, Cotran RS: Overview: The role of endothelial cells in inflammation. *Transplant* 50:537, 1990

248. Austrup F, Vestweber D, Borges E, Lohning M, Grauer R, Herz V, Renz H, Hallmann R, Scheffold A, Rudbruch A, Hamann A: P- and E-selectin mediate recruitment of T helper 1 but not T helper 2 cells into inflamed tissues. *Nature* 385:81, 1997

249. Borges E, Tietz W, Steegmaier M, Moll T, Hallmann R, Hamann A, Vestweber D: P-selectin glycoprotein ligand-1 (PSGL-1) on T helper 1 but not T helper 2 cells binds to P-selectin and supports migration into inflamed skin. *J Exp Med* 185:573, 1997

250. Bradley JR, Pober JS: Prolonged cytokine exposure causes a dynamic redistribution of endothelial cell adhesion molecules to intercellular junctions. *Lab Invest* 75:463, 1996

251. Montesano R, Orci L, Vassalli P: Human endothelial cell cultures: Phenotypic modulation by leukocyte interleukins. *J Cell Physiol* 122:424, 1985

252. Stolpen AH, Guinan EC, Fiers W, Pober JS: Recombinant tumor necrosis factor and immune interferon act singly and in combination to reorganize human vascular endothelial cell monolayers. *Am J Pathol* 123:16, 1986

253. Jutte NHP, Knoof CJ, Heijse P, Balk AHMM, Mochtar B, Claas FHH, Weimann W: Human heart endothelial-cell-restricted allorecognition. *Transplant* 62:403, 1996

254. Damle NK, Doyle LV, Bender JR, Bradley EC: Interleukin 2-activated human lymphocytes exhibit enhanced adhesion to normal vascular endothelial cells and cause their lysis. *J Immunol* 138:1779, 1987

255. Bach FH, Ferran C, Hechenletner P, Mark W, Koyamada N, Miyatake T, Winkler H, Badrichani A, Candinas D, Hancock WW: Accommodation of vascularized xenografts: Expression of "protective genes" by donor endothelial cells in a host T_H2 cytokine environment. *Nat Med* 3:196, 1997

256. Platt AF Jr, Eckman JR: The multidisciplinary management of pain in patients with sickle cell syndromes. *J Am Acad Phys Assist* 2:104, 1989

257. Howard RJ, Gilladoga AD: Molecular studies related to the pathogenesis of cerebral malaria. *Blood* 74:2603, 1989

258. Wautier JL, Wautier MP, Schmidt A-M, Anderson GM, Hori O, Zoukourian C, Capron L, Chappey O, Yan S-D, Brett J, Guillausseau P-J, Stern D: Advanced glycation end products (AGEs) on the surface of diabetic erythrocytes bind to the vessel wall via a specific receptor inducing oxidant stress in the vasculature: A link between surface-associated AGEs and diabetic complications. *Proc Natl Acad Sci USA* 91:7742, 1994

259. Mozarelli A, Hofrichter J, Eaton WA: Delay time of hemoglobin S polymerization prevents most cells from sickling in vivo. *Science* 237:500, 1987

260. Eaton WA, Hofrichter J, Ross PD: Delay time of gelation: A possible determinant of clinical severity of sickle cell disease. *Blood* 47:621, 1976

261. Hebbel RP, Yamada O, Moldow CF, Jacob HS, White JG, Eaton JW: Abnormal adherence of sickle erythrocytes to cultured vascular endothelium. Possible mechanism for microvascular occlusion. *J Clin Invest* 65:154, 1980

262. Wick TM, Moake JL, Udden MM, Eskin SG, Sean DA, McIntire LV: Unusually large von Willebrand factor multimers increase

adhesion of sickle erythrocytes to human endothelial cells under controlled flow. *J Clin Invest* 80:905, 1987

263. Kaul DK, Fabry ME, Nagel RL: Microvascular sites and characteristics of sickle cell adhesion to vascular endothelium in shear flow conditions: Pathophysiological implications. *Proc Natl Acad Sci USA* 86:3356, 1989

264. Wick TM, Eckman JR: Molecular basis of sickle cell-endothelial cell interactions. *Curr Opin Hematol* 3:118, 1996

265. Hebbel RP, Moldow CF, Steinberg MH: Modulation of erythrocyte-endothelial interactions and the vaso-occlusive severity of sickling disorders. *Blood* 58:947, 1981

266. Wautier JL, Wautier MP, Pintigny D, Galacteros F, Courillon A, Passa P, Caen JP: Factors involved in cell adhesion to vascular endothelium. *Blood Cells* 9:221, 1983

267. Sugihara K, Sugihara T, Mohandas N, Hebbel RP: Thrombospondin mediates adherence of CD36⁺ sickle reticulocytes to endothelial cells. *Blood* 80:2634, 1992

268. Brittain HA, Eckman JR, Howard RJ, Wick TM: Thrombospondin from activated platelets promotes sickle erythrocyte adherence to human microvascular endothelium under physiologic flow: A potential role for platelet activation in sickle cell vaso-occlusion. *Blood* 81:2137, 1993

269. Joneckis CC, Ackley RL, Orringer EP, Wayner EA, Parise LV: Integrin $\alpha_4\beta_1$ and glycoprotein IV (CD36) are expressed on circulating reticulocytes in sickle cell anemia. *Blood* 82:3548, 1993

270. Swerlick RA, Eckman JR, Kumar A, Jeitler M, Wick TM: $\alpha_4\beta_1$ -integrin expression on sickle reticulocytes: Vascular cell adhesion molecule-dependent binding to endothelium. *Blood* 82:1891, 1993

271. Setty BNY, Stuart MJ: Vascular adhesion molecule-1 is involved in mediating hypoxia-induced sickle red blood cell adherence to endothelium: Potential role in sickle cell disease. *Blood* 88:2311, 1996

272. Smolinski PA, Offermann MK, Eckman JR, Wick TM: Synthetic double stranded RNA increases sickle red blood cell adherence to endothelium via a VCAM-1/VLA-4 pathway. *Blood* 85:2945, 1995

273. Kumar A, Eckman JR, Swerlick RA, Wick TM: Phorbol ester stimulation increases sickle erythrocyte adherence to endothelium: A novel pathway involving $\alpha_4\beta_1$ integrin receptors on sickle reticulocytes and fibronectin. *Blood* 88:4348, 1996

274. Natarajan M, Udden MM, McIntire LV: Adhesion of sickle red blood cells and damage to interleukin-1 β stimulated endothelial cells under flow in vitro. *Blood* 87:4845, 1996

275. Styles LA, Lubin B, Vichinsky E, Lawrence S, Hua M, Test S, Kuypers F: Decrease of very late activation antigen-4 and CD36 on reticulocytes in sickle cell patients treated with hydroxyurea. *Blood* 89:2554, 1997

276. Brittain HA, Eckman JR, Wick TM: Sickle erythrocytic adherence to large vessel and microvascular endothelium under physiologic flow is qualitatively different. *J Lab Clin Med* 19:538, 1992

277. Swerlick RA, Lee KH, Wick TM, Lawley TJ: Human dermal microvascular endothelial but not human umbilical vein endothelial cells express CD36 in vivo and in vitro. *J Immunol* 148:78, 1992

278. Smolinski PA, Eckman JR, Wick TM: Increased tenacity of sickle erythrocyte-endothelial adherence by involvement of multiple receptor-ligand interactions. *Blood* 86:298a, 1995 (abstr, suppl 1)

279. Kaul DK, Fabry ME, Nagel RL: Vaso-occlusion by sickle cells: Evidence for selective trapping of dense cells. *Blood* 68:1162, 1986

280. Setty BNY, Chen D, Stuart MJ: Sickle red blood cells stimulate endothelial cell production of eicosanoids and diacylglycerol. *J Lab Clin Med* 128:313, 1996

281. Solovey A, Lin Y, Browne P, Choong S, Wayner E, Hebbel RP: Circulating activated endothelial cells in sickle cell anemia. *N Engl J Med* 337:1584, 1997

282. Ockenhouse CF, Tegodhi T, Maeno Y, Benjamin C, Ho M, Kan KE, Thway Y, Win K, Aikawa M, Lobb R: Human vascular endothelial

cell adhesion receptors for *Plasmodium falciparum*-infected erythrocytes: Roles for endothelial leukocyte adhesion molecule 1 and vascular cell adhesion molecule 1. *J Exp Med* 176:1183, 1992

283. Cooke BM, Berendt AR, Craig AG, MacGergor J, Newbold CI, Nash GB: Rolling and stationary cytoadhesion of red blood cells parasitized by *Plasmodium falciparum*: Separate roles for ICAM-1, CD36 and thrombospondin. *Br J Haematol* 87:162, 1994

284. McCormick CJ, Craig A, Roberts D, Newbold CI, Berendt AR: Intercellular adhesion molecule-1 and CD36 synergize to mediate adherence of *Plasmodium falciparum*-infected erythrocytes to cultured human microvascular endothelial cells. *J Clin Invest* 100:2521, 1997

285. Elhassan IM, Hviid L, Satti G, Akerstrom B, Jakobsen PH, Jensen JB, Theander TG: Evidence of endothelial inflammation, T cell activation and T cell reallocation in uncomplicated *Plasmodium falciparum* malaria. *Am J Trop Med Hyg* 51:372, 1994

286. Jakobsen PH, Bate CAW, Taverne J, Playfair JHL: Malaria: Toxins, cytokines and disease. *Parasite Immunol* 17:223, 1995

287. Crandall I, Collins WE, Gysin J, Sherman IW: Synthetic peptides based on motifs present in human band 3 protein inhibit cytoadherence/sequestration of the malaria parasite *Plasmodium falciparum*. *Proc Natl Acad Sci USA* 90:4703, 1993

288. Crandall I, Sherman IW: Cytoadherence-related neoantigens on *Plasmodium falciparum* (human malaria)-infected erythrocytes result from the exposure of normally cryptic regions of the band 3 protein. *Parasitology* 108:257, 1994

289. Chaiyoroj S, Angkasekwinai P, Buranakiti A, Looareesuwan S, Rogerson SJ, Brown GV: Cytoadherence characteristics of *Plasmodium falciparum* isolates from Thailand: Evidence for chondroitin sulfate A as a cytoadherence receptor. *Am J Trop Med Hyg* 55:76, 1996

290. Wautier JL, Panton RC, Wautier MP, Pintigny D, Abadie E, Passa P, Caen JP: Increased adhesion of erythrocytes to endothelial cells in diabetes mellitus and its relation to vascular complications. *N Engl J Med* 305:237, 1981

291. Ross R, Glomset JA: The pathogenesis of atherosclerosis (second of two parts). *N Engl J Med* 295:420, 1976

292. Ross R: The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* 362:801, 1993

293. McGorisk GM, Treasure CB: Endothelial dysfunction in coronary heart disease. *Curr Opin Cardiol* 11:341, 1996

294. Henry PD: Hyperlipidemic endothelial injury and angiogenesis. *Basic Res Cardiol* 1:107, 1994

295. Larrick JW, Wright SC: Hypertension-enhanced monocyte adhesion in experimental atherosclerosis. *FASEB J* 4:3215, 1990

296. Schmidt AM, Hori O, Brett J, Yan SD, Wautier JL, Stern D: Cellular receptors for advanced glycation end products. Implications for induction of oxidant stress and cellular dysfunction in the pathogenesis of vascular lesions. *Arterioscler Thromb* 14:1521, 1994

297. Marui N, Offermann MK, Swerlick R, Kunsch C, Rosen CA, Ahmad M, Alexander RW, Medford RM: Vascular cell adhesion molecule-1 (VCAM-1) gene transcription and expression are regulated through an antioxidant-sensitive mechanism in human vascular endothelial cells. *J Clin Invest* 92:1866, 1993

298. Gimbrone MA Jr: Vascular endothelium: An integrator of pathophysiologic stimuli in atherosclerosis. *Am J Cardiol* 75:67B, 1995

299. Davies P: Flow-mediated endothelial mechanotransduction. *Physiol Rev* 75:519, 1995

300. Malek AM, Izumo S: Control of endothelial cell gene expression by flow. *J Biomechanics* 28:1515, 1995

301. McLenachan JM, Vita J, Fish DR, Treasure CB, Cox DA, Ganz P, Selwyn AP: Early evidence of endothelial vasodilator dysfunction at coronary branch points. *Circulation* 82:1169, 1990

302. Ando J, Kamiya A: Flow-dependent regulation of gene expression in vascular endothelial cells. *Heart J* 37:19, 1996

303. Tsao PS, Buitrago R, Chan JR, Cooke JP: Fluid flow inhibits

endothelial adhesiveness: Nitric oxide and transcriptional regulation of VCAM-1. *Circulation* 94:1682, 1996

304. Grabowski EF, Lam FP: Endothelial cell function, including tissue factor expression, under flow conditions. *Thromb Haemost* 74:123, 1995

305. Khachigian LM, Resnick N, Gimbrone MA Jr, Collins T: Nuclear factor κ B interacts functionally with the platelet-derived growth factor B-chain shear-stress response element in vascular endothelial cells exposed to fluid shear stress. *J Clin Invest* 92:1706, 1993

306. Ohno M, Cooke JP, Dzau VJ, Gibbons GH: Fluid shear stress induces endothelial transforming growth factor beta-1 transcription and production. *J Clin Invest* 96:1169, 1995

307. Lan Q, Mercurius KO, Davies PF: Stimulation of transcription factors NF κ B and AP1 in endothelial cells subjected to shear stress. *Biochem Biophys Res Commun* 201:950, 1994

308. Guba SC, Fink LM, Fonseca V: Hyperhomocysteinemia. An emerging and important risk factor for thromboembolic and cardiovascular disease. *Am J Clin Pathol* 105:709, 1996

309. Rees MM, Rodgers GM: Homocysteinemia: Association of a metabolic disorder with vascular disease and thrombosis. *Thromb Res* 71:337, 1993

310. Mayer EL, Jacobsen DW, Robinson K: Homocysteine and coronary atherosclerosis. *J Am Coll Cardiol* 27:517, 1996

311. Harpel PC, Zhang X, Borth W: Homocysteine and hemostasis: Pathogenetic mechanisms predisposing to thrombosis. *J Nutrition* 71:1285S, 1996

312. Starkebaum G, Harlan JM: Endothelial cell injury due to copper-catalyzed hydrogen peroxide generation from homocysteine. *J Clin Invest* 77:1370, 1993

313. Heinecke JW, Rosen H, Suzuki LA, Chait A: The role of sulfur-containing amino acids in superoxide production and modification of low density lipoprotein by arterial smooth muscle cells. *J Biol Chem* 262:10098, 1987

314. Stamler JS, Osborne JA, Jaraki O, Rabbani LE, Mullins M, Singel D, Loscalzo J: Adverse vascular effects of homocysteine are modulated by endothelium-derived relaxing factor and related oxides of nitrogen. *J Clin Invest* 91:308, 1993

315. Fryer RH, Wilson BD, Gubler DB, Fitzgerald LA, Rodgers GM: Homocysteine, a risk factor for premature vascular disease and thrombosis, induces tissue factor activity in endothelial cells. *Arterioscler Thromb* 13:1327, 1993

316. Rodgers GM, Kane WH: Activation of endogenous factor V by a homocysteine-induced vascular endothelial cell activator. *J Clin Invest* 77:1909, 1986

317. Hajjar KA: Homocysteine-induced modulation of tissue plasminogen activator binding to its endothelial cell membrane receptor. *J Clin Invest* 91:2873, 1993

318. Lentz SR, Sadler JE: Inhibition of thrombomodulin surface expression and protein C activation by the thrombogenic agent homocysteine. *J Clin Invest* 88:1906, 1991

319. Nishinaga M, Ozawa T, Shimada K: Homocysteine, a thrombogenic agent, suppresses anticoagulant heparan sulfate expression in cultured porcine aortic endothelial cells. *J Clin Invest* 92:381, 1992

320. Wang J, Dudman NPD, Wilken DE: Effects of homocysteine and related compounds on prostacyclin production by cultured vascular endothelial cells. *Thromb Haemost* 6:1047, 1993

321. Tsai JC, Perella MA, Yoshizumi M, Hsieh CM, Haber E, Schlegel R, Lee ME: Promotion of vascular smooth muscle cell growth by homocysteine: A link to atherosclerosis. *Proc Natl Acad Sci USA* 91:6369, 1994

322. Lentz SR, Sobey CG, Piegors DJ, Bhopatkar MY, Faraci FM, Malinow MR, Heistad DD: Vascular dysfunction in monkeys with diet-induced hyperhomocyst(e)inemia. *J Clin Invest* 98:24, 1996

323. Van Den Berg M, Boers GHJ, Franken DG, Blom HJ, van Kamp GJ, Jakobs C, Rauwerda JA, Kluit C, Stehouwert CDA:

Hyperhomocysteinemia and endothelial dysfunction in young patients with peripheral arterial occlusive disease. *Eur J Clin Invest* 25:176, 1995

324. Gryglewski RJ, Chlopicki S, Swies J, Niezabitowski P: Prostacyclin, nitric oxide, and atherosclerosis. *Ann NY Acad Sci* 748:194, 1995

325. Ludmer PL, Selwyn AP, Shook TL, Wayne RR, Mudge GH, Alexander RW, Ganz P: Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. *N Engl J Med* 315:1046, 1986

326. Siegel G, Ruckborn K, Schnalke F, Muller J: Endothelial dysfunction in human atherosclerotic coronary arteries. *Eur Heart J* 1:99, 1993

327. Sorensen KE, Celermajer DS, Georgakopoulos D, Hatcher G, Betteridge DJ, Deanfield JE: Impairment of endothelium-dependent dilation is an early event in children with familial hypercholesterolemia and is related to the lipoprotein(a) level. *J Clin Invest* 93:50, 1994

328. Tsurumi Y, Nagashima H, Ichikawa K, Sumiyoshi T, Hosoda S: Influence of plasma lipoprotein(a) levels on coronary vasomotor response to acetylcholine. *J Am Coll Cardiol* 26:1242, 1995

329. Steinberg HO, Chaker H, Leaming R, Johnson A, Brechtel G, Baron AD: Obesity/insulin resistance is associated with endothelial dysfunction. *J Clin Invest* 97:2601, 1996

330. Celermajer DS, Sorensen K, Ryalls M, Robinson J, Thomas O, Leonard JV, Deanfield JE: Impaired endothelial function occurs in the systemic arteries of children with homozygous homocystinuria but not in their heterozygous parents. *J Am Coll Cardiol* 22:854, 1993

331. Woodman OL: Modulation of vasoconstriction by endothelium-derived nitric oxide: The influence of vascular disease. *Clin Exp Pharmacol Physiol* 22:585, 1995

332. Heistad DD, Baumbach GL, Faraci FM, Armstrong ML: Sick vessel syndrome: Vascular changes in hypertension and atherosclerosis. *J Hum Hypertens* 9:449, 1995

333. Celermajer DS, Sorensen KE, Spiegelhalter DJ, Georgakopoulos D, Robinson J, Deanfield JE: Aging is associated with endothelial dysfunction in healthy men years before the age-related decline in women. *J Am Coll Cardiol* 24:471, 1994

334. Celermajer DS, Sorensen KE, Georgakopoulos D, Bull C, Thomas O, Robinson J, Deanfield JE: Cigarette smoking is associated with dose-related and potentially reversible impairment of endothelium-dependent dilation in healthy young adults. *Circulation* 88:2149, 1993

335. Kingwell BA, Tran B, Cameron JD, Jennings GL, Dart AM: Enhanced vasodilation to acetylcholine in athletes is associated with lower plasma cholesterol. *Am J Physiol* 270:H2008, 1996

336. Fish RD, Nabel EG, Selwyn AP, Ludmer PL, Mudge GH, Kirshenbaum JM, Schoen FJ, Alexander RW, Ganz P: Responses of coronary arteries of cardiac transplant patients to acetylcholine. *J Clin Invest* 81:21, 1988

337. Reddy KG, Nair RN, Sheehan HM, Hodgson JM: Evidence that selective endothelial dysfunction may occur in the absence of angiographic or ultrasound atherosclerosis in patients with risk factors for atherosclerosis. *J Am Coll Cardiol* 23:833, 1994

338. Buttery LD, Chester AH, Springall DR, Borland JA, Michel T, Yacoub MH, Polak JM: Atherogenic levels of low-density lipoprotein increase hydrogen peroxide generation in cultured human endothelial cells: Possible mechanism of heightened endocytosis. *J Pathol* 179:197, 1996

339. Holland JA, Ziegler LM, MEyer JW: Atherogenic levels of low-density lipoprotein increase hydrogen peroxide generation in cultured human endothelial cells: Possible mechanism of heightened endocytosis. *J Cell Physiol* 166:144, 1996

340. Naruse K, Shimizu K, Muramatsu M, Toki Y, Miyazaki Y, Okumura K, Hashimoto H, Ito T: Long-term inhibition of NO synthesis promotes atherosclerosis in the hypercholesterolemic rabbit thoracic

aorta. *PGH₂ does not contribute to impaired endothelium-dependent relaxation. Arterioscler Thromb* 14:746, 1994

341. Cooke JP: Role of nitric oxide in progression and regression of atherosclerosis. *West J Med* 164:419, 1996

342. Creager MA, Gallagher SJ, Girend XJ, Coleman SM, Dzau VJ, Cooke JP: L-arginine improves endothelium-dependent vasodilation in hypercholesterolemic humans. *J Clin Invest* 90:1248, 1992

343. Mathew V, Hasdai D, Lerman A: The role of endothelin in coronary atherosclerosis. *Mayo Clin Proc* 71:769, 1996

344. Sawamura T, Kume N, Aoyami T, Moriwaki H, Hoshikawa H, Alba Y, Tanaka R, Miwa S, Katsura Y, Kita T, Masaki T: An endothelial receptor for oxidized low-density lipoprotein. *Nature* 386:73, 1997

345. Boulanger CM, Tanner FC, Bea ML, Hahn AW, Werner A, Luscher TF: Oxidized low density lipoproteins induce mRNA expression and release of endothelin from human and porcine endothelium. *Circ Res* 70:1191, 1992

346. Lerman A, Holmes DR Jr, Bell MR, Garratt KN, Nishimura RA, Burnett JC Jr: Endothelin in coronary endothelial dysfunction and early atherosclerosis in humans. *Circulation* 94:2426, 1995

347. Haak T, Marz W, Jungmann E, Hausser S, Siekmeier R, Gross W, Usadel KH: Elevated endothelin levels in patients with hyperlipoproteinemia. *Clin Invest* 72:580, 1994

348. Effect of simvastatin on coronary atheroma: The Multicentre Anti-Atheroma Study (MAAS). *Lancet* 344:633, 1994

349. Treasure CB, Klein JL, Weintraub WS, Talley JD, Stillabower ME, Kosinski AS, Zhang J, Bocuzzi SJ, Cedarholm JC, Alexander RW: Beneficial effects of cholesterol-lowering therapy on the coronary endothelium in patients with coronary artery disease. *N Engl J Med* 332:4811, 1995

350. Hennig B, Diana JN, Toborek M, McClain CJ: Influence of nutrients and cytokines on endothelial cell metabolism. *J Am Coll Nutr* 13:224, 1994

351. Heitzer T, Just H, Munzel T: Antioxidant vitamin C improves endothelial dysfunction in chronic smokers. *Circulation* 94:6, 1996

352. Levine GN, Frei B, Koulouris SN, Gerhard MD, Kenaey JR Jr, Vita JA: Ascorbic acid reverses endothelial vasomotor dysfunction in patients with coronary artery disease. *Circulation* 93:1107, 1996

353. Chobanian AV, Haudenschild CC, Nickerson C, Hope S: Trandolapril inhibits atherosclerosis in the Watanabe heritable hyperlipidemic rabbit. *Hypertension* 20:473, 1992

354. Overturf ML, Sybers HD, Smith SA: Captopril-induced hyperreninemia in cholesterol-fed rabbits. *Res Commun Chem Pathol Pharmacol* 47:229, 1985

355. Lonn EM, Yusuf S, Jha P, Montague TJ, Teo KK, Benedict CR, Pitt B: Emerging role of angiotensin-converting enzyme inhibitors in cardiac and vascular protection. *Circulation* 90:2056, 1994

356. Stroes ESG, Koomans HA, deBruin TWA, Rabelink TJ: Vascular function in the forearm of hypercholesterolaemic patients on and off lipid-lowering medication. *Lancet* 346:467, 1995

357. Wada H, Mori Y, Kaneko T, Wakita Y, Nakase T, Minamikawa K, Ohiwa M, Tamaki S, Tanigawa M, Kayegama S, Deguchi K, Nakano T, Shirakawa S, Suzuki K: Elevated levels of vascular endothelial cell markers in patients with hypercholesterolemia. *Am J Hematol* 44:112, 1993

358. Hackman A, Abe Y, Insull W Jr, Pownall H, Smith L, Dunn K, Gotto AM Jr, Ballantyne CM: Levels of soluble cell adhesion molecules in patients with dyslipidemia. *Circulation* 93:1334, 1996

359. Morrow JD, Roberts LJ: The isoprostanes. Current knowledge and directions for future research. *Biochem Pharm* 51:1, 1996

360. Reilly M, Delanty N, Lawson JA, FitzGerald GA: Modulation of oxidant stress in vivo in chronic cigarette smokers. *Circulation* 94:19, 1996

361. Antonov AS, Munn DH, Kolodgie FD, Virmani R, Gerrity RG: Aortic endothelial cells regulate proliferation of human monocytes in vitro via a mechanism synergistic with macrophage colony-stimulat-

ing factor. Convergence at the cyclin E/p27^{kip1} regulatory checkpoint. *J Clin Invest* 99:2867, 1997

362. Ruderman A, Williamson N, Brownlee M: Glucose and diabetic vascular disease. *FASEB J* 6:2905, 1992

363. Browlee M, Cerami A, Viassara H: AGEs in tissues and the biochemical basis of diabetic complications. *N Engl J Med* 318:1315, 1989

364. Schmidt A-M, Vianna M, Gerlach M, Brett J, Ryan J, Kao J, Esposito C, Hegarty H, Hurley W, Clauss M, Wang F, Pan Y-C, Tsang T, Stern D: Isolation and characterization of binding proteins for AGEs from lung tissue which are present on the endothelial cell surface. *J Biol Chem* 267:14987, 1992

365. Schmidt A-M, Hori O, Brett J, Yan S-D, Wautier S-D, Stern D: Cellular receptors for AGEs: Implications for induction of oxidant stress and cellular dysfunction in the pathogenesis of vascular lesions. *Arterioscler Thromb* 1521:1528, 1994

366. Khoury J, Thomas C, Loike J, Hickman S, Cao L, Silverstein S: Macrophages adhere to glucose-modified basement membrane via their scavenger receptors. *J Biol Chem* 269:10197, 1994

367. Lander HL, Tauras JM, Ogiste JS, Moss RA, Schmidt A-M: Activation of the receptor for advanced glycation endproducts trigger a MAP kinase pathway regulated by oxidant stress. *J Biol Chem* 272:17810, 1997

368. Schmidt A-M, Hori O, Chen J, Li J, Crandall J, Zhang J, Cao R, Yan S-D, Brett J, Stern D: AGEs interaction with their endothelial cell receptor induce expression of VCAM-1 in cultured human endothelial cells and in mice. *J Clin Invest* 96:1395, 1995

369. Schmidt A-M, Crandall J, Cao R, Hori P, Lakatta E: Elevated plasma levels of vascular cell adhesion molecules-1 (VCAM-1) in diabetic patients with microalbuminuria: A marker of vascular dysfunction and progressive vascular disease. *Br J Haematol* 92:747, 1996

370. Solez K, Axelsen RA, Bendiktsson H, Burdick JF, Cohen AF, Colvin RB, Croker BP, Droz D, Dunnill MS, Halloran PF, Hayry P, Jennette JC, Keown PA, Marcussen N, Mihatsch MJ, Morozumi K, Myers ND, Nast CC, Olsen S, Racusen LC, Ramos EC, Rosen S, Sachs DH, Salomon DR, Sanfilippo F, Verani R, von Willebrand E, Yamaguchi Y: International standardization of criteria for the histologic diagnosis of renal allograft rejection: The Banff working classification of kidney transplant pathology. *Kidney Int* 44:411, 1993

371. Pober JS: Vascular endothelium in transplantation, in Tilney NC, Strom TB, Paul LC (eds): *Transplantation Biology: Cellular and Molecular Aspects*. Philadelphia, PA, Lippincott-Raven, 1996, p 127

372. Strehlau J, Pavlakis M, Lipman M, Shapiro M, Vascincellos L, Harmon W, Strom TB: Quantitative detection of immune activation transcripts as a diagnostic tool in kidney transplantation. *Proc Natl Acad Sci USA* 94:695, 1997

373. Meehan SM, McClusky RT, Pascual M, Preffer FI, Anderson P, Schlossman SF, Colvin RB: Cytotoxicity and apoptosis in human renal allograft: Identification, distribution and quantitation of cells with a cytotoxic granule protein GMP 17 (TIA-1) and cells with fragmented nuclear DNA. *Lab Invest* 76:639, 1997

374. Jutte NHPM, Knoop CJ, Heijse P, Balk AHMM, Mochtar B, Claas FHJ, Weimar W: Human heart endothelial cell-restricted allorecognition. *Transplant* 62:403, 1996

375. Dvorak HF, Mihm MC Jr, Dvorak AM, Barnes BA, Manseau EJ, Galli SJ: Rejection of first-set skin allografts in man. The microvasculature is the critical target of the immune response. *J Exp Med* 150:322, 1981

376. Gao S-Z, Alderman EL, Schroeder JS, Silverman JF, Hunt SA: Accelerated coronary vascular disease in the heart transplant patient: Coronary angiographic findings. *J Am Coll Cardiol* 12:334, 1988

377. Salomon RN, Hughes CCW, Schoen FJ, Payne DD, Pober JS, Libby P: Human coronary transplantation-associated arteriosclerosis: evidence for a chronic immune reaction to activated graft endothelial cells. *Am J Pathol* 138:791, 1991

378. Hurban RH, Beschoner WE, Baumgartner WA, Augustine SM,

Ren H, Reitz BA, Hutchins GM: Accelerated arteriosclerosis in heart transplant recipients is associated with a T-lymphocyte-mediated endothelialitis. *Am J Pathol* 137:871, 1990

379. Dong C, Wilson JE, Winters GL, McManus BM: Human transplant coronary artery disease: Pathologic evidence for fas-mediated cytotoxicity in allograft arteriopathy. *Lab Invest* 74:921, 1996

380. d'Apice AJ, Pearse MJ: Xenotransplantation, in Tilney NC, Strom TB, Paul LC (eds): *Transplantation Biology: Cellular and Molecular Aspects*. Philadelphia, PA, Lippincott-Raven, 1996, p 701

381. Sandrin MS, Vaughan HA, Dabkowski PL, McKenzie IFC: Anti-pig IgM antibodies in human serum react predominantly with Gal (α 1-3) Gal epitopes. *Proc Natl Acad Sci USA* 90:11391, 1993

382. Rooney IA, Liszeuski MK, Atkinson JP: Using membrane band complement regulatory proteins to inhibit rejection. *Xeno* 1:29, 1993

383. Murray AG, Khodadoust MM, Pober JS, Bothwell ALM: Porcine aortic endothelial cells strongly activate T cells: Direct presentation of swine MHC antigens and effective costimulation by swine ligands for human CD2 and CD28. *Immunity* 1:57, 1994

384. Blakely ML, Van Der Werf WJ, Berndly MC, Dalmaso AP, Bach FH, Hancock WW: Activation of intragraft endothelial and mononuclear cells during discordant xenograft rejection. *Transplant* 58:1059, 1994

385. Sultan P, Murray AG, McNiff JM, Lorber MI, Askenase PW, Bothwell ALM, Pober JS: Pig but not human interferon- γ initiated human endothelial cell-mediated rejection of pig tissue *in vivo*. *Proc Natl Acad Sci USA* 94:8767, 1997

386. McCrae KR, Cines DB: Immunologic properties of antibodies to endothelial cells: Relationship to antiphospholipid antibodies, in Cervera R, Khamashta MA, Hughes GRV (eds): *Antibodies to Endothelial Cells and Vascular Damage*. Boca Raton, FL, CRC, 1994, p 47

387. Cines DB, Lyss AP, Reeber MJ, Bina M, DeHoratius R: Presence of complement-fixing anti-endothelial cell antibodies in systemic lupus erythematosus. *J Clin Invest* 73:611, 1984

388. McCrae KR, DeMichele A, Samuels P, Roth D, Kuo A, Meng Q-H, Rauch J, Cines DB: Detection of endothelial cell-reactive immunoglobulin in patients with anti-phospholipid antibodies. *Br J Haematol* 79:595, 1991

389. Cines DB: Disorders associated with antibodies to endothelial cells. *Rev Infect Dis* 11:705, 1989

390. Simantov R, LaSala JM, Lo SK, Gharavi AE, Sammaritano LR, Salmon JE, Silverstein RL: Activator of cultured vascular endothelial cells by antiphospholipid antibodies. *J Clin Invest* 96:2211, 1995

391. Angles-Cano E, Sultan Y, Clauvel JP: Predisposing factors to thrombosis in systemic lupus erythematosus. Possible relation to endothelial cell damage. *J Lab Clin Med* 94:312, 1979

392. Arepally G, Cines DB: Heparin-associated antibodies, in Shoenfeld Y, Peter J (eds): *Autoantibodies*. Amsterdam, The Netherlands, Elsevier, 1996, p 343

393. McCrae KR, Cines DB: Drug-induced thrombocytopenias, in Loscalzo J, Schafer AI (eds): *Thrombosis and Hemorrhage*. Boston, MA, Blackwell Scientific, 1994, p 545

394. Amiral J, Bridey F, Dreyfus M, Vissac AM, Fressinaud E, Wolf M, Meyer D: Platelet factor 4 complexed to heparin is the target for antibodies generated in heparin-induced thrombocytopenia. *Thromb Haemost* 68:95, 1992

395. Visentin GP, Ford SE, Scott JP, Aster RH: Antibodies from patients with heparin induced thrombocytopenia/thrombosis are specific for platelet factor 4 complexed with heparin or bound to endothelial cells. *J Clin Invest* 93:81, 1994

396. Cines DB, Tomaski A, Tannenbaum S: Immune endothelial cell injury in heparin-associated thrombocytopenia. *N Engl J Med* 316:581, 1987

397. Mayet W-J, Csernok E, Szymkowiak C, Gross WL, Meye zum Buschenfelde K-H: Human endothelial cells express proteinase 3, the

target antigen of anticytoplasmic antibodies in Wegener's granulomatosis. *Blood* 82:1221, 1993

398. Damianovich M, Gilburd B, George J, Del Papa N, Afek A, Goldberg I, Kopolovic Y, Roth D, Barkai G, Meroni P-L, Shoenfeld Y: Pathogenic role of anti-endothelial cell antibodies in vasculitis. An idiotypic experimental model. *J Immunol* 156:4946, 1996

399. Savage COS, Pottinger BE, Gaskin G, Lockwood CM, Pusey CD, Pearson JD: Vascular damage in Wegener's granulomatosis and microscopic polyarteritis: Presence of anti-endothelial cell antibodies and their relation to anti-neutrophil cytoplasm antibodies. *Clin Exp Immunol* 85:14, 1991

400. Leung DYM, Havens PL, Moake JL, Kim M, Pober JS: Lytic anti-endothelial cell antibodies in haemolytic-uremic syndrome. *Lancet* 2:183, 1988

401. Carvalho D, Savage COS, Black CM, Pearson JD: IgG antiendothelial cell autoantibodies from scleroderma patients induce leukocyte adhesion to human vascular endothelial cell *in vitro*. *J Clin Invest* 97:111, 1996

402. Sgonc R, Gruschwitz MS, Dietrich H, Recheis H, Gershwin ME, Wick G: Endothelial cell apoptosis is a primary pathogenic event underlying skin lesions in avian and human scleroderma. *J Clin Invest* 98:785, 1996

403. Schett G, Xu Q, Amberger A, van der Zee R, Recheis H, Willeit J, Wick G: Autoantibodies against heat shock protein 60 mediates endothelial cytotoxicity. *J Clin Invest* 96:2569, 1995

404. Leung DYM, Collins T, Lapierre LA, Geha RS, Pober JS: Immunoglobulin M antibodies present in the acute phase of Kawasaki syndrome lyse cultured endothelial cells stimulated by gamma interferon. *J Clin Invest* 77:1428, 1986

405. Cervera R, Navarro M, Lopez-Soto A, Cid M-C, Font J, Esparza J, Reverter JC, Monteagudo J, Ingelmo M, Urbano-Marquez A: Antibodies to endothelial cells in Behcet's disease: Cell-binding heterogeneity and association with disease activity. *Ann Rheumatol Dis* 53:265, 1994

406. Brasile L, Zerbe T, Rabin B, Clarke J, Abrams A, Cerilli J: Identification of the antibody to vascular endothelial cells in patients undergoing cardiac transplantation. *Transplant* 40:672, 1985

407. Boehme MWJ, Schmitt WH, Youinou P, Stremmel WR, Gross WL: Clinical relevance of elevated serum thrombomodulin and soluble E-selectin with Wegener's granulomatosis and other systemic vasculitides. *Am J Med* 101:387, 1996

408. Favaloro EJ: Differential expression of surface antigens on activated endothelium. *Immunol Cell Biol* 71:571, 1993

409. Cotran RS, Gimbrone MA Jr, Bevilacqua MP, Mendrick DL, Pober JS: Induction and detection of a human endothelial activation antigen *in vivo*. *J Exp Med* 164:661, 1986

410. Groger MG, Sarmay E, Fiebirger E, Wolff K, Petzelbauer P: Dermal microvascular endothelial cells express CD32 receptors *in vivo* and *in vitro*. *J Immunol* 156:1549, 1996

411. Cines DB, Lyss AP, Bina M, Corkey R, Kefalides NA, Friedman HM: Herpes simplex virus enhances the expression of receptors for C3 and the Fc portion of immunoglobulin G and human endothelial cells. *J Clin Invest* 69:123, 1982

412. Johnson E, Hetland G: Human umbilical vein endothelial cells synthesize functional C3, C5, C6, C8 and C9 *in vitro*. *Scand J Immunol* 33:667, 1991

413. Brooimans RA, Van der Ark AAJ, Buurman WA, van Es LA, Daha MR: Differential regulation of complement factor H and C3 production in human umbilical vein endothelial cells by IFN- γ and IL-1. *J Immunol* 144:3835, 1990

414. Schmaier AH, Murray SC, Heda GH, Farber A, Kuo A, McCrae K, Cines DB: Synthesis and expression of C1 inhibitor by human umbilical vein endothelial cells. *J Biol Chem* 264:18173, 1989

415. Brooimans RA, Van der Ark AAJ, Tomita M, van Es LA, Daha MR: CD59 expressed by human endothelial cells functions as a

protective molecule against complement-mediated lysis. *Eur J Immunol* 22:791, 1992

416. Venneker GT, van den Hoogen FHJ, Boerbooms AMT, Bos JD, Asghar SS: Aberrant expression of membrane cofactor protein and decay-accelerating factor in the endothelium of patients with systemic sclerosis. A possible mechanism of vascular damage. *Lab Invest* 70:830, 1994

417. Asch AS, Kinoshita T, Jaffe EA, Nussenzweig V: Decay-accelerating factor is present on cultured human umbilical vein endothelial cells. *J Exp Med* 163:221, 1986

418. McNearney T, Ballard L, Seya T, Atkinson JP: Membrane cofactor protein of complement is present on human fibroblast, epithelial, and endothelial cells. *J Clin Invest* 84:538, 1989

419. Nose M, Katoh M, Okada N, Kyogoku M, Okada H: Tissue distribution of HRF-20, a novel factor preventing the membrane attack of homologous complement, and its predominant expression on endothelial cells *in vivo*. *Immunology* 70:145, 1990

420. Daha MR, Miltenburg AMM, Hiemstra PS, Klar-Mohamad N, van Es LA, Van Hinsburg VWM: The complement subcomponent C1q mediated binding of immune complexes and aggregates to endothelial cells *in vitro*. *Eur J Immunol* 18:783, 1988

421. Lozada C, Levin RI, Huie M, Hirschhorn R, Naime D, Whitlow M, Recht PA, Golden B, Cronstein BN: Identification of C1q as the heat-labile serum cofactor required for immune complexes to stimulate endothelial expression of the adhesion molecules E-selectin and intercellular and vascular adhesion molecules 1. *Proc Natl Acad Sci USA* 92:8378, 1995

422. Foreman KE, Vaporciyan BK, Bonish ML, Jones KJ, Glovsky MM, Eddy SM, Ward PA: C5a induced expression of P-selectin in endothelial cells. *J Clin Invest* 94:1147, 1994

423. Kilgore KS, Shen JP, Miller BF, Ward P, Warren JS: Enhancement by the complement membrane attack complex of tumor necrosis factor- α -induced endothelial cell expression of E-selectin and ICAM-1. *J Immunol* 155:1434, 1995

424. Kilgore KS, Schmid E, Shanley TP, Flory CM, Maheswari V, Tramontini NL, Cohen H, Ward PA, Friedl HP, Warren JS: Sublytic concentrations of the membrane attack complex of complement induce endothelial cell interleukin-8 and monocyte chemoattractant protein-1 through nuclear factor- κ B activation. *Am J Pathol* 150:2019, 1997

425. Saadi S, Holznecht RA, Patte CP, Stern DM, Platt JL: Complement-mediated regulation of tissue factor activity in endothelium. *J Exp Med* 182:1807, 1995

426. Tedesco F, Pausa M, Nardon E, Introna M, Mantovani A, Dobrina A: The cytolytically inactive terminal complement complex activates endothelial cells to express adhesion molecules and tissue factor procoagulant activity. *J Exp Med* 185:1619, 1997

427. Hattori R, Hamilton KK, McEver RP, Sims PJ: Complement proteins C5b-9 induce secretion of high molecular weight multimers of endothelial von Willebrand factor and translocation of granule membrane protein GMP-140 to the cell surface. *J Biol Chem* 264:9053, 1989

428. Hamilton KK, Hattori R, Esmon CT, Sims PJ: Complement proteins C5b-9 induce vesiculation of the endothelial plasma membrane and expose catalytic surface for assembly of the prothrombinase enzyme complex. *J Biol Chem* 265:3809, 1990

429. Suttorp N, Seeger W, Zinsky S, Bhakdi S: Complement complex C5b-8 induces PGI₂ formation in cultured endothelial cells. *Am J Physiol* 253:C13, 1987

430. Christiansen VJ, Sims PJ, Hamilton KK: Complement C5b-9 increases plasminogen binding and activation on human endothelial cells. *Arterioscler Thromb Vasc Biol* 17:164, 1997

431. Moake JL: Haemolytic-uraemic syndrome: Basic science. *Lancet* 343:393, 1994

432. Heild GH: Haemolytic-uremic syndrome in practice. *Lancet* 343:398, 1994

433. Forsyth KD, Simpson AC, Fitzpatrick MM, Barratt TM,

Levinsky RJ: Neutrophil-mediated endothelial cell injury in haemolytic uraemic syndrome. *Lancet* 2:411, 1989

434. Morigi M, Micheletti G, Figliuzzi M, Imberti B, Karmali MA, Remuzzi A, Remuzzi G, Zoja C: Verotoxin-1 promotes leukocyte adhesion to cultured endothelial cells under physiologic flow conditions. *Blood* 86:4553, 1995

435. van Setten PA, Monnens LAH, Verstrate RGG, van den Heuvel LPWJ, Van Hinsbergh VWM: Effects of verotoxin-1 on nonadherent monocytes: binding characteristics, protein synthesis, and induction of cytokine release. *Blood* 88:174, 1996

436. Harel Y, Silva M, Weinberg A, Cleary TB, Beutler B: A reporter transgene indicates renal-specific induction of tumor necrosis factor (TNF) by shiga-like toxin. *J Clin Invest* 92:2110, 1993

437. Keusch GT, Acheson DWK, Aaldering L, Erban J, Jacewicz MS: Comparison of the effects of Shiga-like toxin 1 on cytokine- and butyrate-treated human umbilical and saphenous vein endothelial cells. *J Infect Dis* 173:1164, 1996

438. Kaye SA, Obrig TG: Effect of TNF- α , Shiga toxin and calcium ionophore on Weibel-Palade body content of endothelial cells: Possible implications for the hemolytic uremic syndrome. *Thromb Res* 79:415, 1995

439. Bergstein JM, Riley M, Bang NU: Role of plasminogen-activator inhibitor type 1 in the pathogenesis and outcome of the hemolytic uremic syndrome. *N Engl J Med* 327:755, 1992

440. Moake JL, Byrnes JJ: Thrombotic microangiopathies associated with drugs and bone marrow transplantation. *Hematol Oncol Clin North Am* 10:485, 1996

441. Zoja C, Furci L, Ghilardi F, Zilio P, Benigni A, Remuzzi G: Cyclosporin-induced endothelial cell injury. *Lab Invest* 56:455, 1986

442. Gottschall JL, Neahring B, McFarland JG, Wu G-G, Weitekamp LA, Aster RH: Quinine-induced immune thrombocytopenia with hemolytic uremic syndrome: Clinical and serological findings in nine patients and review of the literature. *Am J Hematol* 47:283, 1994

443. Moake JL, Rudy CK, Troll JH, Weinstein MJ, Colanino NM, Azocar J, Seder RH, Hong SL, Deykin D: Unusually large plasma factor VIII: von Willebrand factor multimers in chronic relapsing thrombotic thrombocytopenic purpura. *N Engl J Med* 307:1432, 1982

444. Wada H, Ohiwa M, Kaneko T, Tamaki S, Tanigawa M, Shirakawa S, Koyama M, Hayashi T, Suzuki K: Plasma thrombomodulin as a marker of vascular disorders in thrombotic thrombocytopenic purpura and disseminated intravascular coagulation. *Am J Hematol* 39:20, 1992

445. Glas-Greenwalt P, Hall JM, Panke TW, Kant KS, Allen CM, Pollak VE: Fibrinolysis in health and disease: Abnormal levels of plasminogen activator, plasminogen activator inhibitor, and protein C in thrombotic thrombocytopenic purpura. *J Lab Clin Med* 108:415, 1986

446. Katayama M, Handoa M, Araki Y, Ambo H, Kawal Y, Watanabe K, Ikeda Y: Soluble P-selectin is present in normal circulation and its plasma level is elevated in patients with thrombotic thrombocytopenic purpura and haemolytic uraemic syndrome. *Br J Haematol* 84:702, 1993

447. Wu K, Hall ER, Rosai EC, Papp AC: Serum prostacyclin binding defects in thrombotic thrombocytopenic purpura. *J Clin Invest* 75:168, 1985

448. Kobayashi M, Wada H, Wakita Y, Shimura M, Nakase T, Hiyoyama K, Nagaya S, Minami N, Nakano T, Shiku H: Decreased plasma tissue factor pathway inhibitor levels in patients with thrombotic thrombocytopenic purpura. *Am J Hematol* 73:10, 1995

449. Khong TY, De Wolf F, Robertson WB, Brosens I: Inadequate maternal vascular response to placentation in pregnancies complicated

by preeclampsia and by small for gestational age infants. *Am J Obstet Gynecol* 157:360, 1987

450. Khong TY, Sawyer IH, Heryet AR: An immunohistologic study of endothelialization of uteroplacental vessels in human pregnancy—Evidence that the endothelium is focally disrupted by trophoblast in preeclampsia. *Am J Obstet Gynecol* 167:751, 1992

451. Ferris TF: Preeclampsia and postpartum renal failure: Examples of pregnancy-induced microangiopathy. *Am J Med* 99:343, 1995

452. Fitzgerald DJ, Entmann SSW, Mulloy K, Fitzgerald GA: Decreased prostacyclin biosynthesis preceding the clinical manifestations of pregnancy induced hypertension. *Circulation* 75:956, 1987

453. Taylor RN, Varma M, Teng NN, Roberts JM: Women with pre-eclampsia have higher plasma endothelin levels than women with normal pregnancy. *J Clin Endocrinol Metabol* 71:1675, 1990

454. Lockwood CJ, Peters JH: Increased plasma levels of EDI⁺ cellular fibronectin precede the clinical signs of preeclampsia. *Am J Obstet Gynecol* 162:358, 1990

455. Friedman SA, Schiff E, Emeris JJ, Dekker GA, Sibai BM: Biochemical corroboration of endothelial involvement in severe preeclampsia. *Am J Obstet Gynecol* 172:202, 1995

456. Redman CWG, Beilin LJ, Denson DWE, Bolton FG, Stirrat GM: Factor VIII consumption in pre-eclampsia. *Lancet* 2:1249, 1977

457. Rogers GM, Taylor RN, Roberts JM: Preeclampsia is associated with a serum factor cytotoxic to human endothelial cells. *Am J Obstet Gynecol* 159:906, 1988

458. Tsukimori K, Maeda H, Shingu M, Koyanagi T, Nounaga M, Nakano H: The possible role of endothelial cells in hypertensive disorders during pregnancy. *Obstet Gynecol* 80:229, 1992

459. Endresen MJ, Tosti E, Lorentzen B, Henriksen T: Sera of preeclamptic women are not cytotoxic to endothelial cells in culture. *Am J Obstet Gynecol* 172:196, 1995

460. Zammit VC, Whitworth JA, Brown MA: Preeclampsia: The effects of serum on endothelial cell prostacyclin, endothelin and cell membrane integrity. *Am J Obstet Gynecol* 174:737, 1996

461. Smarason AK, Sargent IL, Redman CWG: Endothelial cell proliferation is suppressed by plasma but not by serum from women with preeclampsia. *Am J Obstet Gynecol* 174:787, 1996

462. Taylor RN, Casal DC, Jones LA, Varma M, Martin JN Jr, Roberts JM: Selective effects of preeclamptic sera on human endothelial cell procoagulant protein expression. *Am J Obstet Gynecol* 165:1705, 1991

463. Lorentzen B, Endresen MJ, Hovig T, Haug E, Henriksen T: Sera from preeclamptic women increase the content of triglycerides and reduce the release of prostacyclin in cultured endothelial cells. *Thromb Res* 63:363, 1991

464. Roberts JM, Edep ME, Goldfein A, Taylor RN: Sera from preeclamptic women specifically activate human umbilical vein endothelial cells in vitro: Morphologic and biochemical evidence. *Am J Reprod Immunol* 27:101, 1992

465. Gallery EDM, Rowe J, Campbell S, Hawkins T: Effect of serum on secretion of prostacyclin and endothelin-1 by decidual endothelial cells from normal and preeclamptic pregnancies. *Am J Obstet Gynecol* 173:918, 1995

466. Poranen A-K, Ekblad U, Uotila P, Ahotupa M: Lipid peroxidation and antioxidants in normal and pre-eclamptic pregnancies. *Placenta* 17:401, 1996

467. Ness RB, Roberts JR: Heterogenous causes constituting the single syndrome of preeclampsia: A hypothesis and its implications. *Am J Obstet Gynecol* 175:1365, 1996



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