Endothelial Apoptosis*
Could It Have a Role in the Pathogenesis and Treatment of Disease?

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Endothelial apoptosis can be found in a number of diseases. This review summarizes the current knowledge about the causes and consequences of endothelial apoptosis, and analyzes its possible role in the pathogenesis and treatment of several diseases. Novel forms of therapy based on the proposed pathophysiologic mechanisms are discussed. (CHEST 2000; 117:841–854)

Key words: apoptosis; atherosclerosis; endothelium; heart failure; hypertension; primary pulmonary hypertension; sepsis

Abbreviations: ACE = angiotensin-converting enzyme; CHF = congestive heart failure; cNOS = constitutive nitric oxide synthase; FGF = fibroblast growth factor; IL-1 = interleukin 1; IL-1 Ra = IL-1 receptor antagonist; LDL = low-density lipoprotein; LPS = lipopolysaccharide; NF-κB = nuclear factor κB; NO = nitric oxide; NOS = nitric oxide synthase; PPH = primary pulmonary hypertension; TNF = tumor necrosis factor; VEGF = vascular endothelial growth factor

Endothelial integrity and normal function are indispensable for the preservation of health. The estimated 1 to 6 × 10^{13} cells of the endothelial monolayer form an approximately 1-kg heavy "organ" in an adult human, and have a central role in the control of vascular tone, permeability, blood flow, coagulation, thrombolysis, inflammation, tissue repair, and growth.1,2 Diseases accompanied by endothelial perturbation are associated with significant morbidity and mortality, and show a similar constellation of findings (Table 1).3–46

ENDOTHELIAL PRO- AND ANTIAPOPTOTIC STIMULI

The endothelial position between tissues and circulating blood assures its simultaneous and constant exposure to a wide variety of stimuli, many of which have the potential to induce or prevent apoptosis (programmed cell death) of its cells (Table 2).47–90 A detailed description of the apoptotic process is beyond the scope of this discussion (see Haunstetter and Izumo91 for review). Briefly, different stimuli, using a variety of receptors and signal transduction pathways, activate a cascade of proteases (caspases) that execute a program of cellular self-destruction by cleaving cellular structures at specific sites in a strictly controlled manner. This leads to disassembly of the cell into small apoptotic bodies without spillage of cellular contents, significant inflammatory response, or damage to surrounding cells. The receptors and exact signal transduction pathways for endothelial pro- and antiapoptotic stimuli are largely unknown, but the available data (Table 2) seem to support one important conclusion: the local balance of pro- and antiapoptotic stimuli decides the survival of each individual endothelial cell. Thus, every antiapoptotic factor does not protect against all proapoptotic stimuli; these responses seem to be dependent on the specific signal transduction pathways used by the individual agents. For example, shear stress can effectively prevent apoptosis induced by tumor necrosis factor (TNF), but it has no effect on apoptotic cell death due to ceramide.85

Several additional facts are important for an understanding of the possible role of the endothelium...
in the pathogenesis of disease. First, there is marked phenotypic variability between endothelial cells from different portions of the vascular tree. They manifest different surface markers and show varying responses to the same stimulus. For example, human macro- and microvascular coronary endothelial cells respond differently to the same concentration of oxidized low-density lipoprotein (LDL) in vitro; these responses could indicate the unequal susceptibility to the development of atherosclerotic changes in those two segments of the same vascular distribution. Second, endothelial cells from different individuals can, even when obtained from the same portion of the vasculature, greatly vary in their susceptibility to apoptosis under the same conditions of cell culture. Whether this is reflective of a genetic predisposition to the development of disease is currently unknown, but these unequal responses have to be considered when interpreting in vitro studies using cells obtained from a single individual. Third, the type of response to a stimulus may be influenced by the duration or intensity of exposure. For example, lipopolysaccharide (LPS) and TNF induce apoptosis of cultured human umbilical vein endothelial cells after an exposure of ≥12 h and apoptosis becomes maximal at 18 h, to be replaced by a predominantly necrotic form of cell death after 24 h of exposure. Fourth, a stimulus may initiate changes in endothelial cells that have opposing effects on the activation of apoptosis. LPS can, for example, activate the transcription of antiapoptotic factors produced by endothelial cells themselves, and the intracellular balance of several opposing influences likely determines the activation of the apoptotic cascade of caspases.

Fifth, the nature, strength, and duration of exposure to a given combination of pro- and antiapoptotic stimuli could perhaps determine the clinical picture by causing a specific distribution and extent of endothelial apoptosis; this may lead, on one end of the spectrum, to localized and low-grade vascular changes that slowly progress over several decades, and could on the other cause widespread apoptosis of a majority of endothelial cells, leading to death within hours to days. The endothelium shows little evidence of perturbation in the absence of disease. If specifically searched for, apoptotic endothelial cells are rarely found in the intima of vessels that show no evidence of vascular disease. Illustrative of this can be the fact that the normal human circulation contains an average number of 2.6 circulating nucleated endothelial cells per milliliter of blood. At least 60% of them were found to be apoptotic using a method that is not sensitive for the earliest changes accompanying apoptosis. The number of approximately 10,000 circulating nucleated endothelial cells (or even one that may be 100 to 1,000 times higher, if one could count all anuclear endothelial cell fragments or apoptotic bodies) in a blood volume of 3.5 to 4 L is negligible compared with the total number of endothelial cells (>10¹¹). This suggests that under normal resting conditions, a predominance of antiapoptotic factors maintains the percentage of apoptotic cells at a very low level (<0.1% in the control group of an animal study). These facts can, at least in part, explain the notorious difficulty in detecting a rare and short-lasting event like apoptosis, in the section of a layer of tissue as thin as the endothelium, especially if it has not been specifically searched for.

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Table 1—Some Diseases Associated With Endothelial Perturbation and Several of Their Accompanying Findings

<table>
<thead>
<tr>
<th>Diseases</th>
<th>Endothelial Injury†</th>
<th>Endothelial Dysfunction†</th>
<th>Endothelial Activation†</th>
<th>Endothelial Apoptosis</th>
<th>Increased Circulating Leukocyte</th>
<th>Increased Endothelial Cell Count</th>
<th>Increased Adrenomedullin</th>
<th>Increased Endothelin-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atherosclerosis</td>
<td>Human</td>
<td>Human</td>
<td>Human</td>
<td>Exp²/hum²</td>
<td>Human</td>
<td>Human</td>
<td>Human</td>
<td>Human</td>
</tr>
<tr>
<td>Allograft vasculopathy</td>
<td>Experimental¹²</td>
<td>Human</td>
<td>Human</td>
<td>Human</td>
<td>Human</td>
<td>Human</td>
<td>Human</td>
<td>Human</td>
</tr>
<tr>
<td>Hypertension</td>
<td>Human¹⁹</td>
<td>Human</td>
<td>Human</td>
<td>Experimental¹¹</td>
<td>Human</td>
<td>Human</td>
<td>Human</td>
<td>Human</td>
</tr>
<tr>
<td>CHF</td>
<td>Human²⁶</td>
<td>Human</td>
<td>Human</td>
<td>Experimental¹¹</td>
<td>Human</td>
<td>Human</td>
<td>Human</td>
<td>Human</td>
</tr>
<tr>
<td>Sepsis and associated</td>
<td>Exp⁵⁵/hum³⁴</td>
<td>Human</td>
<td>Human</td>
<td>Experimental³⁶⁻³⁸</td>
<td>Experimental³¹–³³</td>
<td>Human</td>
<td>Human</td>
<td>Human</td>
</tr>
<tr>
<td>syndromes †</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPH</td>
<td>Human⁴¹</td>
<td>Human</td>
<td>No data</td>
<td>Experimental⁴¹</td>
<td>No data</td>
<td>Human</td>
<td>Human</td>
<td>Human</td>
</tr>
</tbody>
</table>

*Exp = experimental; hum = human.
†The terms endothelial injury, dysfunction, and activation are used throughout the literature with different and partly overlapping meanings. For the purpose of this discussion, the following definitions are used: endothelial injury = microscopically visible endothelial cell shape change or injury, defects in endothelial lining, or elevated soluble markers of endothelial injury; endothelial dysfunction = decreased endothelial-dependent vascular relaxation or NO release, decreased expression or activity of endothelial eNOS; endothelial activation = increased expression or release of endothelial adhesion molecules.
†Includes systemic inflammatory response syndrome, multiple organ dysfunction syndrome, and ARDS.
Changes Accompanying Endothelial Apoptosis

Changes associated with endothelial apoptosis are embedded in a complex array of interdependent events (Fig 1). As detailed in subsequent sections, these changes can affect many aspects of endothelial function, and may be directly linked to some manifestations of disease (Table 1).

Table 2—Some Pro- and Antiapoptotic Stimuli for Endothelial Cells*  

<table>
<thead>
<tr>
<th>Proapoptotic</th>
<th>Antiapoptotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Extracellular) adenosine and ATP</td>
<td>Adrenomedullin</td>
</tr>
<tr>
<td>Age</td>
<td>Albumin</td>
</tr>
<tr>
<td>Angiotensin II</td>
<td>Angiopoietin-1</td>
</tr>
<tr>
<td>Antioxidants</td>
<td>Anti-TNF</td>
</tr>
<tr>
<td>Anti-dsDNA</td>
<td>Apolipoprotein (apo)</td>
</tr>
<tr>
<td>Calcium, increased intracellular concentration</td>
<td>Calcium buffer or chelator</td>
</tr>
<tr>
<td>Cocaine</td>
<td>Cyclosporin A</td>
</tr>
<tr>
<td>Endostatin</td>
<td>Endothelin-1</td>
</tr>
<tr>
<td>Homocysteine/adenosine</td>
<td>Erythropoietin</td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td>Estradiol</td>
</tr>
<tr>
<td>Hypothermia/rewarming</td>
<td>Extracellular matrix components</td>
</tr>
<tr>
<td>Hypertonic stress</td>
<td>FGF</td>
</tr>
<tr>
<td>Hypotonic stress</td>
<td>Forskolin</td>
</tr>
<tr>
<td>Hyponxia</td>
<td>Glutamine</td>
</tr>
<tr>
<td>Interleukin-1β</td>
<td>Hepatocyte growth factor</td>
</tr>
<tr>
<td>Interferon-α, interferon-γ</td>
<td>Insulin-like growth factor</td>
</tr>
<tr>
<td>Intracellular acidosis</td>
<td>Integrins</td>
</tr>
<tr>
<td>Ischemia/reperfusion</td>
<td>Interleukin-10</td>
</tr>
<tr>
<td>LPS</td>
<td>Iron chelators</td>
</tr>
<tr>
<td>Lupus anticoagulant</td>
<td>Monocytes, in direct contact</td>
</tr>
<tr>
<td>2-Methoxyestradiol</td>
<td>N-acetylcysteine</td>
</tr>
<tr>
<td>Natriuretic peptides</td>
<td>NO, endogenous via cNOS</td>
</tr>
<tr>
<td>Neutrophil proteases</td>
<td>NO exogenous (low concentrations)</td>
</tr>
<tr>
<td>NO, exogenous in high doses</td>
<td>NO, via iNOS activity, after &gt;96 h</td>
</tr>
<tr>
<td>NOS inhibition, in confluent cells</td>
<td>Prostaglandin E2</td>
</tr>
<tr>
<td>Oxidized cholesterol</td>
<td>Prostacyclin</td>
</tr>
<tr>
<td>Oxidized LDL</td>
<td>Shear stress</td>
</tr>
<tr>
<td>Peroxynitrite</td>
<td>VEGF</td>
</tr>
<tr>
<td>Proinsulin</td>
<td>Vitamin C</td>
</tr>
<tr>
<td>Protein synthesis inhibitors</td>
<td>Vitamin E</td>
</tr>
<tr>
<td>Radiation</td>
<td></td>
</tr>
<tr>
<td>Serum starvation</td>
<td></td>
</tr>
<tr>
<td>Solid tumor cells, in direct contact</td>
<td></td>
</tr>
<tr>
<td>(Intracellular) Staphylococcus aureus</td>
<td></td>
</tr>
<tr>
<td>TGF-β1</td>
<td></td>
</tr>
<tr>
<td>TNF, soluble</td>
<td></td>
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<tr>
<td>TNF, transmembrane</td>
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</tbody>
</table>

Table 2—Some Pro- and Antiapoptotic Stimuli for Endothelial Cells*  

Apoptotic endothelial cells release interleukin 1 (IL-1), which will have several important effects: (1) IL-1 may enhance apoptosis of endothelial cells; (2) it may enhance endocytosis of apoptotic bodies by surrounding endothelial cells; (3) IL-1 activates neighboring endothelial cells through the activation of nuclear factor κB (NF-κB). While activation of NF-κB may protect the endothelial cell from apoptosis in an environment that is likely to be predominantly proapoptotic, it also leads to the expression and release of adhesion molecules for inflammatory cells and production of proinflammatory cytokines. Leukocytes may adhere, transmigrate, release proteases, cause additional endothelial injury, and lead to the development of inflammatory changes in the vessel wall. Release of adhesion molecules prevents further leukocyte adherence, but may at the same time cause leukocyte activation and lead to release of additional proinflammatory mediators or proteases, thereby giving rise to endothelial injury or apoptosis in nearby or distant endothelial surfaces. The exact nature of soluble adhesion molecule production and release by endothelial cells is presently insufficiently understood; both proteolytic cleavage of membrane-bound forms as well as messenger RNA encoding soluble adhesion molecules have been described in nonendothelial cells. It has been suggested that mediators other than IL-1 participate in the above events. Messenger RNA for several pro- and anti-inflammatory mediators has been found in resting and stimulated endothelial cells, and secretion of TNF by the endothelium has been demonstrated in vitro. Whether release of these mediators participates in the events associated with endothelial apoptosis is presently unknown.

The mitochondrion plays a key role in the initiation and amplification of the apoptotic process. During apoptosis, it releases cytochrome c, which disrupts its electron transport chain. While normal oxidative phosphorylation loses some 1 to 5% of electrons to superoxide production, this percentage increases with loss of cytochrome c from the mitochondria of the apoptotic cell. The freely diffusible nitric oxide (NO) and newly produced superoxide form peroxynitrite, itself a toxic and proapoptotic substance for the endothelium. NO is consumed in this reaction and is not available for its normal role in vasodilatation, inhibition of platelet aggregation, and prevention of endothelial apoptosis, as well as inhibition of vascular smooth muscle cell proliferation and leukocyte adhesion.

Normal antiocoagulant properties of vascular endothelium are lost during apoptosis. The surface of the apoptotic cell exhibits increased tissue factor procoagulant activity, as well as reduced surface thrombo-
modulin, heparan sulfate, and tissue factor pathway inhibitor expression. This leads to increased thrombin formation by both adherent and detached apoptotic endothelial cells. Prostacyclin production is decreased during endothelial apoptosis. This may, apart from a reduced antiapoptotic effect, lead to loss of inhibition of platelet activation. Both activated platelets and thrombin can enhance inflammatory and proapoptotic events through activation of leukocytes and endothelial cells.

Complement activation via the alternative pathway has been observed on apoptotic endothelial cells. While this activation may enhance the clearance of apoptotic cells by phagocytes, it can also activate leukocytes and endothelial cells, and mediate additional endothelial injury or apoptosis.

Not only does endothelial apoptosis lead to the just described changes, but these changes may also, if induced through other mechanisms, lead to apoptosis of the endothelium (Fig 1). To complicate matters further, some inflammatory changes may have pro- and antiapoptotic effects. For example, monocytes can release the proapoptotic TNF but may, in direct contact with the endothelium, prevent apoptosis of its cells in a proapoptotic environment. Neutrophils secrete TNF and proapoptotic proteases, but also release the antiapoptotic vascular endothelial growth factor (VEGF), as well as the anti-inflammatory and likely antiapoptotic IL-1 receptor antagonist (IL-1Ra). These mechanisms may serve to limit the intensity of proinflammatory and apoptotic changes, and prevent their extension to previously unaffected areas of the vasculature.
Adrenomedullin and endothelin-1 levels are elevated in circumstances associated with endothelial perturbation (Table 1). They have opposite effects on vascular tone; endothelin-1 is one of the most potent vasoconstrictors, whereas adrenomedullin causes vasodilatation. Both were found to have an autocrine/paracrine antiapoptotic effect on endothelial cells. Their secretion in circumstances associated with endothelial injury, activation, and apoptosis is controlled independently, and may represent a mechanism that limits endothelial damage and enhances its recovery, while adjusting vascular tone and blood flow in the distribution of disturbed endothelial-derived vasomotor control.

### Circulating Endothelial Cells

Endothelial adherens junction proteins are cleaved during apoptosis. This results in rounding of the normally flat-shaped cell, its retraction from intercellular connections, and, finally, detachment from the underlying basal membrane. When floating in cell culture medium, the apoptotic cell completes its disassembly into smaller apoptotic bodies. As previously mentioned, circulating apoptotic nucleated endothelial cells have been found in the peripheral blood of normal healthy individuals. Circulating endothelial cells and cell carcasses have been isolated and identified using different methods with varying levels of sophistication. Although it has never been directly observed in vivo, it appears possible that they represent those cells that have "desquamated" from the basal membrane in the early stages of apoptosis, and are now completing their disappearance while circulating in the blood. Interestingly, a number of stimuli affecting endothelial apoptosis have been shown to influence the number of circulating endothelial cells (Table 3).

### Table 3—Factors Influencing the Number of Circulating Endothelial Cells or Cell Carcasses*

<table>
<thead>
<tr>
<th>Increased</th>
<th>Decreased</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angina pectoris</td>
<td>Calcium chloride blocker</td>
</tr>
<tr>
<td>ADP</td>
<td>Calcium chloride IV (low</td>
</tr>
<tr>
<td>Adrenergic stimulants</td>
<td>dose)</td>
</tr>
<tr>
<td>(epinephrine, norepinephrine,</td>
<td>Clolibrate</td>
</tr>
<tr>
<td>isoprorenaline)</td>
<td>Digoxin</td>
</tr>
<tr>
<td>Atherosclerosis</td>
<td>Heparin</td>
</tr>
<tr>
<td>CHF</td>
<td>Prostacyclin</td>
</tr>
<tr>
<td>Endotoxin</td>
<td></td>
</tr>
<tr>
<td>Exercise</td>
<td></td>
</tr>
<tr>
<td>Homocysteine</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
</tr>
<tr>
<td>Hypertonic saline solution</td>
<td></td>
</tr>
<tr>
<td>Hypotonic saline solution</td>
<td></td>
</tr>
<tr>
<td>Ischemia</td>
<td></td>
</tr>
<tr>
<td>Lactate</td>
<td></td>
</tr>
<tr>
<td>Protamine</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
</tr>
</tbody>
</table>

*ADP = adenosine diphosphate; ASA = acetylsalicylic acid.

Practically all risk factors for atherosclerosis either cause endothelial apoptosis in vitro or increase the number of circulating endothelial cells in vivo, and some protective factors have the opposite effect (Tables 2, 3). Many interventions known to be effective in the prevention or treatment of atherosclerosis and its complications may in some way reduce apoptosis of the endothelium (Table 4).

It has been suggested that endothelial apoptosis might have a role in the early phases of this disease. Atherosclerosis could begin at an early age with a diffusely and minimally increased percentage of apoptotic endothelial cells throughout the vasculature. Because of the antiapoptotic effects of shear stress, such changes are likely to be most pronounced in areas of low shear stress. These areas are known to be preferential sites for the development of atherosclerotic plaques, and show increased endothelial cell turnover and decreased endothelial-dependent vasodilatation.

The earliest observed alterations in the vessel wall are the fatty streak and monocyte adhesion. The endothelium oxidizes native LDL in a mitochondrial-dependent fashion. This is likely to be enhanced in the presence of increased superoxide production by the mitochondria of the apoptotic endothelial cells, and may not be dependent on an increased availability of LDL. Oxidized LDL is one of the best-described endothelial proapoptotic agents, and apoptosis could amplify itself in this manner. Interestingly, adherent monocytes are anti-apoptotic for the endothelium when the latter is

### Role of Endothelial Apoptosis in Disease

It has been suggested that disturbed endothelial function could account for a large portion of all cardiovascular disease. The subsequent sections will briefly describe the possible role of endothelial apoptosis in several disorders. Some of them are characterized by complex pathophysiologic changes, and the discussion will be limited to only some aspects of the disease that may be related to endothelial apoptosis (see also Table 1, Fig 1).

**Atherosclerosis**

Apoptotic endothelial cells have been demonstrated in experimental and human atherosclerosis.

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exposed to a proapoptotic environment.\textsuperscript{81} They have also been observed to enter and leave the subendothelial space.\textsuperscript{136} Active endothelial participation is required for monocyte adhesion and transmigration to the subendothelium, and one of the first observable changes associated with this process is the expression of endothelial adhesion molecules for mononuclear leukocytes.\textsuperscript{1,2} The early accumulation of monocytes could therefore represent an attempt by the endothelium to reduce the proapoptotic activity of the subendothelial oxidized LDL and assist in its removal. Indeed, it has been hypothesized that the advancement of the atherosclerotic lesion may be occurring as a result of a failure of this monocyte clearance system to remove sufficient lipid.\textsuperscript{136}

Platelet-derived growth factor and endothelin-1 cause vascular smooth muscle cell proliferation and are released in some circumstances associated with endothelial apoptosis.\textsuperscript{2,114,137} Angiotensin II and a reduced availability of NO favor apoptosis in endothelial cells.\textsuperscript{50,72} The exact opposite occurs with smooth muscle cells; their apoptosis is inhibited and proliferation enhanced by angiotensin II and the absence of NO.\textsuperscript{106} This indicates that endothelial apoptosis could be associated with vascular smooth muscle cell proliferation, and smooth muscle cells are an important component of the atherosclerotic plaque.

Both macrophages and smooth muscle cells in the atherosclerotic plaque can produce TNF\textsuperscript{138} and could thereby cause additional apoptosis of the overlying endothelium. Endothelial-derived mediators may be able to activate cells in the plaque.\textsuperscript{74,102} A positive feedback mechanism established in this way could lead to a rapid acceleration of plaque growth. Mediators released in systemic infections and inflammatory processes could lead to an enhancement of endothelial apoptosis and proinflammatory activity inside the atherosclerotic lesion, explaining the well-known association between symptomatic atherosclerosis and systemic inflammation.\textsuperscript{139} In times of predominance of endothelial antiapoptotic stimuli, a reversal of the above process could lead to smooth muscle and inflammatory cell apoptosis,\textsuperscript{91} allowing a reduction of inflammatory changes and possibly even plaque size. The remnants of these cells can accumulate in the depth of the plaque,\textsuperscript{140} and if this process occurs repeatedly through several decades, it could contribute to the formation of high-grade stenotic lesions.

It is difficult to separate endothelial injury and activation from the presence of endothelial apoptosis (Table 1, Fig 1) and soluble markers of endothelial injury or activation could perhaps be viewed as surrogate markers for the presence of endothelial apoptosis. This could explain the observation that soluble endothelial adhesion molecules correlate with the risk of symptomatic atherosclerosis,\textsuperscript{5} and that effective antiapoptotic therapeutic approaches\textsuperscript{8} reduce markers of endothelial injury.\textsuperscript{141} Interestingly, some soluble endothelial adhesion molecules show a diurnal variation in healthy humans,\textsuperscript{142} indicating that mechanisms of endothelial activation or apoptosis might cause the well-documented increased frequency of acute coronary syndromes at certain times of the day.

### Table 4—Some Therapies With Beneficial Effects in Cardiovascular Diseases and Their Relation to Endothelial Apoptosis

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Proven or Possible Effect on Endothelial Apoptosis</th>
</tr>
</thead>
</table>
| Cholesterol lowering | Reduces the substrate for the production of proapoptotic oxidized LDL by endothelial cells.
| Statins increase cNOS activity, which has an antiapoptotic effect. |
| Aspirin | Reduces NF-κB activation, adhesion molecule expression, and leukocyte adhesion.
| NF-κB activity could, if preceding proapoptotic stimuli, lead to reduced endothelial apoptosis. |
| Induction of ferritin production in endothelial cells; this antioxidant and iron-scavenging effect is likely to be antiapoptotic. |
| B-Blockade | Reduces renin production and may thereby decrease proapoptotic angiotensin II. |
| Heparin | Increases cNOS activity, which can have an antiapoptotic effect. |
| Nitrates | May have direct antiapoptotic effects as NO donors. |
| Glucose control in diabetes | Prevents proapoptotic effect of hyperglycemia. |
| ACE inhibitors | Reduce levels of proapoptotic natriuretic factors. |
| Calcium channel blockers | Antiapoptotic effect. |
| Estradiol | Antiapoptotic effect. |
| Vitamins C and E | Antiapoptotic effect. |
| Prostacyclin | Antiapoptotic effect. |
| FGF\textsuperscript{132} | Antiapoptotic effect. |
| VEGF\textsuperscript{133} | Antiapoptotic effect. |
Allograft Vasculopathy

Endothelial apoptosis was demonstrated in human transplant coronary artery disease, and is thought to be caused by cytotoxicity induced by cytolytic T cells. While endothelial changes remain restricted to certain vascular segments in nontransplant atherosclerosis, vessels in transplanted organs are affected diffusely and show vasculitic changes. Similar to in nontransplant atherosclerosis, the endothelium shows evidence of dysfunction and activation. These changes may be an early marker for the severity of allograft vasculopathy and risk of graft failure.

Calcium channel blockers, angiotensin-converting enzyme (ACE) inhibitors, and statins have demonstrated some effect in slowing the progression of allograft vasculopathy, all of them possibly through their antiapoptotic effect on the endothelium (Table 4). Cyclosporin A has shown similar results, apart from its beneficial immunosuppressive effect, it prevents mitochondrial membrane dysfunction and cytochrome c release, and reduces endothelial apoptosis induced by oxidized LDL, TNF, and angiotensin II in vitro (in a model of nontransplant atherosclerosis, it reduced the formation of plaques in vivo).

Hypertension

Hypertension is characterized by capillary rarefaction, a reduction of the number of capillaries per volume of tissue. This rarefaction can be found in young hypertensives and may have a hereditary component. In the established Goldblatt model of hypertension, microvascular rarefaction occurs through apoptosis of the endothelium. Capillary rarefaction was less pronounced in female rats, which could be related to the antiapoptotic effects of estradiol.

Hypertension itself may have a proapoptotic effect on some cell types in vitro and causes endothelial dysfunction accompanied by increased superoxide production; the latter findings are consistent with endothelial apoptosis. Elevated angiotensin II and natriuretic peptides may contribute to endothelial apoptosis. Many treatments used in hypertension also have a direct or indirect antiapoptotic effect (Table 4).

Congestive Heart Failure

In an animal model of aortic insufficiency, increased numbers of circulating endothelial cells were found 28 days after the induction of the hemodynamic abnormality, and this rise was prevented by administration of an ACE inhibitor. In a model of monocrotaline-induced right ventricular failure in rats, the number of apoptotic endothelial cells in skeletal muscle showed a progressive and statistically significant increase over a period of 30 days. These changes were accompanied by an increase in TNF and atrial natriuretic peptide, and the increase in atrial natriuretic peptide showed a significant correlation with the number of apoptotic endothelial and myocyte nuclei. Of note, apoptosis of endothelial cells preceded myocyte apoptosis in this model, which led the authors to conclude that ischemia due to damage or decrease in the number of capillaries may have contributed to the observed myofiber loss. If present in the myocardium, such a process could contribute to the observed apoptosis of cardiomyocytes. Monocrotaline itself causes endothelial apoptosis in vitro, but it appears unlikely that it contributes to such an effect in vivo several weeks after the administration of a single dose.

TNF, natriuretic peptides, angiotensin II, and a reduction of shear stress may contribute to endothelial apoptosis in human congestive heart failure (CHF). Some therapies utilized in CHF could derive part of their benefit from a reduction of endothelial exposure to proapoptotic factors (Table 4). Digoxin may have a “stabilizing” effect on endothelial cells. Carvedilol reduces oxidation of LDL by endothelial cells and prevents endothelial dysfunction in vivo under conditions that are proapoptotic for the endothelium. This suggests that its beneficial effect may, in part, be related to a prevention of endothelial apoptosis. Interestingly, in a recent study in patients with ischemic left ventricular dysfunction, carvedilol showed the greatest benefit in the subgroup of patients with elevated proapoptotic natriuretic peptides in combination with lower norepinephrine levels, suggesting that a possibly present antiapoptotic effect may be of greater importance than its known sympatholytic action.

Sepsis and Associated Syndromes

For the purpose of this discussion, sepsis and associated syndromes includes systemic inflammatory response syndrome, ARDS, and multiple organ dysfunction syndrome.

Endotoxin has been shown to lead to endothelial injury by causing detachment of endothelial cells from the basal membrane, resulting in an increase in the number of circulating endothelial cells in vivo. LPS, TNF and cecal ligation and puncture have been shown to cause endothelial apoptosis in vivo, in association with syndromes that were consistent with sepsis and acute lung injury.

Age, extracellular adenosine triphosphate, angiotensin II, infectious agents and their cellular compo-
nents, hyperglycemia, osmolar stress, ischemia/reperfusion, IL-1, intracellular acidosis, natriuretic peptides, neutrophil proteases, transforming growth factor β1, hypoalbuminemia, decreased antioxidant levels, hypovolemia with an associated hypodynamic state leading to a reduction of shear stress, and possibly other factors may contribute to endothelial apoptosis during the inflammatory response to tissue injury or infection (Table 2). The body’s own anti-inflammatory responses—in the form of soluble TNF receptors, IL-1 Ra, and interleukin 10 release—as well as the development of a hyperdynamic state and the expression of inducible nitric oxide synthase (NOS) in endothelial cells, may help to limit exposure to proapoptotic factors and provide some antiapoptotic stimuli for the endothelium.

Endothelial activation is an integral component of the inflammatory response to tissue injury and infection. It leads to the release of adhesion molecules and their levels correlate with presence of infection, disease severity, and outcome. Endothelial IL-1 and adhesion molecule release, leukocyte activation, and the presence of many of the above-mentioned proapoptotic factors could lead to a dissemination of endothelial activation, apoptosis, and injury, potentially transforming an initially local reaction into a systemic event. Disseminated intravascular coagulation, diffuse microvascular injury and obstruction, increased vascular permeability, perfusion failure, and organ dysfunction in sepsis and the associated syndromes may be related in part to widespread endothelial apoptosis.

Very few therapies utilized in clinical practice are capable of reducing endothelial apoptosis in sepsis, which might partially explain the absence of any significant impact on the outcome of this syndrome despite many years of trials testing anti-inflammatory therapies. Most of the available “antiapoptotic” therapies show a benefit, but remain experimental and are not widely accepted.

Supranormal oxygen delivery is a controversial form of treatment, based on an equally controversial assumption of abnormal oxygen consumption in sepsis. It has shown some benefit when applied prophylactically, before the onset of septic shock or organ dysfunction. Its beneficial effect could be related to an antiapoptotic effect of increased shear stress and to the prevention of adhesion of leukocytes to the endothelium. Both mechanisms may not work if applied too late in the course of disease, when diffuse endothelial injury has already occurred.

The administration of an ACE inhibitor as a low-dose continuous infusion over 5 days to patients with sepsis led to a reduction of soluble markers of endothelial activation and injury, improved oxygenation, reduced neutrophil counts and lactate levels, and decreased incidence of septic shock, and showed a trend toward reduced mortality in a relatively small number of patients. These benefits may be related to the documented reduction of the proapoptotic agent, angiotensin II.

Prostacyclin is an endothelial-derived antiapoptotic agent, the normal functions of which include vasodilatation and platelet inhibition. Its secretion is reduced in endothelial apoptosis. When administered by inhalation, it has effects similar to those of inhaled NO: lowering pulmonary artery pressures and improving oxygenation, but leading additionally to enhanced splanchnic perfusion. A recent multicenter trial evaluated the use of ibuprofen in patients with sepsis. This compound led to a reduction in prostacyclin production, and showed no significant effects on outcome. The beneficial effect of cyclooxygenase inhibition may have been outweighed by the negative effects of inhibited prostacyclin production.

Several other therapies deserve to be mentioned in this context. (1) Antia apoptotic antioxidants have shown some success in ameliorating the inflammatory processes in sepsis. (2) An experimental model demonstrated an impressive success of the antiapoptotic fibroblast growth factor (FGF) in reducing mortality associated with lethal endotoxemia. FGF has been given to humans for other indications, and has not demonstrated prohibitive side effects. (3) Reduced levels of the antiapoptotic VEGF have been found in association with severe complications like sepsis or ARDS in patients with burns and trauma. Reduced secretion of this endothelial survival factor may have contributed to the severity of disease, and its administration in such circumstances could perhaps lead to enhanced endothelial stability and a reduction in tissue injury. Minor side effects have been encountered during VEGF use in human atherosclerosis.

Available data suggests that benefits of any such antiapoptotic therapy appear less likely if it is applied late in the course of disease or in the form of a single agent. The large number of proapoptotic factors present in sepsis and the associated syndromes will probably require a combined blockade of several apoptotic pathways, as well as the use of antiapoptotic endothelial growth factors in order to show a benefit in a process as rapid and currently irreversible as fulminant septic shock.

Primary Pulmonary Hypertension

A number of findings associated with primary pulmonary hypertension (PPH) are consistent with a role of endothelial apoptosis in its pathogenesis. Endothelial apoptosis has been demonstrated in
cells. Endothelial constitutive NOS (cNOS) is a proliferative stimulus for vascular smooth muscle. Increased ACE immuno-reactivity is significantly increased in the endothelium and subendothelial regions of patients with PPH; increased angiotensin II production may cause increased endothelial apoptosis and provide a proliferative stimulus for vascular smooth muscle cells. (2) Endothelial constitutive NOS (cNOS) is reduced in human PPH and its experimental reduction causes pulmonary hypertension in response to hypoxia in mice. A diminished baseline release of NO by the endothelium leads to the reduction of an important endothelial antiapoptotic stimulus and allows enhanced smooth muscle cell growth. (3) Antiphospholipid and antinuclear antibodies are frequently found in association with PPH; the lupus anticoagulant and anti-double-stranded DNA antibody are proapoptotic for the endothelium. The lung tissue metabolizes estrogens and participates in the production of estradiol. A defect in this production could deprive the endothelium of an antiapoptotic substance. Furthermore, some circumstances may lead to an increased production of the proapoptotic 2-methoxyestradiol, but whether or not this occurs in the lung is presently unknown. (5) Increased release of the proapoptotic atrial natriuretic peptide and (6) the presence of hypoxia could exacerbate apoptosis of the endothelium in PPH.

Treatments for PPH include prostacyclin and calcium channel blockers. ACE inhibitors have shown some success in preventing vascular remodeling in an animal model of PPH. All have an antiapoptotic effect on the endothelium (Table 4). Oxygen and diuretics may reduce some proapoptotic influences (hypoxemia and natriuretic factors). Anticoagulation reduces thrombin generation and platelet activation, and could thereby have an indirect effect on endothelial apoptosis (Fig 1).

Other Diseases

Endothelial apoptosis has also been demonstrated in systemic sclerosis, diabetes, and thrombotic thrombocytopenic purpura; it may play a role in systemic lupus erythematosus, the antiphospholipid antibody syndrome, development of metastasis, and treatment of cancer. Although there is no direct evidence of endothelial apoptosis in preeclampsia, the presence of numerous other findings suggest a possible role. A more detailed discussion of these diseases is beyond the scope of this review.

CONCLUSION AND FUTURE PROSPECTS

A number of important questions remain to be answered. Is endothelial apoptosis associated with, contributing to, or causing disease? What is its relation to endothelial activation and injury? Can soluble markers of endothelial activation and/or injury serve as markers for the presence of endothelial apoptosis? Are circulating endothelial cells apoptotic because they lost contact with antiapoptotic components of the vascular wall, or did they detach because they became apoptotic? Do bone marrow-derived endothelial precursors play a role in the recovery after endothelial injury, and could the administration of endothelial growth factors provide a benefit? Can prolonged administration of endothelial antiapoptotic agents influence the growth of malignancies?

Although it cannot be considered proven at this time, the available data point to endothelial apoptosis as the final common pathway through which various insults could contribute to the development of certain diseases. Such a possibility is an opportunity too good not to be taken seriously. The endothelium is ideally positioned and widely exposed to circulating blood. Endothelial cells are easy to collect, culture, and study. Endothelial precursors may be able to provide us with information about individual predisposition to the development of some diseases. Already known pro- and antiapoptotic agents with good side-effect profiles could be evaluated, alone or in combination, as therapeutic agents in a variety of conditions. New pro- and antiapoptotic stimuli are likely to be found in the future and could be tested for their relevance in different diseases. More work in elucidating the mechanisms of apoptosis in endothelial cells needs to be done before we can embark on the path of drug therapy manipulating these processes in ill human beings, but we could be able to do just that by the end of the first decade of the new millennium.

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