Overexpression of Bcl-2 attenuates apoptosis and protects against myocardial I/R injury in transgenic mice

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Chen, Zhongyi, Chu Chang Chua, Ye-Shih Ho, Ronald C. Hamdy, and Balvin H. L. Chua. Overexpression of Bcl-2 attenuates apoptosis and protects against myocardial I/R injury in transgenic mice. Am J Physiol Heart Circ Physiol 280: H2313–H2320, 2001.—To test whether the antiapoptotic protein Bcl-2 prevents apoptosis and injury of cardiomyocytes after ischemia-reperfusion (I/R), we generated a line of transgenic mice that carried a human Bcl-2 transgene under the control of a mouse α-myosin heavy chain promoter. High levels of human Bcl-2 transcripts and 26-kDa Bcl-2 protein were expressed in the hearts of transgenic mice. Functional recovery of the transgenic hearts significantly improved when they were perfused as Langendorff preparations. This protection was accompanied by a threefold decrease in lactate dehydrogenase (LDH) released from the transgenic hearts. The transgenic mice were subjected to 50 min of ligation of the left descending anterior coronary artery followed by reperfusion. The infarct sizes, expressed as a percentage of the area at risk, were significantly smaller in the transgenic mice than in the nontransgenic mice (36.6 ± 5 vs 69.9 ± 7.3%, respectively). In hearts subjected to 30 min of coronary artery occlusion followed by 3 h of reperfusion, Bcl-2 transgenic hearts had significantly fewer terminal deoxynucleotidyl-transferase nick-end labeling-positive or in situ oligo ligation-positive myocytes and a less prominent DNA fragmentation pattern. Our results demonstrate that overexpression of Bcl-2 renders the heart more resistant to apoptosis and I/R injury.

Recent investigations (13, 15) in experimental animal models have demonstrated that apoptosis can be induced by ischemic cardiac injury. Apoptosis in the heart has been implicated in cardiac failure (12), anthracycline-induced cardiotoxicity (22, 41), and overstretching of myocytes (7). Myocardial ischemia-reperfusion (I/R) leads to cell death, which is believed to occur through apoptosis and necrosis. Kajstura et al. (21) showed that apoptosis was the predominant mode of cardiac cell death induced by coronary artery occlusion. Apoptosis is positively and negatively regulated by the Bcl-2 family of proteins (16, 24, 35). Proapoptotic proteins include Bax, Bak, Bcl-XL, Bad, Bid, Bik, Bim, Hrk, and Bok, whereas antiapoptotic proteins include Bcl-2, Bcl-XL, Bcl-w, Mcl-1, and A1/Bfl-1.

Bcl-2 is a 26-kDa protein encoded by a gene involved in 14q11 chromosomal translocation. It is localized to the cytoplasmic face of the mitochondrial outer membrane, endoplasmic reticulum, and nuclear envelope (2, 24). Bcl-2 has been shown to prevent cytochrome c release, caspase activation, and cell death. Regulation of apoptosis is highly dependent on the ratio of antiapoptotic to proapoptotic proteins. For instance, Bcl-2: Bcl-2 or BclXL:Bcl-XL homodimers are antiapoptotic, whereas Bax:Bax homodimers are proapoptotic. Heterodimers can also act to regulate apoptosis; as an example, Bcl-2 can form heterodimers with Bcl-XL or Bax. Previous research (21, 29) has shown that the ratio of Bcl-2 to Bax increases during ischemic adaptation. Moreover, studies have shown that this ratio also increases in failing hearts (12, 32) and in aging hearts (27) due to the upregulation of Bcl-2.

Bcl-2 is capable of preventing p53-induced programmed cell death of neonatal ventricular myocytes (23). To further understand the role of Bcl-2 in apoptosis and I/R injury in a more physiological setting, an animal model that overexpresses Bcl-2 was needed. Toward this end, our experiments were designed to achieve the following goals: 1) to generate transgenic mice bearing extra copies of cloned human Bcl-2 cDNAs under the transcriptional control of a mouse α-myosin heavy chain (MHC) promoter to allow high-level expression of transgenes in the heart, 2) to determine the levels of expressed Bcl-2 in the hearts of these animals, 3) to compare the cardiac functions of hearts from transgenic and nontransgenic animals, and 4) to elucidate the effect of Bcl-2 overexpression on apoptosis.

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MATERIALS AND METHODS

Generation of Bcl-2 transgenic mice. A Bcl-2 expression vector was constructed initially by inserting the SacI to SalI fragment of clone 22 (see Ref. 36; kindly provided by Dr. J. Robbins, University of Cincinnati, Cincinnati, OH), which contains the sequence from the last intron of the mouse β2-MHC gene to exon 3 of the α-MHC gene, into the SacI to SalI sites in plasmid pMSG (Amersham Pharmacia Biotech; Piscataway, NJ). BamHI digestion of the resultant plasmid allowed isolation of the DNA fragment containing SV40 early splicing and polyadenylation sites downstream from the mouse α-MHC sequence. This DNA fragment was then inserted into the BamHI site of plasmid pKS-S, a modified pKS vector (Stratagene; La Jolla, CA) in which the SalI site was destroyed by insertion of an SfiI linker, to generate plasmid pMHC. The full-length human Bcl-2 cDNA, which had previously been flanked by SalI sites using linker ligation, was subsequently inserted into the SalI site in plasmid pMHC. The entire expression sequence was isolated by ClaI plus NotI digestion of the resultant plasmid, and it was utilized in the generation of transgenic mice using fertilized mouse eggs isolated from the mating of B6C3 F1 hybrid mice according to standard procedures.

RNA isolation and RNase protection assay. To detect the expression of human Bcl-2 in different tissues, total RNA was extracted from the heart, lung, kidney, liver, and skeletal muscle of nontransgenic and transgenic mice with the use of the acid guanidinium thiocyanate-phenol-CHCl₃ extraction method (8). A 521-bp fragment corresponding to nucleotides 2,760–3,281 of human Bcl-2 cDNA was used as a template. The Bcl-2 cDNA template was prepared by inserting the EcoRI digestion fragment of human Bcl-2 cDNA (1.9 kb) into the pGEM-7zf(+) vector (Promega; Madison, WI), which was linearized with NciI. RNA (10 μg) was hybridized overnight with a 32P-labeled Bcl-2 riboprobe. The protected mRNAs were resolved on a 5% denaturing polyacrylamide gel.

Immunoblot analysis of Bcl-2, Bax, and heat shock proteins70 and 25. Hearts from nontransgenic and transgenic animals were homogenized in 100 mM Tris-HCl (pH 7.4) containing 15% glycerol, 2 mM EDTA, 2% SDS, and 0.1 mM phenylmethylsulfonylfluoride. Homogenates were heated at 95°C for 10 min, passed through a 23-gauge needle five times, and centrifuged at 12,000 g for 10 min. Protein concentration of the supernatant was determined by bichinchonic acid binding assay (Pierce). Aliquots (100 μg) were electrophoresed on 12% SDS-PAGE (Bcl-2, Bax, and heat shock protein (HSP)25) or 8% SDS-PAGE (HSP70) and transferred onto nitrocellulose membranes. Immunoblot analysis was carried out by incubating the membrane with either a human Bcl-2 antibody (monoclonal, clone Bcl-2/100, 1:600 dilution, BD-PharMingen; San Diego, CA), Bax (monoclonal, 1:1,000 dilution, Santa Cruz Biotechnology; Santa Cruz, CA), HSP70 (monoclonal, 1:1,000 dilution, Calbiochem; San Diego, CA), or HSP 25 (polyclonal, 1:1,250 dilution, StressGen; Victoria, BC, Canada). Horseradish peroxidase-conjugated goat anti-mouse or anti-rabbit antibody was then added. The blot was developed with the use of the enhanced chemiluminescence detection system (Amersham Pharmacia Biotech) and exposed to X-ray films.

Immunocytochemistry. Hearts were removed, and a 2-mm section near the midventricle was sliced, fixed in Bouin’s solution, and embedded in paraffin. Paraffin-embedded myocardial sections (5 μm) were mounted on superfrrost slides and dried at 37°C overnight. Heart specimens were subjected to antigen retrieval by heating in 10 mM citrate (pH 6.0) in a microwave oven at 100% power for 3.5 min and 50% power for 8 min. Immunostaining was carried out with a human Bcl-2 monoclonal antibody (1:50, BD-PharMingen) at 4°C overnight. Antigen-antibody complexes were detected by the Super-sensitive alkaline phosphatase kit (BioGenex; San Ramon, CA) using fast red as a chromogen. Hematoxylin was used as a counterstain.

Global ischemia in vitro. Male B6C3 nontransgenic and transgenic littermates weighing between 25 and 30 g were injected with heparin sodium (500 U/kg body wt ip) 30 min before anesthetization with pentobarbital sodium (120 mg/kg). Hearts were rapidly excised and perfused retrogradely at 60 mmHg by the Langendorff technique with Krebs-Henseleit bicarbonate buffer as described (6). After 30 min of preliminary perfusion, a range of end-diastolic pressures was tested to construct a functional curve. After 40 min of ischemia, hearts were reperfused for 45 min. Coronary effluent was collected for measurement of lactate dehydrogenase (LDH) release. At the end of perfusion, another functional index was measured.

Functional analysis. The ventricular functions of the mouse hearts were measured by inserting a tiny plastic-wraped balloon connected to a pressure transducer into the left ventricle (LV) via the mitral valves. Cardiac functions, such as LV developed pressures (LVDP), maximum rates of pressure development (+dP/dt), heart rates, and coronary flow rates, recorded before ischemia and 45 min after reperfusion, were used for comparison. Percentages of functional recovery were calculated by dividing each of the functional recovery values at the end of reperfusion by their corresponding preischemic values. End-diastolic pressures before ischemia and after 45 min of reperfusion were calculated from the functional curves. The perfusate flowing out of the heart was collected and measured. Coronary flow rate was determined by the amount of perfusate measured in a specific time period.

LDH release. In addition to cardiac function, cardiac injury was assessed by measuring LDH release. Perfusion effluent was collected every 15 min of preischemia and also during reperfusion. LDH released from the heart was determined by a CytoTox 96 assay (Promega) and expressed as milliunits per milligram of protein.

Regional ischemia in vivo. Mice weighing 25–30 g were anesthetized with chloral hydrate (360 mg/kg ip). An endotracheal tube (polyethylene-90) was inserted 5–8 mm from the larynx, and the mice were ventilated with room air (tidal volume of 0.5 ml) with the use of a rodent respirator (Columbus Instruments; Columbus, OH) set at 110–120 beats/min. Left anterior descending (LAD) coronary artery ligation was performed as described previously (6). After 50 min of LAD ligation, the heart was reperfused for 4 h.

Mice were anesthetized with pentobarbital sodium (120 mg/kg ip), and the hearts were perfused as Langendorff preparations for 5 min. The left coronary artery was reocluded, and 1% Evans blue was infused into the aorta and coronary arteries to determine the area at risk. Hearts were transversely cut into five sections, with one section made at the site of the ligature. Macroscopic staining with triphenyltetrazolium chloride (TTC) was used to quantitate the infarct size as described previously (6). The area at risk was expressed as a percentage of the LV, and the area of infarct was expressed as a percentage of the area at risk as described (6).

Nuclear DNA fragmentation by DNA laddering. Hearts were removed and frozen in liquid nitrogen until analysis. DNA fragmentation was performed as described by Bialik et al. (3) with some modifications. Briefly, tissue powders were resuspended in 1 ml of lysis buffer containing 10 mM Tris-HCl (pH 8.0), 100 mM NaCl, 25 mM EDTA, 0.5% SDS,
and 1 mg/ml proteinase K] and incubated at 37°C for 16 h. After the salt concentration was adjusted to 1.2 M, the homogenates were centrifuged at 14,000 g for 30 min. DNA was extracted with phenol-chloroform, precipitated with alcohol, and resuspended in Tris-EDTA buffer. After the DNA was treated with DNase-free RNase A (100 μg/ml) for 30 min at 37°C, the DNA was electrophoresed on a 1.4% agarose gel in the presence of 0.5 μg/ml ethidium bromide. A 100-bp DNA ladder was included as a size marker.

Terminal deoxynucleotidyl-transferase nick-end labeling assays. After 30 min of LAD ligation and 3 h of reperfusion, hearts were removed, and a 2-mm section near the middle part of the area at risk was sliced, fixed in 4% formalin solution, and embedded in paraffin. Myocardial sections (5 μm) were mounted on superfrost slides and dried at 37°C overnight. Immunohistochemical procedures for detecting apoptotic cardiomyocytes were performed by using Cardio-TACS (Trevigen; Gaithersburg, MD) according to the manufacturer’s instructions. In this procedure, nuclei undergoing apoptosis were stained blue. Nuclear fast red was used as a counterstain. Terminal deoxynucleotidyl-transferase nick-end labeling (TUNEL)-positive myocytes were determined by randomly counting 10 fields. The index of apoptosis was then determined (i.e., number of apoptotic myocytes ÷ total number of myocytes counted × 100). Because there was no myocardial necrosis by TTC staining in hearts subjected to 30 min of LAD ligation and 3 h of reperfusion, the results reflected apoptosis in the absence of necrosis. To verify that the apoptosis occurred in the myocytes, immunohistochemical staining of α-sarcomeric actin was carried out with an α-sarcomeric actin antibody (monoclonal, 1:100 dilution, Sigma; St. Louis, MO) at 4°C overnight. Hematoxylin was used as a counterstain.

In situ oligo ligation analysis. In situ staining of DNA strand breaks in the serial section of each specimen was detected by the ApopTag in situ oligo ligation (ISOL) kit using oligo A according to the manufacturer’s instructions (Intergen; Purchase, NY) with some modifications. The endogenous biotin was blocked with an Avidin/Biotin blocking kit (BioGenex). TACS blue label was used as a peroxidase substrate, and nuclear fast red was used as a counterstain (Trevigen). Oligo A was synthesized as a modification of the method described by Didenko et al. (10), in which the sequences of the ten 3′ and ten 5′ bases were complementary and in which a biotin was attached through a triethylene glycol linker inserted between the 13th and 14th bases.

Statistical analysis. Statistical analysis was performed by the Instat software. Significance of differences between means was established by Student’s t-test. Results were expressed as means ± SE. P < 0.05 was considered significant.

RESULTS

We generated a line of transgenic mice that carried a human Bcl-2 transgene under the control of a mouse α-MHC promoter. There were no differences in age, body weight, or heart protein content between the nontransgenic and transgenic groups. In addition, all transgenic mice were healthy and showed no apparent phenotypic differences. Histological analysis by hematoxylin and eosin staining or electron microscopy indicated that the hearts of transgenic animals were normal (results not shown). There was no evidence of tumor development in the hearts from 32-mo-old transgenic mice.

We performed a detailed expression study with the offspring of the founder mouse Tg(Bcl-2)33. Total RNA was isolated from major tissues of nontransgenic or transgenic animals and probed for human Bcl-2 mRNA using a ribonuclease protection assay. Clearly, Bcl-2 transcripts were detected predominantly in the heart (Fig. 1). A significantly lower level of expression was detected in the lung and skeletal muscle. Bcl-2 transcripts were not expressed in the liver or kidney. Western blot analysis showed that the 26-kDa Bcl-2 protein was highly expressed in the transgenic heart (Fig. 2). Overexpression of Bcl-2 did not induce any changes in the expression of Bax, HSP70, or HSP25 (Fig. 2). Immunoreactivity of Bcl-2 demonstrated that it was strongly expressed in the myocytes of transgenic hearts (Fig. 3B), as demonstrated by diffused cytoplasmic staining. Endothelial cells or smooth muscle cells did not show immunoreactivity to Bcl-2 antibody. There was little Bcl-2 staining in the nontransgenic heart (Fig. 3A).
We compared the cardiac parameters of the hearts of Bcl-2 transgenic mice with those of their normal littermates. After 30 min of equilibration perfusion, the cardiac basal parameters were compared (Table 1). \( \pm dP/dt \) during both contraction and relaxation was essentially the same in the two groups. LVDP was 97 ± 11 mmHg in nontransgenic mice and 103 ± 13 mmHg in Bcl-2 transgenic mice. Heart rates and coronary flow rates were similar in both groups.

A comparison of the functional recovery of the nontransgenic and transgenic hearts subjected to 40 min of ischemia and 45 min of reperfusion revealed a significant improvement in the hearts of transgenic mice (Fig. 4). The functional recovery, expressed as heart rate × LVDP, in Bcl-2 transgenic mice was 27 ± 6% after 15 min of reperfusion. At this time point, four of six hearts from the nontransgenic mice failed to recover. After 30 min of perfusion, the functional recovery in Bcl-2 transgenic mice was significantly higher than the functional recovery in nontransgenic mice (60 ± 11 vs. 32 ± 4%, \( P < 0.05 \)). Recovery was 79 ± 7% for Bcl-2 transgenic mice and 47 ± 7% for the nontransgenic group (\( P < 0.05 \)) after 45 min of reperfusion. Percentages of recovery in nontransgenic versus transgenic mice were 64 ± 6 versus 88 ± 6% for \( +dP/dt \), 63 ± 6 versus 85 ± 7% for \( -dP/dt \), and 59 ± 5 versus 80 ± 4% for LVDP, respectively. Heart rates and coronary flow rates were not significantly different between the two groups. End-diastolic pressures of the control hearts increased from 5.6 ± 0.6 to 35.7 ± 9.4 mmHg after 45 min of posts ischemic reperfusion (Fig. 5). Preischemic and posts ischemic end-diastolic pressures of the transgenic hearts were not significantly different (5.2 ± 0.9 vs. 9.4 ± 2.9 mmHg).

Cardiac injury was also assessed by measuring the release of LDH. Total LDH release from the nontransgenic hearts was three times as much as that from the transgenic hearts (Fig. 6). The total release of LDH during 45 min of reperfusion after 40 min of global ischemia of the nontransgenic and transgenic group was 961 ± 172 and 255 ± 60 mU/mg protein, respectively. As for the time point comparison during 45 min of reperfusion, the release of LDH in Bcl-2 transgenic hearts in two 15-min periods was significantly lower than that in the corresponding control hearts.

To study the effect of Bcl-2 overexpression on regional I/R injury in vivo, mice were subjected to 50 min of LAD ligation followed by 4 h of reperfusion (Fig. 7). The area at risk, expressed as a percentage of the LV between the nontransgenic and transgenic hearts, was comparable (30.4 ± 2.3% vs. 30.9 ± 1.9%). Infarct sizes

Table 1. Basal values of nontransgenic and transgenic hearts

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Nontransgenic</th>
<th>Transgenic</th>
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<tr>
<td>(+dP/dt), mmHg/s</td>
<td>2,030 ± 212</td>
<td>1,966 ± 228</td>
</tr>
<tr>
<td>(-dP/dt), mmHg/s</td>
<td>1,484 ± 167</td>
<td>1,417 ± 16</td>
</tr>
<tr>
<td>LVDP, mmHg</td>
<td>97 ± 11</td>
<td>103 ± 13</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>230 ± 16</td>
<td>217 ± 20</td>
</tr>
<tr>
<td>LVDP × HR</td>
<td>21,574 ± 2,058</td>
<td>21,320 ± 1,977</td>
</tr>
<tr>
<td>Coronary flow, ml/min</td>
<td>1.4 ± 0.2</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>5.6 ± 0.6</td>
<td>5.1 ± 0.6</td>
</tr>
<tr>
<td>Body wt, g</td>
<td>31 ± 0.7</td>
<td>32 ± 1.1</td>
</tr>
<tr>
<td>Protein, mg</td>
<td>13 ± 0.4</td>
<td>12 ± 0.2</td>
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All values are means ± SE of six hearts. Measurements of the maximum rates of pressure development (\( \pm dP/dt \)), left ventricular developed pressures (LVDP), left ventricular end-diastolic pressures, (LVEDP), heart rates (HR), and coronary flows were made after 30 min of preliminary perfusion.

Fig. 4. Improvement of the posts ischemic recovery of cardiac function (expressed as a percentage of preischemic value) after 40 min of global ischemia and 45 min of reperfusion in nontransgenic and Bcl-2 transgenic hearts. Values are means ± SE of 6 hearts. *\( P < 0.05 \) vs. nontransgenic hearts. \( +dP/dt \), maximum rate of pressure development; LVDP, left ventricular (LV) developed pressure; HR, heart rate; CFR, coronary flow rate.
of the nontransgenic hearts and transgenic hearts were 21.3 ± 6.2 and 11.3 ± 2.4% of the LV, respectively. Infarct sizes, expressed as a percentage of the area at risk for the nontransgenic and transgenic hearts, were 69.9 ± 7.3 and 36.6 ± 5.0%, respectively. Our results indicate that overexpression of Bcl-2 is able to limit the infarct size in an in vivo regional ischemia model.

To examine whether the functional protection of the Bcl-2 transgenic hearts is related to the antiapoptotic property of Bcl-2, DNA fragmentation analyses were performed on hearts subjected to 30 min of LAD ligation and 3 h of reperfusion. In the apoptosis study, we reduced the ischemia time from 50 to 30 min because no infarction was detected by TTC staining in five nontransgenic hearts after 30 min of ischemia followed by 3 h of reperfusion. This is in contrast to a study by Bolli et al. (17), which showed 60% infarct after 30 min of ischemia and 24 h of reperfusion. The disparity between the two sets of results may be due to a number of differences in the experimental conditions, such as the mouse strains, anesthetic agent used, surgical procedures, site of LAD ligation, and the duration of reperfusion. A prominent nucleosomal ladder was detected from the LVs of nontransgenic hearts, which indicated the occurrence of apoptosis (Fig. 8, lanes 5 and 6). The intensity of the DNA ladder was diminished in Bcl-2 transgenic hearts (Fig. 8, lanes 7 and 8). Nucleosomal DNA ladders were not detected in sham-operated LVs of nontransgenic (Fig. 8, lanes 1 and 2) or transgenic hearts (lanes 3 and 4).

To substantiate the protective effect of Bcl-2 via its antiapoptotic action, TUNEL assays of the hearts were also performed. Heart tissue from sham-operated nontransgenic and transgenic mice exhibited low levels of staining for TUNEL (1.82 ± 0.45%, n = 6, and 1.02 ± 0.15%, n = 6, respectively; Fig. 9A). TUNEL-positive myocytes from the LVs of nontransgenic animals and transgenic animals were 15.8 ± 1.18% (n = 6) and 6.38 ± 0.42% (n = 6, P < 0.05), respectively. Almost all of the TUNEL-positive cells were immunoreactive to

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**Fig. 5.** Improvement of LV end-diastolic pressure (LVEDP) after 40 min of global ischemia and 45 min of reperfusion in nontransgenic and transgenic hearts. Values are means ± SE of 6 hearts. *P < 0.05 vs. nontransgenic hearts; †P < 0.05 vs. preischemic LVEDP.

**Fig. 6.** Release of lactate dehydrogenase (LDH) during reperfusion after 40 min of ischemia. Values represent means ± SE of 6 hearts. *P < 0.05 vs. nontransgenic hearts.

**Fig. 7.** Protective effect of Bcl-2 overexpression on myocardial infarction in mice. Animals were subjected to 50 min of left anterior descending (LAD) coronary artery ligation followed by 4 h of reperfusion. Values are means ± SE of 6 hearts. *P < 0.05 vs. nontransgenic hearts.

**Fig. 8.** Electrophoretic analysis of internucleosomal DNA extracted from nontransgenic and transgenic hearts subjected to 30 min of LAD ligation and 3 h of reperfusion. DNAs from LVs of sham-operated nontransgenic mice (lanes 1 and 2), sham-operated transgenic mice (lanes 3 and 4), nontransgenic mice after ischemia-reperfusion (lanes 5 and 6), and transgenic mice after ischemia-reperfusion (lanes 7 and 8) were isolated and analyzed on a 1.4% agarose gel. A 100-bp DNA ladder was included as a size marker.
a-sarcomeric actin antibodies, indicating that they were myocytes (results not shown).

Because TUNEL analysis is known to detect nonspecific DNA fragmentation due to necrosis, a more specific in situ ligation assay for identification of apoptotic nuclei using hairpin oligonucleotide probes as described by Didenko et al. (11) was performed. Whereas there was little labeling in the nuclei of myocytes of sham-operated animals (0.63 ± 0.12% in sham-operated animals vs. 0.72 ± 0.07% in transgenic animals, n = 6), ISOL-positive myocyte nuclei from the LVs of nontransgenic animals and transgenic animals were 14.43 ± 0.60% (n = 6) and 6.21 ± 0.53% (n = 6, P < 0.05), respectively (Fig. 9B). These results clearly demonstrate that apoptosis in the myocytes was attenuated in the Bcl-2 transgenic mice.

DISCUSSION

Although transgenic mice overexpressing Bcl-2 have been used to understand the protective role of Bcl-2 in the brain (28) and intestine (9), to our knowledge this report is the first study of the cardioprotection of Bcl-2 in both apoptosis and I/R injury. Our results demonstrate that suppression of apoptosis attenuates I/R injury.

We successfully created transgenic mice overexpressing Bcl-2 in the heart driven by the murine α-MHC promoter. The transgenic mice were able to reproduce and were viable until at least 32 mo of age without displaying evidence of any abnormal cardiac development.

Besides its predominant expression in the heart, Bcl-2 is also expressed at lower levels in the lung and skeletal muscle (Fig. 1). The expression in the lung may be attributed to the myocytes located in the pulmonary venous bed (36). In contrast to the lack of expression of α-myosin promoter activity in the muscle (36), our study showed expression of Bcl-2 in the gastrocnemius muscle, which is interesting and warrants further investigation.

Because the overexpression of HSP70 has been shown to protect hearts against I/R injury (20, 34), it is possible that the protective effect of Bcl-2 could be mediated by the activation of HSPs. We demonstrated that there were no alterations in HSP70 or HSP25 in the hearts from our transgenic mice (Fig. 2).

The antiapoptotic role of Bcl-2 is well documented (2, 25, 29). Recently, it has been shown that Bcl-2 is a regulator of mitochondrial transition pores. The mitochondrial permeability transition (PT) pore sites, which promote contacts between the inner and outer membranes of mitochondria, are important in the regulation of apoptosis. There is evidence that, whereas Bax homodimers form and open PT pores, Bcl-2 inhibits pore formation. This suggests that these proteins exert their effects by regulating PT.

The pathogenesis of myocardial reperfusion injury, a multifactorial process involving the interaction of multiple mechanisms, has been extensively researched in the past decade. The exact mechanism for the protective effect of Bcl-2 on I/R injury reported in the present study is not well understood, although recent findings provide some possible explanations. The generation of oxygen-derived free radicals is considered to be one of the mechanisms responsible for I/R injury. Free radicals interacting with other mechanisms can damage cellular components and eventually cause cell death. Superoxide is also known to induce apoptosis (39). In this regard, we and other investigators (1, 6) demonstrated that overexpression of antioxidant enzymes such as manganese superoxide dismutase or glutathione peroxidase protects the heart against I/R injury. Bcl-2 has been hypothesized to be a free radical scav-
en in the antioxidant pathways (19); for instance, Bcl-2 overexpression in neural cells attenuates the generation of reactive oxygen species (4, 31). Although free radicals generated during prolonged ischemia and reperfusion are known to cause cardiac injury, reactive oxygen species such as superoxide or H2O2 generated during brief ischemia or hypoxia have been shown to initiate signal transduction of cardioprotection (5, 37, 38).

In addition, other factors such as ATP depletion and calcium overload also contribute to the pathogenesis of I/R injury. Because of the colocalization of Bcl-2 with Ca2+ pumps and channels on mitochondria, the endoplasmic reticulum, and nuclear membranes (2, 24), Bcl-2 may help to maintain calcium homeostasis in these compartments (14). Recently, overexpression of Bcl-2 in neuroblastoma cells has been shown to increase the mitochondrial Ca2+ load, and it enables the cells to maintain a stable mitochondrial membrane potential to offset the pathological insult (40). Previous studies have demonstrated that Bcl-2 activates nuclear factor (NF)-κB in myocytes by inhibiting the degradation of the cytoplasmic inhibitor IkBα (10). NF-κB is rapidly activated during ischemia in perfused rat hearts (26) and may play an essential role in the cytoprotective effects induced by ischemic preconditioning (30, 33).

Finally, a more plausible explanation of the present study is that PT pores play a critical role in apoptosis as well as I/R injury and that the functional recovery of the hearts can be improved by strengthening the antiapoptotic system. PT induction is believed to be important in both necrosis and apoptosis, depending on the severity of the ischemic damage. Transient pore opening and ATP maintenance of the mitochondria lead to apoptosis during acute myocardial infarction in the mouse localizes to hypoxic regions but occurs independently of p53. J Clin Invest 100: 1363–1372, 1997.


