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

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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+, Cl-, H+ (acidosis), and Ca2+; cytoplasmic, organellar, 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.

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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.4,5 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, senrin, 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.4,5 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 crosslinking 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 Joris7 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
the term “oncosis” to the contrasting pattern of cell death with cell swelling. Thus, necrosis inevitably follows the onset of either apoptotic cell death or oncotypic 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.4 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, oncosis, 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.1-3

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 oncotypic cell death are as follows: (1) morphology, including cell shrinkage versus cell swelling, patterns of nuclear alterations, and ultrastructural features1-3; (2) presence (oncosis) 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 bromide12; (3) DNA gel electrophoresis showing discrete multistranded DNA fragmentation ladders (apoptosis) versus diffuse random DNA fragmentation (oncosis)1,2,6; (4) positive (apoptosis) or negative (oncosis) histochemical detection of double-stranded DNA fragmentation by TUNEL or related methods7; (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 cells10; (7) identification of increased expression of genes and gene products associated with apoptosis4,5; 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 oncosis or apopto-
sis, 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.6 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 oncosis 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 oncotypic 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 oncotypic injury in the 2- and 4-hour reperfusion groups (41±3% and 83±4% positive myocytes, respectively). Irreversible oncotypic 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 oncosis 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 oncotypic 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.

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.6 A histochemical method using Taq polymerase detects single-base 3’ overhangs produced by Ca2+-dependent DNAase I, whereas the method using TdT detects 1-base 3’ overhangs as well as multiple-base 3’ overhangs produced by pH-dependent
TUNEL methods have been reported in a recent study. The sphingolipid signaling cascade with related increase in cellu-
somes and internucleosomal DNA that results in blunt
ends of the digested fragments. A probe produced with Pfu
polymerase labels blunt-ended DNA fragments. The com-
bined 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 oncocy-
tic 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 oncotypic necrosis remains.
Perhaps future work will lead to the development of tech-
niques that more precisely and specifically identify the
specific type of endonuclease activity and DNA fragmenta-
tion 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 consider-
able value. As a corollary to this, investigators should not be
limited by “TUNEL vision” but should apply a broad per-
spective to their studies.

The work of Ohno et al and the considerations raised in this
editorial clearly call into question the extent to which apo-
potis occurs in myocardial infarction in addition to classic
oncotypic 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 apo-
potis or oncotypic 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 suffi-
cient energy to die by apoptosis or, lacking sufficient energy,
will die by oncosis. The ATP level is a 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,
future research is obviously needed to clarify every nuance
of the modes of myocardial cell injury and cell death.

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