review

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.[1] 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.[2-4] The regression of interdigital webs is an example of physiological apoptotic cell death.[1,5,6] The process of apoptosis is an energy-dependent, tightly regulated and genetically encoded mechanism of individual cell disposal. The ubiquitous process of apoptosis is seen in its physiological context, actively deleting cells during embryogenesis, metamorphosis, tissue atrophy, T-cell killing tumour regression and turnover of intestinal epithelial cells.[1,5,7,8] Cellular resistance to apoptosis is exhibited in abnormalities of these processes and particularly in carcinogenesis.[9]

Apoptosis is exhibited pathologically in response to stresses such as ischaemia-reperfusion (IR) hyperthermia, radiotherapy and pharmacological agents.[10-13] However, pathological or inappropriate apoptosis has significant structural and functional implications for the involved end organs. In mature organisms, ongoing apoptosis maintains cellular homeostasis in renewing cell types such as intestinal epithelium and leucocytes. In contrast, apoptosis is not a feature of non-renewable parenchymal cells in the normal course of events. This is particularly so in terminally differentiated cells such as cardiomyocytes. In the myocardium, the finding of apoptosis after reperfusion is particularly significant as it implies late and potentially avoidable cell death.[14-16]

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.[17-21] 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.

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 an increased cell density. The nuclear chromatin condenses, in association with activation of an 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

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.[1,5]

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.[22] 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.[23-25]

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.[26,27]

The regulation of intracellular pH is integrated into the progression of apoptosis, with a fall in pH preceding the terminal subcellular events.[28] Preconditioning of cells using ischaemia protects against cell death through protein kinase C (PKC) mechanisms.[29,30] 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.[30]

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.[31-33] 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.[34] 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.[35] This finding raises the possibility that apoptosis seen in atherosclerotic plaques may contribute to their thrombogenicity in vivo.[20,36-38]

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 photography[36,37]) and may not be detectable beyond 24 hours.[14,38,39] 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.[40] The immunohistochemical finding of apoptosis in the myocardium can be further verified by demonstrating DNA laddering on electrophoresis of comparable specimens.[16]

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.[41,42] 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.[43] 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).[44-46] These cystine proteases, referred to as caspases, are key mediators of apoptosis.[5,44] 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.[39,46-48]

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 polyposis protein APC), DNA repair (DNA-PK, PARP), nuclear envelope (lamins) and cell cycle (retinoblastoma protein) elements.[5] 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.[45-47] Tumour necrosis factor (TNF) and Fas ligand-mediated apoptotic death rely upon caspase activation as a prerequisite to cell deletion.[45] 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).[46,56] Fas-associated death domain (FADD) protein fulfils this role for Fas and DR-4.[47-49] These proteins then interact with caspases, precipitating fatal cellular proteolytic cleavage.

Work by Enari et al[50] 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.[39] Other upstream caspases with long prodromes have regulatory functions.[39]

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.[51,52] This translocation results in the bringing together of the Bcl-2 gene and the immunological heavy chain locus with increased Bcl-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.[53]

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.[54,55] 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.[56] In coronary artery ligation models and in vivo with heart failure, bcl-2 expression is markedly upregulated.[16] 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.[57] The release of mitochondrial proteins, cytochrome c and apoptosis-inducing factor (AIF) into the cytosol activates caspases and thus cell death.[58,59] Caspase -3, -6 and -7 activation are downstream from bcl-2.[60,61] However, bcl-2 blocks the release of cytochrome c and AIF from mitochondria, thus suggesting a potential site for its anti-apoptotic activity.[62,63]

Bcl-2 has also been suggested to function as an anti-apoptotic agent through its anti-oxidative role.[64] 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.[65,66] 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.[13]

The extended Bcl-2 family contains nine members, defined by the presence of three structural motifs in the protein sequence.[65,67] 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.[62] 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.[68,69]

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.[70] The observed reduction in bcl-x seen in experimentally injured arteries may therefore allow bax homodimerisation to occur, precipitating smooth muscle cell apoptosis.[71]

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.[16] 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.[72] 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.[73-78]

In the cardiovascular system, gene transfer-induced p53 overexpression in normoxic cultured rat neonatal cardiomyocytes can precipitate apoptotic cell death.[73] Hypoxia in cultured cardiomyocytes, which induces apoptosis, is also associated with increased p53 expression.[90] However, in p53 deficient mice, apoptosis may also occur in cardiomyocytes by forced entry into the S phase of the cell cycle.[37,74,75,90] 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.[76] Kirshenbaum and de Moissac[77] and Kirshenbaum[78] 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.[77,78]

Nitric oxide

Nitric oxide (NO) is involved in vessel relaxation, inhibition of smooth muscle and endothelial cell proliferation, and reduction of platelet adhesion.[79,80] In the myocardium, recent work has shown that constitutively expressed NO synthase (cNOS), inducible NO synthase (iNOS) and endothelial NO synthase (eNOS) have functional autocrine and paracrine effects similar to their effects on vascular cells.[81] NO release precipitates myocardial depression in response to systemic disturbance and part of this dysfunction is mediated by NO-induced cardiomyocyte apoptosis.[98]

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.[82,83] This was detectable in endothelial cells, cardiomyocytes and infiltrating monocytes.[99] 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.[84] 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.[100]

In vivo gene transfer studies of endothelial cell NO synthase (ecNOS) activity to the myocardium resulted in cardiomyocyte apoptosis.[85] 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.[103] 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.[86-88] Peroxynitrite production results in single strand DNA breaks, with consequent activation of poly ADP-ribose synthetase (PARS) which is cytotoxic.[105] This mechanism has now been shown to cause endothelial cell apoptosis in vitro.[105]

NO appears to play a major role in vessel wall apoptosis by inducing programmed cell death in smooth muscle cells and infiltrating monocytes.[86,87] 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.[88,89] 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-kb dependent.[90,106] Accumulating evidence has shown NF-kb is activated in both vascular cell injury and atherosclerosis.[91-93] 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.[111]

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.[91,92]

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.[93] TNFa has been shown to induce apoptosis in cardiomyocytes in vitro and in vivo.[23,94] Two cell surface receptors for TNFa, the 55kD and 75kD TNFR1 and TNFR2, respectively, are both functionally expressed by cardiomyocytes in the failing myocardium.[95]

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.[96] Fas expression is also upregulated in coronary artery ligation.[18]

Apoptosis in the normal cardiovascular system

In the cardiovascular system, apoptosis is first seen during embryogenesis with remodelling of the bulbis cordis and atrioventricular cushions by programmed cell death.[97] 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.[68] This cardiac remodelling is associated with and facilitated by diminished expression of the anti-apoptotic gene bcl-2.[94] The development of the cardiac conductive system of pathways also employs apoptosis in deleting unnecessary cells.[98] Aberrant 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.[121]

The postnatal development of the vascular system involves cell death by apoptosis in vessel remodelling.[99,100] 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.[101]

Apoptosis in ischaemia-reperfusion (IR)

The vascular endothelium, by virtue of its location at the blood-tissue interface, is in the front line of IR injury. Reperfusion of ischaemic tissues is a consistent aim of clinical therapies. However, as Parks and Granger[102] 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.[103] 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,[43,104] an inflammatory reaction[42,43] and increased mechanical stretch.[105] 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.[44,106,107]

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.[108] This finding is also seen in experimental animal models of IR.[14] 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.[21] Studies have shown that myocardial apoptosis does not occur in purely ischaemic areas, but will occur upon reperfusion of these areas.[14] 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.[109,110] Vascular endothelial cells in vitro are protected from apoptotic cell death in a SIRS analogous model using the amino acid taurine.[111]

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,[43] IR,[14] heart failure[15,16,44,117,119] and myocardial infarction.[18] 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.[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.[44,121] 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 losses.[2,112,113] 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.[135]

Leri et al[114] 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[115] 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 lowered, thus increasing myocyte apoptotic susceptibility.[138] Thus, the possibility arises that apoptosis may be involved in the pathogenesis of overall cardiac remodelling.[135] 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.[4] 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.[15,16,44,131] 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 arrhythmogenic right ventricular dysplasia.[15,44,49,116]

In myocardial ischaemia, apoptosis rather than necrosis has been shown to be the critical determinant of eventual myocardial infarct size and impact.[14,44] In addition, when reperfusion of the myocardium occurs this prompts a wave of apoptotic cell death, aggravating the ischaemic insult.[14]

In vitro studies have shown that cardiomyocytes are primed for apoptosis, which may be triggered by cytokines such as TNFa.[23] TNFa is a particularly clinically relevant cytokine, being present during local and systemic IR, sepsis and SIRS. Chronic infusion of TNFa in vivo has been reported to cause rapid onset of dilated cardiomyopathy with widespread myocyte apoptosis.[118] TNFa levels are elevated in heart failure, as well as in reperfused and infarcted myocardium.[117-119] The finding that oxidative stress induced apoptosis in isolated cardiomyocytes is significant as it suggests a direct mechanism whereby IR can induce apoptosis.[120,121] 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.[91] However, this was followed by widespread apoptosis in the absence of a second adenoviral protein E1B, a structural and functional homologue of bcl-2.[92] Introduction of the bcl-2 homologue allowed cardiomyocyte proliferation and suppressed apoptotic cell death.

The interaction of TNFa with CD95/Apo1/Fas is directly implicated in the death of cardiomyocytes post IR.[166] 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.[19,20,122] Bennett et al[123] 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.[146] 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 enriched with macrophages. This may be as a result of induction of apoptotic pathways via macrophage TNFa release, reactive oxygen species generation or directly by the production of oxidised low density lipoprotein (LDL).[124] LDL penetration of the intima is an initiating pathogenic effect in atherosclerosis.[125,126] 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.[127] 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.[128]

Advanced human atherosclerotic plaques contain sclerotic hypocellular regions. This has led to the suggestion that these areas arise due to apoptotic cell death.[19] 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.[129] 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.[145] Dong et al have also shown Fas-mediated apoptotic cell death to be associated with post-transplant coronary artery disease.[139]

Apoptosis in the vascular endothelium

The endothelial cell fulfils an interactive role inhibiting proliferation of adjoining cells, thrombus formation and leucocyte 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 being displaced from its normal environment.[130] The death of endothelial cells after detachment is beneficial as it prevents detached or abnormal cells from reattaching and growing in a dysplastic fashion.[131]

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.[20,132] 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.[133,134]

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.[106,134] The rate-limiting factor in this context is smooth muscle cell deletion by apoptosis, which intercedes to control cell numbers.[115] The inference, therefore, is that this apoptotic control allows neointimal thickness to remain constant despite a continued smooth muscle cell proliferation response to injury.[135] 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.[82] In neointimal hyperplasia, proto-oncogene controls of apoptosis act as molecular thermostats balancing cell death versus proliferation, which determines cell numbers and survival.[81]

Apoptosis is observed more frequently in restenotic than primary atherosclerotic lesions.[20] 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.[136] 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.[20,37,137]

An even greater frequency of smooth muscle cell proliferation and apoptosis is seen in neointimal hyperplasia complicating stent restenosis.[20,138,148] 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 after angioplasty in an atheromatous rabbit model by inducing apoptosis of smooth muscle cells.[139]

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[140] and late[141,168] in reperfusion as a part of IR injury. The mechanisms involved in these processes are gradually being elucidated in the myocardium and endothelium.[142-144]

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.

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References

1. Kerr JRF, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide ranging implications in tissue cell kinetics. *Br J Cancer* 1972; 26: 239-57.
2. Cheng W, Li B, Kajstura J et al. Stretch induced programmed myocyte cell death. *J Clin Invest* 1995; 96: 2247-59.
3. Beltrami CA, Finato R, Rocco M et al. The cellular basis of dilated cardiomyopathy in humans. *J Mol Cell Cardiol* 1995; 27: 291-305.
4. Olivetti G, Quaini F, Sala R et al. Acute myocardial infarction in humans is associated with activation of programmed myocyte cell death in the surviving portion of the heart. *J Moll Cell Cardiol* 1996; 28: 2005-16.
5. Wyllie A. Apoptosis: an overview. *Br Med Bull* 1997; 53: 451-65.
6. Ballard KJ, Holt SJ. Cytological and cytochemical studies on cell death and digestion in the foetal rat foot: the role of macrophages and hydrolytic enzymes. *J Cell Sci* 1968; 3: 245-62.
7. Bursch W, Oberhammer F, Schulte-Hermann. Cell death by apoptosis and its protective role against disease. *TiPS* 1992; 13: 245-51.
8. Lyons SK, Clarke AR. Apoptosis and carcinogenesis. *Br Med Bull* 1997; 52: 554-69.
9. Williams G. Programmed cell death: apoptosis and oncogenesis. *Cell* 1991; 65: 1097-8.
10. Harmon B, Takano Y, Winterford C, Gobe G. The rôle of apoptosis in the response of cells and tumours to mild hyperthermia. *Int J Radiat Biol* 1991; 59: 489-501.
11. Williams G. Programmed cell death: apoptosis and oncogenesis. *Cell* 1991; 65: 1097-8.
12. Barry M, Behnke C, Eastman A. Activation of programmed cell death (apoptosis) by cisplatin, other anti-cancer drugs and hyperthermia. *Biochem Pharmacol* 1990; 40: 2353-62.
13. Clutton S. The importance of oxidative stress in apoptosis. *Br Med Bull* 1997; 53: 662-8.
14. Gottlieb RA, Burleson K, Kloner R, Babior B, Engler R. Reperfusion injury induces apoptosis in rabbit cardiomyocytes. *J Clin Invest* 1994; 94: 1621-8.
15. Colucci W. Apoptosis in the heart. *New Engl J Med* 1996; 335: 1224-6.
16. Olivetti G, Abbi R, Quaini F. Apoptosis and the failing human heart. *New Engl J Med* 1996; 336: 1131-41.
17. Olivetti G, Melissari M, Balbi T et al. Myocyte nuclear and possible cellular hyperplasia contribute to ventricular remodeling in the hypertrophic senescent heart in humans. *J Am Coll Cardiol* 1994; 24: 140-9.
18. Kajstura J, Cheng W, Reiss K et al. Apoptotic and necrotic cell deaths are independent contributing variables of infarct size

in rats. *Lab Invest* 1996; 74: 86-107.
19. Bjorkerud S, Bjorkerud B. Apoptosis is abundant in human atherosclerotic lesions especially in inflammatory cells and may contribute to the accumulation of gruel and plaque instability. *Am J Pathol* 1996; 149: 367-79.
20. Isner J, Kearney M, Bortman S, Passeri J. Apoptosis in human atherosclerosis and restenosis. *Circulation* 1995; 91: 2703-11.
21. Quaini F, Cigola E, Sala R et al. Apoptosis in the infarcted human heart. *BAM* 1996; 6: 241-9.
22. Arends MJ, Mords RG, Wyllie AH. Apoptosis; The role of the endonuclease. *Am J Pathol* 1990; 136: 593-608.
23. Krown K, Page MT, Nguyen C et al. Tumour necrosis factor alpha induced apoptosis in cardiac myocytes. *J Clin Invest* 1996; 98: 2854-65.
24. Ostling O, Johanson JP. Microelectrophoretic study of radiation induced DNA damage in mammalian cells. *Biochem Biophys Res Commun* 1984; 123: 291-8.
25. Fairbairn DW, Olive PL, O'Neill KL. The comet assay: a comprehensive review. *Mutat Res* 1995; 339: 37-59.
26. Gorczyca W, Gong J, Ardelt B, Traganos F, Darzynkiewicz Z. The cell cycle related differences in susceptibility of HL-60 cells to apoptosis induced by various antitumor agents. *Cancer Res* 1993; 53 (13): 3186-92.
27. Gold R. Differentiation between cellular apoptosis and necrosis by the combined use of in situ tailing and nick translation techniques. *Lab Invest* 1994; 71 (2): 219-25.
28. Meisenholder GW, Martin SJ, Green DR et al. Events in apoptosis. Acidification is downstream of protease activation and BCL-2 protection. *J Biol Chem* 1996; 271 (27): 16260-2.
29. Ytrehus K, Liu Y, Downey JM. Preconditioning protects ischemic rabbit heart by protein kinase C activation. *Am J Physiol* 1994; 266 (3 Pt 2): H1145-52.
30. Gottlieb RA, Gruol DL, Zhu JY, Engler RL. Preconditioning rabbit cardiomyocytes: role of pH, vacuolar proton ATPase, and apoptosis. *J Clin Invest* 1996; 97 (10): 2391-8.
31. Diaz C, Schroit AJ. Role of translocases in the generation of phosphatidylserine asymmetry. *J Membrane Biol* 1996; 151: 1-9.
32. Fadok VA, Savill JS, Haslet C et al. Different populations of macrophages use either the vitronectin receptor or the phosphatidylserine receptor to recognise and remove apoptotic cells. *J Immunol* 1992; 149: 4029-35.
33. Fadok VA, Voeiker DR, Campbell PA et al. Exposure of phosphatidylserine on the surface of lymphocytes triggers specific recognition and removal by macrophages. *J Immunol* 1992; 148: 2207-16.
34. Andree HAM, Reutelingsperger CPM, Hauptinan R et al. Binding of vascular anticoagulant to planar phospholipid bilayers. *J Biol Chem* 1990; 265: 4923-8.
35. Flynn PD, Byrne CD, Baglin TP, Weissberg PL, Bennett MR. Thrombin generation by apoptotic vascular smooth muscle cells. *Blood* 1997; 89: 4278-84.
36. Bebbis M. Studies on cell agony and death: an attempt at classification. In: Ciba Foundation symposium on cellular injury. Eds. AVS Dereuck, J Knight. J&A Churchill, London, 1964: 287-328.
37. Bebbis MC. Death of a cell. New York, NY: Swift motion picture laboratories, 1958.
38. Entman ML, Michael L, Rossen RD et al. Inflammation in the course of early myocardial ischemia. *FASEB J* 1991; 5: 2529-37.
39. Thompson CB. Apoptosis in the pathogenesis and treatment of disease. *Science* 1995; 267: 1465.
40. Narula J, Haider N, Virmani R. Apoptosis in myocytes in end stage heart failure. *New Engl J Med* 1996; 335: 1182-9.
41. Hengartner MO, Horvitz HR. The ins and outs of programmed cell death during *C. Elegans* development. *Phil Trans R Soc Lond B* 1994; 345: 243-8.
42. Ellis HM, Horvitz VR. Genetic control of programmed cell death in the nematode *C. elegans*. *Cell* 1986; 44: 817-29.
43. Hengartner MO, Horvitz HR. C. elegans survival gene ced-9 encodes a functional homologue of the mammalian proto-oncogene bcl-2. *Cell* 1994; 76: 665-76.
44. Wright SC, Wei QS, Zhong J et al. Purification of a 24-kD protease from apoptosis tumour cells that activates DNA fragmentation. *J Exp Med* 1994; 180: 2113-23.
45. Longthorne VL, Williams GT. Caspase activation is required for commitment to Fas-mediated apoptosis. *EMBO J* 1997; 16: 3805-12.
46. Chinnaiyan AM, O'Rourke K, Yu GL et al. Signal transduction by DR3, a death domain containing receptor related to TNFR1 and CD95. *Science* 1996; 274: 990-2.
47. Chinnaiyan AM, O'Rourke K, Tewari M, Dixit VM. FADD, a novel death domain containing protein interacts with the death domain of Fas and initiates apoptosis. *Cell* 1995; 81: 505-12.
48. Kitson J, Raven T, Jiang YP et al. A death domain containing receptor that mediates apoptosis. *Nature* 1996; 384: 372-5.
49. Pan G, O'Rourke K, Chinnaiyan AM et al. The receptor for the cytotoxic ligand TRAIL. *Science* 1997; 276: 111-3.
50. Enari M, Talanian RV, Wong WW, Nagata S. Sequential activation of ICE-like and CPP32-like proteases during Fas-mediated apoptosis. *Nature* 1996; 380: 723-6.
51. Brown R. The BCL-2 family of proteins. *Br Med Bull* 1997; 53 (3): 466-77.
52. Tsujimoto Y, Finger LR, Yunis J, Nowell PC, Croce C. Cloning of the chromosome breakpoint of neoplastic B cells with the t(14;18) chromosome translocation. *Science* 1984; 226: 1097-9.
53. Ellis HM, Horvitz VR. Genetic control of programmed cell death in the nematode *C. elegans*. *Cell* 1986; 44: 817-29.
54. Walton MI, Whysong D, O'Connor PM. Constitutive expression of human bcl-2 modulates nitrogen mustard and campothecin induced apoptosis. *Cancer Res* 1993; 53: 1853-61.
55. Miyashita T, Reed JC. Bcl-2 oncoprotein blocks chemotherapy induced apoptosis in a human leukemic cell line. *Blood* 1993; 81: 151-7.
56. Kajstura J, Mansukhani M, Cheng W et al. Programmed cell death and expression of the proto-oncogene bcl-2 in myocytes during post-natal maturation of the heart. *Exp Cell Res* 1995; 219: 110-21.
57. Yin X-M, Oltvai ZN, Korsmeyer SJ. BH1 and BH2 domains of Bcl-2 are required for inhibition of apoptosis and heterodimerisation with Bax. *Nature* 1994; 369: 321-3.
58. Liu X, Kim CN, Yang J, Jemmerson R, Wang X. Induction of apoptotic program in cell free extracts: requirements for dATP and cytochrome c. *Cell* 1996; 86: 147-57.
59. Zamzami N, Susin SA, Marchetti P et al. Mitochondrial control of nuclear apoptosis. *J Exp Med* 1996; 184: 1331-41.
60. Chinnaiyan AM, Orth K, O'Rourke K et al. Molecular ordering of the cell death pathway: Bcl-2 and bcl-xl function upstream of the CED-3 like apoptotic proteases. *J Biol Chem* 1996; 271(9): 4573-6.
61. Armstrong RC, Aja T, Xiang J et al. Fas induced activation of the cell death related protease CPP32 is inhibited by Bcl-2 and by ICE family proteases. *J Biol Chem* 1996; 271: 16850-5.
62. Susin SA, Zamzami N, Castedo M et al. Bcl-2 inhibits the mitochondrial release of an apoptogenic protease. *J Exp Med* 1996; 184: 1331-41.

63. Kluck RM, Bossy-Wetzel E, Green DR, Newmeyer DD. The release of cytochrome c from mitochondria: a primary site for Bcl-2 regulation of apoptosis. *Science* 1997; 275: 1132-6.
64. Hockenbury DM, Oltavai ZN, Yin XM. Bcl-2 functions in an anti-oxidant pathway to prevent apoptosis. *Cell* 1993; 75: 241-51.
65. Jacobson MD, Raff MC. Programmed cell death and bcl-2 protection in very low oxygen. *Nature* 1995; 374: 814-6.
66. Shimizu S, Eguchi Y, Kosaka H et al. Prevention of hypoxia induced cell death by bcl-2 and bcl-XL. *Nature* 1995; 374: 811-3.
67. Boyd JM, Gallo GJ, Elangovan B. Bik, a novel death inducing protein shares a distinct structural motif with bcl-2 family proteins and interacts with viral and cellular survival promoting proteins. *Oncogene* 1995; 11: 1921-8.
68. Yang E, Korsmeyer SJ. Molecular thanatopsis: a discourse on the bcl-2 family and cell death. Blood 1996; 88: 386-401.
69. Reed JC. Regulation of apoptosis by bcl-2 family proteins and its role in cancer and chemoresistance. *Curr Opin Oncol* 1995; 7: 541-6.
70. Oltavi ZN, Korsmeyer SJ. Checkpoints of dueling dimers foil cell death. *Cell* 1994; 79: 189-92.
71. Pearlman H, Maillard L, Krasinski K, Walsh K. Evidence for the rapid onset of apoptosis in medial smooth muscle cells after balloon injury. *Circulation* 1997; 95: 981-7.
72. Clarke AR, Purdie CA, Harrison DJ et al. Stabilised p53 facilitates ancuploid clonal divergence in colorectal cancer. *Nature* 1993; 362: 849-52.
73. Long X, Boluyt MO, Hipolito M et al. p53 and the hypoxia induced apoptosis of cultured neonatal rat cardiac myocytes. *J Clin Invest* 1997; 99: 2635-43.
74. Kirshenbaum LA, Schneider MD. Adenovirus E1A represses cardiac gene transcription and reactivates DNA synthesis in ventricular cardiomyocytes, via alternate pocket protein and p300 binding domains. *J Biol Chem* 1995; 270: 7791-4.
75. Agah R, Kirshenbaum LA, Abdellatif M et al. Adenoviral delivery of E2F-1 directs cell cycle re-entry and p53 independent apoptosis in post-mitotic adult cardiomyocytes in vitro. *J Clin Invest* 1997; 100: 2722-8.
76. Bialik S, Geenen DL, Sasson IE et al. Myocyte apoptosis during acute myocardial infarction in the mouse localizes to hypoxic regions but occurs independently of p53. *J Clin Invest* 1997; 100: 1363-72.
77. Kirshenbaum LA, de Moissac D. The bcl-2 gene product prevents programmed cell death of ventricular myocytes. *Circulation* 1997; 96: 1580-5.
78. Kirshenbaum LA. Regulators of apoptosis in the heart: a matter of life or death. *Can J Cardiol* 1998; 14: 457-60.
79. Furchgott RF. The discovery of endothelium-derived relaxing factor and its importance in the identification of nitric oxide. *JAMA* 1996; 276(14): 1186-8.
80. Vane JR, Botting RM. The role of chemical mediators released by the endothelium in the control of the cardiovascular system. *Int J Tissue React* 1992; 14(2): 55-64.
81. Balligand J-L, Cannon P. Nitric oxide synthases and cardiac muscle: autocrine and paracrine influences. *Arterioscler Thromb Vasc Biol* 1997; 17: 1864-8.
82. Yang X, Chowdhury N, Cai B et al. Induction of myocardial nitric oxide synthase by cardiac allograft rejection. *J Clin Invest* 1994; 94: 714-21.
83. Szabolcs M, Michler R, Robert E et al. Cellular and molecular cardiovascular disease: apoptosis of cardiac myocytes during cardiac allograft rejection: relation to induction of nitric oxide synthase. *Circulation* 1996; 94: 1665-73.
84. Pinsky DJ, Cai B, Yang X et al. The lethal effects of cytokine induced nitric oxide in cardiac myocytes are blocked by nitric oxide synthase antagonism or transforming growth factor beta. *J Clin Invest* 1995; 95: 677-85.
85. Kawaguchi K, Shin WS, Wang Y et al. In vivo gene transfection of human endothelial cell nitric oxide synthase in cardiomyocytes causes apoptosis like cell death; Identification using Sendai virus coated particles. *Circulation* 1997; 95: 2441-7.
86. Shin WS, Hong Y-U, Peng H-B et al. Nitric oxide attenuates vascular smooth muscle activation by interferon gamma. *J Biol Chem* 1996; 271: 11317-24.
87. Stellar H. Mechanisms and genes of cellular suicide. *Science* 1995; 267: 1445-62.
88. Lawrence R, Chang L-J, Siebenlist U, Bressier P, Sonenshein GE. Vascular smooth muscle cells express a constitutive NF-kB like activity. *J Biol Chem* 1994; 269: 28913-8.
89. Obata H, Biro S, Arima N et al. NF-kB is induced in the nuclei of cultured rat aortic smooth muscle cells by stimulation of various growth factors. *Biochem Biophys Res Commun* 1996; 224: 27-32.
90. Bellas RE, Lee JS, Sonenshein GE. Expression of an NF-kB like activity is essential for proliferation of cultured bovine vascular smooth muscle cells. *J Clin Invest* 1995; 96: 2521-7.
91. Brand K, Page S, Rogler G et al. Activated transcription factor nuclear factor-kB is present in the atherosclerotic lesion. *J Clin Invest* 1996; 97: 1715-22.
92. Lindner V, Collins T. Expression of NF-kB and Ik-B by aortic endothelium in an arterial injury model. *Am J Pathol* 1996; 148: 427-38.
93. Laster SM, Wood JG, Goodling LR. Tumour necrosis factor can induce both apoptotic and necrotic forms of cell lysis. *J Immunol* 1988; 141: 2629-43.
94. Sharov VG, Sabbah HN, Shimoyama H et al. Evidence of cardiocyte apoptosis in myocardium of dogs with chronic heart failure. *Am J Pathol* 1996; 148: 141-9.
95. Torre-Amione G, Kapadia S, Lee J. Tumour necrosis factor-(and tumour necrosis factor receptor in the failing human heart. *Circulation* 1996; 93: 704-11.
96. Yamada T, Wang WZ, Shioi T. Apoptosis in congestive heart failure induced by viral myocarditis in mice. *Circulation* 1996; 94: 1-32.
97. Takeda K, Yu ZX, Nishiwaka T. Apoptosis and DNA fragmentation in the bulbis cordis of the developing rat heart. *J Mol Cell Cardiol* 1996; 28: 209-15.
98. James TN. Normal and abnormal consequences of apoptosis in the human heart: from post-natal morphogenesis to paroxysmal arrhythmias. *Circulation* 1994; 90: 556-73.
99. Cho A, Courtman DW, Langelli BC. Apoptosis in arteries of the neonatal lamb. *Circ Res* 1995; 76: 168-75.
100. Cliff WJ. The tunica media in aging rats. *Exp Mol Pathol* 1970; 13: 172-7.
101. Han DKM, Haudenschild CC, Hong MK. Evidence for apoptosis in human atherogenesis and in a rat vascular injury model. *Am J Pathol* 1995; 147: 267-77.
102. Parks DA, Granger DN. Contributions of ischaemia and reperfusion to mucosal lesion formation. *Am J Physiol* 1986; 250: 749-53.
103. Sies H. Introduction. Oxidative stress. *Academic Press* 1985; 1-7.
104. Kloner RA. Does reperfusion injury exist in humans. *J Am Coll Cardiol* 1993; 21: 537-45.
105. Cheng W, Li B, Kajstura J. Stretch induced programmed myocyte cell death. *J Clin Invest* 1995; 96: 2247-59.
106. Barinaga M. Forging a path to cell death. *Science* 1996; 273: 735-7.
107. Seta Y, Shan K, Bozkurt B. Basic mechanisms in heart fail-

ure: the cytokine hypothesis. *J Cardiac Failure* 1996; 2: 243-9.
108. Saraste A, Pulkki K, Kallajoki M et al. Apoptosis in human myocardial infarction. *Circulation* 1997; 95: 320-3.
109. Hirose K, Longo DL, Oppenheim JJ, Matsushima K. Overexpression of manganese superoxide dismutase promotes the survival of tumour cells exposed to interleukin 1, tumour necrosis factor selected anti-cancer drugs and ionising radiation. *FASEB J* 1993; 7: 361-8.
110. Forrest VJ, Kang YH, McClain DE. Oxidative stress induced apoptosis prevented by Trolox. *Free Radic Biol Med* 1994; 16: 675-84.
111. Wang GH, Redmond HP, Watson RWG, Bouchier-Hayes D. Cellular mechanisms of endothelial cell death during the systemic inflammatory response syndrome. *Surg Forum* 1994; 80: 110-2.
112. Teiger E, Than TV, Richard L. Apoptosis in pressure overload induced heart hypertrophy in the rat. *J Clin Invest* 1996; 97: 2891-7.
113. Hamet P, Richard L, Dam TV et al. Apoptosis in target organs of hypertension. *Hypertension* 1995; 26: 642-8.
114. Leri A, Claudio PP, Qiong L. Stretch mediated release of angiotensin II induces myocyte apoptosis by activating p53 that enhances the local renin angiotensin system and decreases the bcl-2 to bax protein ratio in the cell. *J Clin Invest* 1998; 101: 1326-42.
115. Kajstura J, Cigola E, Malhotra A et al. Angiotensin II induces apoptosis of adult ventricular myocytes in vitro. *J Moll Cell Cardiol* 1997; 29: 859-70.
116. Mallat Z, Tedgui A, Fontaliran F et al. Evidence of apoptosis in arrhythmogenic right ventricular failure. *New Engl J Med* 1996; 335: 1190-6.
117. Herskowitz A, Choi S, Ansari AA. Cytokine mRNA expression in post-ischaemic/reperfused myocardium. *Am J Pathol 1995*; 146: 419-28.
118. Ikeda U, Ohkawa F, Seino Y. Serum interleukin-6 levels become elevated in acute myocardial infarction. *J Moll Cell Cardiol* 1993; 24: 579-84.
119. Levine B, Kalman J, Mayer L. Elevated circulating levels of tumour necrosis factor-alpha in severe chronic heart failure. *New Engl J Med* 1990; 323: 236-41.
120. Aikawa R, Komuro I, Yamazaki T et al. Oxidative stress activates extracellular signal-regulated kinases through src and ras in cultured cardiac myocytes of neonatal rats. *J Clin Invest* 1997; 100: 1813-21.
121. Jeroudi MO, Hartley CJ, Bolli R. Myocardial reperfusion injury: role of oxygen radicals and potential therapy with antioxidants. *Am J Cardiol* 1994; 73: 1621-8.
122. Geng YJ, Libby P. Evidence for apoptosis in advanced human atheroma: co-localization with interleukin-1-(-converting enzyme. *Am J Pathol 1995*; 147: 251-66.
123. Bennett M, Evan G, Schwartz S. Apoptosis of human vascular smooth muscle cells derived from normal vessels and coronary atherosclerotic plaques. *Clin Invest* 1995; 95: 2266-74.
124. Escargueil-Blanc I, Salvayre R, Negre-Salvayre A. Necrosis and apoptosis induced by oxidized low density lipoproteins occurs through two calcium dependant pathways in lymphoblastoid pathways. *FASEB J* 1994; 8: 1075-80.
125. Witztum JL, Steinberg D. Role of oxidized low density lipoprotein in atherogenesis. *J Clin Invest* 1991; 88: 1785-92.
126. Witztum JL. The oxidation hypothesis of atherosclerosis. *Lancet* 1994; 793-5.
127. Dimmeler S, Haendeler J, Nehls M, Zeiher AM. Suppression of apoptosis by nitric oxide via inhibition of interleukin-1beta converting enzyme(ICE)-like and cysteine protease protein (CPP)-32-like proteases. *J Exp Med* 1997; 185: 601-7.
128. Aji W, Ravalli S, Szabolcs M et al. L-arginine prevents xanthoma development and inhibits atherosclerosis in LDL receptor knockout mice. *Circulation* 1997; 95: 430-7.
129. Isner JM, Kearney M, Berdan LG et al. Core pathology lab findings in 425 patients undergoing directional atherectomy for a primary coronary artery stenosis and relationship to subsequent outcome: the CAVEAT study. *J Am Coll Cardiol* 1993; 21: 380.
130. Frish S, Ruoslahti E. Integrins and anoikis. *Curr Opin Cell Biol* 1997; 9: 701-6.
131. Frish SM, Francisa H. Disruption of endothelial cell matrix interactions induces apoptosis. *J Cell Biol* 1994; 124: 619-26.
132. Pickering JG, Weir L, Jekanowski J. Proliferative activity in peripheral and coronary atherosclerotic plaque among patients undergoing percutaneous revascularization. *J Clin Invest* 1993; 91: 1469-80.
133. DeMeester SL, Buchman TG, Qiu Y et al. Heat shock induces IkappaB-alpha and prevents stress-induced endothelial cell apoptosis. *Arch Surg* 1997; 132(12): 1283-7.
134. Clowes AW, Reidy MA, Clowes MM. Kinetics of cellular proliferation after arterial injury, I: smooth muscle growth in the absence of endothelium. *Lab Invest* 1983; 49: 327-33.
135. Obata H, Biro S, Arima N et al. NF-kB is induced in the nuclei of cultured rat aortic smooth muscle cells by stimulation of various growth factors. *Biochem Biophys Res Commun* 1996; 224: 27-32.
136. James TN. Complex causes of fatal myocardial infarction. *Circulation* 1997; 2: 96(5): 1696-700.
137. Isner JM, Kearney M, Bauters C. Use of human tissue specimens obtained by directional atherectomy to study restenosis. *Trends Cardiovasc Med* 1994; 4: 213-21.
138. Kearney M, Pieczek A, Haley L et al. Histopathology of instent restenosis in patients with peripheral artery disease. *Circulation* 1997; 95: 1998-2002.
139. Steg PG, Tahlil O, Aubailly N. Reduction of restenosis after angioplasty in an atheromatous rabbit model by suicide gene therapy. *Circulation* 1997; 96(2): 408-11.
140. Jeremias I, Kupatt C, Martin-Villalba A et al. Involvement of CD95/Apo-1/Fas in cell death after myocardial infarction. *Circulation* 2000; 102: 915-1002.
141. Daemen M, Van'tVeer C, Denecker G. Inhibition of apoptosis induced by ischaemia reperfusion prevents inflammation. *J Clin Invest* 1999; 104: 541-9.
142. Wollert K, Heineke J, Westermann J et al. The cardiac Fas (APO-1/CD95) receptor/Fas ligand system. *Circulation* 2000; 101: 1172-81.
143. Kupatt C, Habazettl H, Goedecke A. Tumour necrosis factor-(contributes to ischaemia- and reperfusion induced endothelial dysfunction in isolated hearts. *Circ Res* 1999; 84: 392-400.
144. Wkaasugi K, Schimmel P. Two distinct cytokines released from a human aminoacyl-t-RNA synthase. *Science* 1999; 284: 147-50.
145. Daemen M, Van'tVeer C, Heineman E. Pro and anti-inflammatory mechanisms in renal reperfusion in mice: modulation by endogenous tumour necrosis factor alpha and interleukin 10. *Transplantation* 1999; 67: 792-800.

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