

APOPTOSIS IN THE FAILING HUMAN HEART

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ABSTRACT

Background Loss of myocytes is an important mechanism in the development of cardiac failure of either ischemic or nonischemic origin. However, whether programmed cell death (apoptosis) is implicated in the terminal stages of heart failure is not known. We therefore studied the magnitude of myocyte apoptosis in patients with intractable congestive heart failure.

Methods Myocardial samples were obtained from the hearts of 36 patients who underwent cardiac transplantation and from the hearts of 3 patients who died soon after myocardial infarction. Samples from 11 normal hearts were used as controls. Apoptosis was evaluated histochemically, biochemically, and by a combination of histochemical analysis and confocal microscopy. The expression of two proto-oncogenes that influence apoptosis, *BCL2* and *BAX*, was also determined.

Results Heart failure was characterized morphologically by a 232-fold increase in myocyte apoptosis and biochemically by DNA laddering (an indicator of apoptosis). The histochemical demonstration of DNA-strand breaks in myocyte nuclei was coupled with the documentation of chromatin condensation and fragmentation by confocal microscopy. All these findings reflect apoptosis of myocytes. The percentage of myocytes labeled with *BCL2* (which protects cells against apoptosis) was 1.8 times as high in the hearts of patients with cardiac failure as in the normal hearts, whereas labeling with *BAX* (which promotes apoptosis) remained constant. The near doubling of the expression of *BCL2* in the cardiac tissue of patients with heart failure was confirmed by Western blotting.

Conclusions Programmed death of myocytes occurs in the decompensated human heart in spite of the enhanced expression of *BCL2*; this phenomenon may contribute to the progression of cardiac dysfunction. (N Engl J Med 1997;336:1131-41.)

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CARDIOMYOPATHY of either ischemic or nonischemic origin is characterized by a progressive loss of myocytes.¹⁻³ Defects in coronary blood flow develop in the overloaded myocardium,^{4,5} resulting in myocyte death and fibrosis at multiple sites in the ventricular wall.^{1-3,5} Recently, apoptosis in myocytes has been demonstrated experimentally after injury due to ischemia

and reperfusion,⁶ myocardial infarction,⁷ cardiac aging,⁸ ventricular pacing,⁹ and coronary embolization.¹⁰ Whether this form of cell death occurs in the failing human heart is not known, however. Apoptosis in the myocardium is complex and thus difficult to recognize. Myocyte apoptosis is scattered across the wall and is restricted to individual cells.^{6,8,9} In the early stages, cell structure is preserved because the damage is limited to the internucleosomal region of DNA, leaving the cytoplasm intact.¹¹ Moreover, the activation of this cellular "suicide program" may be modulated by the expression of the proto-oncogenes *BCL2* (which protects cells from apoptosis), and *BAX* (which opposes the effects of *BCL2*, thereby promoting apoptosis).¹²⁻¹⁴

Studies of apoptosis in diseased hearts have shown great variability in the magnitude of this phenomenon,^{8-10,15-20} thus raising questions about the specificity and sensitivity of DNA end-labeling by the terminal deoxynucleotidyl transferase (TdT) assay, a method commonly used to identify apoptosis. Findings of high levels of apoptosis may be questioned, since the completion of this process may require from 20 minutes to 24 hours.²¹⁻²³ A massive loss of heart tissue may therefore occur over a very short period. In an attempt to clarify these contrasting findings, we used a new approach to the assessment of cell death in myocardial samples obtained from patients with congestive heart failure. Quantitative measurements of apoptotic myocyte nuclei were obtained by the TdT reaction with a fluorescence probe^{7-9,15,17}; this assay was complemented by characterization of the chromatin pattern in the same nuclei by confocal microscopy. This method of analysis combined the histochemical detection of internucleosomal cleavage with the structural definition of chromatin alterations. In addition, DNA laddering was identified in comparable myocardial specimens in order to confirm DNA fragmentation biochemically. Finally, changes in the expression of *BCL2* and *BAX* in the cells were evaluated.

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METHODS

Ventricular Function

Measurements of systolic and diastolic ventricular dimensions and fractional shortening were performed by two-dimensional echocardiography one to six months before transplantation. The stroke-volume index, cardiac output, cardiac index, ejection fraction, right ventricular end-diastolic pressure, and pulmonary-artery wedge pressure were determined by cardiac catheterization.

Tissue Fixation

Samples were collected from the explanted hearts of 36 patients undergoing cardiac transplantation. Three additional samples were obtained two to six hours after death from patients, previously in good health, who died within four days after an acute myocardial infarction. Specimens were fixed in 10 percent buffered formalin or frozen in liquid nitrogen. Control samples of myocardium were obtained within six hours after death from eight patients who died of causes other than cardiovascular disease.²⁴ Three right ventricular endomyocardial specimens obtained before cardioplegic arrest in brain-dead cardiac donors were also used as controls.

TdT Assay

Myocardial sections were incubated with a solution containing 5 units of TdT, 2.5 mM cobalt chloride, 0.2 M potassium cacodylate, 24 mM TRIS-hydrochloride, 0.25 percent bovine serum albumen, and 0.5 nM biotin-16-deoxyuridine triphosphate (dUTP). After exposure to a solution containing 5 μ g of fluorescein isothiocyanate-Extravidin per milliliter, myocytes were stained with α -sarcomeric actin antibody (clone 5C5, Sigma), and the nuclei were visualized with bisbenzimidazole.^{7,9,15,17}

DNA-Strand Breaks in Myocytes

Myocyte nuclei labeled with dUTP were measured by examination with an ocular reticle containing 42 sampling points, covering a minimum of 45 mm² and a maximum of 423 mm² of tissue in each heart. The volume fraction of replacement fibrosis was also evaluated.⁹ The number of myocyte nuclei per unit of area of tissue was determined by counting an average of 50 fields, each 6084 μ m² in size, in each ventricular sample. By combining these data with the estimated numbers of dUTP-labeled myocyte nuclei, the number of apoptotic myocyte nuclei per 10⁶ nuclei was determined.^{7,9,15,17} Myocyte diameter was determined by measuring 100 cells in the region of the nucleus in each left ventricle and averaging these measurements.

Confocal Microscopy

To correlate chromatin alterations with the presence or absence of dUTP labeling, histologic sections were analyzed by confocal microscopy (MRC-1000, Bio-Rad). Chromatin was visualized by staining with propidium iodide (10 μ g per milliliter). Sections were examined at a magnification of 100 (numerical aperture, 1.3). Nine specimens, six from patients with ischemic cardiomyopathy and three from patients with idiopathic dilated cardiomyopathy, were evaluated. In each case, 11 to 16 nuclei that showed only dUTP labeling, both chromatin alterations and dUTP labeling, and chromatin or nuclear damage with no dUTP labeling were collected. A total of 126 myocyte nuclei were analyzed.

DNA Gel Electrophoresis

Fragments of myocardium were homogenized, fixed in 70 percent ethanol, and incubated in 40 μ l of phosphate-citrate buffer (pH 7.8) for one hour. The supernatant was concentrated by vacuum and digested with RNase (1 mg per milliliter) and proteinase K (1 mg per milliliter). Samples were subjected to electrophoresis on 1 percent agarose gel containing 5 μ g of ethidium bromide per milliliter.^{7,9,15,17}

Localization of BCL2 and BAX

Sections were incubated with anti-BCL2 peptide (position of amino acids, 41 to 54) antiserum at a dilution of 1:2000 and anti-BAX peptide (position of amino acids, 43 to 61) antiserum at a dilution of 1:800. After washing with phosphate-buffered saline, sections were incubated for one hour with 2.8 μ g of biotinylated goat antirabbit antibody per milliliter and then with an avidin-biotin complex reagent containing horseradish peroxidase.⁷

Western Blotting

Specimens were lysed, and aliquots containing 50 μ g of protein were fractionated by sodium dodecyl sulfate-polyacrylamide-gel electrophoresis (12 percent gels) and transferred to nitrocellulose filters. Blots were washed with phosphate-buffered saline, treated with 2 percent hydrogen peroxide, and saturated for unspecific binding sites with a buffer containing 10 mM TRIS, 150 mM sodium chloride, and 0.1 percent Tween 20 (pH 7.9; TNT), supplemented with 5 percent nonfat dry milk, 2 percent bovine serum albumen, and 1 percent goat serum. Subsequently, membranes were incubated at 4°C in TNT containing 0.1 to 0.05 percent anti-BCL2 or 0.1 percent anti-BAX antiserum. Blots were washed in phosphate-buffered saline and incubated with peroxidase-conjugated antirabbit IgG. Irrelevant antibodies (rabbit antirat IgG and rabbit antimouse IgG) were used as negative controls.²⁵

Statistical Analysis

All tissue samples were coded, and the code was broken at the end of the studies. Results are presented as means \pm SD. Statistical significance ($P < 0.05$) in comparisons between two measurements and among groups was determined by the two-tailed Student's *t*-test and by analysis of variance with the Bonferroni method, respectively.²⁶

RESULTS

Patients

Twenty of the 39 patients whose hearts were studied had ischemic cardiomyopathy, and 18 had idiopathic dilated cardiomyopathy (Table 1). One patient had mitral stenosis and aortic regurgitation. These patients each had a marked reduction in ejection fraction and a substantial increase in left ventricular diastolic and systolic diameter (Table 2). The ratio of wall thickness to chamber radius was reduced, and left ventricular end-diastolic volume was nearly twice the control value in all patients. At the time of surgery, 20 patients were being treated with intravenous inotropic drugs, and 28 were receiving diuretics. Angiotensin-converting-enzyme inhibitors were administered to 13 patients, and digitalis to 10. Seventeen of the 20 patients with ischemic cardiomyopathy had previously had a myocardial infarction; bypass surgery had been performed in 8.

dUTP Labeling of the Myocardium

We analyzed tissue from 8 control and 15 diseased hearts with dUTP labeling. Control myocardium was restricted to the left ventricle, whereas 15 specimens of the left ventricle and 11 of the right ventricle were available from the 15 diseased hearts. Apoptotic myocyte nuclei were rare in normal myocardium (Fig. 1A and 1B). Scattered dUTP labeling was seen in fail-

TABLE 1. CLINICAL CHARACTERISTICS OF THE PATIENTS WHOSE HEARTS WERE STUDIED.*

| CONDITION OR GROUP | NO. OF PATIENTS | AGE (YR) | SEX (M/F) | DURATION OF DISEASE (MO) | TIME FROM HEART FAILURE TO TRANSPLANTATION (MO) |
|-----------------------------------|-----------------|----------|-----------|--------------------------|---|
| Ischemic cardiomyopathy | 20 | 59±8 | 18/2 | 131±103† | 14±18† |
| Idiopathic dilated cardiomyopathy | 18 | 46±12 | 13/5 | 80±53 | 16±16 |
| Valvular heart disease | 1 | 41 | 1/0 | 192 | 12 |
| Control | 11 | 59±15 | 9/2 | — | — |

*Plus-minus values are means ±SD.

†Data were available for 17 patients.

TABLE 2. ECHOCARDIOGRAPHIC AND HEMODYNAMIC MEASUREMENTS ACCORDING TO THE TYPE OF HEART FAILURE.*

| VARIABLE | ISCHEMIC CARDIOMYOPATHY | IDIOPATHIC DILATED CARDIOMYOPATHY | VALVULAR HEART DISEASE | NORMAL VALUE |
|--|-------------------------|-----------------------------------|------------------------|--------------|
| Left ventricular diameter (mm) | | | | |
| Systolic | 57±13 | 61±15 | 60 | 20–35 |
| Diastolic | 69±10 | 69±12 | 80 | 37–56 |
| Ratio of wall thickness to chamber radius | 0.26±0.12 | 0.28±0.08 | 0.29 | 0.32–0.39 |
| Fractional shortening (%) | 14±5.7 | 12±7 | 25 | 34–44 |
| Left ventricular end-diastolic volume (ml/m ² of body-surface area) | 150±18 | 174±21 | 168 | 44–96 |
| Ejection fraction (%) | 27±9 | 25±13 | 20 | >50 |
| Stroke-volume index (ml/beat/m ²) | 29±5 | 21±9 | 38 | 20–41 |
| Cardiac output (ml/min) | 4133±839 | 3534±753 | 4435 | 5000–7000 |
| Cardiac index (ml/min/m ²) | 2213±492 | 2150±284 | 2240 | 2600–4200 |
| Mean pulmonary-artery wedge pressure (mm Hg) | 26±9 | 14±5 | 23 | 1–10 |
| Right ventricular end-diastolic pressure (mm Hg) | 7±2 | 16±8 | 12 | 0–8 |

*Plus-minus values are means ±SD.

ing hearts (Fig. 1C). At times, DNA-strand breaks affected groups of two to three myocytes (Fig. 1D). Nine of the 15 samples of left ventricular tissue and 4 of the 11 samples of right ventricular tissue from failing hearts included areas of scarring consistent with necrotic cell death. The degree of myocardial fibrosis in these 13 specimens varied from 1 to 44 percent (mean, 10±12 percent). Foci of reparative fibrosis were seen in three of eight control hearts (mean degree of myocardial fibrosis, 2±2 percent). The myocyte diameter was 21±2 μm in control hearts, 26±3 μm (24 percent larger, P=0.0018) in samples from patients with ischemic cardiomyopathy, and

25±3 μm (19 percent larger, P=0.017) in samples from patients with idiopathic dilated cardiomyopathy. There was no correlation between the diameter of myocytes and the degree of apoptosis. Moreover, the distribution of dUTP-labeled myocyte nuclei was independent of the sites of scarring.

Table 3 lists the numbers of dUTP-labeled myocyte nuclei in samples from 8 control hearts and 15 failing hearts. Since the magnitude of DNA-strand breakage in myocytes was similar in the two ventricles, data from the left and right ventricles in diseased hearts were combined. In normal myocardium, apoptotic myocytes were absent or affected at most 28 nuclei

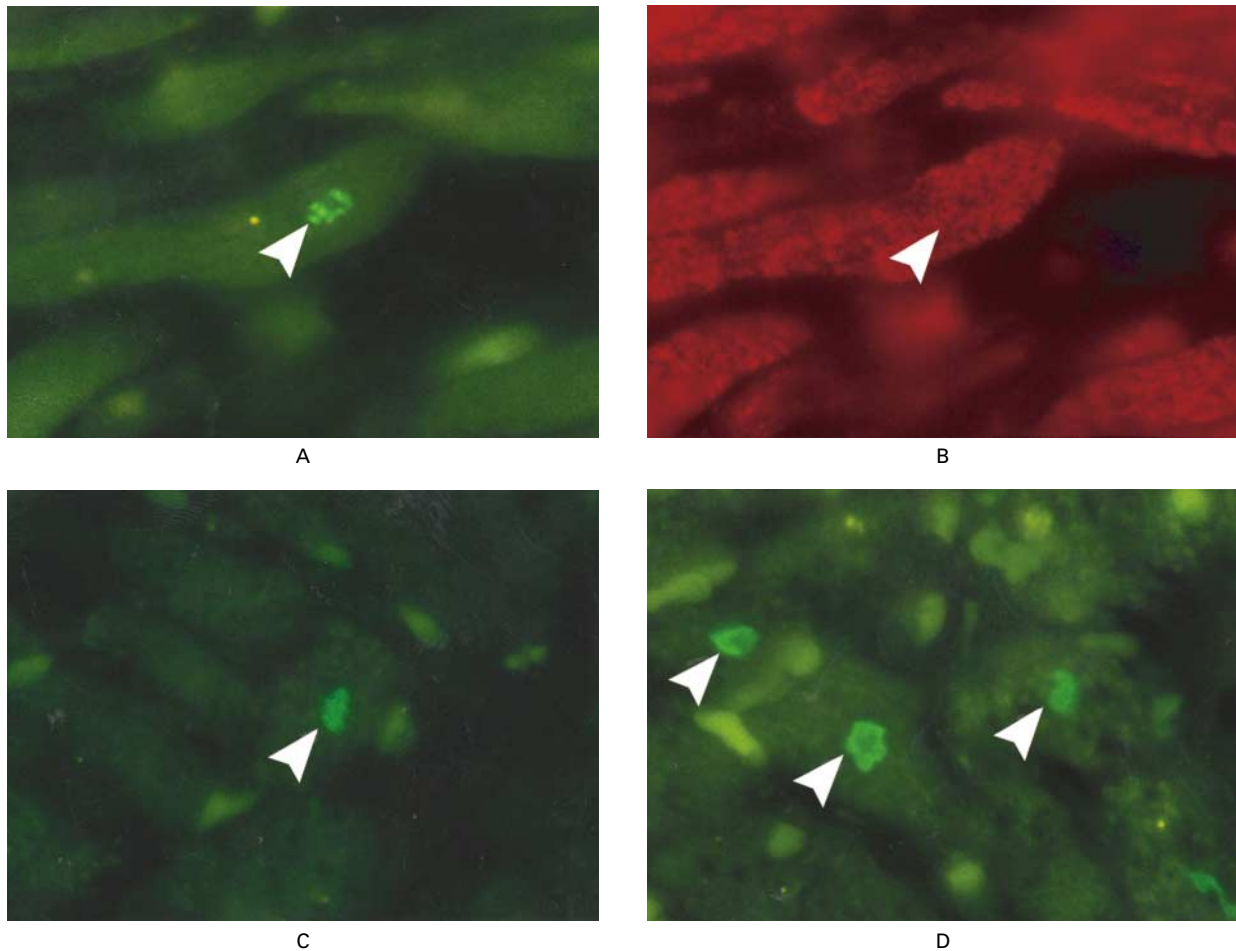


Figure 1. Sections of Left Ventricular Myocardium Showing DNA-Strand Breaks (Arrowheads) in a Myocyte Nucleus (Panels A, B, and C) and in Three Myocyte Nuclei (Panel D) in Control and Diseased Hearts.

Panels A, C, and D show the labeling with deoxyuridine triphosphate; Panel B shows the same microscopical field as Panel A, but after labeling with α -sarcomeric actin antibody (the arrowhead indicates the nuclear region). Panels A and B show tissue from a control heart ($\times 1200$), Panel C tissue from the heart of a patient with ischemic cardiomyopathy ($\times 900$), and Panel D tissue from a patient with idiopathic dilated cardiomyopathy ($\times 900$).

per million. This value was markedly increased in patients with congestive heart failure, from a minimum of 673 to a maximum of 6549 nuclei per million. The average 232-fold increase in the extent of apoptosis in patients with congestive heart failure, as compared with controls, was significant ($P = 0.0036$).

Myocyte Apoptosis as Assessed by Confocal Microscopy

The analysis by confocal microscopy involved 126 myocyte nuclei collected from six samples from patients with ischemic cardiomyopathy (samples 1, 2, 3, 5, 6, and 7 in Table 3) and three from patients with idiopathic dilated cardiomyopathy (samples 2, 4, and 5 in Table 3). Panels A, B, and C in Figure 2 show a myocyte nucleus with preserved chromatin structure

and a smaller nucleus with chromatin that appears condensed, uniform, and smooth in the same microscopical field. These modifications in the characteristics of chromatin were associated with dUTP labeling, indicating DNA-strand breaks and morphologic changes consistent with apoptosis. dUTP-positive nuclei with normal chromatin were also seen in these myocardial samples (Panels D, E, and F in Fig. 2). Nuclear fragmentation in the presence and absence of dUTP labeling was seen as well (Panels G, H, I, and J in Fig. 2). Of the 126 nuclei we studied, 96 exhibited dUTP labeling and chromatin and nuclear alterations, 18 showed dUTP labeling only, and 12 had chromatin or nuclear damage but were negative for dUTP labeling. These values corresponded to 77 ± 5

TABLE 3. DEOXYURIDINE TRIPHOSPHATE LABELING OF MYOCYTE NUCLEI.*

| GROUP AND SAMPLE No. | AREA SAMPLED (mm ²) | NO. OF LABELED MYOCYTE NUCLEI | MYOCYTE NUCLEAR DENSITY (no./mm ²) | NO. OF LABELED MYOCYTE NUCLEI/10 ⁶ NUCLEI |
|--|---------------------------------|-------------------------------|--|--|
| Control hearts | | | | |
| 1 | 68 | 0 | 351 | 0 |
| 2 | 222 | 1 | 304 | 15 |
| 3 | 294 | 3 | 368 | 28 |
| 4 | 140 | 0 | 366 | 0 |
| 5 | 252 | 1 | 340 | 12 |
| 6 | 275 | 1 | 373 | 10 |
| 7 | 206 | 1 | 257 | 19 |
| 8 | 175 | 0 | 351 | 0 |
| All control hearts (n=8) | | | 339±37 | 10±9 |
| Diseased hearts | | | | |
| Ischemic cardiomyopathy | | | | |
| 1 | 98 | 47 | 250 | 1918 |
| 2 | 66 | 102 | 236 | 6549 |
| 3 | 91 | 22 | 156 | 1550 |
| 4 | 45 | 6 | 196 | 673 |
| 5 | 79 | 35 | 205 | 2161 |
| 6 | 241 | 71 | 232 | 1270 |
| 7 | 216 | 310 | 264 | 5436 |
| 8 | 423 | 91 | 158 | 1362 |
| 9 | 156 | 34 | 217 | 1004 |
| | | | 213±36 | 2436±1964 |
| Idiopathic dilated cardiomyopathy | | | | |
| 1 | 71 | 123 | 274 | 6323 |
| 2 | 94 | 51 | 257 | 2111 |
| 3 | 90 | 19 | 246 | 858 |
| 4 | 149 | 71 | 292 | 1632 |
| 5 | 165 | 31 | 207 | 908 |
| | | | 255±29 | 2366±2033 |
| Valvular heart disease | | | | |
| 1 | 90 | 18 | 197 | 1015 |
| All diseased hearts (n=15) | | | 226±39 | 2318±1953 |

*Plus-minus values are means ±SD.

percent, 14±5 percent, and 9±2 percent of all nuclei examined, respectively.

DNA Gel Electrophoresis

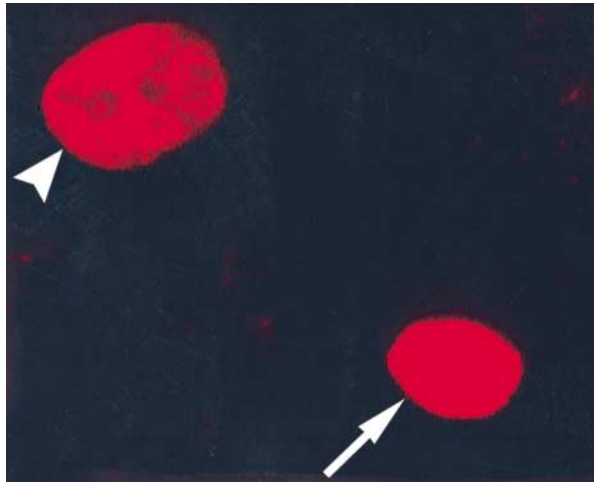
The electrophoretic analysis included tissue from the hearts of three control subjects, five patients with ischemic cardiomyopathy, and six with idiopathic dilated cardiomyopathy. Samples of both ventricles were available from the 11 failing hearts, whereas normal myocardium came only from the left ventricle. DNA nucleosome ladders were present in the myocardium of patients with idiopathic dilated cardiomyopathy and ischemic cardiomyopathy (Fig. 3A and 3B). DNA laddering was seen in all 22 samples examined from diseased hearts. In contrast, no DNA fragments were detected in the three control hearts (Fig. 3A). A diffuse pattern of DNA indicative of cell necrosis was apparent in one heart from a patient with idiopathic dilated cardiomyopathy and

three from patients with ischemic cardiomyopathy (Fig. 3B).

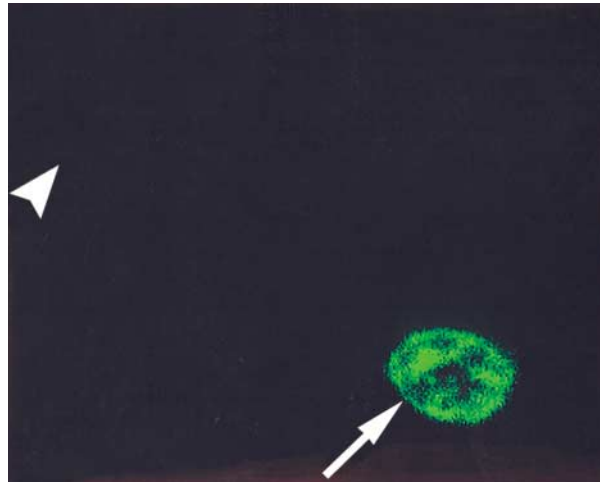
Expression of BCL2 and BAX

BCL2 protein was apparent in the myocyte cytoplasm (Fig. 4A and 4B), and BCL2-positive cells were more numerous in diseased hearts. BAX protein had a similar cytoplasmic localization (Fig. 4C and 4D), but no differences were apparent between control and failing hearts. The results for samples from the left and right ventricles were combined. Heart failure was characterized by a near doubling of the percentage of myocytes labeled with BCL2 (control hearts, 36±11 percent; diseased hearts, 66±18 percent; P<0.001). In contrast, the fraction of myocytes labeled with BAX remained roughly constant (control hearts, 75±11 percent; diseased hearts, 84±13 percent).

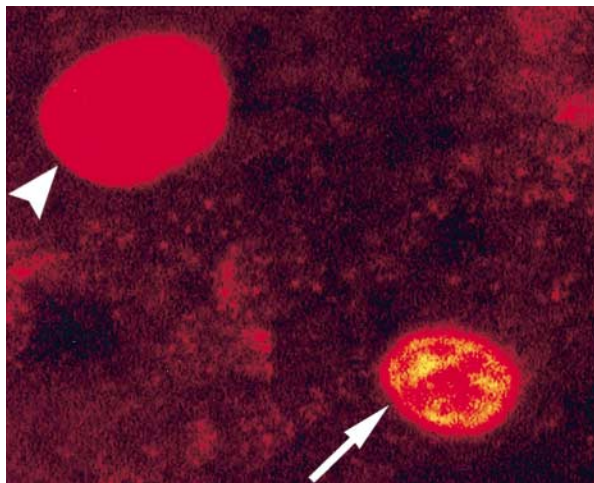
The changes in the expression of BCL2 and BAX



A



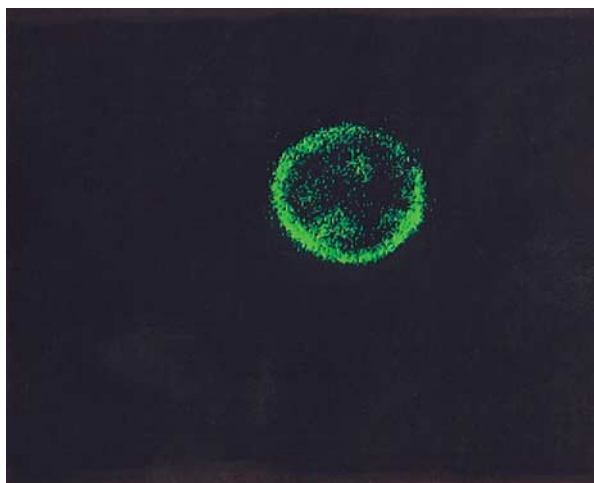
B



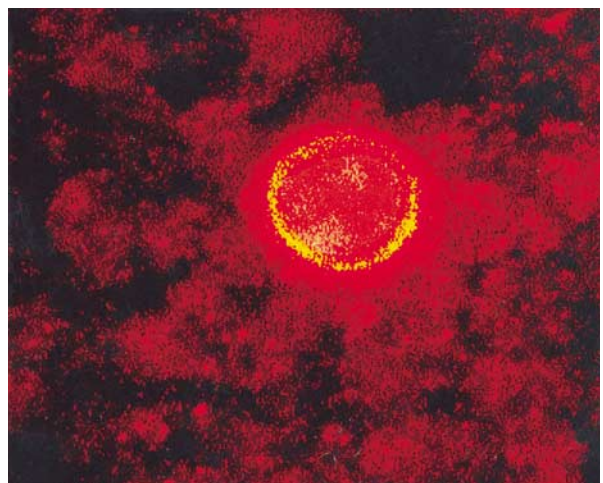
C



D



E



F

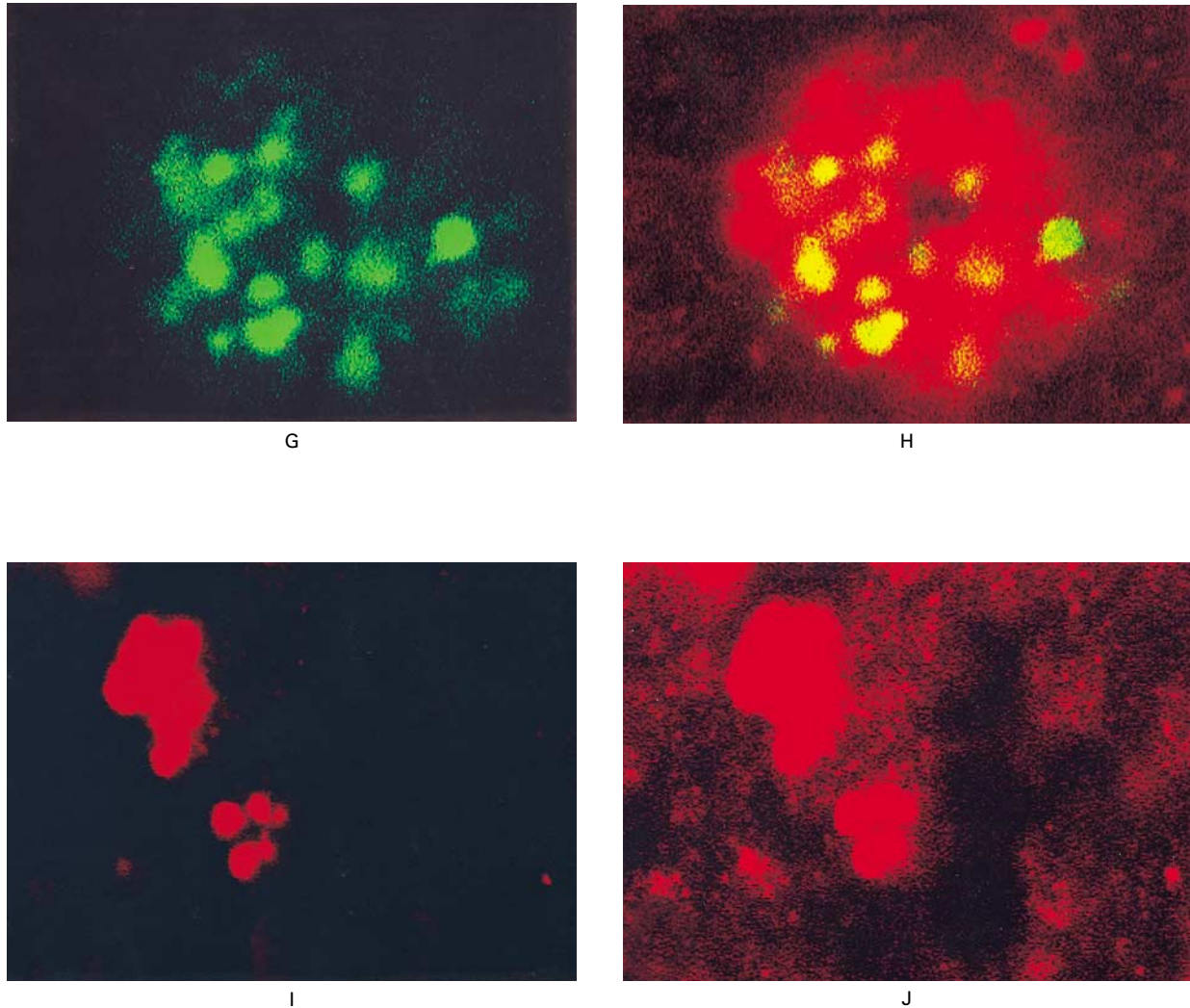


Figure 2. Myocyte Nuclei from Patients with Ischemic and Idiopathic Dilated Cardiomyopathy, Seen on Confocal Microscopy.

In Panel A an apoptotic small, homogeneous, condensed nucleus (arrow) and a normal nucleus (arrowhead) show red fluorescence after propidium iodide staining. Panel B shows the same nuclei after labeling with deoxyuridine triphosphate (dUTP); the apoptotic nucleus is recognizable by its green fluorescence. The combination of propidium iodide and dUTP labeling is shown in Panel C, in which α -sarcomeric actin staining of the myocyte cytoplasm produces red fluorescence. The visualization of α -sarcomeric actin labeling required an increase in the gain of the photomultiplier of the confocal microscope, which resulted in overexposure of the propidium iodide staining of both nuclei in Panel C. Panels D, E, and F show a myocyte nucleus with apparently normal morphologic features after staining with propidium iodide, labeling with dUTP, and the combination of labeling with propidium iodide and dUTP, along with α -sarcomeric actin, respectively. Panels G and H show dUTP labeling of a myocyte nucleus undergoing fragmentation; propidium iodide staining alone is not shown. Panels I and J show a fragmented nucleus as seen with propidium iodide staining alone (Panel I) and in combination with α -sarcomeric actin labeling (Panel J). The negative dUTP labeling of this fragmented myocyte nucleus is not shown. (Panels A through F, I, and J, $\times 1500$; Panels G and H, $\times 3000$.)

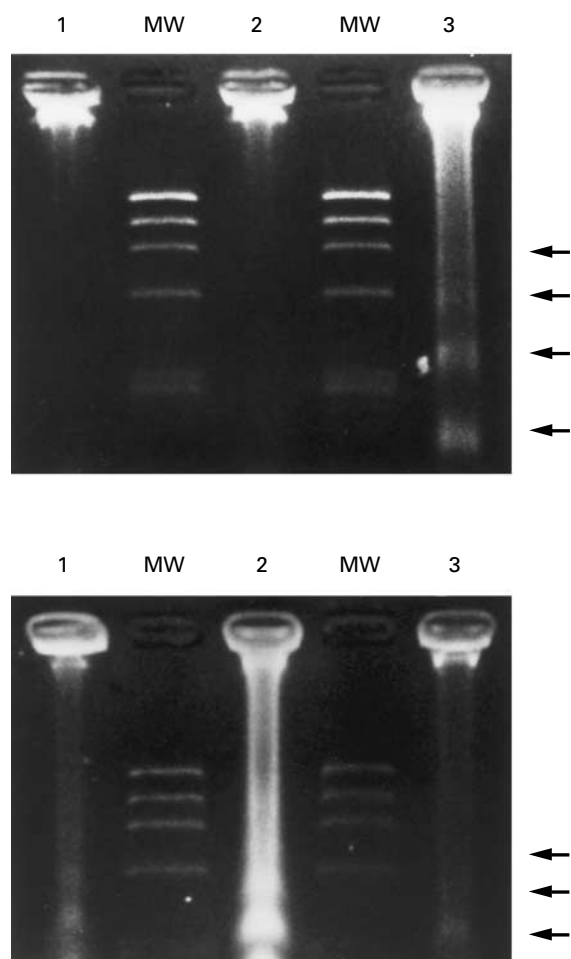


Figure 3. Electrophoretic Pattern of DNA Fragments in Myocytes Extracted from Two Control Hearts (Top Panel, Lanes 1 and 2), from the Heart of a Patient with Idiopathic Dilated Cardiomyopathy (Top Panel, Lane 3), and from the Hearts of Three Patients with Ischemic Cardiomyopathy (Bottom Panel, Lanes 1 through 3).

A combination of DNA laddering and diffusion is apparent in the bottom panel, lane 2. MW denotes markers of molecular weight. The arrows indicate multiples of 180 bp.

in the myocardium were also analyzed by Western blotting. This portion of the study included samples from three control hearts, three hearts from patients with ischemic cardiomyopathy, and three from patients with idiopathic dilated cardiomyopathy. As Figure 5 shows, the amount of BCL2 protein was higher in decompensated hearts than in normal hearts. Similar results were obtained in the two ventricles, and the data were therefore combined. In comparison with control hearts, there was a significant 2.4-fold increase in BCL2 in failing hearts (optical density: control hearts, 30 ± 10 ; hearts from patients with congestive heart failure, 72 ± 18)

($P < 0.01$). However, the expression of BAX protein was not altered by heart failure (optical density: control hearts, 76 ± 9 ; hearts from patients with congestive heart failure, 88 ± 11) (Fig. 5).

DISCUSSION

Heart Failure and Myocyte Death

These results demonstrate that cell death accompanies irreversible congestive heart failure in humans. Myocyte death occurred through apoptosis and necrosis. Apoptosis was documented histologically by the TdT assay and biochemically by DNA agarose-gel electrophoresis. These methods identified double-strand cleavage of the DNA in myocyte nuclei and DNA laddering in the myocardium, respectively. Myocyte necrosis was inferred on the basis of sites of reparative fibrosis in the ventricular wall and a diffuse pattern, resembling a smear, of DNA. The magnitude of ongoing programmed myocyte death was measured quantitatively and found to amount to an average of 2318 cells per 10^6 myocytes. The extent of acute necrotic cell death was not determined in these tissue samples, but the consequences of this form of myocyte loss resulted in scarring of nearly 10 percent of the myocardium. Whether ongoing myocyte necrosis was present in these failing hearts could not be established morphologically.^{7,8,27}

The degree of apoptosis in myocytes varied considerably in recent investigations,^{8-10,15-20} possibly reflecting technical limitations in the procedures used. In the current study, confocal microscopy was used to address this critical issue. With this approach, it was possible to document that both dUTP labeling of the DNA and alterations in the morphologic features of chromatin that are typical of apoptosis²⁸ were present in 77 percent of myocyte nuclei. In addition, 14 percent of myocyte nuclei with normal-appearing chromatin were positive for dUTP. Since the formation of DNA-strand breaks precedes structural damage,¹¹ the degree of dUTP labeling in cells with no apparent loss of morphologic integrity is consistent with the progression of the apoptotic process. Nine percent of myocyte nuclei had severe changes in chromatin but were dUTP-negative. This phenomenon may reflect apoptotic changes with limited digestion of the genomic DNA to 300-kb and 50-kb fragments without the formation of mononucleosomes and oligonucleosomes. Such an occurrence has been demonstrated in hepatocytes and in endothelial and epithelial cells.^{21,29} Even if the 9 percent of cells with apoptotic nuclei that were not detected by dUTP labeling are included in calculating the prevalence of apoptotic cells, the estimated value increases only from 0.23 percent to 0.25 percent. This analysis did not include the contribution of apoptotic bodies, because of the difficulty of recognizing the cell of origin in these late stages of cell death.

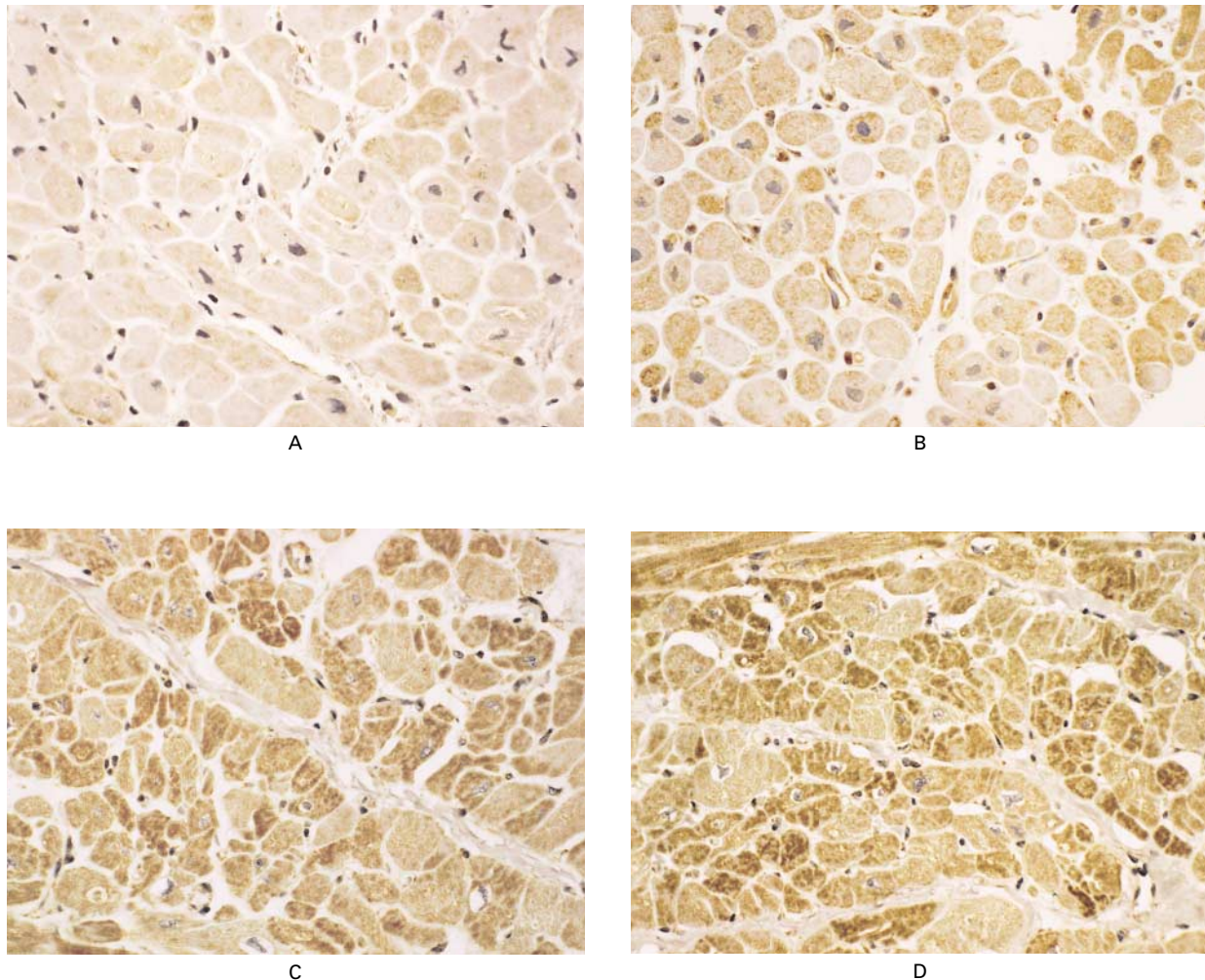


Figure 4. Detection of the Proteins BCL2 (Panels A and B) and BAX (Panels C and D). Panels A and C show tissue from control hearts, and Panels B and D tissue from decompensated hearts. (Panels A and B, $\times 600$; Panels C and D, $\times 450$).

Our results differ from the observations of Narula et al.,¹⁹ who found that the percentage of myocytes affected by apoptosis in patients with ischemic cardiomyopathy and idiopathic dilated cardiomyopathy was 5 to 35.5 percent. These percentages are 20 and 142 times as high as those reported here, raising questions about the actual level of apoptosis in patients with cardiac failure. Since this form of cell death is completed in at most a few hours,^{21,22} such high values would be incompatible with life. Moreover, the histochemical detection of apoptosis in the study by Narula et al. did not include morphologic confirmation of chromatin abnormalities and nuclear damage, suggesting that our estimate of the degree of this process in the decompensated human

heart is more reliable. The inclusion of a larger number of samples allowed us to measure the extent of apoptosis in patients with ischemic cardiomyopathy and idiopathic dilated cardiomyopathy in quantitative statistical terms. We found no significant difference between these two pathologic conditions.

Measurements of the number of myocytes in humans have shown that cell loss occurs in ischemic cardiomyopathy,³⁰ idiopathic dilated cardiomyopathy,² and hypertensive hypertrophy.³¹ Apoptosis was not analyzed in these studies, and necrosis was considered the exclusive mechanism of myocyte death. Similarly, the loss of myocytes with aging has been linked to defects in the oxygenation potential of the aging myocardium and to myocyte necrosis.⁵ How-

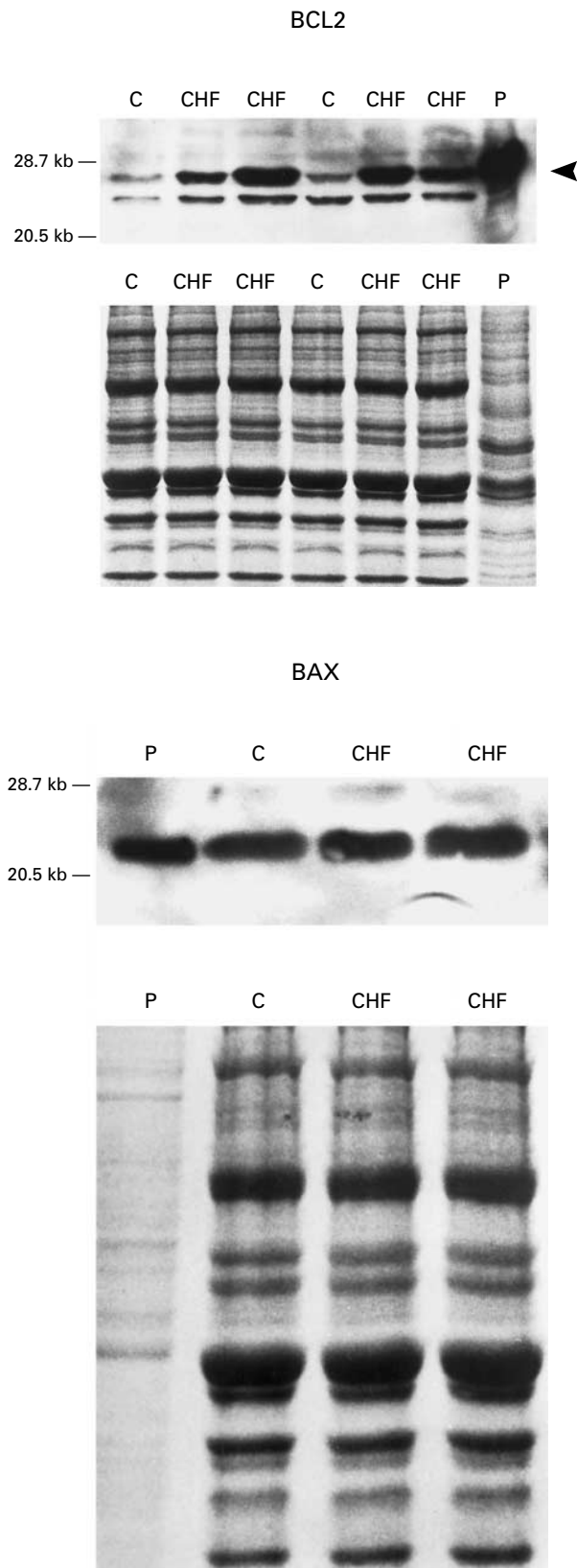


Figure 5. Western Blot Analysis of the BCL2 and BAX Proteins in Human Myocardium.

C denotes control hearts, CHF hearts from patients with congestive heart failure, and P positive control. Protein extracts from the myocardium of mice overexpressing BCL2 (kindly provided by Dr. Richard Kitsis) and from the LG₁₂ lymphoid cell line were used as positive controls for BCL2 and BAX, respectively. Loading of proteins is illustrated by Coomassie blue staining. The upper panels show the Western blot assay for BCL2 (arrowhead) and BAX, and the lower panels show the corresponding loading of proteins.

ever, the contention that cardiac damage is only necrotic in nature has been challenged. Studies *in vitro*^{15,32} and in animal models of transient ischemia^{6,33} and coronary-artery occlusion and myocardial infarction⁷ have demonstrated that myocyte apoptosis is an important component of ischemic myocardial injury. Moreover, this form of cell death may involve the cardiac conduction system, promoting fatal arrhythmias.³⁴ Embolization of the intramural branches of the coronary vasculature in experimental studies¹⁰ and, most important, the infarcted human heart^{18,35} are characterized by myocyte apoptosis and necrosis. Limitations in coronary blood flow may exist in the failing heart^{4,36} in combination with heightened mechanical stress¹⁵; apoptosis triggered by these mechanisms may increase myocyte death in the ventricle, thus contributing to cardiac dysfunction.

Expression of BCL2 and BAX

Our results indicate that alterations in the expression of members of the BCL2 family of proteins occurred in myocytes from the hearts of patients with congestive heart failure; specifically, the level of BCL2 increased and that of BAX remained unchanged. BCL2 promotes cell survival¹³ by forming heterodimers with BAX, a protein that otherwise induces apoptosis.³⁷ The formation of such heterodimers is mediated by three conserved motifs called the BCL2 homology 1 (BH1), BCL2 homology 2 (BH2), and BCL2 homology 3 (BH3) domains.^{13,37-39} The process of heterodimerization is dependent on these BH1 and BH2 domains, and selected mutations within these domains abolish the ability of BCL2 to bind to BAX. If BAX homodimers predominate, cell death will occur, whereas if BCL2-BAX heterodimers prevail, the cell will survive.³⁷ The enhanced expression of BCL2 in the failing heart in the absence of changes in the quantity of BAX strongly suggests that compensatory mechanisms are activated in the overloaded myocardium in an attempt to maintain cell survival.

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