**Abstract**—Traditional thinking accorded a major role to deranged cell proliferation as a determinant of the abnormal cellularity of atheroma. However, studies conducted in several laboratories have documented the occurrence of disordered apoptosis during atherogenesis, leading to the death of lipid-rich foam cells (promoting lipid-core formation) and depletion of vascular smooth muscle cells (fostering fragility of the fibrous cap). A complex interplay of environmental factors and endogenous proteins regulates apoptosis and contributes to the struggle between cell death and procreation in atherosclerosis. In addition to a variety of growth factors, chemically modified lipids, reactive oxygen species, proinflammatory cytokines, and Fas ligand produced by activated immune cells may influence cell viability through a diversity of pathways, including the caspase cascade, the Bcl-2 protein family, and the oncogene/antioncogene system. A clarification of the molecular mechanisms responsible for vascular cell death may aid in the development of novel therapeutic strategies to treat atherosclerosis and its complications, including the acute coronary syndromes.

**Key Words:** atherosclerosis  ■ apoptosis  ■ arteries  ■ caspasess  ■ cytokines

**Progression of Atheroma**

**A Struggle Between Death and Procreation**

Yong-Jian Geng, Peter Libby

Traditional research in the cell biology of atherosclerosis focused on smooth muscle replication. Although <1% of smooth muscle cells (SMCs) in advanced atheroma undergo cell division at a given point in time, a simple calculation will reveal that even this seemingly low rate of replication of SMCs would lead to enormous lesion volume over the time course of human atherogenesis, ie, several decades. This *reductio ad absurdum* has stimulated investigators to probe beyond the primitive concept that cell proliferation holds the key to atherogenesis. Indeed, we should now envisage the progression of atherosclerosis as an ongoing struggle between cell division and cell death, between procreation and demise. In 1992, we hypothesized that “the death of lipid-laden macrophages may not be a random event or simply caused by bursting like an over-inflated balloon due to lipid overload. Rather, this process may resemble apoptosis, a form of programmed cell death.”

Indeed, cells may die in various ways (Table 1). Cell death may occur in a disorganized and chaotic energy-independent manner, associated with swelling, in response to lethal injury, a mode known as necrosis or, more properly, oncosis. In contrast, cell death by apoptosis occurs in a coordinated and highly regulated fashion that involves shrinkage, not swelling. Apoptosis requires the expression of certain groups of genes critical for cellular signal transduction and metabolism. This process provides a major mechanism by which tissues remove unwanted, aged, or damaged cells. Because of its ordered sequence of events, investigators often refer to apoptosis as programmed cell death. Different cell types undergoing apoptosis in tissues and cultures share very similar morphological features, including chromatin compaction and margination, nuclear condensation and fragmentation, and cell body shrinkage and blebbing (Table 1). These characteristic morphological alterations in apoptotic cells, which differ from those seen in oncosic cell death, reflect the self-directed catabolism of cytoskeleton and other intracellular molecules. Complex interactions between extracellular microenvironmental factors and intrinsic gene products occur before apoptosis begins. Once activated, this process can progress in the absence of extracellular insults. This suicidal feature of apoptosis applies to embryonic development and morphogenesis as well as adult tissue turnover. Apoptotic cells or bodies usually retain an intact cellular membrane and undergo prompt removal by tissue macrophages or adjacent cells, thus avoiding damage to neighboring tissue and triggering inflammation. Various methodologies can evaluate apoptosis in vivo and in vitro, including analysis of morphology, DNA fragmentation (eg, in situ labeling of DNA fragments or terminal deoxynucleotidyl transferase–mediated dUTP nick end-labeling [TUNEL] and agarose gel electrophoresis), and immunolocalization of active forms of effector proteins. Many of these methods have significant limitations. For example, avoidance of artifacts with the TUNEL method requires special care. However, because no single validated...
marker for apoptosis exists, its assessment ideally uses a combination of molecular and morphological methods.

In 1995, several laboratories, including our own, provided morphological and biochemical evidence of apoptotic cell death in human advanced atherosclerotic plaques. In addition, we colocalized the prototypical caspase, interleukin-1β-converting enzyme, to sites of apoptosis in human atheroma. These early studies demonstrated the occurrence of apoptosis in macrophages and SMCs, 2 cell types that replicate prominently during atherogenesis. We further showed that the Fas/tumor necrosis factor (TNF) caspase death-signaling pathway operates in human atherosclerotic lesions and may participate in vascular cell death during the progression of atherosclerosis. Experimental atheroma in rabbits fed a high-cholesterol diet also demonstrated apoptosis. Since then, numerous investigations have documented vascular cell apoptosis during the development of atherosclerosis and its complications (eg, the acute coronary syndromes). The present review aims to delineate the relationship of cell proliferation with atherogenesis and the molecular mechanisms that regulate apoptotic vascular cell death during the progression or regression of atheroma and the development of the acute coronary syndromes.

**Proliferation Paradox in Atherogenesis**

Formation of the intimal macrophage-rich fatty streak, the precursor of atherosclerotic lesions, appears ubiquitous in humans. However, not all fatty streaks evolve into advanced lesions, ie, fibrous plaques or atheroma. Thus, the mere accumulation of lipid-laden macrophages within the arterial intima does not ineluctably lead to the formation of complicated atherosclerotic plaques. Depending on the balance between proatherogenic and antiatherogenic factors, some fatty streaks progress into atheroma, and others may regress. The progression of fatty streaks to more complex lesions requires not only inflammatory cell infiltration but also the participation of SMCs, a principal source of the extracellular matrix that ultimately often constitutes the majority of the volume of the advanced atheroma.

For decades, much of the research in the cell biology of atherosclerosis centered on smooth muscle replication. Platelet-derived growth factor (PDGF) received much attention as a potential mitogenic stimulus during atheroma formation. Yet, PDGF in vivo appears more important as a chemoattractant for SMCs than as a mitogen. Moreover, the proliferation of SMCs in human atherosclerotic lesions appears indolent, although bursts of SMC replication may indeed occur during atherogenesis. For example, episodes of localized plaque disruption with mural thrombosis or microvascular rupture within a plaque could stimulate a round of SMC proliferation due to thrombin or PDGF stimulation. However, transforming growth factor-β released from platelet granules might mitigate this mitogenesis because of its cytostatic action. Interferon (IFN)-γ produced by T lymphocytes and other cells within plaques can also retard SMC proliferation by a cytostatic effect. Macrophages proliferate within atheroma as well; this proliferation is no doubt due in part to the action of mitogens and comitogens, such as macrophage colony-stimulating factor (CSF) or granulocyte-macrophage CSF. Despite the operation of proliferation and cytostatic stimuli in atheroma, a full understanding of the regulation of cell number requires consideration of cell death, the topic of the balance of the present review.

**Apoptosis in Regulation of Cellularity of Atherosclerotic Arteries**

Atherosclerotic lesions develop in the tunica intima of the arteries, in which accumulation of cellular components, lipids, and extracellular matrix yields a fibrofatty plaque that focally thickens the artery wall and can ultimately narrow the lumen. By counterbalancing proliferation, apoptosis may limit cell buildup in the intimal compartment. Elimination of lipid-laden foam cells may lead to the regression of atherosclerosis.
In contrast, attenuation of apoptosis may increase the tissue cellularity and promote intimal hyperplasia. Recent studies suggest the possibility that an antiapoptotic mechanism may favor foam cell accumulation in atherosclerotic lesions.\(^{15}\) Despite the harsh environment, many lipid-laden foam macrophages reside in the lipid core of the plaque. Expression of antiapoptotic genes may partially explain the apparent resistance of these foam cells to apoptosis. We have recently found that engagement of the class A scavenger receptor responsible for uptake of chemically modified lipoproteins can protect cultured macrophages from apoptosis induced by oxysterols and oxidized LDLs (oxLDLs).\(^{16}\) The mechanism by which this scavenger receptor inhibits apoptosis in macrophages remains uncertain. Some evidence implies that the scavenger receptor–associated resistance of foam cells to apoptosis involves altered signal transduction, inasmuch as expression of this receptor inhibits the apoptosis induced by sodium fluoride, a global activator of GTP-binding proteins.\(^{17}\)

Apoptosis can occur during different stages of the development and evolution of advanced atherosclerotic lesions or atheroma.\(^{18}\) In addition to lipids and connective tissue, the center of the typical atherosclerotic plaque contains many dead cells or cell debris, hence, the term necrotic or lipid core. Apoptosis causes cell loss as atherosclerosis progresses, often yielding a late or mature lesion containing a dense extracellular matrix with a relatively sparse cell population. Analysis of cell death by in situ end-labeling of DNA fragments (TUNEL technique) and by morphological observation indicates that many cells in the atheromatous lesions bear the markers of apoptosis even though necrotic cell death may also occur in the lipid core area of atherosclerotic lesions.\(^6\) Thus, apoptosis may contribute to the formation of 2 major pathological changes characteristic of advanced atherosclerotic plaques, the “necrotic lipid core” and the hypocellular fibrotic lesion.

Many laboratories have reported a high percentage of apoptotic cells in advanced atherosclerotic plaques. Because the tissue volume should decrease when the levels of apoptosis exceed that of proliferation, this finding poses a paradox. However, the presence of markers of apoptosis may not always be linked with reduction of tissue volume. This situation could occur if the markers used to assess apoptosis did not reflect an increase in the instantaneous rates of cell death. Indeed, accumulation of apoptotic cells in the plaque suggests that the system for scavenging the dead cells operates poorly in atherosclerosis. Some apoptotic cells or bodies in the plaque may remain “mummified” rather than undergo removal by phagocytosis.\(^6\) The following mechanisms might lead to retention of apoptotic cells or bodies in atherosclerotic lesions: (1) intracellular accumulation of lipids may attenuate the ability of macrophages and SMCs to engulf and digest apoptotic cells; (2) phagocytosis may decrease because of increased apoptosis of macrophages in the lesions; (3) cross-linking of macromolecules, such as proteins, nucleic acids, and carbohydrates, may stabilize apoptotic cells in the tissues;\(^{19}\) and (4) increased amounts of chemically modified lipoproteins and other ligands for scavenger receptors may competitively block the receptor-mediated phagocytosis of apoptotic cells by macrophages. In addition, recent studies have provided evidence that increased calcification and fibrosis may occur in atherosclerotic lesions that contain apoptotic cells or bodies.

### Apoptosis and Stability of Atherosclerotic Plaques

Plaque disruption frequently causes the thrombotic complications of atherosclerosis that underlie unstable angina pectoris, myocardial infarction, and other acute syndromes.\(^{20–22}\) Vascular SMCs can synthesize a variety of extracellular matrix macromolecules that stabilize the plaques. Inflammatory cell accumulation characterizes disrupted plaques.\(^{23,24}\) Production of apoptosis-promoting mediators by activated inflammatory or immune cells may lead to the death of vascular SMCs, which may in turn weaken and destabilize the plaques. Indeed, in 1995, we dubbed SMCs “the guardians of the integrity of the plaque’s fibrous cap,” and we originally proposed a role for SMC dropout that was due to apoptosis in the thinning of the fibrous cap.\(^{25}\)

Either apoptotic endothelial cells (ECs)\(^{26}\) or SMCs\(^{27}\) can promote coagulation. Rapid exposure of membrane phosphatidylserine and loss of the anticoagulant membrane components in apoptotic ECs can occur, which may promote a procoagulant environment. Apoptotic vascular SMCs acquire a thrombin-generating capacity due to exposure of phosphatidylserine. Thus, apoptotic cells within atherosclerotic plaques may allow local thrombin activation, thereby contributing to disease progression.\(^{27}\) Increased tissue factor on the surface of apoptotic cells may also contribute to the procoagulant activities.\(^{28}\) These findings illustrate how apoptotic cells or bodies, if not promptly removed, may become thrombogenic or proinflammatory.

Although apoptosis in atherosclerotic arteries usually occurs more frequently in intimal cells, under certain conditions this type of cell death may attack medial SMCs. In association with \(p53\) expression, the medial SMCs of arteries with atherosclerotic plaques exhibit considerable levels of apoptosis.\(^{29}\) Macrophages and T lymphocytes infiltrate the arterial wall in atherosclerotic aortic aneurysms and produce death-promoting proteins (perforin, Fas, and FasL).\(^{30}\) Thus, accelerated apoptosis of medial SMCs represents a cellular mechanism responsible for the development of aortic aneurysms.

### Proapoptotic Factors Operating During Atherogenesis

Vascular SMCs and ECs encounter a broad variety of biologically active environmental factors, such as mechanical force,\(^{31–35}\) oxidative stress,\(^{36}\) radiation,\(^{37–41}\) reactive oxygen or nitrogen species,\(^{42–45}\) lipids (cholesterol and its oxides),\(^{16,41,46–48}\) viral\(^{49,51}\) and bacterial products,\(^{52}\) and inflammatory cytokines.\(^{7,53,54}\) Many of these extracellular environmental and immunologic factors can induce apoptosis in vascular cells (Table 2).

Atherosclerotic lesions contain chemically modified lipoproteins, in particular, oxLDL, a putative instigator of atherosclerosis capable of inducing vascular cell apoptosis.\(^{48}\) Intrinsically, cholesterol and its esters have little proapoptotic action, but they become cytotoxic after oxidation. Some oxysterols\(^{47}\) in oxLDL particles may contribute to the proapo-
TABLE 2. Vascular Cell Apoptosis: Extracellular Triggers and Inhibitors

<table>
<thead>
<tr>
<th>Triggers</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>OxLDL</td>
<td>41, 58, 111–113</td>
</tr>
<tr>
<td>Oxysterols</td>
<td>47, 56, 114</td>
</tr>
<tr>
<td>Reactive oxygen species</td>
<td>36, 44, 76</td>
</tr>
<tr>
<td>Reactive nitrogen species (NO at high levels)</td>
<td>44</td>
</tr>
<tr>
<td>Radiations</td>
<td>38, 40, 84, 115, 116</td>
</tr>
<tr>
<td>Cytokines</td>
<td>7, 8, 45, 117</td>
</tr>
<tr>
<td>Viral and bacterial products</td>
<td>104, 105, 118</td>
</tr>
<tr>
<td>Inhibitors</td>
<td></td>
</tr>
<tr>
<td>Shear stress</td>
<td>31–35</td>
</tr>
<tr>
<td>NO at low levels</td>
<td>66, 119</td>
</tr>
<tr>
<td>PDGF</td>
<td>10, 120</td>
</tr>
<tr>
<td>VEGF</td>
<td>75, 121</td>
</tr>
<tr>
<td>bFGF</td>
<td>81, 122, 123</td>
</tr>
<tr>
<td>iFGF-I</td>
<td>124</td>
</tr>
<tr>
<td>Cowpox virus CrmA</td>
<td>125</td>
</tr>
<tr>
<td>Baculovirus protein p35</td>
<td>126</td>
</tr>
<tr>
<td>IAP protein family</td>
<td>127, 128</td>
</tr>
<tr>
<td>Vitamins C and E</td>
<td>52, 77, 129</td>
</tr>
<tr>
<td>Antioxidant enzymes (eg, SOD and catalase)</td>
<td>36, 43, 86, 87</td>
</tr>
</tbody>
</table>

Apoptotic action of oxLDL. High levels of cholesterol oxides in the cell membrane can trigger foam cell death by apoptosis.55 Oxysterol-mediated apoptosis may foster the formation of a necrotic lipid core, a region with relatively high concentrations of oxysterols.56 Mechanisms by which oxLDLs and oxysterols may trigger apoptosis remain incompletely understood. Recent studies suggest that activation of caspases46 and sphingomyelinase,57 attenuation of Bcl-2,46 and suppression of the nuclear transcription factor-κB58 may contribute to oxLDL-induced apoptosis.

Beyond lipid accumulation, atherogenesis involves innate and acquired immunity. Two major cellular participants in the immune response, macrophages and T lymphocytes, undergo activation in atheroma.59 The antigenic substances may include oxidized lipoproteins and some stress proteins, such as heat shock protein-60. The effector of acquired immunity par excellence, the CD8-positive T lymphocyte, can kill cells by apoptosis via the production of perforin, granzyme, and Fas ligand (FasL).53 However, mediators of innate immunity may also contribute to this process. For example, exposure of human SMCs to a “cocktail” of cytokines found in human atheroma, including interleukin (IL)-1β, TNF-α, and IFN-γ, can induce apoptosis.7 In addition, “priming” of SMCs with cytokines implicated in innate immunity can sensitize them to killing by Fas engagement.8 Thus, the effector limbs of innate and acquired immunity may cooperate in causing the demise of cells within atheroma (Figure 1).

The cytokines produced by activated macrophages and T cells can synergistically induce activation of the sphingomyelin-ceramide signaling pathway60,61 and the L-arginine–NO pathway,7,45,62–65 both of which participate in the regulation of proliferation and apoptosis in vascular SMCs. Hence, proinflammatory cytokines have overlapping biological effects on vascular cell proliferation and death. When combined, they can exhibit synergy in promoting new gene transcription, e.g., the expression of inducible NO synthase.63 This high-capacity isoform of the NO-synthesizing enzyme produces large amounts of NO, a gaseous free radical with multiple biological effects. At high concentrations, NO can attack several important iron-containing enzymes involved in DNA synthesis and mitochondrial respiration, leading to apoptosis of the target cells.64,65 NO can react with superoxide anion (O₂⁻) to form the cytotoxic species peroxynitrite (ONOO⁻), augmenting the proapoptotic action of NO.44 The NO-sensitive iron-containing enzymes in mitochondria include complex I and complex II in the respiratory chain. Because mitochondrial dysfunction critically influences the development of apoptosis (see below), the nitrosylation of iron enzymes may not only inhibit ATP synthesis but also trigger an apoptotic cascade. The proapoptotic effect of NO highly depends on its concentrations and interactions with other reactive species. Low physiological amounts of NO show no harm and, under certain circumstances, demonstrate protective effects against vascular cell apoptosis.32,66,67

In addition to TNF itself, other members of the TNF superfamily can play important roles in the regulation of apoptosis (Figure 1). The fascinating death-signaling protein, Fas/apo-1/CD95, a cell surface–borne protein, belongs to the TNF receptor (TNFR) superfamily. Ligation of Fas by FasL or agonistic anti-Fas antibodies can induce apoptosis in activated T lymphocytes.58 Analysis of markers for apoptosis indicates that many T lymphocytes may undergo apoptosis after they infiltrate atherosclerotic lesions.69 Several lines of evidence implicate the FasL/Fas-caspase death pathway in the induction of apoptosis in atherosclerotic plaques.8,70,71 The inflammatory cells and vascular cells, such as SMCs and ECs, express Fas in atheroma. Although vascular SMCs express Fas, the cells do not undergo apoptosis under usual conditions, even in the presence of FasL or agonistic antibodies.8 However, after pretreatment of SMCs with the cytokines TNF-α, IL-1, and IFN-γ, Fas ligation significantly reduces viability and increases internucleosomal DNA fragmentation. Moreover, priming with these cytokines may enhance the expression of Fas and provide additional death-promoting signals for initiation or acceleration of the apoptotic cascade in plaque cells. In turn, the T lymphocytes (particularly the CD8⁺ cells present in plaque, although they are less abundant than CD4⁺ cells) produce effector molecules (such as FasL, granzymes, and perforin) that can kill other plaque cells by apoptosis.

Interestingly, some vascular cells can also express the ligand for Fas and may thus themselves become triggers for apoptosis.72 The increased expression of FasL in ECs and SMCs may trigger “preemptively” augmented apoptosis of cytotoxic T cells and macrophages, thereby prolonging survival of the vascular cells. This mechanism might explain the recent study reporting that overexpression of FasL in arteries of rabbits with hyperlipidemia promotes the development of atherosclerosis, as a result of intimal SMC accumulation.73 Schaub et al.74 found that signals initiated by regulated Fas-associated death domain protein overexpression in rat
vascular SMCs induce the expression of monocyte-chemoattractant protein-1 and IL-8 and cause massive immigration of macrophages in vivo. Chemokines and other proinflammatory genes in human vascular SMCs elicit the expression of a range of Fas-induced apoptosis, in part through a process that requires IL-1 activation. Thus, apoptotic vascular cells may induce proinflammatory responses and contribute to the pathogenesis of vascular disease.

**Antiapoptotic Factors in Atherosclerosis**

The regulation of apoptosis involves negative as well as positive signaling. Inhibitors of apoptosis relevant to atherosclerosis include shear stress, growth factors, antioxidant drugs or enzymes (eg, superoxide dismutase [SOD]), and pharmacological doses of certain antioxidant vitamins (Table 2). By counteracting the apoptotic effects of cytotoxic factors, these inhibitors may maintain the integrity of the endothelium and increase the cellularity of the vessel wall. Mechanical force generated by blood flow can affect endothelial functions and the structure of the vessel wall. Laminar shear stress found in normal arteries can inhibit apoptosis of human ECs. By contrast, flow disturbances or lack of normal shear stress in regions characteristic of sites of predilection to atheroma may induce the apoptosis of ECs. The inhibitory effect of shear stress on the apoptosis of ECs may be related in part to the increased levels of NO produced by the low capacity endothelial isof orm of NO synthase, a shear stress–activated gene. In contrast to cytokine-induced NO synthase, constitutive endothelial NO synthase generates relatively small amounts of NO that may inhibit caspase activities and protect ECs from apoptosis. Analysis of human atherosclerotic lesions shows more apoptosis in the downstream parts of plaques, where disturbed flow occurs. Laminar shear stress can also induce the expression of apoptosis-regulating genes in human umbilical vein ECs. Bartling et al applied high levels of laminar shear stress (15 and 30 dyne/cm²) to human ECs. They observed a decreased susceptibility of human umbilical vein ECs to apoptosis, whereas low shear stress (1 dyne/cm²) had no effect. These diminished signs of apoptosis occurred in tandem with decreased mRNA expression of apoptosis-inducing Fas. Furthermore, high levels of shear stress induced mRNA and protein expression of the antiapoptotic soluble Fas isof orm FasExo6Del and antiapoptotic Bcl-x(L). Shear stress also induces Bcl-x(L) and Bak mRNA, counteracted by inhibition of endothelial NO synthase.

Much of the early work on growth factors such as PDGF and macrophage CSF focused on their proproliferative actions. However, in the context of contemporary apoptosis research, it has become clear that these factors may act as “survival” factors rather than mediators of mitosis. For example, macrophages deprived of macrophage CSF will undergo apoptosis induced by denatured aggregated LDL. Likewise, PDGF protects SMCs from apoptosis. Vascular EC growth factor can inhibit the apoptosis of bone marrow cells induced by radiation. Basic fibroblast growth factor (FGF) can inhibit the apoptosis of ECs induced by TNF-α. Attenuation of basic FGF expression by an antisense strategy

![Figure 1. Impact of cytokine-induced apoptosis of vascular SMCs on plaque stability. The cells of innate and adaptive immunity, the T lymphocytes and macrophages, secrete various mediators that can promote SMC activation and apoptosis. These mediators include IFN-γ, Fasl, TNF-α, IL-1, and reactive oxygen species, including NO (NO), hydrogen peroxide (H₂O₂), superoxide anion (O₂⁻), and peroxynitrite (ONOO⁻). These various proinflammatory mediators can activate caspases and elicit mitochondrial dysfunction in SMCs. SMC apoptosis may contribute to the decreased numbers of SMCs noted in vulnerable atherosclerotic plaques. Ultimately, such SMC “dropout” leads to reduced synthesis of extracellular matrix proteins such as interstitial collagen. This will ultimately weaken the atherosclerotic plaque, rendering it susceptible to rupture and, hence, thrombosis. In addition, fragments of apoptotic cells may increase thrombogenicity. In particular, SMCs can produce tissue factor, a potent procoagulant that may be released, becoming biologically active, when SMCs undergo apoptosis.

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**Atherogenic Antigens**

<table>
<thead>
<tr>
<th>Proinflammatory MEDIATORS</th>
<th>T Lymphocytes</th>
<th>Macrophages</th>
<th>Free Radicals</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFNγ</td>
<td>Fasl</td>
<td>TNFα</td>
<td>-NO, H₂O₂, O₂⁻, ONOO⁻</td>
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</table>

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**Caspase Activation/Mitochondrial Dysfunction**

- Impaired Cell-to-Cell Connection
- Weakening the Arterial Wall
- Unstable Plaques
can trigger the apoptosis of vascular SMCs. This growth factor also prevents apoptosis induced by serum starvation. Protein kinase C may mediate this protective effect of FGF.

Because oxidative stress can injure vascular cells and promote apoptosis as stated above, antioxidants might mitigate this process. For instance, during 7-ketocholesterol-induced apoptosis of U937 cells, the content of cellular antioxidants falls rapidly. The administration of 2 potent antioxidants, the aminothiolute glutathione and N-acetylcysteine, can protect the monocyctic cells from 7-ketocholesterol–induced apoptotic cell death. In addition, some enzymes with antioxidant properties, such as SOD, can exert protective effects against endothelial apoptosis induced by oxidized lipoproteins. Another antioxidant enzyme, catalase, can prevent vascular SMC apoptosis triggered by hydrogen peroxide. These authors, however, found that superoxide anion stimulates proliferation rather than induces apoptosis in SMCs and that SOD can block the mitogenic effect of this free radical. Thus, different radical species may exert different effects on different cell types.

The interplay of these mitogenic, death-inducing, and survival-promoting factors probably occurs over many years in the history of a given human atherosclerotic lesion. Death of macrophages may lead to accumulation of extracellular lipid and cellular detritus in the necrotic core of the lipid-rich atheroma. Death and disappearance of macrophages and SMCs may account for their virtual absence in certain highly fibrotic and calcified late atherosclerotic lesions. The variety of atherosclerotic lesions, even those residing as neighbors within a given human coronary artery, may result from the outcome of this sustained “tug of war” between cellular death and procreation.

**Cellular Metabolic Pathways for Initiation and Execution of Apoptosis**

Many surface receptors and intracellular proteins or enzyme systems participate in the regulation of apoptosis. Table 3 lists some of these receptors and cellular proteins potentially important for the regulation of vascular cell apoptosis during atherogenesis.

**Caspases**

The cytoplasm contains members of a family of aspartate-specific cysteiny1 proteases known as caspases. All members of the caspase family (Table 3), which now number 14, show a similar substrate cleavage at an aspartyl residue and require processing of a zymogen to attain activity. The cytoplasm contains members of a family of aspartate-specific cysteiny1 proteases known as caspases.

### Table 3. Cellular Functional Protein Families With the Potential of Regulating Vascular Cell Apoptosis During Atherogenesis

<table>
<thead>
<tr>
<th>Death Receptors</th>
<th>Other Names</th>
<th>Ligand</th>
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<tbody>
<tr>
<td>DR3</td>
<td>Apo3, Wst1, TRAMP, LARD</td>
<td>TWEAK</td>
</tr>
<tr>
<td>DR4</td>
<td>Apo2, Trail-R1</td>
<td>Trail</td>
</tr>
<tr>
<td>DR5</td>
<td>Trail-R2, TRICK2</td>
<td>Trail</td>
</tr>
<tr>
<td>Fas</td>
<td>CD95, Apo1</td>
<td>Fasl (CD95L)</td>
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<tr>
<td>TNFR1</td>
<td></td>
<td>TNF</td>
</tr>
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**Caspases**

<table>
<thead>
<tr>
<th>Caspase</th>
<th>Other Names</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caspase-1</td>
<td>ICE</td>
<td>Pro-inflammatory</td>
</tr>
<tr>
<td>Caspase-2</td>
<td>ICH-1</td>
<td>Pro-apoptotic</td>
</tr>
<tr>
<td>Caspase-3</td>
<td>CPP32, YAMA, Apopain</td>
<td>Pro-apoptotic</td>
</tr>
<tr>
<td>Caspase-4</td>
<td>ICERII, TX, ICH-2</td>
<td>Pro-apoptotic</td>
</tr>
<tr>
<td>Caspase-5</td>
<td>ICERIII, TY</td>
<td>Pro-inflammatory</td>
</tr>
<tr>
<td>Caspase-6</td>
<td>Mch2</td>
<td>Pro-apoptotic</td>
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<td>Caspase-7</td>
<td>Mch3, ICE-LAP3, CMH-1</td>
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<td>ICE-Lap6, Mch6, APAF-3</td>
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<td>Mch4</td>
<td>Pro-apoptotic</td>
</tr>
<tr>
<td>Caspase-11</td>
<td></td>
<td>Pro-inflammatory</td>
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<td>Caspase-12</td>
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<td>Pro-inflammatory</td>
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<td>Caspase-13</td>
<td></td>
<td>Pro-inflammatory</td>
</tr>
<tr>
<td>Caspase-14</td>
<td>MICE</td>
<td>Pro-inflammatory/Pro-apoptotic</td>
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</tbody>
</table>

Mitochondrial Bcl2 family. Pro-apoptotic members: Bad, Bak, Bax, Bcl-Xs, Bid, Bim, Bod, Bod, Bok, DIP5, Hrk, Mtd, Nip3; anti-apoptotic members: Bcl-2, Bcl-w, Bfl-1, BHRF-1, Boo, Mcl-1.

and (3) those that act as effectors of apoptosis (caspases 3, 6, and 7).

The precise mechanism by which the activation of caspases kills cells remains unclear. Available evidence largely from the Fas/TNFFR death-signaling model and the mitochondrial death model indicates 2 distinct but related pathways (Figure 2). In pathway 1, Fas-FasL interaction trimerizes the receptor, recruits cofactors (such as Fas-associated protein with death domain and receptor-interacting protein), and facilitates the activation of procaspases 8 and 2 by autoproteolysis and oligomerization. Subsequently, proteolytic activation of the effector caspases 3, 6, and 7 results in the cleavage of a variety of substrates within cells. In pathway 2, various cytotoxic substances may attack mitochondria, translocate proapoptotic members of the Bcl-2 family to mitochondria, alter voltage-dependent channels in the mitochondrial membrane, and release cytochrome c. Bridged by the adaptor molecule Apaf-1 (apoptotic protease activation factor-1), cytochrome c and caspase 9 form a complex, which, in turn, activates the effector caspases and triggers the caspase cascade. Interestingly, in some cells, caspase 8 activation can also activate the mitochondrial death pathway by cleavage of Bid, a proapoptotic member of the Bcl-2 family. Although the 2 pathways of apoptosis can operate in the same cells, studies suggest that certain cell types may use predominantly one or the other mechanism for apoptosis. In general, pathway 1 is more active than pathway 2 in receptor-mediated cell death.
Conversely, pathway 2 is more prominent in death induced by cytotoxic agents.

Caspases play a critical role in apoptosis of vascular cells in atherosclerotic plaques and in cultured vascular cells. However, the biological functions of caspases in atherosclerotic lesions remain uncertain. Caspases can cleave many intracellular proteins. These substrates include regulators of nuclease activity, which play an important role in the final phase of the caspase cascade. Normally, inhibitors of the nucleases protect cells from nucleolysis. Caspase 3 can cleave Flap (DNA fragmentation factor-45), an inhibitor of the nuclease CAD (caspase-activated deoxribonuclease). In addition, activated caspase 3 also cleaves gelsolin, an actin-associated protein that may regulate the DNase I activity. In cultured SMCs, cytokine-induced activation of caspase 3 triggers an apoptosis cascade in which fragmented gelsolin depolymerizes actin, promotes the collapse of cytoskeleton, and induces the activation of nucleases that degrade DNA. Caspase 8 or Flip, the Fas-associated death domain–like IL-1β-converting enzyme inhibitory protein (a naturally occurring caspase-inhibitory protein that lacks the critical cysteine domain necessary for catalytic activity) negatively regulates Fas-induced apoptosis. During differentiation from monocytes, macrophages can express high levels of Flip, a potent inhibitor of the caspase 8 activation. During SMC apoptosis, the expression of this protein expression declines.

Bcl-2 and Mitochondria

The Bcl-2 protein family (Table 3) represents another group of cellular proteins that regulate apoptosis in mammalian cells. The mitochondrial membrane contains abundant Bcl-2. The Bcl-2 family has >15 members. Members of this family fall into 2 subgroups that are based on differences in the regulation of apoptosis. Each group contains 5 function- and structure-related proteins. The first group includes the antiapoptotic proteins, such as Bcl-2, Bcl-X, Mcl-1, Bcl-w, and A1; members of the second group promote apoptosis and include Bax, Bak, Bad, Bik, Hrk, Bid, and Bcl-xs. The mechanism by which Bcl-2 inhibits apoptosis remains incompletely clarified. Bcl-2 may exert an antioxidant effect on stressed cells, prevent the release of mitochondrial cytochrome, and bind to and inactivate proapoptotic molecules such as Bax and Bak.

Rapidly accumulating evidence indicates the pivotal role of mitochondria in apoptosis. Mitochondria can regulate apoptosis in at least 3 ways: (1) inhibition of mitochondrial respiration due to the production of large amounts of NO in cytokine-stimulated vascular cells, (2) production of cytoxic reactive oxygen species or change of cellular reduction-oxidation (redox) potential, and (3) release of proapoptotic molecules including cytochrome c and apoptosis-inducing factor. Many Bcl-2 family proteins reside in the mitochondrial outer membrane, where they form a pore structure that surprisingly resembles certain bacterial toxins that enhance proton extrusion. The release of cytochrome c depends on the mitochondrial inner transmembrane potential. When apoptotic stimuli attenuate Bcl-2 function, a rapid drop or collapse of transmembrane potential may occur, causing rapid release of cytochrome c and other proapoptotic proteins. Cytosolic cytochrome c then forms, together with Apaf-1 and caspase 9, an “apoptosome,” which orchestrates the activation of other caspases and distal effectors of apoptosis.

Several groups have recently reported the expression of some members of the Bcl-2 protein family in atherosclerosis. All studies pointed to an imbalance between proapoptotic and antiapoptotic proteins in the Bcl-2 family. For instance, plaque cells appear to express higher levels of Bax,
a proapoptotic protein of the Bcl-2 family, than inhibitors such as Bcl-2, indicating an increased activity of proapoptotic proteins in the lesions. Regulation of Bcl-2 protein function may be important in vascular cell death. In human EC apoptosis induced by TNF-α, Bcl-2 specifically undergoes degradation by a process sensitive to proteasome inhibitors.\textsuperscript{100} Mutation of the potential ubiquitin-acceptor amino acids of Bcl-2 provides protection against TNF-α and staurosporine-induced Bcl-2 degradation in vitro and in vivo.

\section*{p53 and Oncogenes}

Nuclear proteins, particularly those related to oncogenes or antioncogenes,\textsuperscript{101} such as p53 and c-myc, may also regulate the apoptosis of vascular cells during atherogenesis. The tumor suppressor gene p53 functions as an antioncogene capable of promoting apoptosis (Figure 2). Wild-type p53 arrests cell proliferation and may retain cells with DNA damage in the G1 phase of the cell cycle. During DNA repair, some p53-expressing cells oppose the G1 block and enter the suicide pathway. In atherosclerosis, p53 may mediate the apoptosis of vascular cells.\textsuperscript{102,103} Mutations in p53 or its interaction with viral proteins such as simian virus 40 large-T antigens or the products of human cytomegalovirus can cause dysfunction of p53 and, in turn, inhibit apoptosis.\textsuperscript{104,105} Cells from normal blood vessels express little p53, but vascular cells undergoing apoptosis do contain p53. In vitro studies furnish further evidence that p53 regulates apoptosis in cultured SMCs. Bennett and colleagues\textsuperscript{106–108} reported that p53 mediates the induction of apoptosis by deprivation of growth factors and transfection with c-myc in cultured vascular SMCs.

Different oncogenes may interact in the control of apoptosis. Compared with normal cells, SMCs from atherosclerotic plaques show a higher ratio of the active (hypophosphorylated) to the inactive (phosphorylated) form of the retinoblastoma gene product (Rb, a tumor suppressor gene) and a lower level of E2F transcriptional activity.\textsuperscript{107} Suppression of Rb alone increases the rates of cell proliferation and apoptosis and inhibits the senescence of normal vascular SMCs. Suppression of p53 and Rb together exerts similar effects. In contrast, inhibition of Rb binding to E2F or ectopic expression of E2F-1 in plaque cells induces massive apoptosis, which requires suppression of p53 to rescue cells.\textsuperscript{107} Suppression of Rb and p53 together increases cell proliferation and delays senescence but fails to immortalize plaque cells. Inhibition of p53 alone has a minimal effect on plaque cells but increases the lifespan of normal SMCs.

Arterial injury induces apoptosis as well as proliferation of SMCs. Speir et al\textsuperscript{104} and Tanaka et al\textsuperscript{105} have presented evidence linking arterial injury, cytomegalovirus, and dysregulated apoptosis. They reported that some cells in injured arteries could express wild-type p53, an inducer of apoptosis. They further observed that human cytomegalovirus binds to and inactivates apoptosis-promoter p53, prolonging the life span of SMCs. Expression of the human cytomegalovirus protein IE2-84, but not IE1-72, may protect SMCs from p53-mediated apoptosis.\textsuperscript{105} These observations raise the possibility that attenuation of apoptosis contributes to increased accumulation of cells in the intima and, thus, to intimal expansion after arterial injury.

Using compound mutant mice, Guevara et al\textsuperscript{109} examined the effect of p53 inactivation on atherogenesis in hyperlipidemic apoE\textsuperscript{−/−} mice. Compared with p53\textsuperscript{+/−}/apoE\textsuperscript{−/−} mice, p53\textsuperscript{−/−}/apoE\textsuperscript{−/−} mice developed accelerated aortic atherosclerosis despite similar serum cholesterol levels in response to a high-fat diet. Furthermore, the atherosclerotic lesions in p53\textsuperscript{−/−}/apoE\textsuperscript{−/−} mice had a significant increase in cell proliferation and an insignificant increase in apoptosis compared with those in p53\textsuperscript{+/−}/apoE\textsuperscript{−/−} mice. These observations indicate a role for p53 in controlling arterial cell replication, suggesting a p53-independent mechanism underlying the apoptotic response in these atherosclerotic mice.

The proto-oncogene c-myc can mediate either cell death or proliferation depending on its level of expression. It functions as a nuclear phosphoprotein with certain properties of a transcription factor.\textsuperscript{101} However, in serum-deprived cultures, cells overexpressing c-myc readily undergo apoptosis. Deregulation of c-myc causes apoptosis of the vascular SMCs deprived of growth factors or treated with cytokines such as IFN-γ.\textsuperscript{10} p53 may mediate the proapoptotic effect of c-myc in various cell lines.

\section*{Conclusions}

Apoptosis probably participates in the progression or regression of atherosclerotic lesions, in vascular remodeling, and in complications of advanced atheroma. Many environmental and endogenous factors can influence apoptosis through various signal transduction or enzymatic pathways. The apoptotic mechanisms illustrated above link risk factors (e.g., oxidized lipids) and immune and inflammatory effector mechanisms to disordered cell accumulation, providing a unifying concept situated at the center of current thinking about atherosclerosis. Abnormality of apoptosis may occur in atherosclerosis, favoring the accumulation of intimal cells during some phases of atherogenesis. In advanced atheroma, cell death may contribute to aneurysm formation and to weakening of the fibrous cap, enhance thrombogenicity of the lesion core, and increase the risk of plaque disruption. Future studies will assess whether therapies directed toward apoptosis will modify vascular diseases such as atherosclerosis.

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\section*{References}


66. Dimmeler S, Zeiher AM. Nitric oxide—an endothelial cell survival factor.


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