

APOPTOSIS IN MYOCYTES IN END-STAGE HEART FAILURE

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ABSTRACT

Background Heart failure can result from a variety of causes, including ischemic, hypertensive, toxic, and inflammatory heart disease. However, the cellular mechanisms responsible for the progressive deterioration of myocardial function observed in heart failure remain unclear and may result from apoptosis (programmed cell death).

Methods We examined seven explanted hearts obtained during cardiac transplantation for evidence of apoptosis. All seven patients had severe chronic heart failure: four had idiopathic dilated cardiomyopathy, and three had ischemic cardiomyopathy. DNA fragmentation (an indicator of apoptosis) was identified histochemically by in situ end-labeling as well as by agarose-gel electrophoresis of end-labeled DNA. Myocardial tissues obtained from four patients who had had a myocardial infarction one to two days previously were used as positive controls, and heart tissues obtained from four persons who died in motor vehicle accidents were used as negative controls for the end-labeling studies.

Results Hearts from all four patients with idiopathic dilated cardiomyopathy and from one of the three patients with ischemic cardiomyopathy had histochemical evidence of DNA fragmentation. All four myocardial samples from patients with dilated cardiomyopathy also demonstrated DNA laddering, a characteristic of apoptosis, whereas this was not seen in any of the samples from patients with ischemic cardiomyopathy. Histologic evidence of apoptosis was also observed in the central necrotic zone of acute myocardial infarcts, but not in myocardium remote from the infarcted zone. Rare isolated apoptotic myocytes were seen in the myocardium from the four persons who died in motor vehicle accidents.

Conclusions Loss of myocytes due to apoptosis occurs in patients with end-stage cardiomyopathy and may contribute to progressive myocardial dysfunction. (N Engl J Med 1996;335:1182-9.)

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HEART failure is estimated to affect over 3 million people in the United States.¹ Approximately 400,000 new cases of heart failure are diagnosed each year despite the widespread use of antihypertensive therapy, advances in early intervention during myocardial infarction, the advent of newer cardiotoxic or vasodilator agents, and improved investigative approaches for the early recognition of pathologic states leading

to cardiomyopathy.² Currently, the only potential cure for end-stage heart failure is cardiac transplantation,¹ which is limited by the supply of donor organs and the side effects associated with immunosuppressive therapy.

Heart failure is the final clinical presentation of a variety of cardiovascular diseases, such as coronary artery disease, hypertension, valvular heart disease, myocarditis, diabetes, and alcohol abuse.³ After various pathologic stressors and in response to increased demands for cardiac work, the heart adapts through compensatory hypertrophy of myocytes,⁴ which is characterized by an increase in the size of myocytes, and the expression of contractile and other proteins normally expressed only during fetal development.^{5,6} These short-term adaptive responses to maintain cardiac output eventually become maladaptive.^{3,4} The pathogenetic mechanisms responsible for the transition to cardiac dysfunction and clinical heart failure are not well understood. Active myocardial necrosis is histologically uncommon in cardiomyopathy, and it has been hypothesized that an ongoing process of myocyte dropout, or apoptosis (programmed cell death), may lead to a progressive deterioration in myocardial function, culminating in chronic cardiomyopathy and end-stage heart failure.⁷

Apoptosis is a tightly regulated, energy-requiring process in which cell death follows a programmed sequence of events.⁸⁻¹² Fragmentation of chromosomal DNA is the biologic hallmark of apoptosis.^{13,14} This process of DNA fragmentation is associated with the abnormal expression of genes such as *Fas*,¹⁵ *ICE* [interleukin-1 β -converting enzyme]/*CED-3-CPP-32/Yama*,^{16,17} *p53*,¹⁸ and *c-myc*¹⁹ or a deficiency of other genes, such as *Bcl2*.²⁰ Recognition of the factors responsible for the initiation or prevention of programmed cell death may eventually lead to therapeutic interventions. To determine whether apoptosis occurs in end-stage heart failure, we analyzed the explanted hearts of seven patients undergoing cardiac transplantation. DNA fragmentation was evaluated by gel electrophoresis in frozen myocardial tissue²¹ and by in situ end-labeling of formalin-fixed tissues.^{22,23}

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METHODS

Patients

Explanted hearts from seven patients (age, 18 to 56 years; mean [\pm SE], 44 ± 5 ; six men and one woman) undergoing heart transplantation at Massachusetts General Hospital were used for the analysis of DNA fragmentation. All seven patients had chronic congestive heart failure (New York Heart Association class IV) before transplantation. The duration of the illness ranged from 18 to 77 months (mean, 45 ± 9). Hemodynamic measurements revealed increased mean pulmonary-capillary wedge pressure (22 ± 3 mm Hg) and mean pulmonary-artery pressure (33 ± 5 mm Hg) (Table 1). The mean cardiac index was 1.7 ± 0.2 liters per minute per square meter of body-surface area, and the mean left ventricular ejection fraction was 20 ± 4 percent. Four of the seven patients (Patients 1, 2, 6, and 7) had idiopathic dilated cardiomyopathy (Table 1); Patient 1 also had a restrictive component. None of these four patients had more than 50 percent stenosis of any major epicardial coronary artery on coronary angiography. The remaining three patients (Patients 3, 4, and 5) had clinically significant obstructive coronary lesions and had had one or more prior myocardial infarctions; two of these three patients with ischemic cardiomyopathy had undergone coronary-artery bypass surgery before transplantation. Two patients were admitted for transplantation from home and were receiving a combination of digoxin, diuretics, and angiotensin-converting-enzyme inhibitors (Table 1). The remaining five patients were hospitalized before transplantation and were receiving dobutamine or dopamine (or both). None of these patients had received mechanical circulatory support.

At explantation, the hearts were divided into the apical third and basal two thirds. The apical third was immediately frozen in liquid nitrogen and stored at -80°C for further analysis. The remaining portion was placed in 4 percent buffered formaldehyde fixative. The formaldehyde-fixed ventricles were sectioned at 1.5-cm intervals parallel to the posterior atrioventricular sulcus.

Sections were taken from the anterior and septal walls of the left ventricle for light-microscopical examination after dehydration and embedding in paraffin and staining with hematoxylin and Masson's trichrome.

DNA End-Labeling of Tissue Sections

For in situ detection of apoptosis at the level of a single cell we used a method of end-labeling mediated by deoxynucleotidyl transferase (TdT) (Boehringer Mannheim, Mannheim, Germany).^{22,23} This method involves the addition of deoxyuridine triphosphate (dUTP) labeled with fluorescein to the ends of the DNA fragments by the catalytic action of TdT. All the end-labeling experiments were performed multiple times so that the results for various tissue samples, including prostate, myocardium, and endarterectomy specimens, could be standardized. Thick paraffin sections (4 to 6 μm) were layered on glass slides (Superfrost, Columbia Diagnostics, Springfield, Va.). The tissue sections were deparaffinized with xylene and rehydrated with graded dilutions of ethanol in water. The tissue sections were then treated with 0.05 percent saponin (Sigma Chemical, St. Louis) for 20 minutes at room temperature. The slides were washed four times with double-distilled water for two minutes and immersed in TdT buffer (Boehringer Mannheim). Then TdT (0.3 U per microliter) and fluorescein-labeled dUTP in TdT buffer were added to cover the section, and the samples were incubated in a humid atmosphere at 37°C for 60 minutes. For negative controls, TdT was eliminated from the reaction mixture. The sections were then incubated with antibody specific for fluorescein conjugated to peroxidase. The stains were visualized with a substrate system in which nuclei with DNA fragmentation stained brown. The reaction was terminated by washing the sections twice in phosphate-buffered saline. The nuclei without DNA fragmentation stained blue as a result of counterstaining with hematoxylin.

The types of cell staining positive for DNA fragmentation were characterized with monoclonal antibodies HHH 35 (Dako,

TABLE 1. CLINICAL AND HEMODYNAMIC CHARACTERISTICS OF THE SEVEN PATIENTS BEFORE TRANSPLANTATION.*

PATIENT No.	AGE (Yr)/SEX	DIAGNOSIS	DURATION OF ILLNESS	MEAN RAP	PAP			MEAN PCWP	CI	LVEF	MEDICATIONS		
					SYS-TOLIC	DIA-STOLIC	MEAN				INOTROPIC	DIURETIC	VASODILATOR
			mo	mm Hg			liters/min/m ²		%				
1	46/M	IDCM†	77	18	45	25	32	25	2.3	40	Dopamine, dobutamine, digoxin, milrinone	Bumetanide	—
2	18/M	IDCM	22	5	28	13	20	15	2.1	16	Dobutamine, digoxin	Bumetanide, spironolactone	Captopril
3‡	45/F	ISCM	18	9	60	32	43	24	1.5	19	Digoxin	Furosemide, spironolactone	Captopril
4‡	56/M	ISCM	41	5	27	10	15	11	2.2	19	Digoxin	Furosemide	Enalapril
5	46/M	ISCM	25	11	70	35	45	35	1.7	16	Dobutamine, digoxin, milrinone	Furosemide	Captopril
6	45/M	IDCM	60	14	55	40	45	28	1.0	10	Dobutamine, digoxin	Furosemide	Enalapril, isosorbide dinitrate
7	55/M	IDCM	72	—	38	18	30	18	1.4	17	Dobutamine, digoxin	Furosemide, spironolactone	—

*RAP denotes right atrial pressure, PAP pulmonary-artery pressure, PCWP pulmonary-capillary wedge pressure, CI cardiac index, LVEF left ventricular ejection fraction, IDCM idiopathic dilated cardiomyopathy, and ISCM ischemic cardiomyopathy.

†There was also a restrictive component.

‡This patient was admitted from home for transplantation.

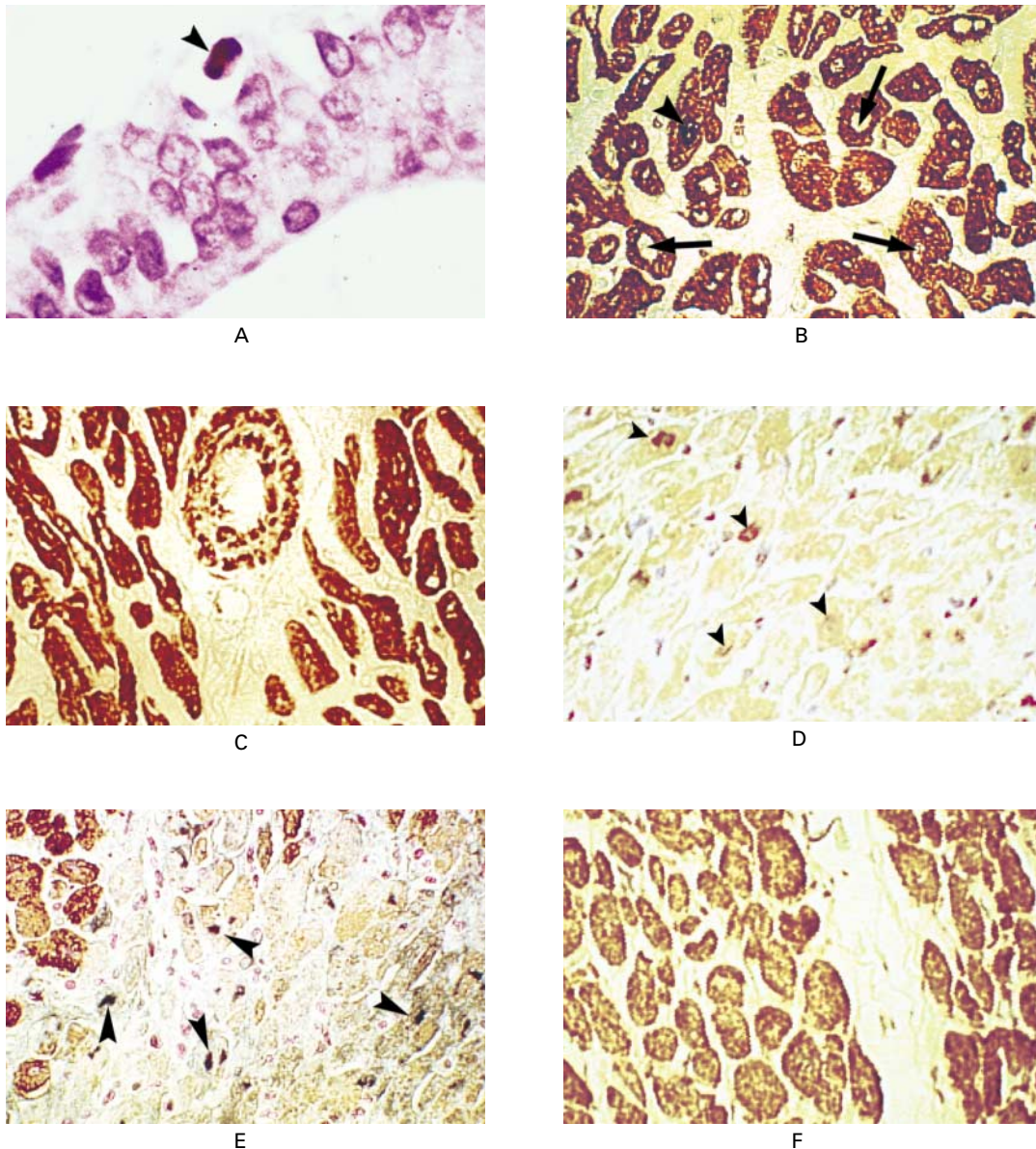


Figure 1. Standardization of in Situ End-Labeling in Rat Prostate and Normal and Infarcted Myocardium from Humans. Panel A shows prostate acini with clear nuclear regions. The sole apoptotic cell has a brown-stained nucleus (arrowhead). (End-labeling for apoptotic nuclei and hematoxylin counterstaining, $\times 750$.) Samples of normal myocardium from persons who died in motor vehicle accidents rarely have apoptotic cells (Panels B and C) (end-labeling for apoptotic nuclei and immunoperoxidase staining for actin, $\times 400$). The samples were stained for both myocyte actin and apoptotic cells; actin stains dark brown, and apoptotic cells blue-gray. Only rare isolated myocytes were apoptotic, as identified by blue-gray nuclei (arrowhead), and they were seen only at the edge of the slide (Panel B); all the other myocytes had clear nuclear regions (arrows). Most of the myocardial regions, as in Panel C, did not show apoptosis in myocytes or blood vessels. Samples for standardization were also obtained from the central infarct zone (Panel D), border infarct zone (Panel E), and a remote myocardial region (Panel F) in a patient who died of acute myocardial infarction. In Panel D, there is focal distribution of apoptotic cells in the infarct center, as identified by their brown nuclei (arrowheads) (end-labeling for apoptotic nuclei and hematoxylin counterstaining, $\times 200$). In the periphery of the infarct (Panel E) apoptotic cells with blue-gray nuclei (arrowheads) are intermixed with viable myocytes (actin-positive [brown] cells in the upper left-hand corner) and necrotic cells. In Panel F, myocardial regions far from the infarct zone contain actin-positive, nonapoptotic cells on double staining. (Panels E and F, end-labeling for apoptotic nuclei and immunoperoxidase staining for actin, $\times 200$.)

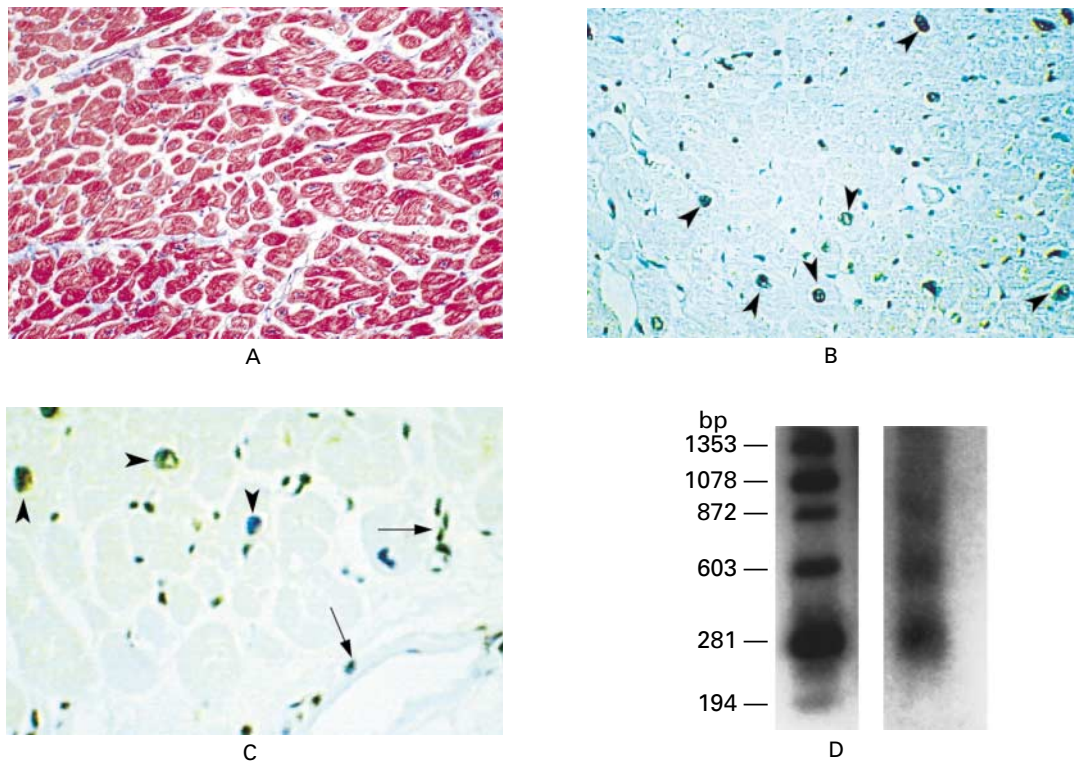


Figure 2. Evidence of Apoptosis in End-Stage Idiopathic Dilated Cardiomyopathy.

In Panel A, a myocardial section from a patient with dilated cardiomyopathy (Patient 6) contains normal myocytes and no interstitial fibrosis (Masson's trichrome staining, $\times 75$). Extensive apoptosis can be seen in myocytes in Panel B (arrowheads). Apoptosis usually occurred in groups of cells, and the severity varied from extensive (Panel B) to mild (Panel C) to absent in different regions of the myocardium. In addition to its presence in myocytes (arrowheads) in Panel C, apoptosis was also observed in vascular smooth-muscle cells of an intramyocardial arteriole as well as in rare interstitial cells (arrows). (Panels B and C, end-labeling for apoptotic nuclei and hematoxylin counterstaining, $\times 250$.) Gel electrophoresis also revealed DNA fragmentation in this patient (Panel D).

Carpinteria, Calif.) and desmin (Ventana Medical Systems, Tucson, Ariz.). Monoclonal antibody HHF 35 is specific for α -actin and γ -actin; desmin recognizes both cardiomyocytes and smooth-muscle cells. Fibroblasts and endothelium are negative for both actin and desmin. Monoclonal antibody HHF 35 was diluted to 1:200, and desmin was obtained prediluted from the manufacturer. Sections were treated with secondary goat antimouse IgG (Ventana), and the color reaction was developed with an avidin-biotin-peroxidase substrate system. For further confirmation of the location of apoptosis, a combination of HHF 35 and end-labeling was used in the same tissue sections. End-labeling was followed by antiluorescein-antibody and chromagen substrate (Vector SK-4600, Vector Laboratories, Burlingame, Calif.). The nuclei with DNA fragmentation stained blue-gray amid the surrounding brown color of actin staining, and nuclei without DNA fragmentation had clear nuclear regions.

For each myocardial specimen, tissue sections were examined microscopically at $40\times$ magnification and at least 200 cells were counted in a minimum of five high-power fields, separately in sub-epicardial, midmyocardial, and subendocardial layers. The percentage of apoptotic cells was determined by means of an apoptotic index; the apoptotic index was calculated by dividing the number of positive-staining myocyte nuclei by the total number of myocyte nuclei and multiplying that value by 100. Stained cells at the edges of the tissues were not counted, and an apoptotic index of 2 or less was considered to indicate the absence of apoptosis.

Standardization of the Staining Procedure in Control Histopathological Specimens

Formalin-fixed tissue sections from the prostate from castrated rats were used as positive controls.²³ In involuting rat prostate, apoptosis was recognized in the epithelial lining of the prostate acini (Fig. 1A). The fraction of apoptotic cells identified by end-labeling and the number identified on the basis of morphologic criteria were similar. Myocardial samples from four persons who died in motor vehicle accidents were used as negative controls. These myocardial specimens showed rare, isolated cells with DNA fragmentation (Fig. 1B and 1C); blood vessels and interstitial cells in the myocardium were normal.

Myocardial tissue samples obtained from patients with acute myocardial infarcts have been shown to contain large populations of apoptotic cells²⁴; therefore, we used four such samples as positive controls for in situ end-labeling. The apoptotic cells in these specimens were observed within the central area of necrosis (Fig. 1D) and rarely in the border zones of the infarcts (Fig. 1E). Normal myocardium far from the site of the infarct did not show evidence of DNA fragmentation (Fig. 1F).

Isolation of Genomic DNA, End-Labeling, and Electrophoresis

Frozen samples of heart tissue were minced and homogenized in extraction buffer (100 mM sodium chloride; 10 mM TRIS-

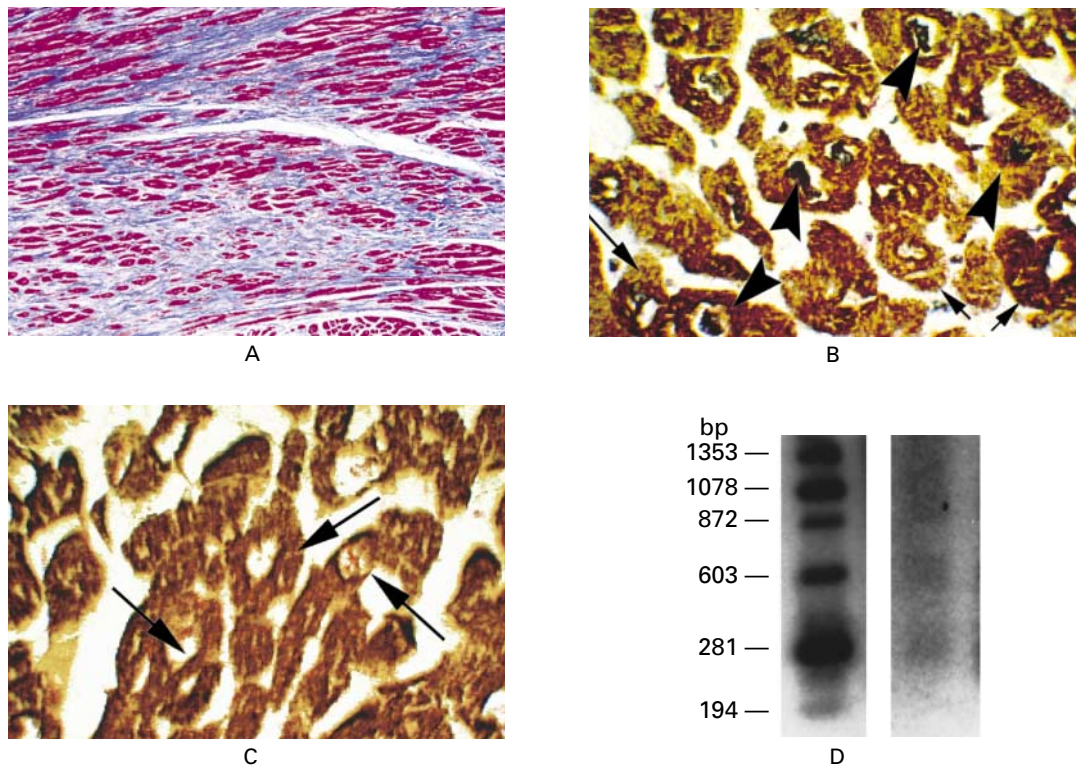


Figure 3. Apoptosis Predominantly Confined to Myocytes in Idiopathic Dilated Cardiomyopathy.

In Panel A, a myocardial section from another patient with idiopathic dilated cardiomyopathy (Patient 1) shows normal myocytes, mild myocardial hypertrophy, and moderate interstitial fibrosis (Masson's trichrome, $\times 75$). Simultaneous staining for apoptotic nuclei and actin was performed (Panels B and C). In Panel B, large numbers of myocytes are apoptotic (arrowheads); only a few cells have clear nuclear regions (arrows). In Panel C, the myocytes appear completely normal, with clear nuclear regions (arrows), demonstrating patchy distribution of apoptosis. (Panels B and C, end-labeling for apoptotic nuclei and immunoperoxidase staining for actin, $\times 350$.) In Panel D, gel electrophoresis also revealed DNA fragmentation, corroborating the histologic evidence of apoptosis.

hydrochloride, pH 8.0; 25 mM EDTA; 0.5 percent sodium dodecyl sulfate; and 0.1 mg of proteinase per milliliter) and incubated at 50°C overnight. Tissue lysates were extracted twice with phenol-chloroform (1:1). To the aqueous layer 0.2 ml of sodium acetate and 5 ml of ethanol were added. DNA was spooled from the solution and washed once with 70 percent ethanol, briefly dried in air, and resuspended in 100 μ l of distilled water. To obviate the possibility of the loss of small DNA fragments during spooling of DNA, total DNA precipitate was collected in another set of experiments by centrifugation at 10,000 revolutions per minute for 15 minutes. In addition, the solution left after spooling the DNA was kept at -20°C for one hour and centrifuged as described above. The pellet was air-dried and resuspended in 50 μ l of distilled water. The supernatant was lyophilized in a vacuum and then resuspended in 50 μ l of distilled water.

We studied nucleosomal fragmentation using the end-labeling method.²¹ Briefly, 1 μ g of genomic DNA was end-labeled in 30 μ l of reaction buffer (10 mM TRIS-hydrochloride, pH 7.5; 5 mM magnesium chloride; and 5 U *Escherichia coli* polymerase I/Klenow; New England Biolabs, Beverly, Mass.) with 0.5 μ Ci of [³²P] α -deoxycytosine triphosphate (3000 Ci per millimole; New England Nuclear-Dupont, Boston) at room temperature for 30 minutes. The reaction was stopped by adding 10 mM EDTA. DNA was precipitated with ethanol and resuspended in 100 μ l of TRIS-EDTA buffer (10 mM TRIS-hydrochloride, pH 8.0; and 0.1 mM EDTA). Approximately 10 μ l of DNA from each sample was loaded on a 1 percent agarose gel for electrophoresis followed

by autoradiography (Kodak, X-OMAT-AR, New York). End-labeled DNA samples were also separated on 5 percent acrylamide gel in a buffer consisting of 89 mM TRIS-borate and 2 mM EDTA, pH 8.0.

RESULTS

The weights of the explanted left and right ventricles along with portions of the atria ranged from 410 to 655 g. In the patients with idiopathic dilated cardiomyopathy, all four chambers were dilated, the ventricles more than the atria. No thrombi were identified. The epicardial arteries, if affected by coronary disease, were narrowed by less than 50 percent. Valvular morphology was normal in three patients; one patient had myxoid degeneration of the mitral valve. Histologic sections from the left ventricles showed absent-to-moderate interstitial fibrosis (Fig. 2A and 3A). There was mild atrophy of the myocytes with focal myofibrillar loss. Inflammatory infiltrates were absent. In the hearts from the three patients with ischemic cardiomyopathy, there was severe epicardial coronary artery disease and evidence of multiple healed infarcts. The ventricles were dilat-

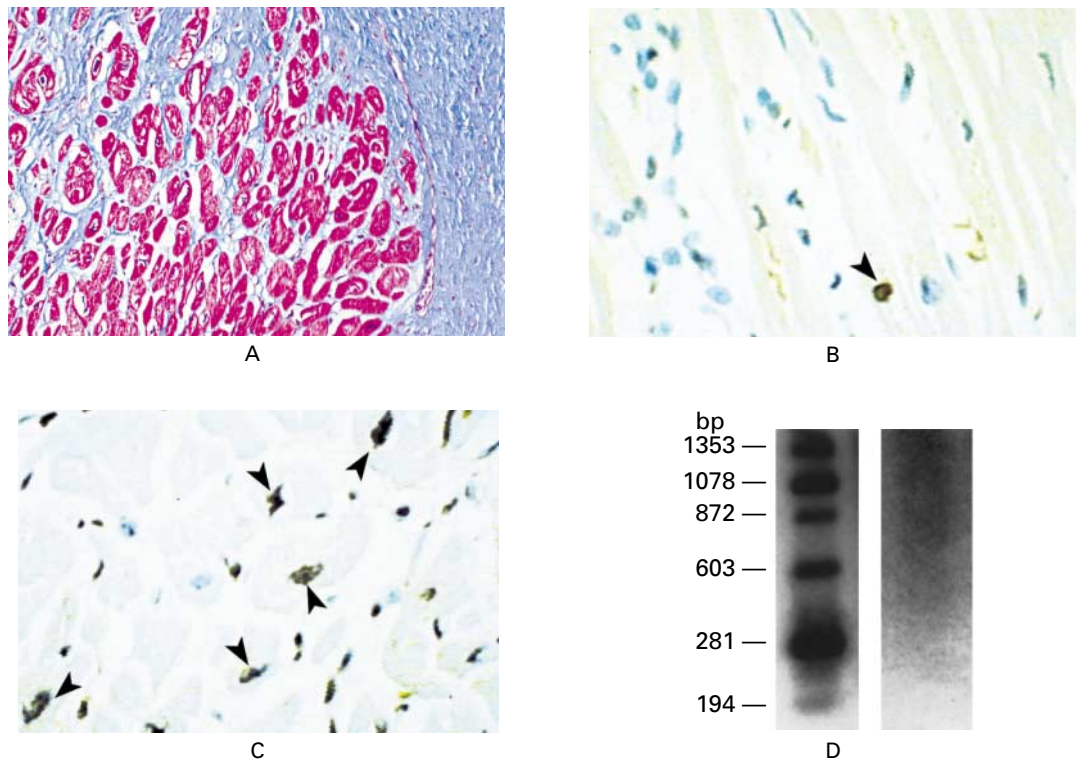


Figure 4. Apoptosis in Ischemic Cardiomyopathy.

In Panel A, a myocardial section from a patient with ischemic cardiomyopathy (Patient 3) shows mild myocardial hypertrophy and extensive interstitial fibrosis (Masson's trichrome, $\times 30$). In Panel B there is a single brown-stained nucleus suggestive of DNA fragmentation (arrowhead), whereas in Panel C, there are more apoptotic cells (arrowheads). Variability in the number of apoptotic cells demonstrates patchy distribution of the apoptotic process. (Panels B and C, end-labeling for apoptotic nuclei and hematoxylin counterstaining, $\times 350$.) In Panel D, gel electrophoresis did not reveal DNA fragmentation.

ed, the left more than the right; no thrombi were identified. Infarcts were further confirmed by histologic examination (Fig. 4A). In areas of transmural infarction, four to five subendocardial layers of myocytes were spared and showed lysis of myofibrils or vacuolar degeneration. No acute necrosis of myocytes was seen.

In situ end-labeling detected apoptosis in myocardial specimens (Table 2) from all four patients with idiopathic dilated cardiomyopathy (Fig. 2B, 2C, 3B, and 3C) and from one of the three patients with ischemic cardiomyopathy (Fig. 4B and 4C). In the patients with idiopathic dilated cardiomyopathy, the apoptotic index ranged from 5 to 35.5. Apoptosis was more predominant in the subendocardium in three patients and in the subepicardial region in the remaining patient. In one patient with ischemic cardiomyopathy, apoptosis was seen predominantly in the subepicardial region away from the area of a healed infarction (apoptotic index, 17.3). Whenever apoptosis was identified, it appeared to occur in small groups of noncontiguous cells rather than

in isolated cells (Fig. 2B, 3B, and 4C). Apoptotic cells were also not seen uniformly throughout areas of one section or in specimens obtained from different ventricular walls. In situ end-labeling performed in combination with staining for actin (with monoclonal antibody HHH 35) confirmed that apoptosis was predominantly confined to the myocytes. Apoptosis was rare in the smooth-muscle cells of the intramyocardial arterioles or in interstitial cells. There was no correlation between the apoptotic index and either the degree of impairment of left ventricular function or the severity of hemodynamic abnormalities.

With every set of end-labeling experiments, one section from every myocardial sample was used as a negative control (TdT was intentionally omitted from the incubation). All these sections were negative for nuclear staining.

All four patients with idiopathic dilated cardiomyopathy had evidence of DNA fragmentation on agarose-gel electrophoresis, which was represented by a characteristic laddering pattern of DNA fragments

TABLE 2. EVIDENCE OF APOPTOSIS ON AGAROSE-GEL ELECTROPHORESIS AND IN SITU END-LABELING.*

PATIENT No.	DIAGNOSIS	EXTENT OF FIBROSIS (PREDOMINANT DISTRIBUTION)	LADDERING OF DNA FROM APICAL MYOCARDIUM	IN SITU END-LABELING			
				INTER-VENTRICULAR SEPTUM	LV ANTERIOR WALL	APOPTOTIC INDEX	PREDOMINANT DISTRIBUTION
1	IDCM	Moderate	+	+	—	35.5	Subendocardial
2	IDCM	Mild (subendocardial)	+	+	—	15.2	Subepicardial
3	ISCM	Severe (transmural)	—	—	+	17.3	Subepicardial
4	ISCM	Severe (transmural)	—	—	Rare	—	—
5	ISCM	Severe (transmural)	—	—	—	—	—
6	IDCM	Mild	+	—	+	17.8	Subendocardial
7	IDCM	Moderate	+	+	—	5.0	Subendocardial

*LV denotes left ventricular, IDCM idiopathic dilated cardiomyopathy, and ISCM ischemic cardiomyopathy. Plus signs denote positive responses, and minus signs negative responses.

(size, 270 bp to 1 Kb) (Fig. 2D and 3D, and Table 2). None of the three patients with ischemic cardiomyopathy had DNA laddering (Fig. 4D). No DNA fragmentation was observed on acrylamide-gel electrophoresis of the lyophilized residual solution after DNA extraction by the spooling method.

DISCUSSION

There are two general mechanisms of cell death: necrosis and apoptosis.⁸⁻¹² Apoptosis is physiologically important in the maturation of organ systems (such as the deletion of autoreactive T cells and thymic involution) and the renewal of mature cells (such as leukocytes), as well as in senescence (such as late prostatic regression).²⁵⁻²⁷ Terminally differentiated cells such as myocardial or neuronal cells are not believed to undergo apoptosis under natural conditions. However, recent evidence suggests that apoptosis can be induced in cardiomyocytes by hypoxia, ischemia, and other insults.^{24,28,29}

Necrosis of myocytes is characterized by the depletion of ATP, damage to intracellular organelles, cell swelling, and rupture of cell membranes.⁸⁻¹² The extrusion of intracellular contents results in an inflammatory reaction.¹² Apoptosis, on the other hand, is an energy-requiring process that involves active intracellular signaling pathways. It involves the loss of surface contact of the index cell from the neighboring cells, cell shrinkage, and the condensation of chromatin into crescentic caps at the nuclear periphery. Eventually, endonucleolytic digestion of nuclear DNA results in the accumulation of oligonucleosomes of 180 bp or multiples of 180 bp.^{13,14} In apoptotic cells, mitochondrial DNA is not fragmented.³⁰ Apoptotic cells then undergo extracellular degeneration or phagocytosis by macrophages and neighboring cells.⁸⁻¹²

We found evidence of DNA fragmentation in all four patients with idiopathic dilated cardiomyopathy on the basis of both in situ end-labeling and electrophoresis, and in one of the three patients with ischemic cardiomyopathy on the basis of in situ end-labeling. DNA fragmentation was not observed by electrophoresis in the patients with ischemic cardiomyopathy. This discrepancy could result from the fact that different regions of myocardium were used for these studies. Furthermore, in the case of electrophoresis, DNA from apoptotic myocytes could be diluted with DNA from normal myocytes and nonmyocytes, resulting in an underestimation of the extent of apoptosis. Apoptotic cells were seen focally in noncontiguous cells, and other areas of the myocardium appeared essentially normal. Rare apoptosis of the interstitial cells as well as of the vascular smooth-muscle cells was also seen. The evidence of apoptosis obtained by either method suggests that apoptosis of myocytes may play a part in the progression of cardiomyopathy to end-stage heart disease. The inexorable decline in cardiac function seen in dilated cardiomyopathy despite the absence of an active inflammatory process may be partially explained by apoptosis.

Recent reports of the response of myocytes to a variety of stress factors lends credence to the association of apoptosis with the progression of cardiomyopathy. Transient myocardial pressure overload induces the expression of proto-oncogenes, which leads to compensatory hypertrophy of myocytes.^{31,32} However, the persistence of growth factors may result in apoptosis.¹⁹ Furthermore, an increased sarcoplasmic calcium concentration, which is a consistent feature of dilated cardiomyopathy,³³ may activate endonucleases involved in the apoptotic cascade. An elevated intracellular calcium concentration has been

linked to apoptosis in tumor cells,³⁴ and calcium-channel blockers have been shown to delay apoptosis.³⁵ In thymocytes, increases in the concentrations of calcium, cyclic AMP, and calcium ionophore have been shown to induce apoptosis.^{36,37} In addition to the persistent expression of proto-oncogenes and intracellular calcium overload, relative hypoxia of myocytes due to left ventricular hypertrophy³⁸ or dilatation may also perpetuate apoptosis. The possibility of the role of inotropic agents in the induction of apoptosis cannot be excluded, especially since all the patients with idiopathic dilated cardiomyopathy were receiving catecholamines. However, the focal occurrence of apoptosis argues against a major role for catecholamine-induced apoptosis.

Our results support the hypothesis that apoptosis is one of the mechanisms leading to end-stage heart disease.⁷ Larger studies with explanted hearts and serial endomyocardial biopsies are required to pinpoint the prevalence of apoptosis and the part that apoptosis may play in the progression of myocardial hypertrophy to overt heart failure. Although apoptosis appears to be irreversible, it has been suggested that it can be modulated by growth factors, or cytokines.^{18,19,39,40} If apoptosis is involved in the death of myocytes, this knowledge may be useful in finding a way to prevent progressive left ventricular dysfunction.

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