The Role of Cell Death in Heart Failure

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In this issue of Circulation Research, Guerra and colleagues report that myocyte death in the failing human heart is gender dependent. This work is in continuation of Dr Anversa’s numerous reports on the occurrence of apoptosis, necrosis, and proliferation in the failing human heart, as well as under various experimental conditions including, among others, aging and myocardial infarction in rats as well as insulin-like growth factor (IGF) transgenic mice. In their recent work, the authors demonstrate levels of necrosis 7-fold greater than that of apoptosis in patients of either sex with cardiac failure. Interestingly, cell death was 2-fold higher in men than in women. Necrosis comprised 1.2% (male heart) and 0.5% (female heart), and apoptosis was either 0.16% (Taq or terminal deoxynucleotidyl transferase (TdT)) or 0.25% (electron microscopy) in men and 0.076% (Taq or TdT) or 0.08% (electron microscopy) in women. With respect to baseline, myocyte necrosis was 13-fold higher in female and 27-fold higher in male hearts. Compared with control, apoptosis was 85-fold higher in males and 35-fold higher in female hearts. The conclusion was drawn that both necrosis and apoptosis “affect the decompensated heart, each contributing to the evolution of heart failure.”

This publication provokes many questions about the role of cell death in failing hearts. The problems listed below are involved in the interpretation of the data presented:

1. Specificity of techniques
2. Interpretation of cell numbers obtained
3. What is the mechanism for either necrosis or apoptosis in cardiomyopathy?
4. What is the role of cell death in heart failure?

Specificity of Techniques
The TUNEL method (also called TdT labeling) is the most widely used technique for the detection of apoptotic cells. In addition, based on the work of Didenko and Hornsby, the Taq and Pfu labeling techniques have been developed. Taq polymerase-generated probes identify single-base 3′ overhangs in fragmented DNA caused by endonucleases typical of apoptosis that are not present in the blunt DNA ends resulting from exonuclease activity in necrotic cell death. The latter are labeled by Pfu polymerase-generated probes. The principles of the different labeling techniques have been outlined carefully in the present article. The apoptotic rates reported for the Taq polymerase or TUNEL labeling techniques were almost identical, confirming a previous observation by the same group.

Several limitations are inherent to these methods: (1) It is not yet known how many DNA strand breaks will result in a positive signal. If only 10% of all DNA fragmented were required for a positive signal, the rate for apoptosis or necrosis may be grossly overestimated than it would be if 100% DNA fragmentation is required. Additionally, positivity for DNA strand breaks, be it due to necrosis or apoptosis, may persist after complete DNA fragmentation has occurred, contributing to falsely high levels of cell death. (2) Recent reports from Dr Fujiwara’s group have shown by electron microscopy that not only apoptotic but also necrotic cardiomyocytes may be labeled by the TUNEL method. Furthermore, the same group demonstrated TUNEL labeling of cardiomyocytes undergoing DNA repair, ie, living cells, rendering the use of this method even more questionable. Despite the apparent weaknesses of the TUNEL method, it is widely used for the identification and quantitation of apoptosis because it is commercially available as an easy-to-use kit and offers the possibility of identifying the type of cell affected by suicidal cell death and relating it to the total number of such cells.

Electron microscopy provides the gold standard for the identification of both apoptosis and necrosis. On the basis of previous reports reviewed by Majno and Joris, it is postulated that cardiomyocytes undergoing apoptotic cell death exhibit nuclear condensation without affecting mitochondria or sarcolemma. Incomplete or delayed fixation of the tissue studied may disturb the ultrastructural appearance of presumably apoptotic cells, as is the case in the present work of Guerra et al. The term “necrosis” implies ischemic cell death, and the ultrastructural criteria of ischemic injury as described by Jennings et al and others have been used frequently to identify necrotic cardiomyocytes by electron microscopy. In contrast to apoptotic cardiomyocytes, necrotic cells exhibit irregular chromatin clumping, mitochondrial changes including the occurrence of flocculent densities, and a fragmented sarcolemma. Prominent nucleoli are confused easily with aggregated chromatin (see Figure 11A), illustrating that the diagnosis of cell death by electron microscopy provides some pitfalls as well. Furthermore, this technique is extremely labor-intensive and does not lend itself easily to quantitative evaluation of phenomena that involve small numbers of myocytes such as apoptosis.

Guerra et al tried to confirm necrotic cell death by vinculin staining, which was assumed to be a marker of sarcolemmal integrity. Because, however, vinculin labels only the costameres and not the entire sarcolemma, it is
not a good measure of membrane integrity. A protein that is known to occur along the entire cell membrane such as dystrophin would be preferable for this purpose.

**Interpretation of Cell Numbers Obtained**

Considering that heart failure is a chronic process developing over months and years, rates of apoptosis as well as necrosis should be calculated for a certain time period to assess the true pathophysiological importance of any such numbers. Assuming that apoptosis takes 24 hours to be completed, an apoptotic rate of 0.16% as reported in Guerra et al.1 means that 58.4% of all cardiomyocytes would disappear within 1 year. Recent studies using adult rat cardiomyocytes in culture exposed to H2O2, however, showed that apoptosis needs only 14 hours from stimulation to DNA fragmentation, which means that the rate of disappearance of cells would be almost doubled.16 The same consideration applies to the estimation of the rate of necrosis, which was indicated in the present study as 1%, ie, 365% in 1 year or 30% in 1 month. These numbers calculated would be even higher when a logarithmic scale would be used, taking into account the decreasing absolute numbers of cells. This problem has been discussed by the authors of the present work, and two arguments were brought forward: (1) myocyte proliferation occurs in the failing heart and (2) these findings represent the “end-stage, preterminal condition of the disease.” Myocyte proliferation ought to occur at an extremely high rate to compensate at least partially for cell loss, which is highly improbable in failing myocardium. The mitotic rate of 0.0131% and 0.0152% in dilated and ischemic cardiomyopathy, respectively, as reported by Anversa’s group,3 is much too low to compensate at least partially for the high rates of cell loss caused by apoptosis and necrosis described here.

The second statement classifies the occurrence of cell death as an epiphenomenon that is not related to the evolution of heart failure and lacks therefore any mechanistic significance. Again, the time factor is important in this consideration.

**What Is the Mechanism for Either Necrosis or Apoptosis in Cardiomyopathy?**

In the case of necrosis, it is difficult to perceive of single-cell ischemia leading to necrosis in dilated cardiomyopathy because the diagnosis of dilated cardiomyopathy as a disease, by definition, excludes any ischemic alterations. Alcohol toxicity, pregnancy-associated nutritional defects, a genetic defect, and postviral myocarditis are currently discussed as pathogenetic mechanisms.17 The question again arises: If there are truly necrotic cells present, ie, cells with irreversible injury of the major cellular organelles, at what stage of DNA fragmentation will labeling with the Pfu-generated probe be positive and how long will it persist? Also, reversible injury precedes irreversible injury or necrosis. Will reversibly damaged cells then be labeled as well? In normothermia, ischemic cell death develops within a time span of 45 to 60 minutes.18 This time factor has not been taken into consideration by Guerra et al.1

In the case of ischemic cardiomyopathy, cellular degeneration and accomplished necrosis due to ischemia are com-

mon.19 In the present study, the reader would expect differences in the occurrence of ischemic cell death between both types of cardiomyopathy. However, the choice of sampling site plays a crucial role in ischemic cardiomyopathy, and given that this had not been defined in detail in the present study,1 predictions as to the presence of necrosis are difficult to establish. Both types of cardiomyopathy were found to exhibit the same rates of necrosis and apoptosis and were therefore pooled, which is difficult to justify in view of the different pathogenetic mechanisms. Measurements of troponin I release into the patients’ blood would have provided an independent indicator of ongoing cell damage.20

As possible causes for apoptosis, angiotensin II release and stimulation of DNAse I by [Ca2+], have been mentioned. The most pressing question, however, has not been answered: Why do some cardiomyocytes die of necrosis, ie, ischemic cell death, and others of apoptosis, and why do the majority survive?

Protection against either necrotic or apoptotic cell death in failing hearts has been attributed to estrogens and an associated upregulation of insulin-like growth factor-I receptor. An explanation, however, for the cause of heart failure in female hearts, despite the low rate of cell death, is still lacking.

**What Is the Role of Cell Death in Heart Failure?**

From the present study, it would appear that necrosis and apoptosis play major roles in the development of heart failure. However, despite the affirmative statement in their Abstract, the authors discuss their data in a more cautious manner and rather regard their numbers of cell death presented as well as those previously reported21 as values that are too low and “that may challenge the impact of this phenomenon on the final outcome of the pathologic state,”1 rendering the present report purely descriptive without mechanistic evidence.

A recent review on apoptosis and its implications for cardiovascular disease22 pointed out that the role of apoptosis in heart failure needs substantiation because the values of incidence vary so widely and mechanistic evidence is still lacking. Heart failure represents an exceedingly complex pathophysiologic entity involving structural changes, such as loss of myofilaments and disorganization of the cytoskeleton,23 as well as disturbance of Ca2+ homeostasis, alterations of receptor density and signal proteins, the occurrence of fibrosis and left ventricular remodeling, and many other phenomena recently reviewed in detail by Mann.24 As stated in this review, knowledge of the presence of apoptosis in mild-to-moderately failing hearts and preferably also in human hearts with compensated hypertrophy is needed to interpret these data. Furthermore, the apoptotic cascade, including receptors at the cellular membrane, signal transduction, mitochondrial mechanisms, and role of caspases, must be studied carefully before any conclusion about the significance of apoptosis in heart failure can be reached.

The pioneering, provocative work of Anversa and his group concerning the role of proliferation of apparently terminally differentiated cells, cardiomyocyte necrosis, and apoptosis in myocardium under different pathophysiological conditions is widely recognized. However, the time has come
to evaluate carefully each hypothesis presented, to examine critically the advantages and weaknesses of the techniques used, and to interpret the findings in relation to the pathophysiology of the disease in order to clarify the issue of cell death in the failing heart.

References


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