Role of apoptosis in atherosclerosis and its therapeutic implications

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ABSTRACT
Atherosclerotic plaques develop as a consequence of the accumulation of circulating lipid and the subsequent migration of inflammatory cells (macrophages and T-lymphocytes) and VSMCs (vascular smooth muscle cells). Advanced plaques consist of a lipid-rich core, separated from the lumen by a fibrous cap composed of VSMCs, collagen and extracellular matrix. Plaque enlargement ultimately narrows the lumen (stenosis) causing angina. However, recent studies have emphasized that acute coronary syndromes (unstable angina/myocardial infarction) are caused by lesion erosion/rupture with superimposed thrombus formation on often small non-stenotic plaques. Thus current therapies work predominantly on stabilization of plaques rather than plaque regression. Apoptosis (programmed cell death) is increasingly observed as plaques develop, although the exact mechanisms and consequences of apoptosis in the development and progression of atherosclerosis are still controversial. Increased endothelial cell apoptosis may initiate atherosclerosis, whereas apoptosis of VSMCs and macrophages localizes in 'vulnerable' lesions, i.e. those most likely to rupture, and at sites of rupture. This review will focus on the regulation of apoptosis of cells within the vasculature, concentrating on the relevance of apoptosis to plaque progression and clinical consequences of vascular cell apoptosis.

PATHOBIOLOGY OF ATHEROSCLEROSIS
The arterial wall is composed of an innermost layer of endothelial cells resting on a thin basal lamina membrane; beneath this is the intima consisting mainly of elastin and collagen fibres and some SMCs (smooth muscle cells). Below this is the media layer consisting mainly of SMCs; and finally the outer adventitia layer of connective tissue, collagen and elastic fibres embedding the entire vessel within its surroundings. Atherosclerosis is a systemic disease involving the intima of the large and medium arteries. Atherosclerotic lesions develop from an initial ‘fatty streak’, a region of intimal thickening in response to the presence of inflammatory cells, lipid deposition and lipid-containing intimal macrophages (foam cells). These early lesions are present in most people under the age of 30 and are asymptomatic. The development of fatty streaks into more advanced lesions and plaques (Figure 1) is dependent upon further inflammatory processes, including circulating monocytes infiltrating the lesion, these monocytes differentiating into foam cells, intimal macrophages and progressing into foam cells.

Key words: apoptosis, atherogenesis, growth factor, inflammation, oxidative stress, plaque rupture, signalling pathway.
Abbreviations: Apaf-1, apoptotic protease-activating factor-1; Ang, angiotensin; EC, endothelial cell; FADD, Fas-associated death domain; bFGF-2, basic fibroblast growth factor-2; Fas-L, Fas ligand; IAP, inhibitor of apoptosis protein; ICAM-1, intercellular cell-adhesion molecule-1; IGF-1, insulin-like growth factor-1; IL-1β, interleukin-1β; FLICE, FADD-like IL-1β-converting enzyme; LDL, low-density lipoprotein; MCP-1, macrophage chemotactic protein-1; NO, nitric oxide; NOS, NO synthase; eNOS, endothelial NOS; iNOS, inducible NOS; O$_2^-$, superoxide; ox-LDL, oxidized LDL; PI3K, phosphoinositide 3-kinase; PS, phosphatidylserine; ROS, reactive oxygen species; SMC, smooth muscle cell; statin, 3-hydroxy-3-methylglutaryl-CoA reductase inhibitor; TNF, tumour necrosis factor; VEGF, vascular endothelial growth factor; VSMC, vascular SMC.
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Figure 1  Diagramatic representation of the development of the atherosclerotic lesion (atherogenesis)

Phase 1 is the development of the fatty streak from a region of isolated macrophages, ox-LDL and foam cells into a significant fatty streak with multiple small lipid cores (LC) and some SMCs. During phase 2, the accumulation of lipid expands the core and the increase in SMCs and collagen deposition develops the fibrous cap (FC). However, the fibrous cap can rupture to form a thrombus (Th) and change the geometry of the lesion (phase 3). Repeated ruptures increase stenosis and possible angina or MI or occlusion of the vessel (phases 4 and 5).

REGULATION AND INDUCTION OF APOPTOSIS

Programmed cell death is a highly regulated process, initiated by the absence of survival factors or presence of death-promoting factors, whereas extrinsic factors such as hypoxic/ischaemic and osmotic insults contribute to necrosis. All cells contain components of the death machinery, ready to initiate self-destruction unless signalled not to do so. Thus death is often the default program unless the cell is actively signalled to survive, for example by cell–cell or cell–matrix contact. The terms programmed cell death and apoptosis are often interchanged; however, apoptosis is a morphological description of mode of death [1] and, although the term apoptosis has only been used for the last 25 years, morphology typical of apoptosis had been noted for many years by embryologists [2] and in descriptions of atherosclerosis [3].

Cells undergoing apoptosis shrink and retract from neighbouring cells; this is followed by condensation of the chromatin at the nuclear membrane, whilst other organelles appear normal. The cell membrane forms blebs and vesicles, and finally there is nuclear fragmentation and the formation of a number of dense apoptotic bodies [4]. The latter are membrane-bound structures containing fragments of nuclear chromatin, which may be phagocytosed by adjacent cells, thereby preventing spillage of cellular contents and, consequently, apoptotic cells are removed without significant inflammation or
Figure 2  Diagramatic representation of the apoptosis signalling pathways mediated by death receptors and mitochondria featuring Fas, the prototypic member of the TNF death receptor family

The recruitment of the adapter molecule FADD and pro-caspase 8 results in the activation of the latter. Caspase 8 activation directly activates downstream caspases (3, 6 and 7), which result in DNA fragmentation and cleavage of cellular proteins. Cells with insufficient caspase 8 require additional amplification of the death-inducing signal via the parallel pathway involving the mitochondria controlled by either the pro-apoptotic (Bax, Bid and Bik) or anti-apoptotic (Bcl-2 and Bcl-XL) Bcl-2 family.  

Non-death receptor stimuli, such as growth factor withdrawal or activation of p53, act through this mitochondrial pathway. Fas-induced apoptosis can also be blocked by expression of several intracellular proteins, including FLIPs (FLICE-inhibitory proteins) and IAPs.

Formation of scar tissue. This is in contrast with necrosis which is characterized by a progressive loss of cytoplasmic membrane integrity, the rapid influx of Na⁺, Ca²⁺ and water, cytoplasmic swelling and nuclear pyknosis, leading to cellular fragmentation and release of cellular contents with resulting inflammation.

Complex interactions with the extracellular environment and intrinsic gene products of the cell occur before apoptosis begins and, once activated, the apoptosis process continues without further extracellular stimuli. The morphological changes which characterize apoptosis reflect the activation of a cascade of cysteine protease enzymes (caspases). These caspases are present as inactive pro-enzymes, activated by proteolytic cleavage, with caspase 8, 9 and 3 pivotal to the death process [5]. This cascade culminates in the selective cleavage of cellular substrates (many of the caspase targets are involved in the formation and regulation of the cytoskeleton [6–8], internucleosomal fragmentation of DNA by DNases and the characteristic apoptotic morphology). The subcellular events regulating apoptosis have been extensively reviewed elsewhere (for example, see [9,10]).

Briefly, there are two major signalling pathways for the regulation of apoptosis (Figure 2). The first extrinsic pathway concerns membrane-bound death receptors of the TNF-R (tumour necrosis factor-receptor) family, such as Fas/CD95, TNF-R1 and the death receptors DR3, DR4 and DR5, binding their trimerized ligands (reviewed in [11–13]). The binding of the death ligand to its receptor results in receptor aggregation, and subsequent recruitment of a number of adapter proteins, including FADD (Fas-associated death domain), through protein–protein interactions [14]. In turn, these adapter proteins bind to death domains of the receptor [15] and RIP (receptor-interacting protein) and recruit caspases, such as caspase 8/FLICE [FADD-like IL-1β-converting enzyme]. The resulting oligomerization proteolytically activates caspase 8 [16], facilitating the subsequent activation of terminal effector caspases, such as caspases 3, 6 and 7 [5].

The second intrinsic pathway is dependent on the release of cytochrome c from mitochondria into the cytoplasm. Once released, cytochrome c associates with Apaf-1 (apoptotic protease-activating factor-1) and pro-caspase 9, transforming this pro-caspase into its active form, which, in turn, activates downstream caspases. This release of cytochrome c is mediated by the relative expression and activity of the Bcl-2 family of pro- or anti-apoptotic proteins, as well as the tumour suppressor gene p53. There is cross-talk between these two parallel pathways, such as seen with the cleavage of the Bcl-2 family member BID by caspase 8 in response to TNF-α.
Apoptosis is seen as an active form of death as it involves energy-requiring steps such as the formation of the ATP-dependent proteases and the pro-caspases. There is evidence that cells triggered to undergo apoptosis can be forced to die instead of undergoing necrosis when energy levels are reduced [19]. Apoptosis and necrosis have also been shown to co-exist under certain pathological conditions [20], an observation that is likely to be relevant to the pathology of atherosclerosis.

REGULATION OF VASCULAR CELL APOPTOSIS IN ATHEROSCLEROSIS

Apoptosis of vascular cells
ECs (endothelial cells) form the inner lining of all blood vessels and function to maintain vascular tone and anticoagulant properties of vessels. EC apoptosis or dysfunction (the loss of vasomotor tone, alteration in pro-coagulant activity and inflammation) is therefore important in many disease states, including atherosclerosis. EC apoptosis also regulates apoptosis of VSMCs (vascular SMCs) as EC products promote VSMC survival [21]. VSMCs within the vessel wall are normally able to proliferate, migrate and synthesize/degrade extracellular matrix upon receiving appropriate stimuli. However, the normal adult artery shows very low levels of VSMC turnover, and apoptotic and mitotic indices are low [22]. In diseased tissue, additional factors present (such as inflammatory cytokines) substantially alter the balance of cell proliferation and apoptosis to varying degrees. Multiple stimuli can trigger apoptosis in cells, but in vascular disease, it is also likely that specific alterations within the cell itself elicit sensitivity to a specific disease-associated stimulus. Therefore, although the regulation of vascular apoptosis is a combination of both systemic and local factors, the importance of any particular pathway regulating apoptosis depends upon the cell type and the stimulus for apoptosis. Thus different pathways may be responsible for inducing apoptosis in vascular cells in different disease states, at different stages of the disease or in VSMCs compared with ECs.

Regulation of apoptosis in early atherosclerotic lesions
Following the initial EC damage, monocytes adhere to endothelial fibronectin via the CD11/CD18 integrins. The ensuing inflammatory responses stimulate lipid accumulation [23], initiating a cascade effect of increasing lipid accumulation and exposure to other atherogenic components. This results in changes to the oxidative state of the EC, and changes to the expression of adhesion molecules such as ICAM-1 (intercellular cell-adhesion molecule-1) [24] and MCP-1 (macrophage chemotactic protein-1) [25]. The subsequent inflammatory cell recruitment mediates further monocyte–EC interactions [26,27], culminating in increased EC apoptosis from the low level which is seen in normal vessels that have little cellular turnover [28]. In addition, membrane vesicles from apoptotic cells contain biologically active oxidized phospholipids that can induce further monocyte adhesion to the endothelium [29,30]. In this way, the inflammatory infiltrate within lesions perpetuates and amplifies the increased apoptosis within lesions. As the disease progresses, apoptosis increases and is significantly elevated in advanced lesions, predominant in the lipid core or equally divided between the lipid core and the region underlying the plaque rupture [28].

Traditional hypotheses of atherogenesis have suggested that injury of vascular ECs is critical for the development of atherosclerosis [31]. The sites where plaques develop may be associated with increased EC turnover rate, suggesting a mechanical link with susceptibility to atherosclerosis, perhaps as an increase in apoptosis [32]. It has been reported that regenerated endothelial cells do not function correctly [33]; therefore, if the endothelial monolayer has to be repaired due to damage, the dysfunctional cells will be unable to provide sufficient atheroprotective activity [e.g. synthesis of NO (nitric oxide)]. Increased apoptosis may be due to a variety of systemic factors; for example, high glucose [34,35], increased oxidative stress and Ang II (angiotensin II) [36] and ox-LDL (oxidized low-density lipoprotein) [37–39] all induce endothelial apoptosis and are known risk factors for atherosclerosis. In contrast, atheroprotective factors such as NO and laminar flow (shear stress) are more localized and reduced protection may trigger local endothelial cell apoptosis. These are discussed in more detail below.

Role of cell–cell and cell–matrix signalling
Haemodynamic/mechanical stresses on blood vessels are exerted either by blood pressure and transmitted across the entire vessel wall or through blood flow creating shear stress at the luminal endothelial surface. Exposure to positive shear stress reinforces EC contacts, with a subsequent increase in survival signals through integrin signalling to intracellular kinases [40]. For example, the extracellular matrix protein fibronectin transmits survival signals via FAK (focal adhesion kinase), the phosphorylation of which inactivates the tumour suppressor gene p53 (a potent pro-apoptotic stimulus), therefore allowing cell survival [41]. Certain areas of the arterial tree, such as branch points, exhibit low shear stress and/or flow disturbances with resultant EC apoptosis and changes in gene expression [42,43]. Shear stress also induces anti-apoptotic protein expression of Bcl-Xα, and Bak mRNA and the anti-apoptotic soluble form of Fas [44] and enhances endothelial expression of the anti-atherogenic NO
synthase (NOS) eNOS (endothelial NOS) [45,46]. Haemodynamic forces are also important in more advanced plaques, where low or turbulent flow surrounding plaques is associated with a high incidence of apoptosis [47]. As for ECs, the signals generated by cell–cell and cell–matrix interactions are important for VSMC survival as fibronectin and the integrin α/β are of great importance for the maintenance of the survival pathways [48–51].

**Growth/survival factors**

The absence of growth factors is a potent inducer of apoptosis in both ECs and VSMCs. The growth/survival factors for ECs include VEGF (vascular endothelial growth factor), angiopoietin-1 and bFGF-2 (basic fibroblast growth factor-2). VEGF is produced by cultured VSMCs and, as well as acting as a potent angiogenic factor, is also a survival factor for ECs in vasculogenesis and angiogenesis, probably by stimulating endothelial NO production and inhibiting leucocyte recruitment [52]. The VEGF survival signals include the up-regulation of Bcl-2, the Bcl-2 homologue A1 [53] and the expression of IAPs (inhibitor of apoptosis proteins) [54], and bFGF-2 inhibits serum-deprivation-induced EC apoptosis through both Bcl-2-dependent and -independent pathways [55]. Also, integrins function in conjunction with VEGF to promote EC survival; for instance, angiopoietin-1 (action also via the PI3K (phosphoinositide 3-kinase)/Akt pathway) has been shown to bind to EC-specific tyrosine kinase receptor Tie-2 [56].

Many known growth factors of VSMCs can act as survival factors [57–59]. In particular, IGF-1 (insulin-like growth factor-1) is a potent anti-apoptotic stimulus for VSMCs [60,61], whose action may be lost in VSMCs from atherosclerotic plaques, pre-disposing them to apoptosis [62] via reduced expression of the type I IGF-1 receptor. Importantly, ox-LDL (oxidized-LDL) can suppress expression of this receptor [63], which may be one mechanism by which oxidized lipids induce VSMC apoptosis in atherosclerosis. Other factors now known to down-regulate IGF-1 and consequently decrease VSMC viability and promote plaque instability include TNF-α [64]. Significantly, the use of the signalling pathway involving PKC (protein kinase C) inhibition can be one mechanism of inducing apoptosis in human coronary artery SMCs, even in the presence of the survival factor IGF-1 [65].

**Oxidative-sensitive mechanisms**

Oxidative stress occurs when cells are exposed to excessive levels of O₂ or its derivatives, i.e. ROS (reactive oxygen species), the most prominent being O₂⁻ (superoxide). O₂⁻ has a number of effects, including lipid peroxidation, proliferation of VSMCs and apoptosis of ECs [66–68]. Platelets, neutrophils, monocytes, lymphocytes and erythrocytes all possess the ability to produce O₂⁻, especially neutrophils which release large amounts and co-adhere with platelets and monocytes, thereby releasing other substances that can promote death of ECs [69]. In the vasculature, the major source of O₂⁻ is NADPH oxidase, which is known to be overexpressed in the atherosclerotic and diabetic vasculature [70]. One important regulator of the expression of NADPH oxidase is the vasoconstrictor Ang II, which stimulates the enzyme via the Ang II type 1 receptor [70,71]. Ang II promotes oxidative-stress-induced endothelial dysfunction and apoptosis [36].

The relationship between serum cholesterol and atherosclerosis has been established for over 50 years, with major effects being attributed to LDL-cholesterol [72]. Increased formation of oxygen radicals leads to LDL oxidation and peroxidation within the vessel wall [73,74]. Ox-LDL is a potent inducer of apoptosis, signalling death by intrinsic pathways and also sensitizing to extrinsic pathways [75–77], in part by inducing the degradation or blocking activation of the pro-survival protein kinase Akt [78,79].

Apoptotic macrophages have been identified within the lipid-rich core of atherosclerotic lesions [80] and macrophage apoptotic death is significantly higher at sites of plaque rupture and thrombosis in patients with sudden coronary death [81]. Ox-LDL induces macrophage death via a mitochondrial pathway, therefore contributing to the development of the lesion necrotic core [82–84]; abundant cholesterol crystals in advanced atherosclerosis lesions or plaques, especially in macrophages loaded with modified cholesterol, have also been observed [85]. These in vitro observations have been recently verified in a mouse model of reduced oxidative stress in which both vascular cell apoptosis and atherogenesis declined [86].

**NO**

Another important multifunctional regulator of apoptosis in the vasculature is the intermediary molecule NO which has already been mentioned in passing in this review. NO is hydrophobic and a highly diffusible free radical generated from the oxidation of L-arginine by a family of NADPH-dependent isoenzymes, the NOSs. In the endothelium, eNOS is constitutively expressed and transiently activated by the increase in intracellular concentration of free Ca²⁺. The cytokine-inducible (i) NOS is transcriptionally regulated, resulting in NO production at basal Ca²⁺ levels. It is highly reactive and can react with oxygen to form ONOO⁻ (peroxynitrite) which can not only induce ATP depletion and necrosis [87], but can also cause lipid peroxidation [88].

Physiological levels of NO produced by eNOS protect against apoptosis through the prevention of caspase 3 activation [89,90] or by decreasing the non-specific permeability of the inner mitochondrial membrane thereby preventing cytochrome c loss [91]. Furthermore, the
beneficial effects of L-arginine in experimental and human studies confirm the importance of the anti-atherogenic effects of NO [86,92]. In contrast, Delikouras et al. [93] have demonstrated that iNOS expression induced in primary porcine ECs increased Bcl-2 and Bcl-XL; this expression was associated with an acquired protection of ECs from TNF-α-induced apoptosis. Physiologically, the anti-apoptotic pathway induced by shear-stress involves the phosphorylation of Akt/PKB (protein kinase B), the release of NO and the inhibition of caspase 3 activation [94,95].

The high levels of NO produced by iNOS in macrophages induce a burst of reactive nitrogen species that promote oxidative damage [96], and can also induce the release of cytochrome c, the up-regulation of p53 expression and the activation of the Bel-2-dependent pathways [97]. In particular, incubation of ECs with high levels of NO increases apoptosis by decreasing the levels of Bcl-2 and increasing levels of the pro-apoptotic protein Bax. NO can also react with O₂⁻ to form other cytotoxic species that augment the pro-apoptotic action of NO [98]. Furthermore, there are many examples of NO action magnifying the effect of other apoptosis inducers; for example, Shichiri et al. [99] demonstrated that serum deprivation and NO donors together increased the rate of apoptosis of rat VSMCs.

Death receptor signalling and role of inflammation

Macrophages and T-lymphocytes within the complex environment of the atherosclerotic plaque are potent inducers of apoptosis. CD4⁺ and CD8⁺ T-cells express well-characterized cytotoxic mediators, including perforin (which produces membrane damage) and Fas-L (Fas ligand) [100]; macrophages express both membrane-bound Fas-L [101] and secrete TNF-α. In addition, the production of tissue metalloproteinases by macrophages may accelerate cell death by degrading the extracellular matrix from which VSMCs derive survival signals [102,103]. Observations of increased inflammatory markers and cellular infiltration in atherosclerotic lesions provide indirect evidence for the role of death receptors and other inflammatory mechanisms of vascular cell death [104–106].

The role of death-receptor-mediated apoptosis in ECs is presently controversial. Although Fas/Apo-1 activation may induce apoptosis of VSMCs in vitro under specific conditions and is expressed in atherosclerosis (including ECs) [107–110], ECs are normally resistant to Fas-L [111,112]: furthermore, Fas-L may protect ECs from invading inflammatory cells by inducing Fas-mediated apoptosis in the latter cells [113,114]. VSMCs are also usually resistant to Fas-induced apoptosis, but both ECs and VSMCs can be induced to undergo Fas-mediated apoptosis following priming of the cells with cycloheximide, proteasome inhibitors or activation of p53. In the latter two cases, this manipulation results in reduced internalization of surface Fas or increased movement of internal Fas to the cell surface respectively [115,116].

In contrast with Fas, stimulation of human ECs with the cytokine TNF-α directly results in both pro- and anti-apoptotic signals; TNF-α-induced apoptosis of ECs is mediated, in part, by the degradation of Bel-2 and the activation of caspase 3 [117]. TNF-α is also capable of protecting against apoptosis acting through the transcription factor NF-κB (nuclear factor κB) via the induction of Ang-1 [118]. TNF-α, in combination with other inflammatory cytokines, including IFN-γ (interferon-γ) and IL-1β, rapidly induces apoptosis of VSMCs, but each cytokine is ineffective in isolation. In general, human VSMCs generate a concentration of NO an order of magnitude lower with the same cytokine combination. However, this cytokine combination can also sensitize VSMCs to Fas-induced apoptosis [110], reinforcing the role of inflammatory cells, especially macrophages, in atherosclerosis and may partly explain the observation that VSMC apoptosis co-localizes with areas of high inflammatory cell content [119,120].

ROLE OF APOPTOSIS IN ATHEROGENESIS AND PLAQUE RUPTURE

Many studies have identified increased apoptosis of vascular cells in atherosclerotic plaques compared with normal vessels [80,121–123]. As discussed above, what is more speculative are the mechanisms of vascular apoptosis, as well as the direct effects of vascular apoptosis, rather than other aspects of plaque biology, such as inflammation. Although there are many possible sequelae of vascular cell apoptosis, depending upon cell type and lesion stage, direct evidence for the effect of apoptosis in vivo is often lacking.

Plaque rupture

Plaques tend to rupture at sites of increased macrophage and reduced VSMC contents, suggesting that VSMC apoptosis, possibly triggered by macrophages, promotes plaque rupture [124]. There is also recent evidence of mast cell involvement in VSMC death within the fibrous cap [125]. Indeed, there is an increased level of VSMC apoptosis in symptomatic plaques [126–128] in patients presenting with unstable versus stable angina, and apoptotic macrophages co-localized with sites of plaque rupture [81].

Although these studies imply a direct causal relationship between plaque rupture and apoptosis, only recently has direct evidence for this emerged. In an animal model of plaque rupture in mice, direct induction of VSMC apoptosis in the fibrous cap of mouse lesions induces plaque rupture and thrombosis of plaques [129]. This demonstrates directly that VSMC apoptosis, particularly
if localized and of moderate frequency, can induce plaque rupture and luminal thrombus similar to that seen in human lesions.

The specific role of macrophages in plaque rupture is more controversial. Macrophages and macrophage apoptosis co-localize with sites of rupture, suggesting a direct causal role in rupture. However, to date, this has not been proven in vivo, even though killing of SMCs by monocytes/macrophages has been demonstrated in vitro [130]. Any reduction in macrophage numbers in advanced lesions could therefore improve plaque stability, due to the resulting increase in metalloproteinase activity and the decreased breakdown in collagen [131]. However, a decrease in macrophages would reduce the scavenging of apoptotic SMCs and macrophages, allowing the cells to undergo secondary necrosis thereby increasing thrombogenicity of the plaque.

**Alteration of coagulation**

An additional contributory factor in the initiation of atherogenesis as well as plaque rupture is an alteration in the procoagulant state of apoptotic ECs. Normal unperturbed ECs exhibit anticoagulant properties that include the release of the inhibitor of platelet aggregation prostacyclin; however, exposure to inflammation and atherogenic factors induces pro-coagulant activity [132]. Moreover, apoptosis of ECs increases the expression of PS (phosphatidylserine) and the loss of anticoagulant components of the EC membrane, as PS exposure enhances tissue factor activity, which is highly thrombogenic. Certainly, extracellular tissue factor expression is increased in and around apoptotic monocyte/lymphocyte cells in necrotic regions of plaques. Apoptosis of vascular cells is also the basis for the generation of microparticles within the circulation, which act as potent procoagulant substrates both locally and systemically [133,134]; these particles are increased in patients with unstable coronary disease, and account for the vast proportion of the pro-coagulant activity of the plaque.

**THERAPY TO TARGET APOPTOSIS**

The above discussion has emphasized that apoptosis in the vasculature in postnatal life is generally detrimental to vascular integrity. This suggests that anti-apoptotic therapy may reduce the initiation, progression or clinical consequences of atherosclerosis. Therapy may be aimed either at the triggers of apoptosis or the machinery used to execute apoptosis, and there is increasing evidence for the beneficial use of both. Therapies may need precise targeting, as the apoptotic machinery is conserved across evolution, and systemic therapy may block apoptosis in multiple cell types with deleterious effects. Importantly, therapies aimed at particular aspects of atherosclerosis, for example oxidation, inflammation and cholesterol accumulation, may also exert beneficial effects via an actual reduction in apoptosis.

**Antioxidant therapy**

Antioxidant-based therapies, which have been extensively used, are designed to reduce ROS and the associated vasculopathy [135]. Antioxidants used include vitamins C and E, probucol, NADPH oxidase inhibitors and SOD (O2−-dismutase) mimics. For instance, the oxygen-radical scavenger enzyme catalase prevents VSMC apoptosis induced by H2O2 [75], and vitamin E analogues protect against vascular apoptosis and atherogenesis [136]. Oxidation of LDL is a major trigger for both the initiation and progression of atherosclerosis and is a potent inducer of vascular cell apoptosis. However, it is difficult to establish a direct causative role of ox-LDL in the induction of vascular cell apoptosis in vivo as antioxidant intervention will exert effects via direct anti-apoptosis and other non-atherogenic effects of ox-LDL. More importantly, the role of oxidation and antioxidants in regulating vascular cell apoptosis has been undermined by recent randomized trials that have shown no significant benefit of antioxidants, including vitamins C and E, for example [137] (The Heart Protection Study 2002, available at www.hpsinfo.org).

**Statins (3-hydroxy-3-methylglutaryl-CoA reductase inhibitors)**

Statins have been shown in numerous clinical trials to reduce death and myocardial infarction in both primary and secondary prevention studies. The fact that these effects occur with little reduction in overall plaque burden has emphasized that alterations in plaque stability, including plaque cell apoptosis, underlie their efficacy. It is thought that statins have both a direct effect on cell viability and also reduce inflammation within the lesions that would otherwise trigger apoptosis. Statins prevent EC apoptosis via the induction of eNOS [138]. In addition, statin intermediates act as antioxidants and also decrease platelet aggregation and cytokine release from macrophages consequently reducing EC apoptosis [139]. Although statins in vitro may promote apoptosis of VSMCs at high concentrations [140–142], statins in vivo and cholesterol reduction decrease both VSMC and macrophage apoptosis in both experimental animals and humans [143,144]. Importantly, this effect of statins may be independent of their ability to lower cholesterol [145].

**Apoptosis signalling pathways**

Any direct induction of apoptosis may reduce cellular accumulation after injury to vessels. Such manipulations include inhibiting survival pathway signalling through Bcl-xL [146], induction of apoptosis through Fas-L gene transfer [114,147] and adenovirus-mediated
overexpression of TIMP-3 (tissue inhibitor of metalloproteinases-3) [148,149]. In all cases, such induction of VSMC apoptosis results in reduced intima formation over the short term in either animal vessels or human saphenous vein grafts. The anti-apoptotic affect of IGF-1 has also been illustrated by adenoviral overexpression of the IGF-1 receptor, suggesting a strategy to stabilize plaques [60]. Other recent therapies aimed at targeting apoptosis (e.g. prevention of restenosis by Fas-induced apoptosis [150] or reducing myocardial infarction by caspase inhibitors [151,152]) are promising.

The induction of apoptosis using mechanical and physical approaches is also being tried. An example of this is the use of local photodynamic therapy combined with systemic sensitizers, such as motexafin lutetium (which generates cytotoxic singlet oxygen), to induce macrophage and VSMC apoptosis and to reduce neointima formation in animals [153]: there is currently a phase 1 dose-ranging clinical trial in subjects undergoing coronary artery stent implantation.

Importantly, all of these studies rely on the assumption that induction of VSMC apoptosis can be beneficial, reducing cellular accumulation and, thus, stenosis of vessels. However, after vessel injury, such as that occurring after angioplasty and stenting, there is massive apoptosis of both intimal and medial VSMCs, peaking within hours/days after injury. Repopulation of the media of the vessels then occurs over the subsequent weeks and may contribute to negative remodelling seen after injury. Of interest, inhibition of medial VSMC apoptosis using caspase inhibitors can reduce neointima formation after injury in animal models [154], suggesting that protection against apoptosis after injury may be beneficial.

CONCLUSIONS

Apoptosis occurs in all cells of the atherosclerotic plaque, becoming increasingly frequent and important as the plaque develops. Apoptosis contributes to plaque growth, lipid core development, plaque rupture and thrombosis, although the extent to which apoptosis regulates these processes is unknown. Apoptosis is regulated by both local and systemic factors with different factors responsible for apoptosis in different cell types. Current therapies shown to improve patient outcomes in atherosclerosis reduce apoptosis in atherosclerosis as part of their action. However, the next decade will see trials of agents that specifically manipulate apoptosis in atherosclerosis as their primary mode of action.

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