Review

Apoptotic cell death in heart failure

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1. Introduction

Progressive deterioration of left ventricular (LV) function is a characteristic feature of the failing heart. This hemodynamic deterioration, often accompanied by worsening of clinical symptoms, occurs despite the absence of clinically apparent intercurrent adverse events [1,2] and, invariably, culminates in the syndrome of congestive heart failure. The exact mechanisms that drive this process are not known. For years, a so-called ‘vicious circle’ postulate was adopted to explain this phenomenon whereby compensatory mechanisms, elicited to maintain homeostasis, themselves become factors that exacerbate the heart failure state. Such compensatory mechanisms included LV hypertrophy, LV chamber dilation, and enhanced and sustained activity of the sympathetic nervous system and renin–angiotensin system [3–6]. In recent years, we and others have put forward a working hypothesis that progressive LV dysfunction in heart failure may result, in part, from ongoing loss of cardiomyocytes. The notion that progression of heart failure may be due, in part, to ongoing loss of functional cardiac units was based on the presence of ultrastructural degenerative changes of cardiomyocytes in the failed human heart as well as in hearts of animals with experimentally-induced heart failure [7–10]. Structural abnormalities included myofibrillar disruption and disarray [8], abnormalities of mitochondria characterized by disruption of the internal and external membranes, hyperplasia and reduced organelle size [7], and abnormalities of the cardiac interstitium characterized by accumulation of collagen [9]. These observations provided some support, albeit indirect, to the concept that ongoing myocyte degeneration and loss may occur in the failing heart. Objective evidence in support of this concept, however, was only recently put forward [11]. Studies in dogs with heart failure produced by intracoronary microembolization showed, for the first time, that progressive LV dysfunction was associated with increased volume fraction of replacement fibrosis [11] or, in other words, increased proportion of scarred to viable myocardium suggesting ongoing myocyte loss. While these studies provided support to the concept of ongoing loss of viable myocardium in the failing heart, the means by which myocytes were lost was not identified. Even before these studies where completed, investigations in dogs with heart failure [12] and in explanted failed human hearts [13,14] showed that cardiomyocyte death through apoptosis occurs in advanced heart failure, a finding that supports the original working hypothesis. In this review, results of research in experimental animals as well as in humans on the identification of cardiomyocyte apoptosis in heart failure will be discussed. The discussion will also address potential molecular and pathophysiological triggers that may drive this process of cell death in the failing heart. Finally, an attempt will be made to address a central theme of whether cardiomyocyte apoptosis plays an important role in the progression of LV dysfunction that is characteristic of the heart failure state.

Unlike necrosis, apoptosis is an active, precisely regulated, energy requiring process which appears to be orchestrated by a genetic program [15] and hence the interchangeable use of the terms ‘apoptosis’ and ‘programmed cell death’. Apoptosis plays a crucial role in the regulation of proliferating cell populations in adult tissues and in normal tissue development [16,17]. Cells such as neurons and cardiac myocytes, even though terminally differentiated contain the genes and signal transduction pathways necessary for programmed cell death and thus retain the ability to die by apoptosis [18]. In humans and

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other mammals, adult cardiac myocytes are thought to have, at best, a very limited capacity for self-renewal [19], and are intended to survive and actively function for the entire life of the organism. Viewed from this perspective, death of a significant number of adult cardiac muscle cells can have lasting adverse consequences on overall cardiac performance.

2. Cardiomyocyte apoptosis and predisposing factors for heart failure

2.1. Myocardial infarction

Myocardial ischemia and infarction represent the major etiologies that underscore the development of congestive heart failure. Cardiomyocyte loss secondary to prolonged ischemia has long been thought to result from overt necrosis. While this form of cell death remains a primary cause of tissue injury, recent studies have suggested that cardiomyocyte loss after acute myocardial infarction can also be caused by apoptosis [20±25]. In studies in rats with myocardial infarction, internucleosomal DNA fragmentation, evidenced by DNA laddering on agarose gel electrophoresis, was detected as early as 3 h after coronary artery occlusion and was also present for up to 1 month after coronary ligation primarily in regions adjacent to infarcted tissue and to a lesser extent in myocardial regions remote from the infarction [22]. Cardiomyocyte apoptosis has also been observed in humans following acute myocardial infarction [23,24]. In hearts obtained from patients with acute myocardial infarction who died within 10 days of the onset of symptoms, Olivetti et al. [24] reported DNA strand breaks suggestive of apoptosis in 12% of myocytes in the infarct border zone and in 1% of constituent myocytes of myocardial regions remote from the infarction. In a study by Saraste et al. [23], hearts from patients who died of an acute myocardial infarction and had patent infarct-related arteries at autopsy, also showed extensive DNA strand breaks in cardiomyocytes. In this same study, apoptotic cardiomyocytes were observed primarily in myocardial regions that bordered the infarction [24]. As will be discussed in subsequent parts of this review, the observation of a high prevalence of cardiomyocyte apoptosis in the peri-infarct border region in comparison to myocardial regions remote from the infarction is also evident in myocardium of both humans with chronic heart failure secondary to ischemic cardiomyopathy as well as in animal models of chronic heart failure produced by intracoronary microembolizations [12,26].

2.2. Ventricular hypertrophy

Heart failure can result from sustained pressure overload as in long-standing hypertension or aortic valvular stenosis. Ventricular hypertrophy is associated with loss of cardiomyocytes that result in focal sites of replacement fibrosis traditionally attributed to necrosis [27]. Recent studies, however, have shown that experimentally-induced LV hypertrophy is associated with myocyte apoptosis [28±30]. In rats with LV hypertrophy produced by aortic banding, Teiger et al. [28] identified myocyte apoptosis during the first 7 days after instituting aortic banding. Studies from other laboratories suggested that cardiomyocyte apoptosis may be important in the transition from compensated hypertrophy to heart failure [29]. In spontaneously hypertensive rats (SHR) with symptoms of heart failure, Li et al. [29] showed a near five-fold increase in the number of cardiac myocytes undergoing apoptosis compared to nonfailing SHR rats. In this rat model, the transition to heart failure was accompanied by features characteristic of the heart failure state including cardiac pump dysfunction [31], myocardial fibrosis [32], and reduction in the volume fraction of cardiac myocytes [33]. The incidence of apoptotic myocyte nuclei in failed SHR was ~40 cells per 100 000 nuclei compared to ~8 per 100 000 nuclei in non-failing SHR rats. In age-matched WKY rats the incidence of apoptotic nuclei of myocyte origin was ~2 per cells per 100 000 nuclei [29]. These results, while interesting, do not establish a cause and effect relationship between apoptosis and transition to heart failure.

2.3. Ventricular dilatation

Left ventricular chamber enlargement is a characteristic adaptation of the failing heart regardless of etiology. Chronic ventricular enlargement and failure can result from long-standing volume overload as in aortic or mitral valve insufficiency or the development of large conduit vessel arterio-venous fistulas. As with ventricular hypertrophy, LV chamber dilation is associated with loss of cardiac myocytes that result in focal sites of fibrosis. Recent studies have shown that passive myocardial stretch is also associated with cardiomyocyte apoptosis [34]. In vitro studies by Cheng et al. [34] showed a 21-fold higher incidence of myocyte DNA strand breaks in rat posterior papillary muscles exposed to high tension levels as a result of overstretch compared to papillary muscles exposed to lower tension levels. DNA laddering studies using extracts from muscles exposed to high stretch also revealed the presence of DNA fragments consistent with apoptosis while degradation of DNA was not observed in non-overstretched papillary muscles [34]. These data suggest that myocardial stretch alone, as can occur under conditions of acute or chronic volume overload, may be associated with cardiomyocyte loss through apoptosis.

2.4. Autoimmunity

In inflammatory heart muscle disease, autoimmunity is considered to play a role in the pathogenesis of impaired
cardiac performance [35]. Marked depression of cardiac function occurs in patients with dilated cardiomyopathy in the absence of extensive loss of viable myocardium. In a subset of this population, the etiology and pathogenesis points to an inflammatory origin as in myocarditis. Under such circumstances, the immune response to the invading pathogen is the major determinant of the severity, course and progression of the disease. Secretory products of immune cells such as macrophages and other infiltrating cells could well contribute to abnormalities of contraction and relaxation that are seen, for instance, in myocarditis [35]. Proinflammatory cytokines such as tissue necrosis factor-α (TNF-α), interleukin (IL)-1, IL-2 and IL-6 are antigen-nonspecific glycoproteins that are synthesized rapidly and released locally by immune cells in response to injury [36]. Cytokines have been shown to reduce the positive inotropic response of isolated cardiac myocytes to adrenergic agonists [37]. TNF-α and IL-1 have also been shown to uncouple agonist-occupied receptors from adenylate cyclase in isolated cardiac myocytes [37]. Of particular importance to the present discussion is the potential role for proinflammatory cytokines, and in particular TNF-α, in the induction of cardiomyocyte apoptosis [38]. TNF-α is overexpressed in patients with heart failure regardless of etiology [39]. Plasma levels of TNF-α were shown to be elevated in patients with NYHA class I–III heart failure compared with age-matched controls and were progressively elevated in relation to decreasing functional status [40]. Transgenic mice with cardiac-specific overexpression of TNF-α have been shown to manifest lymphohistocytic myocarditis, cardiomegaly and congestive heart failure [41]. In this transgenic mouse, overexpression of TNF-α was associated with increased incidence of cardiomyocyte apoptosis shown by both DNA laddering and terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) assay. The increased incidence of myocyte apoptosis in this model was also associated with activation of multiple members of the apoptosis pathway that included Fas and Bax [42]. The fact that TNF-α is a potent inducer of apoptosis is not surprising. Cardiomyocytes express functional TNF type-1 receptors and have been shown to undergo apoptosis after stimulation with TNF-α in vitro [38]. Clinical trials are currently underway to determine whether chronic inhibition of TNF-α is beneficial in the treatment of patients with heart failure.

3. Evidence for cardiomyocyte apoptosis in animal models of heart failure

Even though the exact mechanisms that underly the process of progressive LV dysfunction in heart failure is not known, the possibility that this process results, in part, from ongoing loss of viable cardiomyocytes is no longer relegated to the realm of pure speculation. Studies in myocardium of animal models of experimentally-induced heart failure as well as studies in end-stage explanted failed human hearts have provided strong evidence for the existence of cardiomyocyte apoptosis. The very fact that myocyte apoptosis occurs in chronic heart failure, albeit at a pace yet to be defined and with a magnitude yet uncertain, supports the concept of ongoing loss of functional cardiac units. Cardiomyocyte apoptosis was identified in dogs with heart failure produced by multiple sequential intracoronary microembolization [12]; a model that manifests many of the classic sequelae of heart failure seen in humans, including marked and sustained depression of LV systolic function, LV hypertrophy and dilation and enhanced activity of the sympathetic nervous system [43]. In dogs with heart failure, the existence of cardiomyocyte apoptosis was established by uncovering ultrastructural features of this form of cell death as well as by immunohistochemical staining for nuclear DNA strand breaks using the TUNEL assay. Electron microscopic evidence of cardiomyocytes at various stages of apoptosis were identified in LV tissue obtained from dogs with heart failure but not in LV tissue obtained from normal dogs [12]. Features of early stages of apoptosis included compaction of nuclear chromatin into dense masses that abut on the nuclear envelop. In these cells, the inner organelles were preserved and the sarcolemma was intact. Features of cardiomyocytes in advanced stages of apoptosis included an intact sarcolemma in the presence of highly disorganized inner organelles with only remnants of myofibrils and Z-band material that allows the recognition of these bodies as cardiomyocytes in origin. Myocytes in advanced stages of apoptosis also manifested sarcolemmal blebbing or severe endocytosis along with cell shrinkage [12,44]. Apoptotic cardiomyocytes were primarily localized to LV regions bordering old infarcts, were frequently encircled by large amounts collagen and had an intact sarcolemma with absence of associated inflammation, the latter being a key distinguishing feature of apoptosis compared to necrosis [12]. In the same dog model, the number of cardiomyocytes undergoing apoptosis was quantified histomorphometrically based on an assessment of the number of myocytes positively labeled for nuclear DNA strand breaks per 1000 cardiomyocytes [11]. Assessments were made in LV regions bordering old infarcts or scar tissue as well as in LV regions remote from any infarcts. As in previous studies [12], the number of cardiomyocytes undergoing apoptosis was significantly higher in LV regions bordering old infarcts compared to LV regions remote from any infarcts (4.0±0.5 vs. 0.5±0.3 nuclear DNA fragmentation events per 1000 cardiomyocytes). This observation further suggests that in the failing heart, the peri-infarct border region may be a primary site of myocyte loss through apoptosis, a finding consistent with...
the observation of profound ultrastructural abnormalities of cardiomyocyte in these regions [12,45]. Cardiomyocyte apoptosis was also examined in dogs with heart failure produced by rapid ventricular pacing [46]. DNA laddering, with stretches of DNA equivalent to 160 bp and 320 bp being most abundant, was detected in myocardium of dogs with heart failure but not in sham-operated control dogs [46]. Consistent with this finding an average of 3700 myocyte nuclei per million was detected as undergoing apoptosis based on positive dUTP-labeling. Also consistent with observations in the microembolization model of heart failure, groups of apoptotic myocytes were frequently found in proximity of small areas of replacement fibrosis [46].

Age is an independent risk factor for the development of congestive heart failure. Interestingly, the aging process in humans and in animals is also associated with a significant loss of cardiac myocytes [47–49]. It is estimated that the aging process may itself contribute to loss of nearly 30% of all of LV myocytes [48,49]. This ongoing cell loss may be partly responsible for the increased risk of development of ventricular dysfunction and failure in the elderly. Several studies have evoked apoptosis as a contributory process to the overall loss of cardiomyocyte in the aging heart [29,49]. Kajstura et al. [49] used Fischer 344 rats of varying age from 3 to 24 months to quantify cardiomyocyte necrosis and apoptosis. Results showed that in the LV free wall, the extent of both myocyte necrosis and apoptosis increased with increasing age. Cell necrosis, based on in vivo labeling with antimyosin antibody occurred in less than two myocytes/1000 at 3 months of age and increased to nearly 13 myocytes/1000 at 12 months of age. Cardiomyocyte apoptosis was evident in nearly 10 myocytes/million at age 3 and increased to over 80 myocytes/million at 24 months of age. In this rat model, the progressive increase in apoptotic and necrotic cell death was associated with the development of ventricular dysfunction and failure which became clinically apparent between 16 and 24 months of age [49]. An interesting finding of this study was the observation that, in contrast to cell necrosis, which tended to peak early and level off between 12 and 24 months of life, apoptosis tended to increase sharply between 12 and 24 months of life suggesting, perhaps, that triggers of both forms of cell death may be different. This temporal difference between necrosis and apoptosis can also be explained on the basis of formation of islets of scar tissue. It is quite likely that cell necrosis, which preceded apoptosis, may have led to discrete sites of replacement fibrosis and trigger apoptosis in viable myocardial regions that border the fibrous scar as seen in dog models of heart failure described earlier as well as in explanted failed human hearts [12,26,46]. The exact mechanism or mechanisms responsible for apoptosis in peri-infarct regions remain uncertain. One possible explanation is that myocardial regions that border infarcts are susceptible to hypoxia, a physiologic condition believed to promote apoptosis.

4. Evidence for cardiomyocyte apoptosis in humans with heart failure

Studies in tissues obtained from explanted hearts of patients with end-stage heart failure have confirmed the presence of cardiomyocyte apoptosis [13,14,26,50]. Narula et al. [13] examined tissue from seven explanted failed human hearts for evidence of cardiomyocyte apoptosis. Four of seven patients in whom heart failure was due to idiopathic dilated cardiomyopathy (IDC) had immuno- histochemical evidence of cardiomyocyte nuclear DNA fragmentation by TUNEL technique and demonstrated DNA laddering consistent with apoptosis. In three of seven patients with ischemic cardiomyopathy (ICM) [13] one patient had evidence of cardiomyocyte nuclear DNA fragmentation by TUNEL technique and none were positive for apoptosis based on DNA laddering. This small and largely inconclusive study, was followed by a much larger and more convincing study by Olivetti et al. [14]. In tissue samples from eight normal donor hearts, Olivetti and colleagues reported TUNEL positive cardiomyocyte nuclei in only 10 nuclei/million. In 15 failed hearts, this value increased from a minimum of 673 to a maximum of 6549 nuclei/million. There was no difference in the number of apoptotic myocyte nuclei in patients with IDC (2366 nuclei/million, n=5) compared to patients with heart failure due to ICM (2436 nuclei/million, n=9) [14]. In contrast to the results of the study by Narula et al. [13], they showed DNA laddering in myocardium of patients with IDC and ICM [14]. In a more recent study, a higher incidence of cardiomyocyte apoptosis was reported in failed hearts of patients with IDC compared to patients with ICM (0.31 vs. 0.10 apoptotic myocytes/1000) [26]. The higher number of apoptotic myocytes in ICM was due largely to a near three-fold higher incidence of apoptosis in regions bordering old infarcts compared to myocardial regions remote from any infarcts (0.50 vs. 0.06 apoptotic myocytes/1000) [26]. In patients with acromegaly induced cardiomyopathy, Frustaci et al. [51] reported a near 500-fold increase in apoptosis of cardiomyocytes compared to that observed in myocardial tissue samples obtained from papillary muscle of patients undergoing mitral valve replacement. Biochemically, apoptosis is characterized by internucleosomal cleavage of DNA by Ca2+ and Mg2+- dependent endonuclease whose activity increases during apoptosis [52]. Yao et al. [50] showed that deoxyribonuclelease I (DNase I), which is indistinguishable from endonuclease [53], is significantly increased in myocardium of patients with end-stage heart failure compared to myocardium of non-diseased hearts. The above studies in human hearts, when considered in aggregate, strongly suggest that
apoptosis of cardiomyocytes occurs in heart failure regardless of the predisposing factor.

5. Molecular triggers of apoptosis in heart failure

The multigene family of Bcl-2-like proteins, some of which such as Bcl-2 itself inhibits apoptosis and others such as Bax which promote apoptosis is one of the best known regulators of the apoptotic process [54–56]. The ratio of Bcl-2 to Bax, the so-called ‘death switch’ is often used as an indicator of apoptosis. An increase in this ratio is used to signify attenuation of the apoptotic process whereas a decrease in the ratio is used to signify exacerbation of the apoptotic process. In viable myocardium of rats with myocardial infarction and ventricular failure, Cheng et al. [22] reported a decrease in the expression of Bcl-2 and an increase in the expression of Bax, an imbalance that favors apoptosis. In myocardium of SHR with heart failure, expression of Bcl-2 was unchanged compared to non-failing SHR rats [29]. In explanted failed human hearts, Olivetti et al. [14] reported a near doubling of the expression of Bcl-2 in cardiac tissue without changes in the expression of Bax, a situation that favors protection from apoptosis. Another factor involved in apoptosis is the tumor suppressor p53 protein implicated in cell cycle arrest through upregulation of p21/WAF-1, a cyclin-dependent kinase (Cdk) inhibitor [56]. The p53 protein is believed to induce apoptosis in response to DNA damage [57] and other signals such as increased expression of c-myc in a manner independent of cell cycle arrest [58]. In myocardium of SHR rats with heart failure, Li et al. [29] showed a significant increase WAF-1 mRNA levels in comparison to levels in myocardium of non-failing rats.

A family of cysteine proteases known as interleukin-converting enzymes (ICE) more recently referred to as ‘caspases’ have recently taken a front and center seat as primary regulators of apoptosis. Studies in rats with acute myocardial infarction [59,60], have suggested that ICE-like proteases modulate apoptosis based on the ability of certain pharmacologic inhibitors, such as z-VAD-fmk, a non-specific peptide caspase inhibitor, to block the apoptosis process. In a recent study, the expression of caspase-3 was examined in LV tissue obtained from failing human hearts [61]. In this study, caspase-3 was strongly induced in myocytes bordering recent infarcts and to a lesser extent in failing hearts due to dilated cardiomyopathy. Co-localization of caspase-3 with apoptotic cardiomyocytes has been reported in rats following myocardial ischemia and reperfusion [60]. The release of cytochrome c or the release of ‘apoptosis inducing factor’ from mitochondria may be an important pathway for the activation of caspasers with resulting apoptosis in the failing heart [62] (Fig. 1). Cytochrome c release from mitochondria has been shown to precede caspase activation in apoptotic cardiomyocytes during ischemia in the rat [63]. Expression of Bcl-2 appears to prevent activation of the ICE protease cascade [64], possibly by preventing release of cytochrome c. Mitochondrial abnormalities have been described in patients and in dogs with heart failure that include structural disruption of the inner membrane, hyperplasia, and reduced organelle size [7]. In myocardium of dogs with chronic heart failure we have also shown a marked decrease in mitochondrial respiratory parameters compared to normal dogs [65]. While requiring further confirmatory studies, the role of mitochondria in mediating myocyte apoptosis in the failing heart could be central indeed given its crucial in apoptosis of other cell types. A cell surface antigen Fas, a member of the TNF family, is also involved in the regulation of apoptosis by acting as a receptor for the ligand FasL which induces apoptosis; a sequence that has become known as the ‘death domain’. Recent studies have shown that circulating levels of soluble Fas, a molecule that lacks the transmembrane domain of Fas and, therefore, inhibits apoptosis, is increased in patients with congestive heart failure [66,67]. In contrast to these studies, other studies have reported increased circulating levels of soluble Fas ligand (FasL) in patients with congestive heart failure [68]. Soluble FasL is a molecule that promotes binding between Fas and FasL and favors apoptosis.

Abnormal cell cycle events, cell cycle progression in the face of DNA damage and forcing cell cycle reentry in terminally differentiated cells are all potent inducers of apoptosis [69–72]. Cardiac hypertrophy and failure are associated with DNA synthesis in myocytes and upregulation of molecular markers of cell cycle progression [42,73]. An increase in proliferating cell nuclear
antigen (PCNA), a nuclear protein necessary for DNA synthesis and cell cycle progression [74] was reported in myocardium of dogs with heart failure induced by rapid ventricular pacing [46]. Coordination of events that occur during the cell cycle is also dependent on a series of cyclin-dependent kinases (Cdks) that, as active complexes, appear to be important for the progression from G1 to mitosis [75]. Progression through G1 also requires inactivation of several tumor suppressor genes including p53, p21, p16, p15 and p27 and the retinoblastoma gene Rb, that inhibit the kinase activity of the cyclin/Cdk complexes [75]. In a recent study, Burton et al. [76] showed a decrease in the expression of p21 in myocardium of patients with end-stage heart failure compared to normal donor hearts, a finding consistent with initiation of cell cycle activation. Studies by Anversa and Kajstura [77] suggested that adult cardiac myocytes are able to divide and that this capacity increases during cardiac disease including heart failure. However, the overall frequency of such cell division, if true, remains very low, and its significance in modulating the disease state is, at the present time, uncertain. Another possibility is that cardiomyocytes stimulated to divide are driven toward apoptosis. Evidence for this can be found in studies in which DNA synthesis induced in cardiomyocytes transfected with EIA gene, resulted in apoptosis rather than cell division [78]. The above discussion only briefly describes those signal transduction pathways that are potentially relevant to cardiomyocyte apoptosis in the failing heart. A more detailed treatment of the apoptotic signaling pathways can be found in many comprehensive reviews on the subject [79–88].

6. Pathophysiological triggers of apoptosis in heart failure

There is ample evidence to suggest that pathophysiological conditions, common to the heart failure state, are important regulators of cardiac myocyte apoptosis (Fig. 2). It is often suggested that apoptosis may be induced by the same agents that produce necrosis with the type of cell death being dependent on the severity of the insult rather than its qualitative nature [89]. The observation that increased cytosolic calcium concentration can lead to apoptosis [90] is consistent with this concept. Other factors implicated as triggers of cardiomyocyte apoptosis include the formation of oxygen free radicals [91], exposure to hypoxia [92,93], excess levels of angiotensin-II (A-II) [94], excess levels of norepinephrine [95] and increased levels of specific cytokines such as TNFα [38,39]. Among these, the role of A-II, norepinephrine and limited oxygenation of the myocardium have received considerable attention in recent years and for good reason. Enhanced and sustained activity of the renin–angiotensin system and the sympathetic nervous system as well as localized or even global hypoxia, are in many respects characteristic features of the failing heart and have long been implicated in the progression of the disease. Exposure of isolated adult rat cardiomyocytes to A-II was shown to cause a near five-fold increase in apoptosis [94]. When cardiomyocyte were exposed to A-II in the presence of losartan, a selective AT1-receptor antagonist, apoptosis was completely blocked [94]. Consistent with this finding, we observed an attenuation of cardiomyocyte apoptosis in dogs with microembolization-induced heart failure treated long-term with the angiotensin-converting enzyme (ACE) inhibitor enalapril [96]. ACE inhibition has also been shown to attenuate apoptosis in rats with heart failure [29]. Exposure of isolated adult rat cardiomyocyte to norepinephrine caused a near two-fold increase in apoptosis [95]. When myocytes were exposed to norepinephrine in the presence of the mixed β1- and β2-adrenergic antagonist propranolol, the effect was completely blocked [95]. Consistent with these findings in isolated rat myocytes, we observed a marked reduction of cardiomyocyte apoptosis in dogs treated long-term with the β1-selective blocker metoprolol [97]. The attenuation of myocyte apoptosis with metoprolol was associated with an increase in the Bcl-2 to Bax ratio, a finding that favors cell survival. Several studies provide support for the role of hypoxia as a potential inducer of cardiomyocyte apoptosis. Exposure of cultured rat neonatal cardiac myocytes to hypoxia was shown to induce apoptosis as evidenced by positive labeling for nuclear DNA fragmentation [92]. In the same study, enhanced expression of Fas antigen was also noted in response to hypoxia. ICE-like proteases have also been shown to be involved in the hypoxia-induced apoptosis in cardiac myocytes [98]. Hypoxic stress has also been suggested to increase the expression and nuclear accumulation of specific proto-oncogenes such as c-fos, c-jun and c-myc that also have been implicated in the induction of cell cycle progression and apoptosis [99–101].
7. Importance of cardiomyocyte apoptosis in the progression of heart failure

At present, there appears to be sufficient evidence based on studies in the end-stage failed human heart as well as studies in animal models of heart failure to support the concept that cardiomyocyte apoptosis occurs in heart failure. While important, the significance of this finding in the context of the overall pathophysiology of this disease state and, in particular, in relation to the progressive deterioration of LV function, remains uncertain. A key question is as yet unanswered namely, does cardiomyocyte apoptosis play an important role in the progression of heart failure? While answers to this question are still lacking at present, it is possible to address this issue if certain assumptions are made. If one assumes that (1) the human LV contains approximately $6 \times 10^9$ myocytes, (2) a cardiomyocyte apoptosis rate, remote from any scar tissue, of $1/10,000$, (3) one myocyte for each nucleus positively labeled for nuclear DNA fragmentation, (4) a period of 24 h for myocyte death to occur from apoptosis, and (5) no cardiomyocyte division, one can project a near 4% loss of LV cardiomyocytes per year from apoptosis. Admittedly, some of the above assumptions are somewhat speculative and require confirmation. Nevertheless, if the proportion of cardiomyocyte loss due to apoptosis over the course of 1 year is even in the range of 1–5%, such a magnitude of loss of viable myocardium can certainly have a significant adverse impact on global LV performance. An accurate determination of the rate at which myocytes are lost in the failing heart as a result of apoptosis will help resolve many of the current uncertainties. While difficult, future studies must be directed toward obtaining answers to this question. Alternatively, or even simultaneously, chronic studies in animal models of heart failure can be undertaken to determine if direct inhibition of apoptosis with specific pharmacologic probes prevents progressive LV dysfunction. Only after these questions have been thoroughly addressed will the importance of cardiomyocyte apoptosis in the pathophysiology of heart failure be fully appreciated.

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