The role of apoptotic cell death in cardiovascular disease

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Abstract

Background Programmed cell death, or apoptosis, is a distinct, managed form of cell death. It is fundamentally different from necrosis. It is a genetically controlled, energy-dependent method of cellular deletion without inflammation. In the cardiovascular system, apoptosis occurs as a primary and secondary event in disease pathogenesis. This review addresses our current understanding of the initiation, propagation and significance of apoptosis in the cardiovascular system, as well as assessing therapeutic potentials arising therefrom.

Methods A Medline search was performed and relevant publications reviewed. Further articles were obtained from the references of these publications.

Results and conclusions Apoptotic cell death is a key element in the pathogenesis and progression of ischaemia-reperfusion (IR) injury, cardiac failure, myocardial infarction, atherosclerosis, endothelial dysfunction and the clinical syndromes which these situations produce. Our increased understanding of the role of apoptosis in the pathogenesis of cardiovascular disease offers potential to develop new therapeutic strategies.

Introduction

The term and concept of apoptotic or programmed cell death first appeared in the medical literature in 1972. Since then, abnormalities of programmed cell death, or manipulation of the processes of apoptosis, have become recognised as being of central importance in a multitude of pathological states. The concept that cells possess a modifiable capacity to dictate whether or not they enter into a controlled lethal process is significant in understanding cellular responses to adverse circumstances.

Apoptosis is a physiological process for the deletion of unwanted or senescent cells without inflammation. Apoptosis works synchronously with, but opposite to, mitosis in counterbalancing and regulating tissue kinetics. The regression of interdigital webs is an example of physiological apoptotic cell death. Apoptosis is exhibited pathologically in response to stresses such as ischaemia-reperfusion (IR) injury, cardiac failure, myocardial infarction, atherosclerosis, endothelial dysfunction and the clinical syndromes which these situations produce. Our increased understanding of the role of apoptosis in the pathogenesis of cardiovascular disease offers potential to develop new therapeutic strategies.

Pathological characteristics of apoptosis

Apoptotic cell death is a morphologically characteristic process identified by a series of discrete distinguishable steps. Initially, detachment of the affected cell from its surrounding cells occurs. Apoptosis proceeds in an orderly controlled fashion with characteristic cell volume reduction followed by convolution and blebbing of the cell membrane. Condensation of the cytoplasm is reflected in increased cell density. The nuclear chromatin condenses, in association with activation of endogenous endonuclease, and gathers beneath the nuclear membrane. The entire cell may now split into membrane-bound structures with preserved organelle structures. These apoptotic bodies may be visualised within tissues at this stage. The cell now becomes subject to tissue phagocytic cell recognition and action. Membrane integrity is maintained throughout the process, in contrast to the swelling and subsequent

myocardium, the finding of apoptosis after reperfusion is particularly significant as it implies late and potentially avoidable cell death.

In cardiovascular disease, developments in molecular biology have facilitated an increasing appreciation of the role of apoptosis, not only in heart failure and myocardial infarction but also in IR injury, ageing, vascular wall remodelling and atherosclerotic plaque destabilisation. Apoptosis is now recognised to not only contribute to the initiation of these disease states but also to influence long-term outcome in terms of eventual myocardial pump failure or arterial occlusion. Apoptosis occurs acutely but also persists after an initial inflammatory or ischaemic insult, thus perpetuating the injury sustained. As apoptosis is a process that can be potentially attenuated, recognition of its contribution to cardiovascular disease opens exciting new therapeutic avenues.
rupture of a cell dying by necrosis. The destructive spilling of intracellular contents into the surrounding tissues evoking the classical inflammatory responses of necrosis is absent in apoptosis. Apoptotic cells are phagocytosed by neighbouring cells without the inflammation associated with, or need for, neutrophil recruitment.14

The process is an inducible phenomenon with a key biological event being the activation of an endogenous endonuclease, resulting in internucleosomal cleavage of genomic DNA. The extensive nucleosomal DNA fragments resulting from this endonuclease activation are 180-200 bp oligonucleosomal units, or multiples thereof, and are a pathological hallmark of apoptosis, which may be detected as a ladder on gel electrophoresis.15 Single cell microgel electrophoresis, the 'Comet' method, can also demonstrate these distinctive DNA strand breaks in individual apoptotic cells by their migration into a specific 'tail' pattern.16

The specific cleavage pattern of genomic DNA by endonucleases yields strand breaks that can be identified by labelling of the free 3′-OH DNA ends with modified nucleotides. Terminal deoxynucleotidyl transferase (TdT) catalyses polymerisation of nucleotides to free 3′-OH DNA ends in a template manner in order to label these breaks in the TUNEL (TdT-mediated dUTP nick end labelling) assay for apoptosis.17

The regulation of intracellular pH is integrated into the progression of apoptosis, with a fall in pH preceding the terminal subcellular events.18 Preconditioning of cells using ischaemia protects against cell death through protein kinase C (PKC) mechanisms.19,20 The PKC protective mechanism involves diminution of intracellular acidification and inhibition of this pathway in vitro. This has been shown to protect cardiomyocytes from apoptotic death in response-simulated IR injury.21

Although the apoptotic cell does not evoke inflammatory responses to stimulate its removal, it does signal its death to resident tissue phagocytes and neighbouring cells. This is achieved by cell membrane surface expression of the normally exclusively cytoplasmic facing aminophospholipid phosphatidylserine.22,23 This phosphatidylserine extrusion facilitates phagocytic removal of the apoptotic cell. It also facilitates phosphatidylserine detection on the cell surface by Annexin V, thus allowing for another method of identifying apoptotic cells.24 As phosphatidylserine surface expression is an early event in the apoptotic pathway, Annexin V labelling allows identification of apoptotic cells at this timepoint. Cell surface phosphatidylserine expression promotes thrombin generation in vitro.25 This finding raises the possibility that apoptosis seen in atherosclerotic plaques may contribute to their thrombogenicity in vivo.26,27

Myocyte apoptosis is difficult to recognise as it affects individual myocytes and is scattered across the muscle or myocardium. The process of apoptosis is rapidly completed in as little as 34 minutes (as assessed by time lapse photography28,29) and may not be detectable beyond 24 hours.28,29 Given this fact, the detection of a relatively low prevalence (0.2%) of apoptotic myocytes in the myocardium at a given time point is highly significant, implying that if sustained for one year this would lead to an unsustainable loss of more than 50% of the myocardium. Various investigators have reported great variability in the extent of apoptosis in diseased myocardium, some reporting as high as 35% in ischaemic cardiomyopathies.30 The immunohistochemical finding of apoptosis in the myocardium can be further verified by demonstrating DNA laddering on electrophoresis of comparable specimens.

Biochemical control of apoptosis

The process of apoptosis is orchestrated by the interactions of numerous proto-oncogenes and environmental factors. Initial clues about the genetic command structure of apoptosis were gained from landmark studies on the tiny nematode Caenorhabditis elegans showing that there are 1,090 cell births and 131 cell deaths in the construction of the adult worm.31 Further work identified two genes ced-3 and ced-4 which are essential for the programmed death of these 131 cells. Mutations of these genes permit these cells, which would normally die, to survive. A third gene, ced-9, is necessary to sustain life through tonic inhibition of the pro-apoptotic genes ced-3 and ced-4.32 The ced-9 gene shares structural and functional homology with the human bcl-2 gene.

Caspases

The C. elegans ced-3 gene equivalent product in humans is a family of cystine proteases associated with interleukin (IL) 1B-converting enzyme (ICE).33 These cystine proteases, referred to as caspases, are key mediators of apoptosis.34 A characteristic feature of the caspases (of which 10 have been identified to date) is the presence of a cysteine residue within a highly conserved pentameric sequence in the catalytic centre.35,36

The caspase family appears to play a key role in the initiation of apoptotic cell death, being present during apoptosis and their inhibitors protecting against apoptotic cell death. Caspase activation produces the structural nuclear changes in apoptosis via protein cleavage of cytoskeletal (actin, fodrin and the familial polyoma protein APc), DNA repair (DNA-PK, PARP), nuclear envelope (lamins) and cell cycle (retinoblastoma protein) elements.37 In addition, one of the endonucleases thought to be involved in the chromatin changes of apoptosis is activated by proteolytic cleavage.

The activation of caspases may be initiated via receptor-dependent mechanisms, specifically Fas for two members of the family, caspase-2 and -8.38 Tumour necrosis factor (TNF) and Fas ligand-mediated apoptotic death rely upon caspase activation as a prerequisite to cell deletion.39 There are a number of characterised death receptors, including Fas, TNFR1, death receptor (DR)-3 (ApO-3) and TNF-related, apoptosis-inducing ligand (TRAIL) receptors DR-4 and -5. Binding of the respective ligands to these receptors results in recruitment of dedicated intracellular adapter proteins to the cellular membrane. For TNFR1 and DR3, this protein is TNFR-associated death domain protein (TRADD).40 Fas-associated death domain (FADD) protein fulfils this role for Fas and DR-4.41 These proteins then interact with caspases, precipitating fatal cellular proteolytic cleavage.

Work by Enari et al. on apoptosis induced by Fas receptor interaction suggested that the caspases are sequentially activated. The ultimately lethal proteolytic effects of caspase activation are mediated downstream by caspases -3 and -7.42 Other upstream caspases with long prodomes have regulatory functions.

Bcl-2 family

The Bcl-2 (B-cell lymphoma-2) gene was isolated from the breakpoint of a translocation between chromosomes 14 and 18. The gene is found in 90% of follicular lymphomas and 20% of diffuse non-Hodgkin's lymphomas.43 This translocation results in the bringing together of the Bcl-2 gene and the immunoglobulin heavy chain locus with increased Bel-2 production and expression. Bcl-2 expression in myeloid precursors and pro-B cells dramatically improves survival on withdrawal of growth factors. The C. elegans equivalent to bcl-2 was found to be ced-9, whose loss of function mutation precipitated apoptosis, thus characterising it as a negative regulator of apoptosis.44
Bcl-2 gene expression in humans correlates with resistance to apoptotic cell death. Bcl-2 overexpression confers resistance to the action of gamma radiation and chemotherapeutic drugs that act by inducing DNA damage. It does not prevent damage to the cells from these agents, but rather inhibits apoptosis occurring in response. In the heart, diminished bcl-2 expression in the right ventricle has been associated with apoptotic cell death assisting the transition to an adult circulatory system. In coronary artery ligation models and in vivo with heart failure, bcl-2 expression is markedly upregulated. This upregulation is suggested to be a compensatory mechanism by the myocardium in the face of widespread cell death. The exact mechanism whereby bcl-2 inhibits apoptosis is uncertain; however, its co-localisation with bax on the mitochondrial membrane suggests this may be a key site. The release of mitochondrial proteins, cytochrome c and apoptosis-inducing factor (AIF) into the cytosol activates caspases and thus cell death. Caspase -3, -6 and -7 activation is downstream from bcl-2. However, bcl-2 blocks the release of cytochrome c and AIF from mitochondria, thus suggesting a potential site for its anti-apoptotic activity.

Bcl-2 has also been suggested to function as an anti-apoptotic agent through its anti-oxidative role. However, bcl-2 is also anti-apoptotic under anaerobic cell culture conditions and apoptosis occurs in similar circumstances without reactive oxygen species being present bcl-2 still remains protective. Therefore, bcl-2 cannot function through purely antioxidant-dependent mechanisms, nor can the generation of reactive oxygen species be an exclusive effector of programmed cell death.

The extended Bcl-2 family contains nine members, defined by the presence of three structural motifs in the protein sequence. These proteins may be pro- or anti-apoptotic and appear to interface after cell surface signals induce caspase activation. Bax is a pro-apoptotic member of this family with 45% homology to bcl-2. It appears that the formation of Bax/Bcl-2 heterodimers is an important control point in apoptosis. Mutations of Bcl-2 may allow it to evade the blocking effect of binding to Bax and thus allow anti-apoptotic signals to assume dominance within the cell. Thus, if the Bax/Bcl-2 ratio is altered, this allows the dominant pro- or anti-apoptotic influence to prevail. The overexpression of bcl-2 and the anti-apoptotic proto-oncogene bcl-xL have been shown to be associated with an alteration in the onset of toxin-induced apoptosis.

The bcl-x member of this family offers protection from apoptosis by binding to bax, thus preventing the formation of bax homodimers which accelerate the apoptotic process. The observed reduction in bcl-x seen in experimentally injured arteries may therefore allow bax homodimerisation to occur, precipitating smooth muscle cell apoptosis. Bcl-2 expression is markedly upregulated in humans with heart failure. However, Bax expression is unaffected. This upregulation occurs in the context of an increase in cardiomyocyte apoptosis. The increase in Bcl-2 expression appears in this context to be a secondary compensatory mechanism to promote survival of the remaining cardiomyocytes.

**p53**

The tumour suppressor gene p53 is pro-apoptotic. Loss of p53 function is the result of the most commonly mutated gene in human malignancies. It acts as a cellular sensor of DNA damage, inappropriate oncogene activation, hypoxia and the presence or absence of certain cytokines, and induces apoptosis in response to such stimuli. p53 has a role in chaperoning DNA, inducing apoptotic death when defects emerge.

In the cardiovascular system, gene transfer-induced p53 overexpression in normoxic cultured rat neonatal cardiomyocytes can precipitate apoptotic cell death. Hypoxia in cultured cardiomyocytes, which induces apoptosis, is also associated with increased p53 expression. However, in p53 deficient mice, apoptosis may also occur in cardiomyocytes by forced entry into the S phase of the cell cycle. In these p53 deficient mice, ligation of the left coronary artery does not alter the degree of apoptosis in hypoxic areas when compared with wild type mice. Kirshenbaum and de Moissac and Kirshenbaum have shown that p53 interacts dynamically with other proto-oncogenes involved in apoptotic regulation. p53 expression causes an upregulation of pro-apoptotic Bax, but this effect and its own apoptotic potency are downregulated by bcl-2 expression.

**Nitric oxide**

Nitric oxide (NO) is involved in vessel relaxation, inhibition of smooth muscle and endothelial cell proliferation, and reduction of platelet adhesion. In the myocardium, recent work has shown that constitutively expressed NO synthase (eNOS), inducible NO synthase (iNOS) and endothelial NO synthase (eNOS) have functional autocrine and paracrine effects similar to their effects on vascular cells. NO release precipitates myocardial depression in response to systemic disturbance and part of this dysfunction is mediated by NO-induced cardiomyocyte apoptosis.

In a rat model of heterotopic cardiac transplantation, it was shown that rejection was associated with significant induction of the mRNA, protein and enzyme activity of iNOS. This was detectable in endothelial cells, cardiomyocytes and infiltrating monocytes. Further work expanded the understanding of the role of NO in this context by demonstrating that cytokine induction of iNOS was associated with NO-mediated death of cardiomyocytes in vitro. Szabolcs et al subsequently confirmed that the mechanism whereby NO-induced cardiomyocyte death in vivo was indeed apoptotic, thereby demonstrating directly the link between NO and cardiac apoptosis.

In vivo gene transfer studies of endothelial cell NO synthase (eNOS) activity to the myocardium resulted in cardiomyocyte apoptosis. The suggestion from this study was that the inflammation and cytokine release associated with myocardial infarction, myocarditis and cardiomyopathies may result in similar pro-apoptotic effects on cardiomyocytes via NO-dependent mechanisms.

In the myocardium, NO activates cGMP-dependent protein kinases and cGMP-modulated phosphodiesterases, thus regulating calcium current and contraction. In the course of oxidative injury (such as IR), the simultaneous production of NO and superoxide leads to the formation of peroxynitrite. It now appears that peroxynitrite is a significant mediator of injury previously attributed solely to NO or superoxide. Peroxynitrite production results in single strand DNA breaks, with consequent activation of poly ADP-ribose synthetase (PARS) which is cytotoxic. This mechanism has now been shown to cause endothelial cell apoptosis in vitro.

NO appears to play a major role in vessel wall apoptosis by inducing programmed cell death in smooth muscle cells and infiltrating monocytes. NO activity is reduced in the vessel wall in hypercholesterolaemia suggesting a potential mechanism whereby diminished apoptotic control of cellular homeostasis allows progression of atherosclerosis.

**Nuclear factor kappa beta (NF-kb)**

NF-kb has a potent anti-apoptotic role. In the vascular endothelium, it is expressed constitutively and can be upregulated by stimuli such as IL-6, thrombin, platelet-derived...
growth factor (PDGF) and basic fibroblast growth factor.\textsuperscript{99} In addition, it is essential for in vitro proliferation of vascular smooth muscle cells. Expression of vascular cell adhesion molecule (VCAM)-1 is also NF-\kappa b dependent.\textsuperscript{99-101} Accumulating evidence has shown NF-\kappa b is activated in both vascular cell injury and atherosclerosis.\textsuperscript{99-101} In a rat model of balloon catheter carotid injury, NF-kb regulated genes, monocyte chemoattractant protein (MCP)-1 and VCAM-1 were apparent in smooth muscle cells within four hours. This expression occurred in parallel with macrophage infiltration. This finding links NF-kb to the injury and lesion formation responses of the endothelium.\textsuperscript{101}

NF-kb plays a key role in regulating vascular smooth muscle cell proliferation in normal and damaged endothelium. Abnormalities in the regulatory processes of smooth muscle and endothelial cell proliferation clinically produce neointimal hyperplasia. Its anti-apoptotic role in such circumstances may ultimately be harmful. Its upregulation in atherosclerotic plaques also suggests a potential role in their pathogenesis.\textsuperscript{99,102}

**Tumour necrosis factor**

Tumour necrosis factor (TNF) is a potent inducer of apoptotic cell death in many cell types, subsequent to binding to its receptor.\textsuperscript{103} TNF\alpha has been shown to induce apoptosis in cardiomyocytes in vitro and in vivo.\textsuperscript{104} Two cell surface receptors for TNF\alpha, the 55kD and 75kD TNF\alphaR1 and TNF\alphaR2, respectively, are both functionally expressed by cardiomyocytes in the failing myocardium.\textsuperscript{105}

Fas expression in the murine myocardium has been found to be enhanced in models of viral congestive cardiac failure and this was associated with apoptosis of inflammatory cells and cardiomyocytes.\textsuperscript{106} Fas expression is also upregulated in coronary artery ligation.\textsuperscript{106}

**Apoptosis in the normal cardiovascular system**

In the cardiovascular system, apoptosis is first seen during embryogenesis with remodelling of the bulbus cordis and atrioventricular cushions by programmed cell death.\textsuperscript{107} Postnatal cardiac apoptotic cell death is expressed preferentially in the right ventricle, with the resultant reduced right chamber muscle mass accompanying the transition to an adult circulatory system.\textsuperscript{108} This cardiac remodelling is associated with and facilitated by diminished expression of the anti-apoptotic gene bcl-2.\textsuperscript{109} The development of the cardiac conductive system of pathways also employs apoptosis in deleting unnecessary cells.\textsuperscript{110} Persistent atrioventricular conductive pathways, which would normally be removed by apoptosis, have been suggested as a potential cause of abnormalities such as Wolff-Parkinson-White syndrome.\textsuperscript{111}

The postnatal development of the vascular system involves cell death by apoptosis in vessel remodelling.\textsuperscript{110,112} In an analogous situation, the vascular endothelial post-injury response relies on homeostasis between proliferation and remodelling by apoptotic cell deletion in order to prevent excessive neointimal hyperplasia.\textsuperscript{112}

**Apoptosis in ischaemia-reperfusion (IR)**

The vascular endothelium, by virtue of its location at the blood-tissue interface, is in the first line of IR injury. Reperfusion of ischaemic tissues is a consistent aim of clinical therapies. However, as Parks and Granger\textsuperscript{113} showed, reperfusion is a double-edged sword which can exacerbate the initial hypoxic injury. Reactive oxygen species such as superoxide, NO, hydrogen peroxide, lipid peroxides and the hydroxyl radical generated in excess during IR injury can subject a cell to oxidative stress and subsequent apoptosis.\textsuperscript{114} In addition to the reactive oxygen species, other elements of the reperfusion injury which are known to cause apoptosis include alterations in intracellular calcium homeostasis,\textsuperscript{115} an inflammatory reaction,\textsuperscript{116,117} and increased mechanical stretch.\textsuperscript{118} The presence of similar mediators in other contexts leads on to a potential role for apoptosis in mediating some of the clinically relevant effects of local and systemic IR injury, as well as the systemic inflammatory response syndrome (SIRS). The synergistic dual effect produced under such circumstances results in initial vascular endothelial damage and ultimately end organ injury. An analogous situation occurs in the failing myocardium where reactive oxygen species, inflammatory cytokines, NO, hypoxia, reperfusion and mechanical stretch exert pro-apoptotic influences upon the endothelium and cardiomyocyte.\textsuperscript{119,120}

Autopsy studies of post-myocardial infarction patients, who had initially successful thrombolysis and therefore a patent infarct-related artery, showed a clear subset of cardiomyocytes dying by apoptosis as a result of IR.\textsuperscript{121} This finding is also seen in experimental animal models of IR.\textsuperscript{122} Because apoptosis after infarction is an energy-dependent process relying on circulatory nutrient supply, it will occur primarily in reperfused or watershed areas of the infarct.\textsuperscript{123} Studies have shown that myocardial apoptosis does not occur in purely ischaemic areas, but will occur upon reperfusion of these areas.\textsuperscript{122} Therefore, while one may attempt to deal with an atherosclerotic thrombus after the event occurs, acute apoptotic cardiomyocyte death, while it may be prompted, begins when flow is partially or fully restored. Therefore, reperfusion by recanalisation, thrombolysis or angioplasty provides a window from which time onwards the contribution of apoptotic cell death to the pathological process is potentially amenable to attenuation.

Antioxidants and free radical scavengers such as superoxide dismutase have been used to counteract the pro-apoptotic effects of oxidative stress on tissues exposed to IR.\textsuperscript{124,125} Vascular endothelial cells in vitro are protected from apoptotic cell death in a SIRS analogous model using the amino acid taurine.\textsuperscript{126}

**Apoptosis in the diseased myocardium**

Cardiomyocyte cell loss and scar formation are integral components of cardiac dysfunction from numerous aetiologies. Experimental and autopsy studies have confirmed that cardiomyocytes undergo cell death by apoptosis as a component of hypoxia,\textsuperscript{127} heart failure\textsuperscript{128} and myocardial infarction.\textsuperscript{128} Following on from this work, the traditional viewpoint of heart failure as a purely haemodynamic continuum has been modified by an increasing awareness of the fact that the interaction of cytokines, neurohormones and apoptotic mediators play a significant role in the evolution and progression of this disease process.\textsuperscript{130} As the myocardium has no regenerative capacity, prevention of apoptosis-induced cardiomyocyte loss has potentially significant clinical implications.

The changes in myocardial loading that accompany heart disease of ischaemic or non-ischaemic origin activate various cellular responses. Apoptotic myocyte death is a sequel of such physical stresses.\textsuperscript{127,129} Animal studies of pressure overload hypertrophy models, such as aortic banding, loading of isolated papillary muscle and genetically determined hypertension, have all demonstrated resultant apoptotic cardiomyocyte loss.\textsuperscript{119,126,130} The progression of cardiac hypertrophy resulting from pressure overload involves myocyte loss with hypertrophy of the remaining myocytes and proliferation of non-muscle cells. In a rat model of pressure overload, the secondary cardiac hypertrophy and remodelling was initiated by a wave of apoptotic cardiomyocyte death, thus implicating apoptosis in the pathogenesis of these events.\textsuperscript{131}
Leri et al have suggested a mechanism whereby myocyte stretch-induced autocrine release of angiotensin II is associated with activation of p53, thus resulting in prolonged upregulation of myocyte apoptosis. This suggestion was reinforced by Kajstura et al who noted an increased incidence of apoptosis after treatment of isolated adult cardiomyocytes with angiotensin II. Furthermore, in myocytes subjected to stretch, the bcl-2 to bax ratio was lower, thus increasing myocyte apoptotic susceptibility. Thus, the possibility arises that apoptosis may be involved in the pathogenesis of overall cardiac remodelling. Postmortem examination studies of human hearts 10 days post-myocardial infarction showed an incidence of apoptosis of 0.7% in areas distant from the infarct. There was no apoptosis in control non-infarcted hearts. This study implicates apoptosis in a wider myocardial role, not limited to just the infarcted or hypoxic area. The ventricular myocardial adaptation and remodelling that occurs in response to pathological stimuli, while it may be compensatory in the short term, may initiate changes leading ultimately to pump failure. Recently, evidence has been accumulating that apoptotic cellular deletion participates, and may be a significant determinant, in this pathological transition process. If this rationale holds true, then the gradual progression of myocardial dysfunction and ultimate failure could potentially be halted by the arrest of apoptosis in the myocardium.

The range of cardiac diseases in which inappropriate apoptosis has been demonstrated also includes cardiomyopathic failure of ischaemic, viral and idiopathic origin, as well as in arthrythmogenic right ventricular dysplasia. In myocardial ischaemia, apoptosis rather than necrosis has been shown to be the critical determinant of eventual myocardial infarct size and impact. In addition, when reperfusion of the myocardium occurs this prompts a wave of apoptotic cell death, aggravating the ischaemic insult.

In vitro studies have shown that cardiomyocytes are primed for apoptosis, which may be triggered by cytokines such as TNFα. TNFα is a particularly clinically relevant cytokine, being present during local and systemic IR, sepsis and SIRS. Chronic infusion of TNFα in vivo has been reported to cause rapid onset of dilated cardiomyopathy with widespread myocyte apoptosis. TNFα levels are elevated in heart failure, as well as in reperfused and infarcted myocardium. The finding that oxidative stress induced apoptosis in isolated cardiomyocytes is significant as it suggests a direct mechanism whereby IR can induce apoptosis. Therefore, cardiomyocytes are primed for apoptosis which may be triggered by cytokines, mechanical stretch or IR. Interestingly, it has been suggested that the readiness with which cardiomyocytes undergo apoptosis may also explain the rare incidence of primary cardiac tumours.

Investigators attempting to promote cellular regeneration in the myocardium have used recombinant adenoviruses, delivering the adenoviral protein 12S E1A, and have induced DNA synthesis in terminally differentiated cardiomyocytes. However, this was followed by widespread apoptosis in the absence of a second adenoviral protein E1B, a structural and functional homologue of bcl-2.

Introduction of the bcl-2 homologue allowed cardiomyocyte proliferation and suppression of apoptotic cell death.

The interaction of TNFα with CD95/Apo1/Fas is directly implicated in the death of cardiomyocytes post IR. The evidence above suggests that the expression of apoptosis in the myocardium is a frequent pathological event with significant clinical implications.

**Apoptosis in atherosclerosis**

Atherosclerotic plaque destabilisation has been shown to be associated with apoptotic cell death, principally of inflammatory cells and subendothelial smooth muscle cells in the fibrous cap. Bennett et al have shown that human vascular smooth muscle cells derived from normal and atherosclerotic endothelium have different thresholds for expression of apoptosis in vitro. Apoptosis in normal vascular endothelial smooth muscle cells occurred only on removal of serum growth factors. However, cells from atherosclerotic plaques died even with high serum conditions. In addition, bcl-2 expression induced by gene transfer and the cytokines IGF and PDGF protected against smooth muscle cell apoptosis. This suggests that the prevailing influences in determining the occurrence of apoptosis in vascular smooth muscle cells include proto-oncogene expression and local cytokine interactions.

The highest concentration of apoptotic cells in an atherosclerotic plaque appears to be in regions associated with macrophages. This may be as a result of induction of apoptotic pathways via macrophage TNFα release, reactive oxygen species generation or directly by the production of oxidised low density lipoprotein (LDL). LDL penetration of the intima is an initiating pathogenic effect in atherosclerosis. Its subsequent oxidative modification by macrophages and other cells precedes foam cell formation. The effect of oxidised LDL in directly precipitating apoptosis in cultured endothelial cells is significant as it implies a pathway for the participation of apoptosis in the initial pathogenesis and progression of atherosclerosis. This effect has been exploited therapeutically in LDL receptor knockout mice by using the NO precursor L-arginine to prevent and induce regression of atherosclerosis in hypercholesterolaemic animals, possibly by an antioxidant effect or alternatively directly via NO.

Advanced human atherosclerotic plaques contain sclerotic hypocellular regions. This has led to the suggestion that these areas arise due to apoptotic cell death. As part of the Coronary Angioplasty Versus Excisional Atherectomy Trial (CAVEAT), 93% of the primary atherosclerotic plaques obtained by directional atherectomy were found to be hypocellular. In addition, apoptotic cell death of proliferating smooth muscle cells has been shown to control their numbers after injury such as atherectomy.

Fas is widely expressed in atherosclerotic lesions, suggesting a potential receptor-mediated mechanism of apoptotic death within such lesions. Dong et al have also shown Fas-mediated apoptotic cell death to be associated with post-transplant coronary artery disease.

**Apoptosis in the vascular endothelium**

The endothelial cell fulfills an interactive role inhibiting proliferation of adjoining cells, thrombus formation and leukocyte adhesion while simultaneously regulating vasomotor tone in response to tissue requirements or systemic stimuli. Endothelial dysfunction contributes to abnormalities of these processes.

In the endothelium, loss of integrin-mediated cell matrix contact results in apoptotic cell death known as ‘anoikis’. The term anoikis is derived from the Greek word for homeless and is used in this context to denote a cell dying by apoptosis having been displaced from its normal environment. The death of endothelial cells after detachment is beneficial as it prevents detached or abnormal cells from reattaching and growing in a dysplastic fashion.

Neutrophil endothelial interaction is a key element in the development of the adult respiratory distress syndrome (ARDS), often as a component of SIRS. The endothelial dysfunction,
manifested as increased capillary permeability in SIRS, is partly as a consequence of endothelial cell apoptosis.\textsuperscript{11,15} It has been suggested that, as a therapeutic strategy in sepsis and SIRS, attenuation of the cellular response to this process may be more beneficial than dealing with the extracellular mediators of this process. It has been demonstrated in vitro that induction of heat shock proteins can protect endothelial cells against subsequent apoptosis in response to a SIRS insult model.\textsuperscript{12,13,19}

**Apoptosis in neointimal hyperplasia**

The vascular endothelium response to injury involves smooth muscle cell proliferation and neointimal formation. This proliferation continues for up to 12 weeks. If abnormally regulated proliferation proceeds unchecked, then an overexuberant thickening of the intima will result. However, the total number of cells present after such injury is typically maximal at two weeks.\textsuperscript{20,21} The rate-limiting factor in this context is smooth muscle cell deletion by apoptosis, which intercedes to control cell numbers.\textsuperscript{15} The inference, therefore, is that this apoptotic control allows neointimal thickness to remain constant despite a continued smooth muscle cell proliferation response to injury.\textsuperscript{22} Pearlman et al showed that extensive apoptosis is detectable in an animal model of intimal injury as early as 30 minutes post-injury. However, no apoptosis was seen in normal vessels.\textsuperscript{23} In neointimal hyperplasia, proto-oncogene controls of apoptosis act as molecular thermostats balancing cell death versus proliferation, which determines cell numbers and survival.\textsuperscript{24}

Apoptosis is observed more frequently in restenotic than primary atherosclerotic lesions.\textsuperscript{25} This is again consistent with the dynamic interaction of cell proliferation and apoptosis exerting homeostatic control on cell numbers in the context of endothelial repair and remodelling. This increased frequency of apoptosis in restenotic rather than primary atherosclerotic lesions is supported by autopsy reports of patients dying after multiple angioplasty procedures.\textsuperscript{26} Studies of specimens obtained by percutaneous transluminal atherectomy have provided further evidence of hyperplasia being homeostatically counterbalanced by apoptosis, with both elements being more prominent in restenotic lesions.\textsuperscript{27-29}

An even greater frequency of smooth muscle cell proliferation and apoptosis is seen in neointimal hyperplasia complicating stent restenosis.\textsuperscript{30,31,32} The combined significance of these observations is that if apoptosis can be upregulated within these lesions, then the homeostatic mechanisms will dampen down the excessive proliferation of smooth muscle cells. Gene therapy delivering the thymidine kinase gene via an adenoviral vector has been successfully used to reduce restenosis in angioplasty in an atheromatous rabbit model by inducing apoptosis of smooth muscle cells.\textsuperscript{33}

There is now clear evidence that apoptosis plays a key role in the pathogenesis of cardiovascular disease. Its effects range from reduced apoptosis contributing to neointimal hyperplasia to excessive apoptosis inducing cardiac failure.

The process of apoptosis may be modulated to attenuate, delay, avoid or precipitate cell death. These modulations are used by neoplastic and viral infected cells to promote their own survival. However, in the context of cardiovascular apoptotic cell death, similar manipulations have the potential to be used to induce favourable clinical outcomes. Genetic transfers of particular cellular profiles of proto-oncogene expression, such as bcl-2 and p53, hold the potential to suppress undesirable apoptosis in cardiac ischaemia or to beneficially promote apoptosis in neointimal hyperplasia.

The inflammatory response is a dynamic process and apoptosis is recognised as being a key element in this continuum. It occurs early\textsuperscript{34} and late\textsuperscript{35,36} in reperfusion as part of IR injury. The mechanisms involved in these processes are gradually being elucidated in the myocardium and endothelium.\textsuperscript{37,38}

In the myocardium and vascular endothelium, reactive oxygen species, inflammatory cytokines, NO, hypoxia, reperfusion and mechanical stretch exert pro-apoptotic influences upon cells. The clinical syndromes produced by these influences have established apoptotic contributions to their pathogenesis and significance. The apoptotic component contributing to these disease processes is potentially amenable to beneficial therapeutic manipulation.

This study was funded by a Health Research Board clinical research fellowship and the North-Eastern Health Board.

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