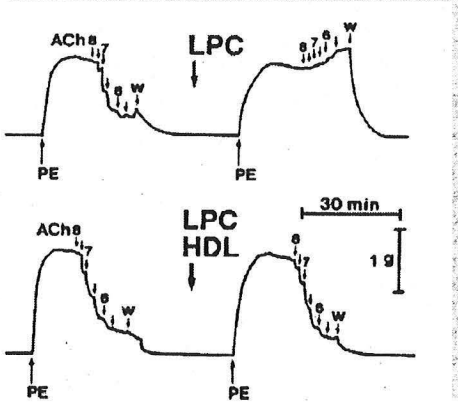
# Endothelial Dysfunction:

## Mechanism, Syndrome, Artifact or Myth?

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*Vascular endothelial cells, critically situated at the blood-tissue interface, exert important effects on vascular tone and permeability, regulate the coagulation and fibrinolytic systems, mediate translocation of inflammatory cells to the tissue compartment and modulate proliferation of vascular smooth muscle cells. As the physiology of the endothelium has been defined, defects in endothelial function have been identified in association with human disease, and a syndrome of dysfunctional endothelium has been described. While it remains debateable whether a coherent syndrome of endothelial dysfunction exists, disordered endothelial biology appears to contribute to the pathophysiology of several important human diseases. Identification of specific molecular mechanisms offers potential targets for novel therapeutic interventions, including genetic modification of endothelial cells in vivo.*

The endothelium, long envisioned as a passive vascular lining, plays important roles in the regulation of vascular tone, maintenance of hemostatic integrity and the modulation of immune and inflammatory responses. As the physiology of vascular endothelium has been defined, functional abnormalities have been identified in association with a number of important human diseases, including hypertension, atherosclerosis and intravascular thrombosis. Over time, a loosely conceptualized syndrome of *endothelial dysfunction* has taken form, in which vasoconstricting, pro-inflammatory and pro-thrombotic alterations in endothelial physiology result from diverse endothelial "injuries", and dysfunctional endothelium has been invoked as an important pathogenic mechanism. From this perspective, I intend to review selected aspects of endothelial biology, attempting to highlight i) alterations in endothelial function associated with clinical and experimental disease states, ii) alterations in the pattern of endothelial gene expression in response to injury, and iii) the concept of the activated endothelial cell as an important participant in the pathophysiology of human disease. I hope to address, in at least a preliminary way, the following questions:

> Do endothelial cells respond to injury with changes in phenotype that contribute to the development and progression of human vascular disease?
>
> What mechanisms operate to alter the endothelial phenotype?
>
> Do these mechanisms offer the potential for novel therapeutic interventions?

I believe these questions are timely. In particular, the development of somatic cell gene transfer as an investigational and, increasingly, therapeutic tool, offers the potential for novel approaches to vascular disease if appropriate molecular targets can be defined. At least by virtue of accessibility, vascular endothelium offers particularly attractive targets.

## PHYSIOLOGY OF VASCULAR ENDOTHELIUM

Situated critically at the blood-tissue interface, the principal physiologic functions of the endothelium are those required for the maintenance of circulatory integrity and homeostasis, and control of transit between the blood and tissue compartments.

### *Regulation of Vascular Tone*

Vascular endothelial cells synthesize a number of compounds with vasodilator (nitric oxide, prostacyclin, EDHF) or vasoconstrictor (Thromboxane $A_2$, $PGH_2$, endothelin) activity. Secretion of vasoactive factors by the endothelium has effects on basal vascular tone, and is the most important mechanism mediating alterations in vascular tone in response to circulating, paracrine and physical stimuli.

#### Nitric Oxide

In response to acetylcholine, platelet release products including histamine and serotonin, thrombin and sheer stress, endothelial cells release a potent, very short-acting vasodilator, endothelium-derived relaxing factor (EDRF). The active principal of EDRF

is now identified as nitric oxide (Palmer, 1987), but whether the free radical NO is released directly from endothelial cells, or is released by extracellular degradation of a secreted nitrosylated precursor (Myers, 1990) remains uncertain.

Labeling studies demonstrate that secreted nitric oxide is formed by oxidation of the guanidine-nitrogen of L-arginine (Palmer, 1988), and the arginine analogue L-NMMA (L-$N^G$-monomethyl arginine) reversibly inhibits endothelial release of both nitric oxide and EDRF activity (Richard, 1990).

The enzyme catalyzing production of nitric oxide from L-arginine, nitric oxide synthase, is a cytoplasmic enzyme structurally resembling cytochrome P450 reductase, and requires both $Ca^{++}$-calmodulin and NADPH (Bredt, 1990; 1991). Both inducible and constitutive forms of the enzyme exist, and isoforms of NO synthase have been identified in platelets, smooth muscle cells and macrophages in addition to endothelial cells (Radomski, 1990). The endothelial, constitutive NO synthase has been cloned, and cells transfected with the NO synthase cDNA secrete EDRF activity (Bredt, 1991).

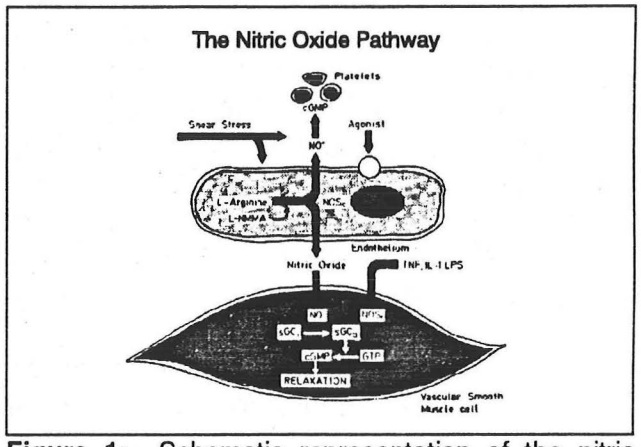


**Figure 1:** Schematic representation of the nitric oxide pathway for modulation of vascular tone.

Because, under physiologic conditions, EDRF has a very short half life ($t_{1/2}$~6 seconds), it is a local vasodilator. Both superoxide and hemoglobin accelerate inactivation of EDRF, and antagonize endothelium-dependent vascular relaxation.

The vasodilatory activity of EDRF is mediated by activation of guanylate cyclase. Vascular relaxation in response to EDRF are associated with an increase in smooth muscle cGMP levels (Rapoport, 1983), and methylene blue, an inhibitor of soluble guanylate cyclase, antagonizes EDRF-induced smooth muscle relaxation (Figure 1). Exogenous NO directly activates guanylate cyclase in both platelets and smooth muscle cells (Nguyen, 1991).

Cyclo-oxygenase Products

Prostacyclin, or $PGI_2$, is the principal vasodilatory product of cyclo-oxygenase in vascular endothelium, and the principal cyclo-oxygenase product in arterial endothelial cells (Moncada, 1979). Synthesis of prostacyclin is, under basal conditions, substrate-limited, and is stimulated by effectors which release the precursor, arachidonic acid, from membrane phospholipid. In general, effectors that stimulate NO synthesis, simultaneously increase endothelial production of prostacyclin. While, in pharmacologic concentrations, prostacyclin is a potent vasodilator, under physiologic conditions, the contribution of prostacyclin to endothelium-dependent relaxation appears minor in comparison to that of NO (Dohi, 1991).

In contrast to arteries, veins exposed to exogenous arachidonic acid demonstrate endothelium-dependent contraction which is inhibited by the cyclo-oxygenase antagonist indomethacin (Yang, 1991). Three products appear to contribute to this cyclo-oxygenase

derived contracting activity, Thromboxane $A_2$ and prostaglandin $H_2$, and a byproduct of the cyclo-oxygenase pathway, superoxide anion, which inactivates endothelium-derived NO (Katusic, 1989).

Endothelin

Endothelial cells produce and secrete the potent vasoconstricting peptide endothelin. While three endothelin peptides, 1-3, encoded by different genes have been identified, endothelial cells appear to produce exclusively endothelin-1 (Figure 3) (Luscher, 1993). Expression of ET-1 is regulated at the level of gene transcription, and is induced by a number of physiologic stimuli including thrombin, epinephrine, interleukin-1, angiotensin-II, arginine vasopressin and transforming growth factor-β (Yanagisawa,1988; Boulanger, 1990). Processing of the translation product, preproendothelin, to the mature vasoactive peptide is catalyzed by an endothelin converting enzyme found in endothelial cells (Kimura, 1988).

Endothelin-1 binds specifically to $ET_A$ and $ET_B$ receptors on vascular smooth muscle cells, indirectly activating voltage sensitive $Ca^{++}$ channels and evoking potent and sustained vasoconstriction (Yang, 1990) (Figure 2). Circulating endothelin-I levels under basal conditions are low (Suzuki, 1989), perhaps reflecting preferential abluminal secretion of the peptide from endothelial cells (Boulanger, 1990), and suggesting that under basal conditions endothelin is predominantly a locally-acting vasoconstrictor.

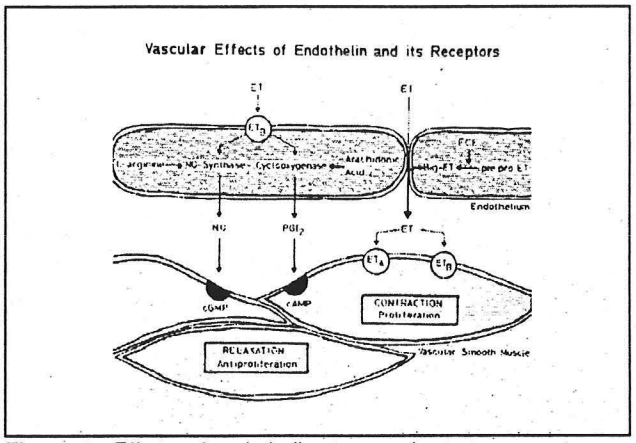


**Figure 2:** Effects of endothelin on vascular tone.

Interaction of Endothelial Products in the Regulation of Vascular Tone

Endothelium-dependent regulation of vascular tone is complex. First, agonists stimulating vasodilating and vasoconstricting factors accumulate simultaneously under several physiologic conditions. For example, during thrombus formation, platelet activation results in release of histamine and serotonin, which stimulate nitric oxide synthase activity. Activation of the coagulation cascade, however, results in generation of thrombin, a potent inducer of endothelin gene expression.

Secondly, cooperative and feedback mechanisms operate to modulate the effects of endothelial products on vascular tone (Figure 3). At low concentrations, endothelin has vasodilatory activity (Kasuya, 1989), reflecting induction of prostacyclin synthesis mediated by activation of endothelial $ET_B$ receptors (Dohi, 1991). Prostacyclin potentiates the effects of nitric oxide on vascular smooth muscle cells, and nitric oxide inhibits the vasoconstrictor activity of ET on vascular smooth muscle cells by a cGMP-dependent mechanism (Boulanger, 1990).

In addition, the pattern of vasoactive factors released from the endothelium varies with location in the vascular tree. In arteries, exogenous arachidonic acid stimulates

release of prostacyclin, while, in venules, the vasoconstrictors thromboxane $A_2$ and $PGH_2$ are the predominate cyclo-oxygenase products (Moncada, 1979). Similarly, the antagonistic effects of nitric oxide on endothelin induced vasoconstriction are more potent in the arterial circulation (Luscher, 1990). These differences suggest biases in the endothelial modulation of vascular tone, toward vasodilation in the arterial tree, and vasoconstriction in the venous circulation.

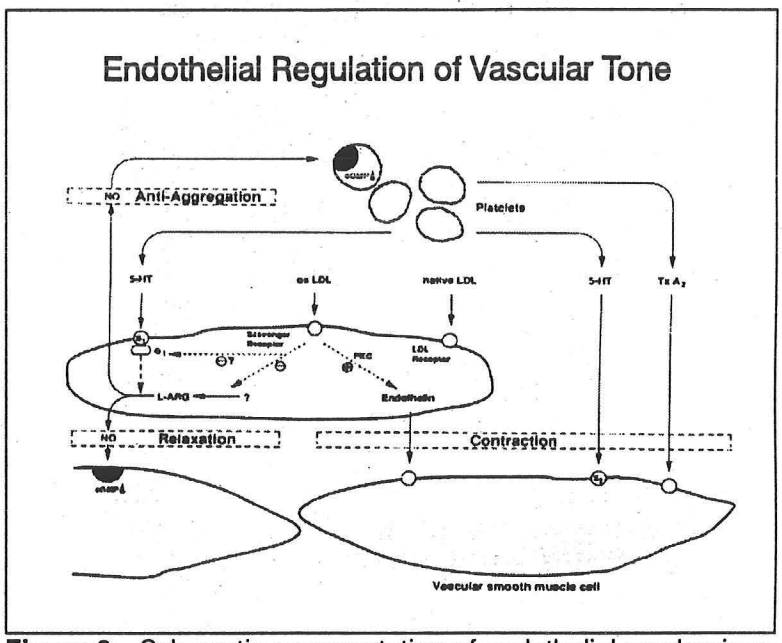


**Figure 3:** Schematic representation of endothelial mechanisms operating to regulate vascular tone.

Effects of Endothelium on Basal Arterial(olar) Tone

Muscular arteries and pre-capillary resistance vessels denuded of endothelium undergo vasoconstriction (Luscher, 1993). Inhibition of nitric oxide synthase, and, to a lesser extent, cyclo-oxygenase, induces vasoconstriction in the coronary circulation in experimental animals (Yang, 1991; Amezcua,1989; Chu, 1991) and humans (Richard, 1990). Infusion of L-NMMA, an inhibitor of nitric oxide synthase, into experimental animals is associated with sustained increases in blood pressure (Rees, 1989). These observations suggest that the endothelium exerts significant tonic vasodilatory effects in medium sized arteries and precapillary resistance vessels.

*Endothelial Permeability*

The earliest recognized function of the vascular endothelium was its role as a barrier, modulating the transport of fluid, solutes and cells between the vascular and tissue compartments. "Permeability" of the endothelial monolayer actually reflects several processes by which water and dissolved solutes, macromolecules and leukocytes transit the endothelial layer, processes which are tightly controlled by vascular endothelial cells.

Permeability to water, solutes and macromolecules

Transit of water and dissolved molecules across the endothelial monolayer has been most extensively studied in microvasculature. Physiologic studies have defined two classes of "pores" through which serum constituents exit the vascular space. Water and molecules <10nm in effective radius transit the endothelial monolayer via the "small pore" system, while macromolecules >10nm transit via a separate "large pore" mechanism. These "pores" have been thought to be located at intercellular junctions, and that the permeability of the endothelial monolayer was determined by the junctional sieve

(Manjo,1961). In response to a variety of stimuli, endothelial cells contract, resulting in the formation of intercellular "gaps", with a resulting increase in vascular permeability (Shasby, 1982).

More recent experimental evidence has suggested that this model of endothelial permeability is not, at least for transit of macromolecules under basal conditions, accurate. Ultrastructural studies have failed to demonstrate intercellular pores, but rather show transcytotic vesicles and channels forming from the endothelial plasmalemma. Molecular probe experiments suggest that macromolecules of both size classes transit the endothelial barrier primarily via these transcytotic vesicles/channels (Palade, 1992). Whether endothelial contraction opens a second pathway for macromolecular transmigration or is associated with enhanced transcytotic transport is not clearly defined. Nonetheless, vascular permeability is clearly mediated by endothelial cell processes.

A variety of physiologic stimuli, including histamine, thrombin, bradykinin, platelet activating factor and hydrogen peroxide alter vascular permeability. In response to these stimuli, intracellular $Ca^{++}$ and endothelial cell protein kinase C activity are increased (Oliver, 1990), triggering endothelial cell contraction (Montesano, 1985) and rearrangement of cytoskeletal actin filaments (Nimi, 1992). Effectors which increase endothelial cell cyclic AMP or GMP levels result in "relaxation", and are associated with a reduction in the permeability of the endothelial monolayer (Suttorp, 1993).

The role of the endothelium extends beyond mediating changes in vascular permeability, however, as several endothelial cell products exert regulatory effects. Endothelin-1 increases vascular permeability in the coronary circulation of rats *in vivo* via an $ET_A$ receptor mediated mechanism (Filep, 1992). Prostaglandin $E_1$, which increases intracellular cyclic AMP levels, antagonizes the increase in vascular permeability produced by histamine. Recently, EDRF (NO) has been demonstrated to alter vascular permeability. In cultured endothelial cells, nitrates decrease and nitric oxide synthase inhibitors enhance leakage of small molecules across the monolayer (Oliver, 1992). In intact vasculature the response is more complex, as L-NMMA reduces capillary leakage but antagonizes the increase in permeability induced by histamine (Yuan, 1993; Filep, 1993). In aggregate, these observations suggest that autocrine and/or paracrine loops may act to modulate changes in vascular permeability in response to exogenous signals.

Transmigration of Leukocytes

Adhesion of leukocytes to the endothelial monolayer and transmigration into and through the vessel wall is an important component of physiologic (e.g. inflammation) and pathophysiologic (e.g. atherosclerosis) processes. Leukocytes actively penetrate the vessel wall via intercellular junctions after adhesion to endothelial cells mediated by specific ligand-receptor interactions. Many of these ligand-receptor pairs have now been identified, providing insight into the processes controlling leukocyte

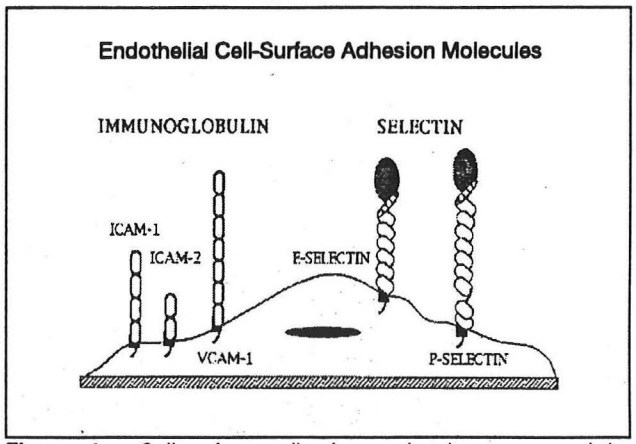


**Figure 4:** Cell-surface adhesion molecules expressed by endothelial cells.

migration.

Endothelial cells express two principal classes of leukocyte receptor, the selectins and members of the immunoglobulin superfamily (reviewed in Springer, 1990; Bevilacqua, 1991; Williams, 1992) (Table I and Figure 4):

> The selectins, of which E- and P-selectin are expressed on endothelium, have affinity for carbohydrates, Lewis-X and asialyl-Lewis-X ($\alpha_{1\text{-}3}$fucosylpolylactose), present on the surface of neutrophils and monocytes. Binding is mediated by N-terminal lectin-binding and EGF homology domains (Springer, 1991; Polley, 1991).
>
> The Ig-superfamily receptors ICAM-1, ICAM-2 and VCAM-1 are comprised of variable numbers of repeated immunoglobulin-like domains. These molecules have affinity for the leukocyte integrins, a family of immunoglobulin binding proteins. The integrins are heterodimeric molecules formed by noncovalent association of $\alpha$ and $\beta$ chains. Six $\beta$ and at least 14 $\alpha$ chain gene products have been identified, providing potential for a large number of binding specificities. Two classes of integrins, $\beta_2$ (the $CD_{11}/CD_{18}$ molecules LFA-1, Mac-1 and P150) and $\beta_1$ (VLA-4, $\alpha_1\beta_4$ ) function as ligands for the Ig-superfamily receptors (Rouslahti, 1991).

Endothelial cells modulate leukocyte adherence by regulating expression of adhesion molecules on the cell surface. P-selectin and ICAM-2 are expressed constitutively, and mediate transient attachment (rolling) under basal conditions and adherence early in the course of an inflammatory response (Lawrence, 1991). Targeted disruption of the P-selectin gene severely reduces both leukocyte rolling and extravasation in transgenic mice (Mayadas, 1993).

| EC RECPTOR | STRUCTURE | LEUKOCYTE(S) | LIGAND |
|---|---|---|---|
| **Selectins** | **Lectin-EGF-CRP** | | **Carbohydrate** |
| E-Selectin | | PMN/MONO | (Sialyl)-Lewis X |
| P-Selectin | | PMN/MONO | (Sialyl)-Lewis X |
| **Ig Superfamily** | **Ig domain repeats** | | **Integrins** |
| ICAM-1 | 5 Ig domains | ALL | LFA-1 (CD11/CD18) (Beta-2) |
| ICAM-2 | 2 Ig domains | PMN/MONO | Mac-1, P150 (Beta-2) |
| VCAM-1 | 6-7 Ig domains | LYMPH/MONO | VLA-4 (CD29) (Beta-1) |

**Table I:** Ligand-receptor pairs mediating leukocyte-endothelial interactions.

In contrast, expression of E-selectin (Bevilacqua, 1989), ICAM-1 (Staunton, 1988) and VCAM-1 (Osborne, 1989) are regulated at the level of gene transcription, and are induced by exposure of endothelial cells to the inflammatory cytokines interleukin-1, tumor necrosis factor (Prober, 1993) and variably interferon-$\gamma$, and by exposure to bacterial lipopolysaccharide (endotoxin) (reviewed in Williams, 1992). The latter adhesion molecules mediate primarily the stable adhesion of leukocytes which precedes transmigration.

The pattern of expression of adhesion molecules by endothelial cells in response

to inflammatory stimuli also influences the time course of inflammatory cell accumulation. P-selectin is pre-stored in secretory granules, secreted rapidly in response to the early inflammatory mediators thrombin and histamine, and mediates attachment of neutrophils and monocytes. Synthesis E-selectin, which also mediates PMN and monocyte attachment, peaks within hours of induction. Synthesis of VCAM-1, which functions as the primary adhesion molecule for lymphocyte attachment, peaks 18-24 hours after induction, while ICAM-1 reaches peak levels by 18-24 hours and these peak levels persist as long as the inducing stimulus remains (Bevilacqua, 1989; Osborn, 1989; Prober, 1986).

*Endothelial Modulation of Coagulation and Fibrinolysis*

Vascular endothelium functions to maintain hemostasis and the fluidity of blood through critical thromboregulatory effects on platelets, the coagulation cascade and the fibrinolytic system.

Platelet - Endothelium Interactions

Under basal conditions, endothelial cells produce several products which inhibit platelet activation. Nitric oxide (EDRF) activates platelet guanylate cyclase and inhibits both platelet degranulation (Nguyen, 1991) and aggregation (Durante, 1992). Prostacyclin also exhibits potent inhibitory activity mediated by cAMP (Nolte, 1991), and the inhibitory effects of NO and $PGI_2$ on platelets are synergistic (Bowen, 1991). While activated platelets do not adhere to intact endothelium under basal conditions, inhibition of cyclo-oxygenase with indomethacin (Curwen, 1980) or nitric oxide synthase with L-NMMA renders the endothelial monolayer susceptible to platelet adherence, suggesting that endothelial cells exert important tonic antiplatelet activity. In addition, endothelial cells respond to released products of platelet activation in a manner which may provide feedback inhibition of platelet aggregation. Histamine, serotonin and ADP released from platelet granules during activation stimulate endothelial production of EDRF and prostacyclin (Adams, 1989) (Figure 5).

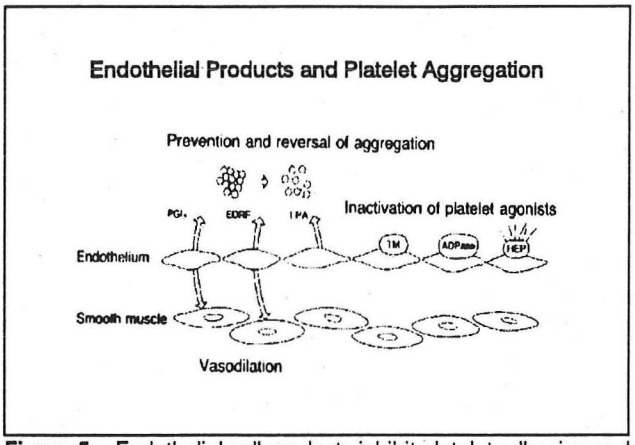


**Figure 5:** Endothelial cell products inhibit platelet adhesion and aggregation.

Endothelial cells also express cell-surface products which antagonize platelet aggregation by sequestering or inactivating mediators of platelet activation. Thrombomodulin, an

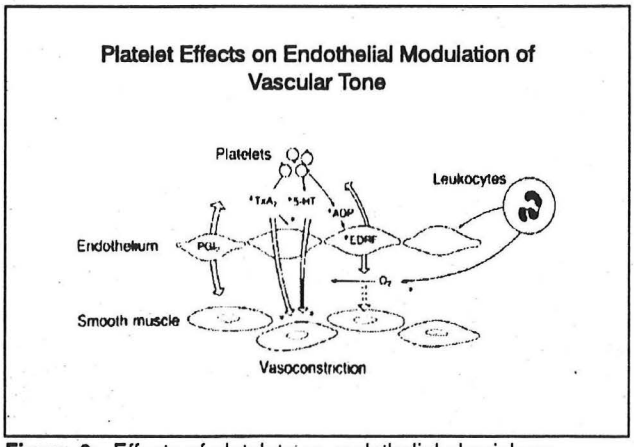


**Figure 6:** Effects of platelets on endothelial physiology.

endothelial cell-surface glycoprotein, binds the potent platelet activator thrombin with high affinity (Dittman, 1990). Similarly, an ecto-ADPase on the endothelial cell surface degrades ADP to inactive metabolites (Marcus, 1991).

Activated platelets and inflammatory cells, however, release several mediators which can alter endothelial cell physiology in a manner which promotes platelet aggregation. Von Willebrand factor, a multimeric extracellular glycoprotein synthesized and secreted by endothelial cells, binds to the platelet membrane glycoproteins GPIIb/IIIa and GPIb, and forms a matrix for platelet aggregation (Pytela, 1986; Cheresh, 1987; Titani, 1987; Hedner, 1989). Transforming growth factor-β, histamine and serotonin released from activated platelets, and tumor necrosis factor and interleukin-1 released from leukocytes stimulate secretion of vWf from endothelial cells (Hamilton, 1987; Penny, 1991). Activation of endothelial cells is also associated with secretion of platelet activating factor (PAF), which stimulates platelet aggregation through a receptor-mediated increase in cytosolic $Ca^{++}$ (Prescott, 1990).

Endothelial Modulation of the Coagulation Cascade

Endothelial cells may function indirectly to stimulate the coagulation cascade through stimulation of platelet activation and resulting thrombin release, but the principal effects of the endothelium on the coagulation cascade are inhibitory. Heparin and related mucopolysaccharides on the endothelial cell surface function as cofactors for antithrombin III (Stern, 1985). In addition to inactivating thrombin, AT-III exerts an inhibitory effect on coagulation factors IXa and Xa (Rosenberg, 1989).

Thrombin is also removed from the coagulation cascade by binding to thrombomodulin, and endothelial cell surface glycoprotein. Thrombomodulin associated thrombin demonstrates an altered catalytic specificity, acquiring protein C activating activity (Esmon, 1983; Johnson, 1983). Activated protein C complexes with protein S (Owen, 1981; Harris, 1985) bound to the endothelial cell surface (Hackeng, 1993), and complexed protein C inactivates coagulation factors Va and VIIIa (Walker, 1980; Walker, 1981; Stern, 1986). Protein S, a vitamin K dependent factor, is, like thrombomodulin, and endothelial cell product (Fair, 1986) (Figure 7).

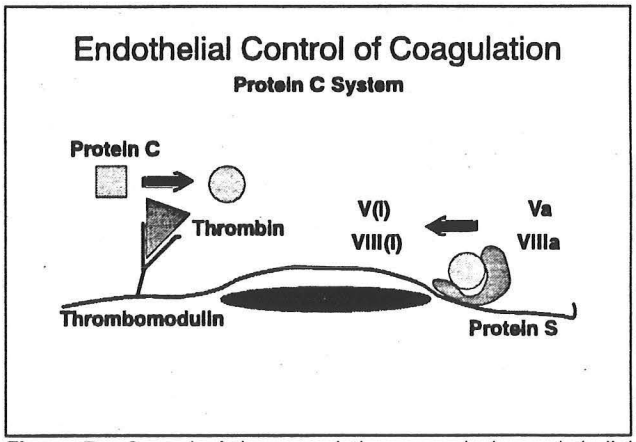


**Figure 7:** Control of the coagulation cascade by endothelial proteins thrombomodulin and protein S.

Endothelium and Fibrinolysis

In addition to exerting important regulatory effects on the coagulation cascade, vascular endothelium plays an central thromboregulatory role by modulating clot dissolution or fibrinolysis. Fibrin is degraded by the serine protease plasmin, which is generated by enzymatic cleavage of the circulating proenzyme plasminogen. In blood, plasminogen activation is catalyzed primarily by tissue plasminogen activator (tPA), an Mr 70,000 serine protease synthesized and secreted by vascular endothelial cells. Because plasminogen circulates at relatively

high concentration (2μM), concentration of active tPA is the rate-limiting determinant in initiation of the fibrinolytic system (Castellino, 1984). Endothelial cells modulate the availability of active tPA by three mechanisms: i) regulated synthesis and release of tPA from vascular endothelial cells; ii) binding of tPA to specific endothelial cell-surface receptors; and iii) production of a specific plasminogen activator inhibitor (reviewed in Gerard, 1989).

Synthesis of tPA is regulated primarily at the level of gene transcription. Thrombin, basic fibroblast growth factor, histamine and sheer stress increase, and transforming growth factor-β inhibits expression of the human tPA gene and the rate of secretion of tPA from endothelial cells (Hanns, 1987; Dichek, 1989; Diamond, 1989).

In solution, tPA activates plasminogen slowly as the $K_M$ for plasminogen (16-65 μM) is substantially greater than the circulating plasminogen concentration. In the presence of fibrin, thrombospondin or a specific endothelial cell surface receptor, tPA and plasminogen form a ternary complex in which the $K_M$ for plasminogen is greatly reduced (0.16μM), and the rate of plasminogen activation increased by several orders of magnitude (reviewed in Gerard, 1989). Vascular endothelial cells express a specific, saturable cell-surface receptor which binds tPA with high-affinity ($K_d$=9x10$^{-9}$M) (Hajjar, 1986). This receptor is an Mr 40,000 intrinsic membrane protein, present in approximately 8x10$^5$ sites/cell, which binds tPA in an active-site independent manner (Hajjar, 1990) (Figure 8). Recently, this same endothelial membrane protein has been demonstrated to bind plasminogen with high affinity, thus providing a mechanism for efficient generation of cell-surface fibrinolytic activity (Hajjar, 1991). Receptor-bound tPA is resistant to inactivation by specific inhibitors (see below), suggesting that a small cell-surface pool of tPA could contribute significantly to the antithrombotic activity of the intact endothelium by initiating pericellular fibrinolysis.

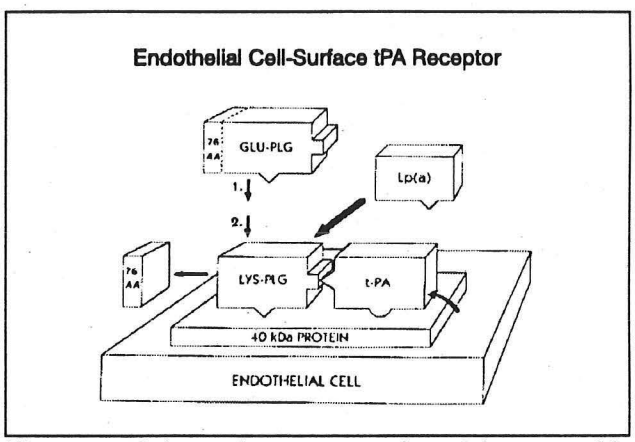


**Figure 8:** Schematic representation of the assembly of the ternary tPA-Plg-tPAR complex on the endothelial cell surface.

In contrast, in circulation tPA is inactivated by specific inhibitors. While several plasma proteins inactivate tPA slowly, the principal physiologic inhibitor in blood is plasminogen activator inhibitor-1 (PAI-1), a serine protease inhibitor or serpin synthesized and secreted by endothelial cells that rapidly and specifically inactivates tPA by serving as a suicide substrate. From the kinetics of inactivation of tPA and the concentrations of tPA and PAI-1 in plasma, the estimated half-life of active tPA in circulation is approximately 100 sec; within minutes of secretion the majority of tPA in plasma is converted to the inactive tPA-PAI-1 complex, and under basal conditions only 1-10% of circulating tPA exists in a catalytically active form.

Like tPA, the secretion of PAI-1 into the circulation is regulated in response to several physiologically important effectors. Thrombin, TGF-β, TNF, IL-1α and bacterial endotoxin all induce expression of the PAI-1 gene and secretion of PAI-1 from endothelial

cells (Hanns, 1989; Gelehrter, 1986; Reido, 1990). Additionally, PAI-1 is stored in the α-granules of platelets and released by platelet activation (Kruithof, 1986).

Several observations suggest that PAI-1 is an important physiologic modulator of plasminogen activator activity. Elevated PAI-1 levels have been observed in patients with familial thrombotic syndromes, and have been associated with risk for venous thrombosis, acute or recurrent myocardial infarction, and early thrombotic graft occlusion following coronary bypass surgery (reviewed in Gerard, 1989).

*Effects of Endothelium on Vascular Smooth Muscle Cell Proliferation*

In addition to short-acting effects on vascular physiology, endothelium plays a role in adaptation to chronic demands entailing changes in the structure of the vasculature. Ignoring angiogenesis as beyond the scope of this discussion, endothelium appears to influence the proliferative state of vascular smooth muscle cells.

In cell culture, endothelial cells secrete several factors which inhibit smooth muscle cell proliferation, including transforming growth factor-β, interferon-γ and heparin/heparan sulfate (reviewed in Davies, 1986, De Mey, 1993). Vascular smooth muscle cells co-cultured with endothelial cells are maintained in a contractile rather than synthetic phenotype (Chamley-Campbell, 1981).

A static effect of endothelium on vascular smooth muscle is supported by observations in organ culture, in which gentle removal of the endothelial monolayer is associated with an increase in intra-arterial DNA synthesis (De May, 1991). The stabilizing effect of the endothelium is not mimicked by NO, nor do nitric oxide synthase inhibitors or free radical scavengers reduce the inhibitory effects of an intact endothelium on smooth muscle proliferation. In contrast, exposure of denuded vessel segments to TGF-β or prostacyclin (Iloprost) reduces the rate of $^{3}$H-thymidine incorporation (De May, 1991b), suggesting that these products may mediate the static effect of endothelium on vascular smooth muscle.

In experimental animals, denudation of the endothelium with a balloon catheter is associated with the development of a neointima composed primarily of smooth muscle cells (Stemerman, 1977; Clowes, 1983; Clowes, 1986). This intimal thickening results from i) an early proliferative response in medial smooth muscle cells, ii) migration of medial smooth muscle cells to the intimal layer, and iii) subsequent intimal proliferation and secretion of extracellular matrix glycoproteins (Clowes, 1983; Schwartz, 1987). In several different experimental models of vascular injury, investigators have observed that the degree of neointima formation is related to the severity of vessel wall injury. In the absence of significant medial dissection, endothelial regrowth occurs rapidly (48-96 hours), and significant neointimal proliferation does not occur (Fishman, 1977). Even in the presence of an established response, smooth muscle cell proliferation appears to cease when a mechanically denuded area is reendothelialized (Walker, 1983).

In part, the inhibitory effect of endothelium on smooth muscle proliferation may result from the composition of the extracellular matrix. Endothelial cells produce a heparin-like substance which inhibits smooth muscle cell proliferation in vitro (Castellot, 1981). Endothelial regeneration after injury is associated with accumulation of heparan-sulfate containing glycosaminoglycan (Richardson, 1980), which is inhibitory for smooth muscle cell growth (Ausprunk, 1981). The relative content of heparan sulfate in the walls of vessels denuded of endothelium is reduced.

The effects of endothelial cells on smooth muscle cell proliferation are not unidirectional, however. Endothelial cells synthesize and secrete PDGF-A (Collins, 1987) and B (c-sis, Wilcox, 1988) chains, potent smooth muscle cell mitogens, Release of mitogenic factors from endothelial cells is dramatically enhanced in response to lethal injury (Fox, 1984), suggesting the potential for a bimodal response in which intact endothelium is growth inhibitory for smooth muscle cells, but local endothelial injury triggers a focal proliferative effect.

## DISRUPTION OF ENDOTHELIAL PHYSIOLOGY

Ip et al. (1990) have described three forms of injury to the vessel wall: i) dissection through the internal elastic lamina, ii) denudation of the endothelium, and iii) sublethal injury to the endothelium producing physiologic but not anatomic disruption, that is, a syndrome of endothelial dysfunction. Sublethal injury could, in theory, disrupt two types of endothelial responses to physiologic stimuli. The first are acute reactive processes, for example, NO mediated alterations in vascular tone or antiaggretory effects on platelets mediated by prostacyclin, in which the proximate effectors or signals are produced via the pre-existing cellular machinery. Endothelial insults which disrupt these processes act by inhibiting specific biochemical reactions or by inactivating critical proteins. In general, removal of the noxious stimulus results in rapid recovery of homeostatic function, as inhibitory effects decay and critical proteins are resynthesized. Disruptions to these processes should, therefore, be transient.

### *Activated Endothelium and Regulation of Endothelial Gene Expression*

The second type are longer term responses, dependent upon de novo mRNA and protein synthesis, and reflecting effects on endothelial cell gene expression. As mechanisms operating to control the expression of genes encoding important endothelial cell proteins have been defined, it has become apparent that several of these genes share common regulatory elements. Under basal conditions, the net effects of vascular endothelium are vasodilatory, antithrombotic and antiproliferative. Overlapping control mechanisms, however, can produce coordinated changes in the pattern of endothelial gene expression, altering the endothelial phenotype to one in which vasoconstrictor, pro-inflammatory, pro-thrombotic and mitogenic effects predominate.

### Transcriptional Regulation of Gene Expression

The regulatory, or promoter regions of eukaryotic genes contain DNA sequence elements which interact with the transcriptional machinery of the cell, functioning as specific binding sites for proteins involved in the process of gene transcription. These elements are of two types: i) sequences interacting with the core transcriptional machinery and present in most genes, and ii) elements found in genes transcribed only in a specific tissue or in response to specific stimuli. The latter sequences function as binding sites for transcription factors, regulatory proteins which alter the activity of the core transcriptional apparatus. The activity of these transcription factors is regulated by one of two general mechanisms: i) controlled synthesis such that the factor is produced on demand, and ii) regulated activity of a pre-synthesized factor. Transcription factors controlled by each of these mechanisms regulate the expression of important endothelial genes. Moreover, because transcription factors can interact with and influence

expression of multiple genes simultaneously, coordinated patterns of gene expression in response to a single stimulus are common (reviewed in Mitchell, 1989).

Fos/Jun

In response to basic fibroblast growth factor, tumor necrosis factor, interleukin-1 and a number of other cytokines and intercellular signalling molecules which activate protein kinase C, expression of the nuclear proto-oncogene c-fos is rapidly induced. The c-fos gene product complexes with another transcription factor, jun, forming a fos-jun heterodimer which binds specifically to cis-acting regulatory sequences termed AP-1 sites (TGACT) present in several important endothelial genes. The bound heterodimer interacts with the core transcriptional apparatus to stimulate transcription (reviewed in Angel, 1991) (Figure 9). Because the genes encoding ICAM-1, VCAM-1, and E-selectin all contain AP-1 sites and are induced by c-fos, stimulation of a single signalling pathway can produce coordinated upregulation of several cell-surface adhesion molecules.

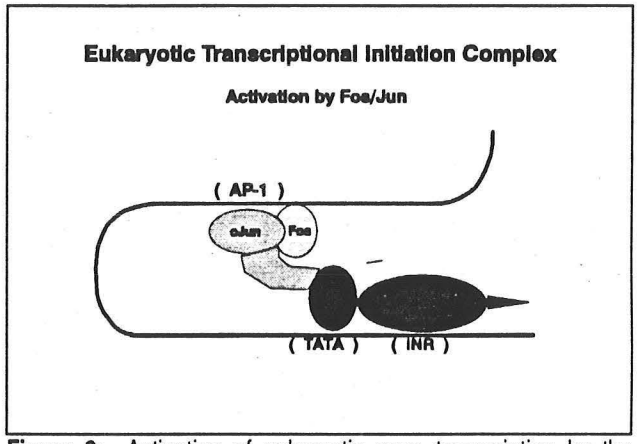


**Figure 9:** Activation of eukaryotic gene transcription by the fos/jun heterodimeric transcription factor.

NF-κB

NF-κB is a constitutively expressed factor with regulated transcription-enhancing activity. In its most common form, NF-κB is most commonly a heterodimer of p65 and p50 chains. This heterodimer complexes with another protein, IκB, which retains the NF-κB in the cytoplasm. In response to cytokines including TNFα and IL-1, or mediators of inflammation including bacterial lipopolysaccharide and reactive oxygen species, IκB is phosphorylated and dissociates from the NF-κB complex. Free NF-κB is translocated to the nucleus where it binds to a decameric recognition element (GGGRNNYYCC) and activates transcription (reviewed in Collins, 1993) (Figure 10). NF-κB recognition elements

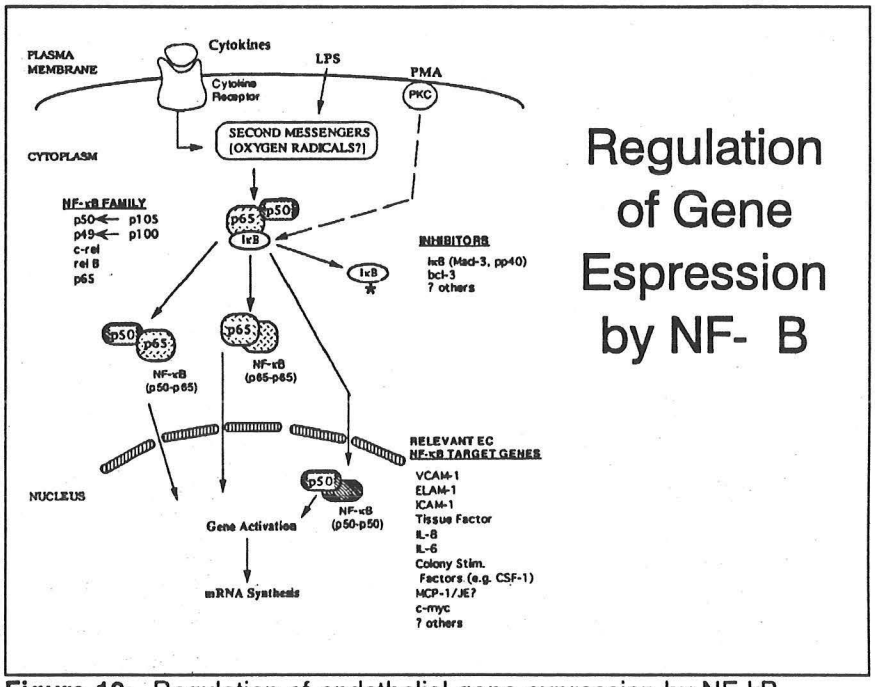


**Figure 10:** Regulation of endothelial gene expression by NF-kB.

are found in the promoter regions of the ICAM-1, VCAM-1, and E-selectin genes, and in the genes encoding urokinase, IL-6 and MHC I.

Inflammation and the Effects of Leukocytes on Endothelial Function

Complex physiologic stresses can alter both acute regulatory functions of endothelium and the pattern of endothelial gene expression. The interaction of activated leukocytes with vascular endothelium provides the best characterized example. Exposure of endothelial cells to activated leukocytes is associated with defective endothelium-dependent vasodilation, an increase in vascular permeability, progressive increases in leukocyte adherence, adherence of platelets and intraluminal thrombus formation. Two types of leukocyte products appear to mediate these effects, reactive oxygen species including superoxide and hydrogen peroxide, and cytokines, of which tumor necrosis factor is the most clearly implicated (Figure 11).

Activated leukocytes release superoxide, which inhibits EDRF release and inactivates nitric oxide *in vitro* (Lefer, 1993). Sub-injurious concentrations of hydrogen peroxide have been demonstrated to impair the endothelial barrier function (Berman, 1993), and to increase cell surface expression of ICAM-1 and MHC class I molecules (Bradley, 1993). The latter effect apparently reflects specific induction of gene transcription, as the cellular content of mRNA encoding ICAM-1, but not E-selectin or VCAM-1, is increased by hydrogen peroxide. Induction of an intercellular adhesion molecule by products of activated leukocytes creates the potential for a positive feedback loop, but hydrogen peroxide also antagonizes induction of ICAM-1, VCAM-1 and E-selectin expression by TNF or interleukin-1 (Bradley, 1993) (Figure 12). Independently, TNFα and IL-1 induce not only expression of adhesion molecules, but increase endothelial production of von Willebrand factor, tissue factor and plasminogen activator inhibitor-1 (Penny, 1991; Reido, 1990), favoring platelet aggregation and activation of the coagulation cascade.

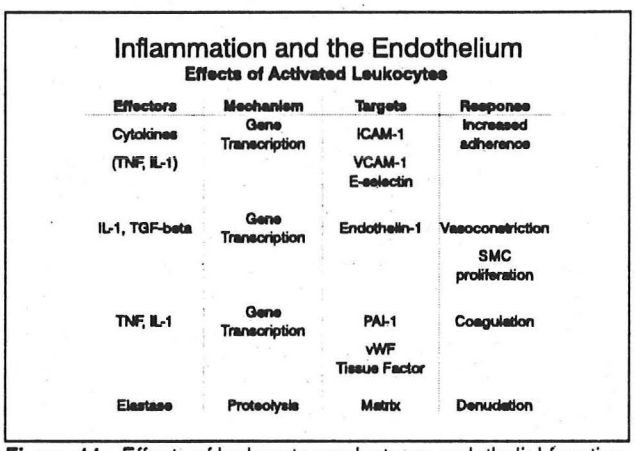


**Figure 11:** Effects of leukocyte products on endothelial function.

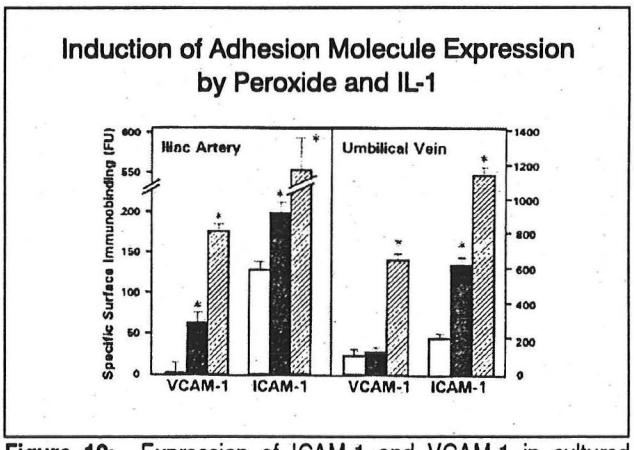


**Figure 12:** Expression of ICAM-1 and VCAM-1 in cultured endothelial cells exposed to hydrogen peroxide or interleukin-1.

The importance of these mechanisms *in vivo* has been demonstrated in models of ischemia-reperfusion injury (Lehr, 1993). Inhibition of leukocyte adhesion by interleukin-8 or transforming growth

factor-β preserves endothelium-dependent relaxations, reduces edema and intraluminal thrombosis (Lefer, 1993), and prevents the progressive endothelial injury mediated by neutrophil elastase that can result in endothelial denudation (Westlin, 1993).

*Endothelial Dysfunction in Human and Experimental Disease*

Hypercholesterolemia

Humans with hypercholesterolemia demonstrate blunted vasodilation in response to acetyl choline (Drexler, 1991) at a time prior to the development of detectable atherosclerosis. The vasodilator response to nitrates remains intact, and infusion of L-arginine restores endothelium-dependent relaxation. In experimental animals, hypercholesterolemia is similarly associated with reduced endothelium-dependent relaxations. Evidence suggests that both defective nitric oxide production and accelerated degradation of nitric oxide contribute to this effect.

In cultured endothelial cells and the intact arteries of experimental animals, LDL does not reduce EDRF release or endothelium dependent relaxation. Oxidized LDL or lysolecithin, a component thereof, however, reduce nitric oxide release in response to acetyl choline, serotonin or thrombin (Tanner, 1991; Kugiyama, 1990) (Figure 13). As in clinical hypercholesterolemia, the defect in EDRF release induced by oxidized LDL can be overcome by L-arginine, suggesting that nitric oxide synthase is intact, and that the defect induced by oxidized LDL is one of arginine availability (Tanner, 1991). This effect appears to be mediated via the scavenger receptor, as the effect of oxidized LDL is antagonized by dextran sulfate.

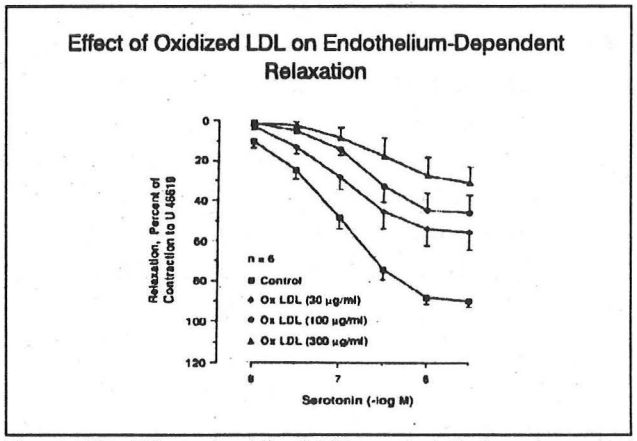


**Figure 13:** Effect of oxidized LDL on endothelium-dependent relaxations.

Oxidized LDL also appears to exert important effects on the pattern of endothelial gene expression. Cultured endothelial cells exposed to oxidized LDL accumulate mRNA encoding endothelin-1 (Boulanger, 1990) (Figure 14), and release of endothelin-1 from porcine aorta is enhanced. Oxidized LDL also induces expression of the intercellular adhesion molecule VCAM-1 in cultured endothelial cells, an effect mimicked by lysophosphatidylcholine (Kume, 1992). Rabbits fed a high cholesterol diet demonstrate focally increased expression of VCAM-1 by aortic endothelial cells after as little as 1 week of hypercholesterolemia (Li, 1993, Cybulski, 1991). Focal expression of VCAM-1 has been observed at sites of fatty streak formation and

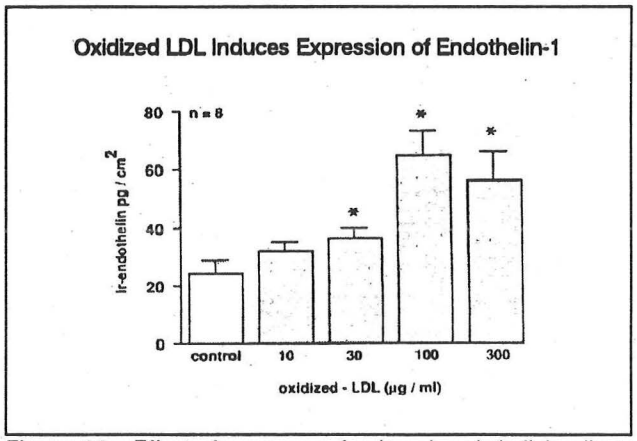


**Figure 14:** Effect of exposure of cultured endothelial cells to oxidized LDL on secretion of immunoreactive endothelin-1.

mononuclear cell accumulation in the aortic wall in cholesterol fed animals (O'Brien, 1993), and appearance of immunoreactive VCAM-1 precedes the development of histologic abnormality (Li, 1993). These observations suggest that expression of VCAM-1 in response to LPC/oxidized LDL may play an important role in the development of early atherosclerotic lesions.

Atherosclerosis

In comparison to subclinical hypercholesterolemia, established atherosclerosis is associated with more extensive disruption of normal endothelial physiology. In addition to defective EDRF release, the vasodilatory response of atherosclerotic arteries to nitric oxide is defective (Bossaller, 1987). This defect appears to result from accelerated inactivation of nitric oxide, by superoxide derived from associated inflammatory cells and by oxidized lipids within the plaque (Yamamoto, 1988, ).

Increased production of vasoconstricting factors may also contribute to the abnormal regulation of vascular tone in patients with atherosclerosis, as release of thromboxane is increased (FitzGerald, 1984). Additionally, in arteries with advanced atherosclerosis, stimuli which are normally vasodilatory can have vasoconstricting activity; both acetyl choline and serotonin can elicit vasospasm in diseased vessels (Ludmer, 1986, Golino, 1991, McFadden, 1991).

Expression of the adhesion molecules ICAM-1 and VCAM-1 are increased in atherosclerotic plaques. The endothelium of microvessels within developed lesions also shows an abnormal pattern of gene expression, including high levels of VCAM-1 (O'Brien, 1993), and PDGF-A chain (Wilcox, 1989). The latter observation suggests that, in addition to mitogenic factors produced by leukocytes accumulating in the atherosclerotic vessel wall, endogenous production of growth factors by activated endothelial cells may contribute to the proliferation of smooth muscle cells.

The composition of the extracellular matrix secreted by endothelial cells in diseased arterial segments is also altered. An increase in chondroitin and dermatin sulfate and a reduced content of heparin/heparan sulfate have been described, alterations potentially associated with reduced anticoagulant activity and a loss of antiproliferative effects on smooth muscle cells (Wright, 1989).

Hypertension

Vascular reactivity is abnormal in both clinical and experimental hypertension. In comparison to normal controls, the fall in forearm vascular resistance with acetylcholine is reduced in patients with primary hypertension. Vasodilator responses to exogenous nitrates and adenosine are preserved, suggesting that the abnormality lies in the nitric oxide generating system. Interestingly, while L-NMMA reduces endothelium-dependent relaxation in normal persons, the abnormal response of hypertensive patients is not aggravated (Panza, 1993a). In contrast to abnormalities of the nitrovasodilator system in atherosclerosis, infusion of L-arginine do not improve the abnormal acetylcholine response (Panza, 1993b). These observations suggest that synthesis of nitric oxide/EDRF by the endothelium of hypertensive patients is not substrate limited.

Administration of serotonin, or exposure of the endothelium to aggregating platelets results in vasoconstriction (Luscher,1986). In spontaneously hypertensive rats, similar abnormalities have been observed, even before the development of overt hypertension.

The endothelium-dependent vasoconstrictor activity in SHR rats is sensitive to inhibition of cyclo-oxygenase, but there are conflicting data with regard to the effects of blockade of $PGH_2/TxA_2$ receptors (Jameson,1993; Kato 1990).

Diabetes Mellitus

In human coronary arteries, insulin potentiates release of EDRF, while endothelium-dependent relaxation is defective after sustained hyperglycemia (Thom, 1988). The finding that advanced glycosylation products accumulating in uncontrolled diabetics quench nitric oxide may explain this defect (Bucala, 1991). Consistent with these findings, vasodilatory responses to nitrates are preserved in diabetic patients with reduced acetyl choline induced vasodilation (De Tejada, 1989).

In a rabbit model of diabetes, endothelial production thromboxane $A_2$, which promotes vasoconstriction and platelet aggregation, is increased (Tesfamariam, 1989).

Homocystinuria

The most compelling evidence of a role for dysfunctional endothelium in the pathophysiology of human disease exists for cystathionine β-synthase deficiency, or homocystinuria. Patients homozygous for CBS deficiency accumulate high levels of homocystine and its derivative thiolactone in serum, show accelerated atherosclerosis characterized by exuberant intimal proliferation, and suffer a high incidence of thrombotic and thromboembolic complications (reviewed in Mudd, 1985). More recently, milder abnormalities of sulfur-containing amino acid metabolism, characterized by methionine intolerance without overt baseline increases in circulating homocystine or mild hyperhomocystinemia, have been associated with an increased incidence of cerebral, peripheral and coronary vascular disease (Malinow, 1989; Boers, 1985; Genest, 1990). In studies of patients presenting with signs of vascular occlusive disease before the age of 50, as many as 1 in 3 demonstrate elevated circulating homocystine levels or an abnormal response to methionine loading (Boers, 1985). Abnormal endothelium-dependent forearm blood flow increases have been demonstrated in children with homozygous CBS deficiency prior to the development of overt vascular disease, suggesting impaired endothelial function in early life (Celermajer, 1993).

In high concentration, homocystine is toxic to endothelial cells, producing progressive morphologic changes and loss of cell viability as assessed by $^{51}Cr$ release. Cells cultured from patients heterozygous for cystathionine β-synthase deficiency, however, demonstrate toxic effects at substantially lower concentrations of homocystine than normal endothelial cells (DeGroot, 1991). The observation that inhibition of homocystine uptake into endothelial cells is protective suggests that intracellular accumulation of homocystine is required for the toxic effects (Hajjar, 1993). In this light, the greater sensitivity of endothelial cells from obligate heterozygotes is likely to reflect deficient endothelial cell CBS activity, and thus a relative inability to detoxify homocystine by conjugation with serine to form cystathionine.

Exposure of endothelial cells to sub-lethal concentrations of homocystine produces a number of physiologic disruptions which are likely to contribute to the pathogenesis of the clinical syndrome. Endothelial cells exposed to homocystine for more than 3 hours demonstrate defective secretion of EDRF (Stamler, 1993), consistent with the observed clinical abnormality of endothelium-dependent vasodilation. Platelet adherence to cultured

endothelial cells is increased in the presence of homocystine in the absence of demonstrable abnormalities of platelet function (Clarke, 1991). Additionally, in the presence of copper, homocystine generates reactive oxygen species, superoxide and hydrogen peroxide, which both reduce EDRF release and inactivate nitric oxide (Heinecke, 1987). By a similar mechanism, homocystine can potentiate oxidation of low density lipoprotein.

Homocystine modifies endothelial physiology to promote thrombus formation by several mechanisms. Factor Va activity is increased on the surface of endothelial cells cultured in the presence of homocystine (Rodgers, 1986). Both induction of an endothelial activator and a reduction in endothelial cell-surface protein C activity (Rodgers, 1990) apparently contribute to this increase. The deficiency in endothelial protein C activity appears to result from a defect in thrombomodulin cofactor activity (Hayashi, 1992). This defect does not appear to reflect reduced synthesis, as both mRNA encoding thrombomodulin and total cellular immunoreactive thrombomodulin are increased. Instead, the defect in thrombomodulin cofactor activity may result from a broad defect in transporting proteins to the cell surface. Like thrombomodulin, secretion of von Willebrand factor by cultured endothelial cells is reduced by homocystine. Maturation of asparagine-linked oligosaccharide and cleavage of pro-vWF are defective in the presence of homocystine, resulting in retention of the immature protein in the endoplasmic reticulum and accelerated degradation (Lentz, 1993). Homocystine also reduces endothelial cell-surface binding of tissue plasminogen activator to its intrinsic membrane receptor (Hajjar, 1993). Binding of plasminogen, which associates with an independent site on the same protein, is not altered, implying that the defect is functional rather than the result of deficient expression.

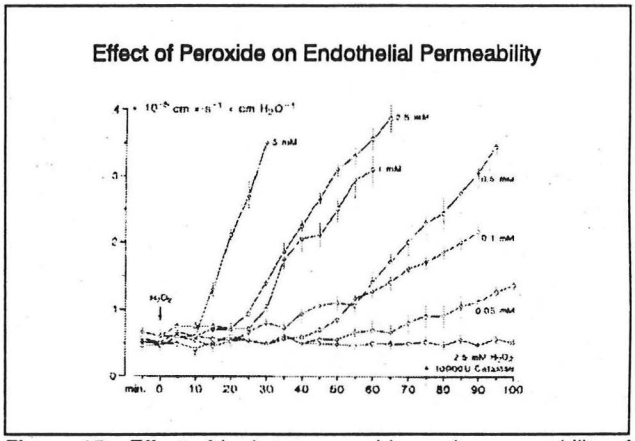


**Figure 15:** Effect of hydrogen peroxide on the permeability of endothelial monolayers to water.

In summary, endothelial cells exposed to homocystine, particularly in the setting of defective endothelial cystathionine β-synthase activity, demonstrate physiologic disruptions which reduce vasodilatory activity, promote platelet adherence, enhance lipid uptake and smooth muscle cell proliferation, activate coagulation and reduce fibrinolytic potential; these defects are associated with a clinical syndrome of accelerated atherosclerosis and intraluminal thrombosis.

*Intervention for Endothelial Dysfunction*

Modification of Endothelial Function

Several of the physiologic abnormalities of endothelium associated with human disease appear reversable. Defective EDRF release is improved by L-arginine. Induced expression of cell surface adhesion molecules is dependent upon generation of oxidized LDL in patients with hypercholesterolemia. The endothelial abnormalities in heterozygous cystathionine β-synthase deficiency are dependent upon exposure to elevated levels of

sulfur containing amino acids.

In some cases, available therapeutic interventions may have beneficial effects on endothelial function. Production of oxidized LDL can be reduced by aggressive efforts to lower circulating LDL in patients with hypercholesterolemia, with the potential, in the absence of established atherosclerosis, to reverse abnormalities of nitric oxide release and expression of cell-surface adhesion molecules. In addition, HDL appears to antagonize the adverse effects of hypercholesterolemia on endothelial function (Matsuda, 1993) (Figure 16), suggesting that current secondary prevention strategies may have salutary effects on endothelial function. Inhibitors of cyclo-oxygenase can prevent release of vasoconstricting prostenoids. Aggressive control of blood glucose in diabetic patients can reduce production of advanced glycosylation products suggested to inactivate nitric oxide.

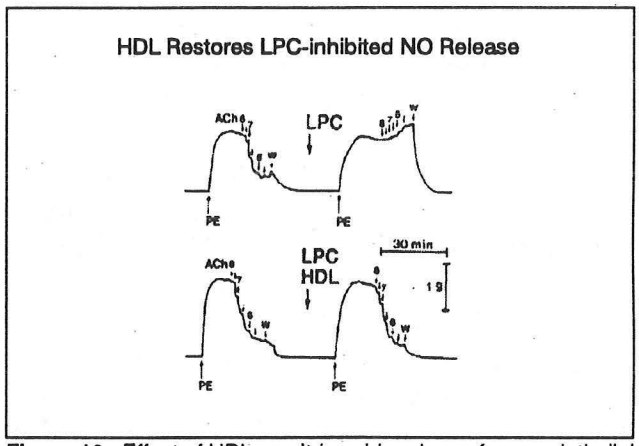


**Figure 16:** Effect of HDL on nitric oxide release from endothelial cells pre-treated with lysophosphatidylcholine.

A number of the defects in endothelial function associated with human disease, however, are not amenable to currently available therapeutic approaches. Recently, however, genes have become therapeutic agents. While the initial trials of human gene therapy have targeted inherited monogenic defects or advanced malignancies, experimental studies suggest that somatic cell gene transfer offers the potential to intervene in complex and common human diseases for which conventional therapeutic approaches have shown limited efficacy. Of specific interest in cardiovascular medicine and biology, techniques for genetic modification of vascular endothelial cells have been developed.

## Genetic Modification of Endothelial Cells

The walls of blood vessels, by virtue of both accessibility and involvement in clinically important disease, are attractive targets for gene-based therapeutic strategies. While a number of laboratories have reported very efficient expression of foreign genes in the vasculature using cell-based gene transfer strategies (Nabel et al, 1989; Wilson et al, 1989), for most clinically significant applications denudation of the endothelium or culture of autologous endothelial cells prior to the reintroduction of genetically modified cells is impractical.

Efforts to achieve efficient direct gene transfer into the vessel wall using retrovirus vectors or liposome encapsulated DNA have proven disappointing, although biologically significant results have been obtained using retrovirus when high efficiency

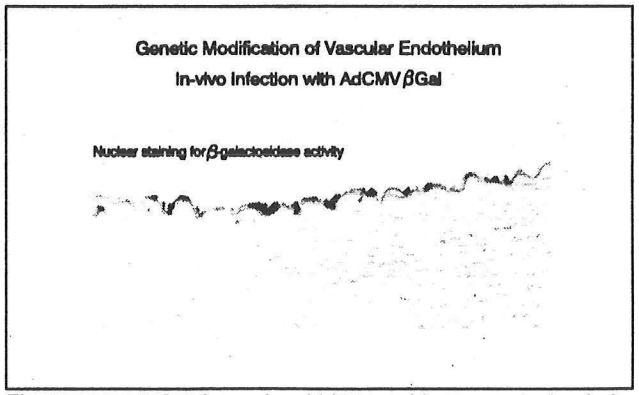


**Figure 17:** Section of rabbit carotid artery stained for galactosidase activity after infection with AdCMVBgal.

transduction is not required (Nabel et al, 1992). In contrast, early efforts at direct gene transfer into vascular endothelium using recombinant adenoviral vectors have demonstrated relatively efficient gene transfer. Lemarchand et al (1992) have reported that perfusion of isolated vessel segments with a recombinant adenovirus encoding a nuclear-localizing variant of β-galactosidase resulted in expression in essentially all of the endothelial cells. Expression of a physiologically important protein, human α1-antitrypsin, by endothelial cells infected *in situ* in isolated vessel segments with a recombinant adenovirus was also reported, although no physiologic effects were sought or described.

Efficient genetic modification of vascular endothelial cells infected *in vivo* by direct intraluminal instillation of recombinant adenoviruses, or infusion of recombinant virus through a double balloon catheter has been accomplished (Willard et al, 1992) (Figure 17), providing a potential approach to modifying the pattern of gene expression and phenotype of endothelium in selected vessel segments.

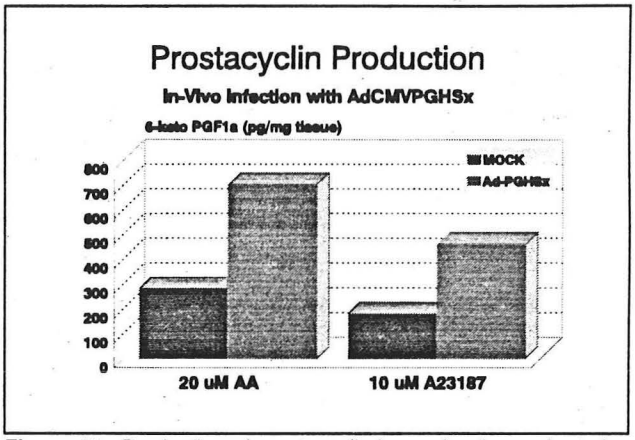


**Figure 18:** Production of prostacyclin in porcine femoral arteries infected in vivo with AdCMVPGHSx.

Dr. Kenneth Wu and colleagues at the University of Texas Medical School at Houston have demonstrated one approach by which genetic modification of vascular endothelium *in vivo* might be employed to inhibit intravascular thrombus formation (Figure 18). These investigators have employed a recombinant adenovirus encoding human prostaglandin H synthase to infect the endothelium of porcine femoral arteries, and demonstrated significantly enhanced release of prostacyclin (Zoldhelyi, 1993). Similar strategies to increase expression of prostacyclin synthase, thrombomodulin, tissue plasminogen activator or its cell-surface receptor, or nitric oxide synthase might be expected to have potential therapeutic utility. While studies to evaluate these, or other potential approaches, are not yet available, the technology to achieve efficient genetic modification of vascular endothelium is rapidly evolving and holds considerable promise.

## Gene Transfer to Reduce Cardiovascular Risk

Gene based therapeutic strategies also have the potential to modify the metabolic environment in which endothelial dysfunction arises. Drs. Herz and Gerard (1993) at this institution have demonstrated that administration of a recombinant adenovirus encoding human LDL receptor to normal mice resulted in overexpression of human LDLR in liver.

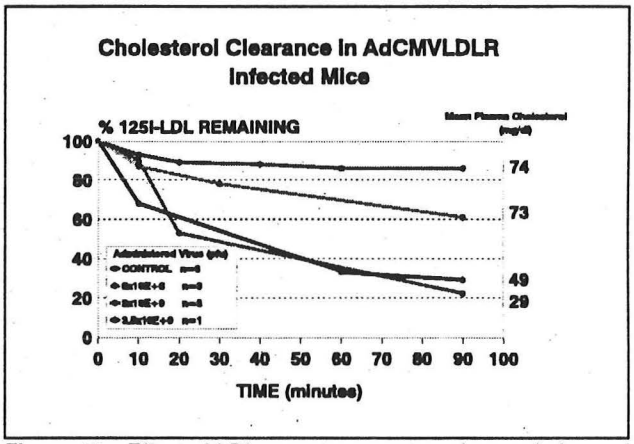


**Figure 19:** Effect of LDL receptor gene transfer on cholesterol clearance in normal mice.

Animals infected with this virus showed accelerated clearance of $^{125}$I-LDL, and plasma cholesterol levels were reduced by approximately 50%. Infection of LDLR-deficient transgenic mice with the recombinant adenovirus restored expression of the LDL receptor, increased the rate of $^{125}$I-LDL clearance, and normalized the lipoprotein profiles in these mice (Ishibashi, 1993). Dr. Kopfler and others in our Division have similarly demonstrated that infection of mice with a recombinant adenovirus encoding human apolipoprotein A-I resulted in accumulation of immunoreactive human ApoA1 in serum and a 34% increase in circulating HDLc levels (Table II). In view of the adverse effects of elevated LDLc

**Table II:** Serum ApoA1 and HDLc levels in AdCMVApoA1 infected mice.

| VIRUS | Human ApoA1 (mg/dl) | Cholesterol (mg/dl) | HDLc (mg/dl) |
|---|---|---|---|
| AdCMVLuc (n=6) | 0 | 92 +/- 7 | 61 +/- 5 |
| AdCMVApoA1 (n=4) | 194 +/- 14 | 143 +/- 13 | 81 +/- 4 |

levels on endothelial function, and the protective effects of high HDLc levels against oxidized LDL induced endothelial injury and the development of intimal hyperplasia following balloon arterial injury in animal models, these findings suggest gene based therapeutic strategies with the potential to prevent or ameliorate endothelial dysfunction. A similar approach might be employed to increase cystathionine β-synthase activity in deficient patients (Michel, 1992).

## SUMMARY

Endothelial cells are the focal point of vascular biology and medicine, exerting critical regulatory effects on vessel tone, permeability, inflammatory cell adhesion, coagulation, fibrinolysis and the growth state of vascular smooth muscle. Alterations in endothelial cell function accompany and contribute to the pathophysiology of important human disease. While the vision of a monolithic syndrome of endothelial dysfunction appears an oversimplification, common patterns of altered endothelial physiology and gene expression are associated with several different human diseases. Identification of the molecular basis for defects in endothelial function opens the potential for novel therapeutic strategies.

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