When the concept of apoptosis was introduced in the 1970s, it attracted only limited attention. However, less than two decades ago, Horvitz and colleagues identified its essential genetic components in the roundworm, Caenorhabditis elegans, and apoptosis emerged as a significant research front. The explosion of knowledge that took place is represented by the accumulation of >25,000 studies in the last 5 years alone. It is now clear that apoptosis is an important aspect of normal organ development and cellular regulation and that it plays a role in a wide variety of physiological and pathological conditions. However, there is still much debate and controversy concerning the role of apoptosis in heart failure. To address the issues of its presence in, significance for, and overall contribution to heart failure, we will review the currently available literature and then discuss its implications for future research and treatment strategies in heart failure.

Evidence of Apoptosis in Animal and Human Models of Heart Failure

The etiology of heart failure involves multiple agents and conditions, but the progressive loss of cardiac myocytes is one of the most important pathogenic components. During the past few years, there has been accumulating evidence in both human and animal models suggesting that apoptosis may be an important mode of cell death during heart failure (Table 1). Therefore, the possibility of limiting cardiac myocyte loss by inhibiting apoptosis has potentially important implications in the treatment of heart failure.

The numerous animal models of heart failure encompass a spectrum of species and a variety of apoptotic inducers. These studies suggest that the rate of occurrence of apoptosis can vary widely and depends on the model used and the area at risk examined. For example, in acute ischemia and reperfusion, apoptosis can be high as 14% in the area at risk. In contrast, the rate of apoptosis associated with chronic stimuli, such as pressure overload, is <1% in nontransgenic models when measured by terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) staining. But even though the rate of apoptosis in heart failure is relatively low in absolute numbers, it is significantly higher than that in the normal heart, which has essentially negligible baseline apoptosis.

In human heart failure, the data are limited to postmortem samples or tissue samples from patients undergoing heart transplantation. Although the initial studies reported unrealistically high levels of apoptosis in failed hearts (as much as 35%), more recent studies showed apoptosis rates of <1% (TUNEL-positive cells) during heart failure. The most common forms of heart failure associated with apoptosis are idiopathic dilated cardiomyopathy and ischemic cardiomyopathy, but apoptosis has been observed in other forms of heart failure as well.
<table>
<thead>
<tr>
<th>Animal model</th>
<th>Species</th>
<th>TUNEL</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ligand-activatable caspase-8 overexpression</td>
<td>Tg mice</td>
<td>NR</td>
<td>Inhibition of cardiac myocyte apoptosis by broad-spectrum caspase inhibitor6</td>
</tr>
<tr>
<td>$G_{sa}$ overexpression</td>
<td>Tg mice</td>
<td>0.6%</td>
<td>Decreased myocardial damage and preserved cardiac function by β-adrenergic blocker6,7</td>
</tr>
<tr>
<td>Gp130 knockout + TAC</td>
<td>Tg mice</td>
<td>3% (baseline)</td>
<td>Apoptosis rate increased to 34% in Gp130 knockout8</td>
</tr>
<tr>
<td>Cardiac allograft rejection in NOS2−/− mice</td>
<td>Tg mice</td>
<td>2.5× control</td>
<td>Antiapoptosis model (decreased caspase-3 activity, p53, and Bcl-xl, increased Bcl-2/Bax ratio9)</td>
</tr>
<tr>
<td>$G_{sa}$ overexpression + pregnancy</td>
<td>Tg mice</td>
<td>26%</td>
<td>Increased p38 and JNK10</td>
</tr>
<tr>
<td>IGF-1 overexpression + coronary ligation</td>
<td>Tg mice</td>
<td>4.2× control</td>
<td>IGF-1 overexpression decreased apoptosis and infarct size11</td>
</tr>
<tr>
<td>Viral myocarditis (3 mouse strains)</td>
<td>Mice</td>
<td>NR</td>
<td>Increased apoptosis and ANF in only 1 mouse strain12</td>
</tr>
<tr>
<td>Pressure overload by TAC</td>
<td>Rat</td>
<td>LVH 0.08%</td>
<td>Increased Bax, decreased Bcl-213</td>
</tr>
<tr>
<td>MI model</td>
<td>Rat</td>
<td>2× control</td>
<td>Increased JNK and lipid peroxidation activity14</td>
</tr>
<tr>
<td>Ischemia/reperfusion</td>
<td>Rat</td>
<td>11.1%</td>
<td>Apoptosis rate decreased to 3.1% by caspase inhibitor, zVAD.fmK15</td>
</tr>
<tr>
<td>Failing spontaneous hypertensive rats</td>
<td>Rat</td>
<td>0.04%</td>
<td>Decreased apoptosis to 0.009% by ACE inhibitor16</td>
</tr>
<tr>
<td>MI model</td>
<td>Rat</td>
<td>0.07%</td>
<td>Decreased Bcl-2, increased Bax17</td>
</tr>
<tr>
<td>Pacing-induced DCM</td>
<td>Dog</td>
<td>4× control</td>
<td>IGF-1 partially block apoptosis18</td>
</tr>
<tr>
<td>Acute chagasic myocarditis</td>
<td>Dog</td>
<td>0.27% (baseline)</td>
<td>Decrease rate of apoptosis to 0.08% with ACE inhibition20</td>
</tr>
<tr>
<td>Ischemic HF by intracoronary microembolism</td>
<td>Dog</td>
<td>0.41%</td>
<td>Positive EM for apoptosis21</td>
</tr>
<tr>
<td>Ischemic HF by intracoronary microembolism</td>
<td>Dog</td>
<td>0.37%</td>
<td>Increased PCNA and Fas22</td>
</tr>
<tr>
<td>Pacing-induced DCM</td>
<td>Rabbit</td>
<td>13%</td>
<td>Inhibition of apoptosis and infarct size by caspase inhibitor23</td>
</tr>
<tr>
<td>Ischemia/reperfusion</td>
<td>Rabbit</td>
<td>14.7%</td>
<td>Decreased apoptosis by carvedilol treatment (3.4%)24</td>
</tr>
<tr>
<td>MI model</td>
<td>Pig</td>
<td>0.02%</td>
<td>Hibernating myocardium25</td>
</tr>
<tr>
<td>Ischemic HF by intracoronary microembolism</td>
<td>Sheep</td>
<td>NR</td>
<td>Increased DNase 1, caspase-2, -3 activities26</td>
</tr>
<tr>
<td>Coxsackievirus myocarditis</td>
<td>Orangutan</td>
<td>NR</td>
<td>Case report27</td>
</tr>
<tr>
<td>End-stage HF + LV assist device (8 DCM, 2 IHD)</td>
<td>NA</td>
<td>NR</td>
<td>Increased Bcl-xl, decreased pro-ANP, DNA fragmentation with LV assist device placement28</td>
</tr>
<tr>
<td>End-stage HF (21)</td>
<td>NA</td>
<td>NR</td>
<td>Decreased serum eNOS expression29</td>
</tr>
<tr>
<td>Ischemic HF (7)</td>
<td>NA</td>
<td>35× (female), 85× (male) control</td>
<td>Necrosis increased by ×13 (female) and ×27 (male) vs controls30</td>
</tr>
<tr>
<td>End-stage HF for transplant</td>
<td>NA</td>
<td>0%–3.9% IHD</td>
<td>Increased DNA fragmentation in areas with increased Fas staining31</td>
</tr>
<tr>
<td>End-stage HF for transplant</td>
<td>NA</td>
<td>0%–30.5% DCM</td>
<td>Different rate of apoptosis for different causes of heart failure32</td>
</tr>
<tr>
<td>Explanted hearts (21 DCM, 14 IHD)</td>
<td>NA</td>
<td>NR</td>
<td>Presence of cytosolic cytochrome c and increased caspase-3 activity33</td>
</tr>
<tr>
<td>Acronegalic cardiomyopathy (10)</td>
<td>NA</td>
<td>0.26%</td>
<td>Case series35</td>
</tr>
<tr>
<td>Myocarditis (16)</td>
<td>NA</td>
<td>0.36%</td>
<td>Increased soluble FasL36</td>
</tr>
<tr>
<td>Ischemic HF for transplant (36)</td>
<td>NA</td>
<td>0.23%</td>
<td>Increased Bcl-2, no change in Bax37</td>
</tr>
<tr>
<td>Hypertrophic CM (1)</td>
<td>NA</td>
<td>NR</td>
<td>Case report38</td>
</tr>
<tr>
<td>End-stage HF (4 DCM, 3 IHD)</td>
<td>NA</td>
<td>5%–35%</td>
<td>Increased DNA fragmentation39</td>
</tr>
<tr>
<td>Arhythmogenic RV dysplasia (6)</td>
<td>NA</td>
<td>0%–28%</td>
<td>Increased caspase-3 expression40</td>
</tr>
</tbody>
</table>

ACE indicates angiotensin-converting enzyme; ANF, atrial natriuretic factor; ANP, atrial natriuretic peptide; DCM, dilated cardiomyopathy (CM); EM, electron microscopy; eNOS, endothelial constitutive NO synthase; HF, heart failure; IGF-1, insulin-like growth factor-1; IHD, ischemic heart disease; JNK, c-Jun N-terminal kinase; LVD, left ventricular dilatation; LVH, left ventricular hypertrophy; MI, myocardial infarction; NA, not applicable; NOS, NO synthase; NR, not reported; PCNA, proliferating cell nuclear antigen; RV, right ventricular; TAC, transverse aortic constriction; and Tg, transgenic.
**Problems With Interpreting the Presence of Apoptosis in Heart Failure**

Despite the wealth of published data, there are still many controversies surrounding the presence and the significance of apoptosis in heart failure. These controversies stem largely from the limitations of the technique used to detect apoptosis and the difficulties in translating these findings to the ultimate significance of apoptosis in heart failure.

Acute insults, such as myocardial infarction and ischemia/reperfusion, and chronic conditions, such as ischemic and dilated cardiomyopathies, have been linked to increased apoptotic cell death in human and animal hearts. But not all models of heart failure are associated with apoptosis and the difficulties in interpreting these findings (however well done), the use of TUNEL alone to detect the presence of apoptosis is not sufficient to define the role of apoptosis in heart failure. We need more studies using in vitro models of cardiac myocyte apoptosis to decipher and to understand the molecular mechanism of apoptosis in cardiac myocytes. In addition, we need “interventional studies” using transgenic and cardiac-specific gene knockout mice models to study the consequences of genetic manipulation of proapoptotic and antiapoptotic genes in vivo. These should be complemented by studies in larger animal models (eg, pigs or dogs) that can mimic human clinical conditions much better than murine models, as well as by pharmacological studies to modulate apoptosis.

**Models of Heart Failure From Cardiac Apoptosis**

Recently, animal models of heart failure incorporating transgenic technology have confirmed that myocyte apoptosis...
itself is sufficient to induce heart failure. Kitsis et al generated transgenic mice with cardiac-specific overexpression of ligand-activatable cysteine aspartate proteinase (caspase-8), which is an artificial (or engineered) fusion protein consisting of the FK506 binding protein and the catalytic domain of caspase-8. Transgenic mice expressing this construct appeared normal at birth, but administration of the divalent dimerizer FK1012 activated caspase-8 and -3, resulting in overwhelming cardiac myocyte apoptosis and rapid death of the animal. Perhaps not surprisingly, even in the absence of a dimerizer, adult mice with high expression of the protein manifest spontaneous cardiac myocyte apoptosis, leading over time to a lethal dilated cardiomyopathy. These changes were ameliorated by a broad-spectrum caspase inhibitor (R. Kitsis, personal communication, 2000). Although it is not clear whether endogenous caspase-8 is an important regulator of apoptosis in heart, the study of Kitsis et al

nevertheless demonstrates that the induction of apoptosis can be achieved in the heart. It also shows that slow induction of apoptosis alone can result in dilated cardiomyopathy.

Another mouse model of heart failure uses cardiac-specific knockout of gp130, a common subunit of the interleukin-6 family of cytokine receptors that have been shown to promote cell survival in the presence of an apoptotic stimulus in vitro. Under baseline conditions, these mice showed a grossly normal phenotype with normal cardiac structure and function. However, when gp130 knockout mice were exposed to acute pressure overload by surgical constriction of the transverse aorta, they developed significant cardiac apoptosis (≈34%), and >90% died by dilated cardiomyopathy in a few weeks. In contrast, normal wild-type mice exposed to similar pressure overload developed compensatory cardiac hypertrophy without heart failure. Of particular interest is that aortic-banded gp130 knockout mice did not develop hypertrophy. Because gp130 has been shown to provide important survival signals in the cardiac myocyte during cardiac hypertrophy via cardiotoxin-1, this model provides important clues to the relationship between hypertrophy and apoptosis. For example, for adaptive cardiac hypertrophy to occur in response to mechanical stress, it is necessary to have antiapoptotic or survival factors, such as gp130, present in heart. The notion that cardiac hypertrophy is a favorable adaptation to stress and that hypertrophied cells can be more resistant to an apoptotic stimulus is also supported by other transgenic models of cardiac hypertrophy. Overexpression of calcineurin confers a protective effect on cardiac myocytes both in vitro and in vivo when they are exposed to apoptotic stimuli. Also, cardiac hypertrophy by overexpression of insulin-like growth factor-1 in animals has been shown to limit infarct size by limiting apoptotic cell death.

On the other hand, the overexpression of heterotrimeric G proteins, such as G_{s} or G_{a}, may promote apoptosis in cardiac myocytes. For example, transgenic mice with overexpression of G_{s} signaling develop compensatory hypertrophy at baseline. However, when transgenic females become pregnant, they develop lethal dilated cardiomyopathy, resembling human peripartum cardiomyopathy, within 1 week after delivery. TUNEL staining of the heart revealed markedly increased levels of apoptosis (≈26%). Also, G_{a} overexpression results in increased sensitivity to apoptotic stimulation and leads to cardiomyopathy. This was confirmed by blocking the β-adrenergic receptor, which prevented myocyte damage, decreased cardiac apoptosis, and preserved cardiac function in G_{a} transgenic mice. In addition, other important hypertrophic signaling molecules, such as angiotensin II, have also been shown to promote apoptosis in vitro, and several studies show that administration of angiotensin-converting enzyme inhibitors blocks cardiac apoptosis in vivo.

These models of heart failure in mice demonstrate that apoptosis does occur during heart failure and could play a significant role in the development of heart failure in certain settings. However, whether hypertrophy renders cardiac myocytes more sensitive or resistant to apoptosis is still controversial. Some hypertrophic signaling factors, such as cardiotoxin-1 via gp130, insulin-like growth factor-1 via phosphoinositide-3 kinase, and calcineurin via the nuclear factor of activated T cells, seem to be protective. On the other hand, hypertrophic signaling via heterotrimeric G proteins and angiotensin II seems to render cardiac myocytes more sensitive to apoptosis. These findings are important because they provide possible strategies to modulate cardiac apoptosis. However, we believe further studies, especially during the transition from compensated hypertrophy to heart failure, are needed to better understand the complex and delicate balance that exists among hypertrophy, apoptosis, and heart failure.

**Regulation of Cardiac Apoptosis**

The major apoptotic pathway is initiated by the release of cytochrome c from mitochondria in response to an apoptotic stimulus. Released cytochrome c, in the presence of dATP, forms an activation complex with apoptotic protein-activating factor-1 and caspase-9 that activates downstream caspases to execute the final morphological and biochemical alterations. This pathway is tightly regulated by a group of antiapoptotic proteins, such as Bcl-2, and proapoptotic proteins, such as Bax; further regulation occurs downstream by various inhibitors of caspases. There is recent evidence to suggest that cardiac myocytes also use a mitochondrial-dependent apoptotic pathway. Cytoolic cytochrome c and the activation of caspases have been observed in both human and animal models of heart failure. The level of Bcl-2 is upregulated soon after acute coronary occlusions, especially in the salvageable myocardium, but is decreased after chronic heart failure by pressure overload. Also, the overexpression of Bcl-2 in the heart effectively reduces myocardial reperfusion injury by reducing cardiac myocyte apoptosis. Moreover, some studies suggest that the balance between Bcl-2/Bax may promote apoptosis in cardiac myocytes. These findings are supported by the reversal of the Bcl-2/Bax ratio in the heart after left ventricular assist device placement.

It has been suggested that the cardiac myocyte could also use an alternative apoptotic pathway that activates downstream caspases via “death receptors” (eg, Fas, tumor necrosis factor [TNF] receptor) and caspase-8. Expression of the death receptor Fas is upregulated in cardiac myocytes during myocardial ischemia and heart failure, and
increased levels of soluble Fas ligand and TNF-α have been reported in patients with end-stage heart failure.73 Also, in immune-mediated cardiomyopathies, cardiac apoptosis is associated with an augmented Fas/FasL system.50 However, cardiac-specific overexpression of both TNF-α and FasL did not result in increased cardiac myocyte apoptosis.47,49 Therefore, we speculate that although a death receptor–mediated pathway may be important in certain situations, notably in immune-mediated heart failure, this may not be the main pathway in more common forms of heart failure, such as ischemic and dilated cardiomyopathy.

**Is There a Potential for Antiapoptotic Therapy for Heart Failure?**

Several strategies can be used to inhibit apoptosis in heart, and various agents and factors have been shown to be effective in experimental models.74 For example, caspase inhibitors significantly decrease apoptosis in the area at risk, with subsequent reduction in infarct size in rat hearts during experimentally induced ischemia and reperfusion.16,24 Furthermore, the long-term beneficial effects of angiotensin-converting enzyme inhibitors and carvedilol in the treatment of heart failure may involve, at least in part, inhibition of cardiac apoptosis.17,21,25

Even though the therapeutic targeting of apoptotic pathways has potential in the treatment of heart failure, several important questions still need to be answered. First, it has not yet been shown whether inhibition of apoptosis could delay or prevent the development of heart failure. It is possible that inhibiting apoptosis may simply result in the activation of another mode of cell death, such as necrosis, which may have more deleterious effects on neighboring cells and ultimately a worse outcome. Although the early studies on animal models of heart failure have been encouraging, the long-term consequences of inhibiting apoptosis in the heart are not known. Second, the safety of antiapoptotic therapy has not been tested. Apoptosis is needed for the normal functioning of other cell systems, such as the immune system, and an excessive inhibition of apoptosis is associated with lymphoma or autoimmune disorders. Therefore, the chronic systemic inhibition of apoptosis may have significant deleterious consequences in noncardiac organs. Third, antiapoptotic therapy for heart failure may not apply to all types of heart failure. We speculate that an antiapoptotic strategy for heart failure due to persistent pressure overload will remain controversial for a while, because chronic and complete inhibition of apoptosis may be very difficult to achieve with the current repertoire of drugs. The role of antiapoptotic therapy in heart failure associated primarily with inflammation (eg, viral myocarditis) may also remain controversial because the removal of virally infected cells is likely to be a necessary step toward recovery. The most ideal conditions for antiapoptotic intervention, in our opinion, occur in transient and acute insults, such as reperfusion. During reperfusion, cardiac myocyte apoptosis occurs at a high rate during a defined time period; thus, a short treatment period may be highly effective. Moreover, a short therapeutic course has the additional benefit of minimizing the possible deleterious side effects arising in other organ systems.

**Conclusion**

It is clear that apoptosis plays an important role in a variety of physiological and pathological states. However, in the cardiovascular system, we have only begun to clarify the role of apoptosis and to start exploring the therapeutic potential associated with its inhibition. Still more work is necessary to understand the molecular mechanisms that govern these processes and the significance of apoptosis in heart failure. Apoptosis must be demonstrated by multiple criteria, not just TUNEL staining alone. Genetic interventional studies should be explored further by use of mouse models, but pharmacological studies using large animal models should be encouraged. The initial analytical work must be carried out in well-defined experimental frameworks that are tissue-targeted and time specific, with clear quantitative end points. Only then will we be able to conduct meaningful human studies to answer whether the inhibition of apoptosis in heart failure will translate into clinical benefit.

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**References**


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Peter M. Kang and Seigo Izumo

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