The manifestations and mechanisms of myocardial cell injury and cell death in response to impaired coronary perfusion and thrombosis continue to be the collective subject of ongoing investigation because of intrinsic scientific interest and relevance for the diagnosis and treatment of patients with ischemic heart disease. An extensive body of evidence has documented the cellular and subcellular alterations that accompany the progressive reduction in high-energy ATP in response to oxygen and substrate deprivation affecting all cell types, including cardiac myocytes.\(^1\)\(^-\)\(^3\) The characteristic pattern of ischemic cell injury involves fluid and electrolyte alterations, with loss of \(K^+\) and \(Mg^{2+}\) and accumulation of water, \(Na^+\), \(Cl^-\), \(H^+\) (acidosis), and \(Ca^{2+}\);\(^2\) cytoplasmic, organelar, and cellular swelling with plasma membrane blebbing; and margination and clumping of nuclear chromatin. These cellular changes are due to progressive impairment of membrane composition, structure, and function.\(^1\) The transition from reversible to irreversible injury is characterized by the development of a severe membrane permeability defect that allows the unregulated influx of divalent and trivalent cations, including calcium.\(^1\) Subsequently, the swollen cells develop physical defects (holes) in their cell membranes and rupture. These features of cell injury with cell swelling have been shown to involve cardiac myocytes subjected to hypoxia in vitro and cardiac muscle during the evolution of myocardial infarction in vivo.\(^1\)\(^,\)\(^3\) Myocardium undergoing ischemic death ultimately exhibits some variant of coagulation necrosis and elicits an inflammatory response with an initial influx of neutrophils.\(^3\) The underlying membrane damage to ischemic myocytes is the basis for the diagnosis of myocardial infarction by pathology laboratory and nuclear cardiology methods.

Recently, considerable attention has been directed to another form of cell death, referred to as apoptosis.\(^1\)\(^,\)\(^2\) Although apoptosis was initially characterized as a counterbalancing physiological process to mitosis, in which internal signals activate a death program (programmed cell death), apoptosis subsequently has been recognized as a process that also can be induced by external stimuli, either physiological or pathological.\(^1\)\(^,\)\(^5\) Apoptosis is characterized morphologically by a pattern of nuclear pyknosis (highly condensed chromatin, segregated into sharply defined bodies within an intact nuclear envelope), cytoplasmic condensation, and cell shrinkage, followed by nuclear and cellular fragmentation and rapid phagocytosis of apoptotic bodies by adjacent cells in the absence of exudative inflammation.\(^1\)\(^,\)\(^2\) Chromatin fragmentation in apoptosis involves activation or de novo synthesis of endonucleases and is at least in part calcium dependent. The result is cleavage of DNA at linking regions between nucleosomes to form a series of double-stranded fragments that are multiples of 180 to 200 base pairs in length. These fragments give a characteristic DNA ladder pattern on gel electrophoresis.\(^6\) Of several cytochemical approaches for the detection of double-stranded DNA fragmentation, the most extensively used method has been the terminal deoxynucleotidyl transferase (TdT)–mediated biotinylated dUTP nick end-labeling (TUNEL) method.\(^7\)

Extensive recent research has provided insight into the pathogenesis of apoptosis.\(^1\)\(^,\)\(^5\) The fundamental process involves activation of a cascade of cytosolic aspartate–specific cysteine proteases (caspases), including interleukin converting enzyme and caspase-3. Initiation of the caspase cascade can occur by activation of the Fas (apo1)/TNFR-1 (tumor necrosis factor receptor-1) signaling pathway by tumor necrosis factor and other stimuli. A novel ubiquitin-like protein, sentrin, can block apoptosis by binding to the death domain of Fas/TNFR-1.\(^8\) Other stimuli for apoptosis include processes that lead to ceramide synthesis or release.\(^9\) Several normal and mutated gene products, including \(bcl-2\), c-myc, and p53, regulate whether or not a cell becomes apoptotic.\(^1\)\(^,\)\(^5\) Mitochondria also participate in apoptosis.\(^10\) Mitochondrial alteration by free radicals or other mechanisms leads to leakage of cytochrome c, binding of cytochrome c to apoptotic protease activating factor-1 (Apaf-1), and subsequent apoptosis; this process is blocked by overexpression of \(bcl-2\), whose protein product localizes to mitochondrial membranes.\(^5\) Alterations in cell volume and shape (cell shrinkage) are related to activation of proteases and transglutaminase, resulting in cross-linking of cytoplasmic proteins. The apoptotic process also involves changes in the composition of the cell membrane, including increased expression of phosphatidyl serine in the outer leaflet.\(^11\) These changes trigger rapid phagocytosis of apoptotic bodies without exudative inflammation.

Apoptosis and necrosis generally have been considered to represent the 2 fundamental forms of cell death. However, Majno and Joris\(^7\) have pointed out some logical inconsistencies with this analysis. Necrosis, precisely defined, refers to the sum of degradative changes that follow any type of cell death. Apoptosis is characterized by cell death with cell shrinkage and fragmentation. Majno and Joris have applied

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**Editorial**

**Modes of Myocardial Cell Injury and Cell Death in Ischemic Heart Disease**

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Myocardial Cell Injury and Death in Ischemic Heart Disease

the term “oncysis” to the contrasting pattern of cell death with cell swelling. Thus, necrosis inevitably follows the onset of either apoptotic cell death or oncycytic cell death.

Recently, intense interest has been focused on the role of apoptosis in normal cardiac development and in the pathology of cardiovascular disease, including chronic heart failure and various manifestations of ischemic heart disease.1–3 Apoptosis has been reported to involve the majority of myocytes during the first few hours of the evolution of myocardial infarction in a rat model, when the majority of myocytes undergo irreversible injury after coronary occlusion.12 However, there are inconsistencies, with some studies describing the occurrence of apoptosis in ischemic myocardium during coronary occlusion and others describing the development of apoptosis only during reperfusion of previously ischemic myocardium.12–14 Furthermore, oncysis, ie, cell injury and death with cell swelling, has been repeatedly described as the dominant pattern of myocardial ischemic and hypoxic injury, based on extensive experimental evidence.1–5

Resolution of the issues regarding modes of cell injury and death in ischemic myocardium requires careful analysis, application of established parameters, and, probably, the development of new approaches. The parameters that can be used to distinguish between apoptotic and oncycytic cell death are as follows: (1) morphology, including cell shrinkage versus cell swelling, patterns of nuclear alterations, and ultrastructural features1–3; (2) presence (oncysis) or absence (apoptosis) of membrane permeability defects as determined by various injected tracers such as antimyosin antibody (in vivo) or in vitro markers such as propidium or ethidium bromide; (3) DNA gel electrophoresis showing discrete multistranded DNA fragmentation ladders (apoptosis) versus diffuse random DNA fragmentation (oncysis)1–5; (4) positive (apoptosis) or negative (oncysis) histochemical detection of double-stranded DNA fragmentation by the TUNEL method;7 (5) detection in apoptotic cells of increased expression of phosphatidyl serine in the outer leaflet of the cell membrane11; (6) detection of caspase activation in apoptotic cells12; (7) identification of increased expression of genes and gene products associated with apoptosis13; and (8) identification of an activated endonuclease specific for apoptosis (see below).

The complexities of the different modes of cell injury and death present difficulties in definitively differentiating these processes. Nevertheless, many studies have focused on differences in patterns of DNA fragmentation and have identified apoptotic cells exclusively or primarily on the basis of a ladder pattern of double-stranded DNA fragmentation on gel electrophoresis coupled with histochemical detection of double-stranded DNA fragmentation by the TUNEL method. However, there is evidence that TUNEL positivity and DNA laddering are not absolutely specific for apoptosis because cell death leading to necrosis, whether via oncysis or apoptosis, may have a transient stage of TUNEL positivity.7 Another consideration is that detection of DNA laddering with DNA electrophoresis is extremely sensitive, such that double-stranded oligonucleosomal fragments can be detected when as few as 2% apoptotic cells can be observed morphologically in an experimental cell system.5 DNA gels from ischemic myocardium often show atypical patterns, which likely represent mixtures of DNA smearing and some laddering. Also, there is the issue of identification of the TUNEL-positive cells as cardiac myocytes, nonmyocytic interstitial cells, or inflammatory cells. Detection of the presence or absence of sarcolemmal membrane permeability by use of injected tracers in vivo is also prone to variability, particularly in the first few hours after coronary occlusion.

The issues discussed above raise legitimate concern regarding the true extent of apoptosis versus oncysis in ischemic myocardial damage. In this issue of Circulation, Ohno et al15 present experimental evidence that reinforces this concern. Using a rabbit model of coronary occlusion and reperfusion, Ohno et al investigated whether light microscopic (LM)–TUNEL-positive infarcted myocytes have apoptotic or oncocytic ultrastructural features when evaluated by electron microscopic (EM)–TUNEL, a technique that allows for simultaneous observation of the ultrastructure and DNA fragmentation of the same myocytes. Different groups of rabbits were subjected to 30 minutes’ coronary occlusion followed by 0, 30 minutes, 2 hours, or 4 hours of reperfusion. Only in the 2- and 4-hour reperfusion groups did DNA electrophoresis show a ladder pattern, and the LM-TUNEL positivity involved 6±2% and 11±3%, respectively, of the myocytic nuclei in the infarcted region. However, EM-TUNEL positivity, based on significant accumulation of immunogold particles, was observed only in myocytes with features of severe oncycytic injury in the 2- and 4-hour reperfusion groups (41±3% and 83±4% positive myocytes, respectively). Irreversible oncycytic injury was characterized by cell swelling, inhomogeneously clumped chromatin in nuclei, dense bodies in mitochondria, and/or ruptured plasma membranes. These features, which are those of oncystosis and classic ischemic injury, were already seen in the 0- and 30-minute reperfusion groups without TUNEL-positive myocytes. Ohno et al concluded that DNA fragmentation occurs in myocytes subsequent to their development of ultrastructural features of oncysis but not apoptotic cell injury and death; that DNA fragmentation itself does not always mean apoptosis; and that “apoptotic” infarcted myocytes belong to a category of cell death other than apoptosis. Gottlieb and coworkers15 demonstrated that extensive DNA fragment labeling could be imparted to myocardial tissue sections by DNase I treatment, further demonstrating that in situ nick end-labeling does not absolutely distinguish between nucleosomal cleavage of apoptosis and nonspecific DNA degradation.

Further consideration of the biochemistry of DNA degradation is warranted because considerable reliance has been placed on differences in DNA alterations in the differential detection of apoptosis and oncycytic necrosis. Double-stranded DNA is subject to the activity of endonucleases, which can induce cuts with staggered ends and blunt ends.16 The characteristic pattern of apoptosis is double-stranded breaks at internucleosomal DNA sites such that the breaks have staggered ends with 3’ overhangs comprising 1 or 2 bases, or longer overhangs involving 4 bases.16 A histochemical method using Taq polymerase detects single-base 3’ overhangs produced by Ca2+-dependent DNase I, whereas the method using TdT detects 1-base 3’ overhangs as well as multiple-base 3’ overhangs produced by pH-dependent
DNAase II and possibly other DNAases. Although the TdT-based TUNEL assay is less specific, comparable results of the Taq polymerase—based in situ ligation and TdT-based TUNEL methods have been reported in a recent study. In onecytic necrosis, there is more generalized activation of endonucleases and exonucleases leading to cleavage of nucleosomal and internucleosomal DNA that results in blunt ends of the digested fragments. A probe produced with Pfu polymerase labels blunt-ended DNA fragments. The combined use of the 3 histochemical probes, Taq polymerase, TdT, and Pfu polymerase, holds the promise of providing increased accuracy in distinguishing different patterns of DNA fragmentation associated with apoptosis and onecytic necrosis. Also, the single-cell electrophoresis (comet) assay has been proposed as superior to the TUNEL assay on the basis of putative absolute specificity for double-stranded DNA breaks. However, the issue of possible transient double-stranded DNA breaks in onecytic necrosis remains. Perhaps future work will lead to the development of techniques that more precisely and specifically identify the specific type of endonuclease activity and DNA fragmentation characteristic of apoptosis. Nevertheless, as pointed out by Collins and associates, identification of apoptosis requires a concordance of distinctive ultrastructural features, DNA degradation pattern, and circumstances of occurrence, which should be correlated, if possible, with distinctive molecular events; the inclusion of quantitative and temporal data to define the kinetics of the cellular events is also of considerable value. As a corollary to this, investigators should not be limited by “TUNEL vision” but should apply a broad perspective to their studies.

The work of Ohno et al and the considerations raised in this editorial clearly call into question the extent to which apoptosis occurs in myocardial infarction in addition to classic onecytic ischemic cell injury. Nevertheless, there is credible evidence that significant apoptosis does occur in certain forms of myocardial perturbation, including activation of the sphingolipid signaling cascade with related increase in cellular ceramide level. The rate of decline of ATP may play an important role in determining whether myocytes enter apoptosis or onecytic injury in response to ischemia or other stimuli. Interestingly, Apaf-1 has an ATP-binding site, which might explain why the ATP level in an injured cell may play an important role in determining whether the cell has sufficient energy to die by apoptosis or, lacking sufficient energy, will die by onecosis. The ATP level is an critical determinant of the fate of injured cardiac myocytes. It is also important to determine the extent of apoptosis in myocardial ischemia because of the potential for the development and use of new forms of therapy, such as caspase inhibitors. Reports of amelioration of myocardial metabolic and ischemic injury with caspase inhibitors have appeared. Because of potential diagnostic and therapeutic benefits and intrinsic interest, further research is obviously needed to clarify every nuance of the modes of myocardial cell injury and cell death.

References


Key Words: Editorials ■ apoptosis ■ ischemia ■ myocytes
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