ORIGINAL ARTICLE

Bax Ablation Protects Against Hepatic Ischemia/Reperfusion Injury in Transgenic Mice

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Apoptosis appears to be a central mechanism of cell death following reperfusion of the ischemic liver. The aim of this study was to determine the effect of decreased expression of the proapoptotic Bax gene on hepatic apoptotic warm ischemia/reperfusion (I/R) injury. Three groups of mice were studied: homozygotic knockout mice ($Bax^{-/-}$); heterozygotic ($Bax^{+/-}$); and wild type ($Bax^{+/+}$). Isolated mouse livers were subjected to 90 minutes of ischemia (37°C) followed by 15 minutes of reperfusion. Bax and Bcl-2 expression in liver tissue homogenates was measured by Western blot. Serum liver enzyme levels were measured and intrahepatic caspase-3 activity was determined by fluorimetric assay. Oil red O (ORO) staining was performed for fat detection. Apoptotic cells were identified by morphological criteria, immunohistochemistry for caspase-3, and terminal deoxynucleotidyl transferase-mediated 2′-deoxyuridine 5′-triphosphate nick-end labeling (TUNEL) assay. At 1 minute of reperfusion, the ischemic ($Bax^{-/-}$) livers were characterized by statistically significantly lower liver enzyme levels and lower caspase-3 activity than the ischemic ($Bax^{+/+}$) livers ($P < 0.05$ for both). The reduction in postischemic apoptotic hepatic injury in the ischemic $Bax^{-/-}$ livers group was confirmed morphologically, by the significantly reduced microvesicular steatosis as determined by ORO staining, fewer apoptotic hepatocyte cells detected ($P < 0.05$); immunohistochemically, by the significantly weaker activation of caspase-3 compared to the ischemic group ($P < 0.05$); and by TUNEL assay ($P < 0.05$). Similar levels of antiapoptotic Bcl-2 protein expression were detected in all 3 groups of ischemic livers on Western blots. Bax protein was not expressed in Bax-deficient livers and was detected in $Bax^{+/+}$ normal livers. In the $Bax^{+/-}$ livers, levels of the damage markers were moderate. In conclusion, The better tolerance of Bax knockout livers to I/R injury suggests that the Bax gene may serve as a potential target for therapeutic intervention in hepatic I/R injury. ***Liver Transpl 13:1181–1188, 2007.***

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Warm ischemia/reperfusion (I/R) injury during liver transplantation is the most important cause of primary nonfunction of liver grafts. Despite the significant improvement in clinical outcome during the last decade, the dramatic shortage of organs has led to the consideration of marginal grafts, which have a higher susceptibility to I/R injury.

Warm and cold hepatic ischemia followed by reperfusion leads to necrotic cell death. The mechanism underlying necrosis is related to an adenosine-triphosphate-depletion-dependent pathway.[1] However, recent studies suggest a major role for apoptosis as well after I/R injury. Rudiger et al.[2] reported that apoptosis is a central mechanism of cell death following reperfusion of the ischemic liver, and that apoptosis is associated with a caspase-dependent pathway.[1,2]

The apoptotic pathway in the ischemic liver is complex, involving the release of tumor-necrosis factor al-

Abbreviations: I/R, ischemia/reperfusion; ORO, Oil red O; TUNEL, terminal deoxynucleotidyl transferase-mediated 2′-deoxyuridine 5′-triphosphate nick-end labeling; DNA, deoxyribonucleic acid.

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pha, several caspases, and cytochrome c from the mitochondria, resulting in the activation of caspase-3 and deoxyribonucleic acid (DNA) fragmentation.[2]

Bcl-2 is a mitochondrial membrane protein that acts at various levels of the apoptotic cascade. The Bcl-2 family is composed of both antiapoptotic (Bcl-2) and proapoptotic (Bax) molecules.[3,4] Although hepatocytes do not express Bcl-2 under normal circumstances, Bcl-2 overexpression was reported in ischemic mouse livers after reperfusion, protecting the liver against the ischemic injury by inhibiting apoptosis.[5,6] Bcl-2 suppresses cytochrome c release[7] whereas Bax stimulates its release, and thereby, caspase-3 expression; this reportedly occurs in isolated mitochondria in vivo and in intact cells following heterologous expression.[7-9] Bax alone has been shown to be sufficient for induction of apoptosis.[10] Rentsch et al.[11] found that the induction of apoptosis in rat liver transplantation was associated with caspase 3 activation and Bax expression, during graft cold storage and after cold I/R. We have recently reported that myocytes lacking the Bax gene had reduced myocardial I/R injury through prevention of the necrotic and apoptotic pathways.[12]

The aim of the present study was to determine the effect of a decreased expression of the proapoptotic Bax gene using Bax knockout mice, on hepatic apoptotic injury induced by I/R.

MATERIALS AND METHODS

Animals

Bax-deficient mice, originally inbred from C57B1, were kindly provided by Dr. S.J. Korsmeyer (Howard Hughes Medical Institute, Washington University School of Medicine, St. Louis, MO).[13] Experiments were performed in accordance with the guidelines established by the Institutional Animal Care and Use Committee at Tel Aviv University (Tel Aviv, Israel).

As the male mice proved infertile, we mated the hemizygote for the Bax gene ($Bax^{+/-}$). To verify genetic identity for genotyping, DNA was isolated from the tail using the high pure template preparation kit (Roche Mannheim, Germany) and then subjected to standard polymerase chain reaction. The primers used were as follows: 5′-GTTGACCAGATGGCGTAGG-3′ (Bax in), 5′-GAGCTGATCAGAACCATFATG-3′ (Bax ex), and 5′-CCGCTTCCATTGCTCAGCGG-3′ (Neo R) for the exogenous neo gene.[13]

Isolated Perfused Liver Preparation

Adult male mice weighing 23-28 gm were anesthetized by intraperitoneal injection of chloral hydrate (10 mg/100 gm body weight). Laparotomy was performed, and the livers were prepared as previously described.[14] Briefly, 14-gauge cannulas were inserted into the portal vein and suprahepatic inferior vena cava to serve as ports of flow and for pressure measurements. The liver was then placed in an environmental chamber. The intrahepatic inferior vena cava, gastroepiploic vein, and hepatic artery were ligated. Temperature was monitored with a thermistor located under the right lobe, and an infrared lamp was used to maintain a constant liver temperature of 37°C. The liver was perfused via the portal vein with hemoglobin-free oxygenated modified Krebs-Henseleit solution containing NaCl 118 mmol/L, KCl 4.7 mmol/L, $NaHCO_3$ 25 mmol/L, $CaCl_2$ 2.5 mmol/L, $MgSO_4$ 1.2 mmol/L, KH_2PO_4 1.2 mmol/L, and α-D-glucose 11 mmol/L, at a rate of 3 mL/minute^{-1}/gm^{-1} liver weight in an open circuit system. The perfusate was maintained at a constant temperature (37°C) and equilibrated with 95% O_2/5% CO_2 to achieve an influent PO_2 of 500 mmHg or more and PCO_2 of 30-35 mmHg at pH 7.34-7.46.

General Experimental Protocols

A total of 30 mice (12-15 weeks old) were divided into 3 ischemic groups (n = 10 each), as follows: transgenic homozygotic knockout mice ($Bax^{-/-}$), transgenic heterozygotic mice ($Bax^{+/-}$), and wild-type homozygotic mice ($Bax^{+/+}$).

All livers were allowed to stabilize for 30 minutes and were then made globally ischemic, wherein perfusion was stopped for 90 minutes, followed by reperfusion.

Determination of Liver Viability and Data Collection

Liver viability was assessed by monitoring changes in the following parameters: perfusate PO_2, PCO_2, and pH, tissue color, and perfusion pressure on the portal vein. Liver efferent perfusate samples were collected for laboratory tests at stabilization and at 1 and 15 minutes of reperfusion.

Serum Enzyme Analysis

Efferent perfusate samples were kept on ice until processed. Levels of aspartate transaminase, alanine transaminase, and lactate dehydrogenase were determined in duplicate using commercial kits, according to the manufacturers' protocols.

Caspase-3 Activity

Snap-frozen mice liver samples that were obtained at the stabilization and at the end of the reperfusion period (15 minutes), homogenized with a polytron homogenizer and centrifuged at 16,000*g* for 20 minutes. Protein content was measured in the supernatant using a commercial kit (BCA protein assay reagent kit 23227; Pierce, Rockford, IL). The synthetic fluorogenic substrate N-acetyl-Asp-Glu-Val-Asp (AC-DEVD; Biomol, Plymouth Meeting, PA) at concentrations of 50 μmol/L in 60 μg liver protein extracts was used to measure caspase-3 activity, according to the manufacturer's instructions. Briefly, the fluorogenic substrate produces a blue fluorescence that can be detected by exposure to ultraviolet light at 360 nm. It is labeled with the fluorochrome 7-amino-4-methyl coumarin, which is released upon cleavage by caspase-3, producing a blue fluores-

cence that is measured by a fluorometer at 460 nm (Fluostar BMG Labtech, Offenburg, Germany). Pancaspase inhibitor (Z-Asp-2,6,dichlorobenzoyloxymethyl ketone; Biomol) is added to the mixture (50 μmol/L) 30 minutes after AC-DEVD–7-amino-4-methyl coumarin to determine the specificity of the caspase reaction.

Pathological Evaluation

Specimens from the mice livers in all three ischemic groups were fixed in formalin, embedded in paraffin, and stained with hematoxylin-eosin. Oil Red O (ORO) staining for fat in frozen sections was also performed in the standard manner. Pathological findings were assessed by one of the authors blinded to the group allocations.

Apoptosis Assay

Apoptotic cells were identified by morphological criteria (cell shrinkage, chromatin condensation, margination, and apoptotic bodies).

Terminal Deoxynucleotidyl Transferase-Mediated 2′-Deoxyuridine 5′-Triphosphate Nick-End Labeling (TUNEL) Assay

Apoptotic cells in the formalin-fixed, paraffin-embedded liver tissue sections were also identified with the classic in situ DeadEnd™ fluorimetric TUNEL assay (Promega, Madison, WI), according to the manufacturer's protocol. This assay is designed to specifically detect the fragmented DNA of apoptotic cells by catalytically incorporating fluorescein-12-2′-deoxyuridine 5′-triphosphate[a] at the 3′-OH DNA ends using the terminal deoxynucleotidyl transferase enzyme, which forms a polymeric tail. The fluorescein-12-2′-deoxyuridine 5′-triphosphate–labeled DNA can then be visualized directly by fluorescence microscopy. Hepatocytes with nuclear positivity for DNA fragmentation were counted in 50 ± 5 fields (original magnification ×10).

Caspase-3 Immunohistochemistry

For activated caspase-3 immunostaining, 5 μM sections of liver samples were dewaxed and hydrated through graded ethanols, cooked in 25 mmol/L citrate buffer pH 6.0 in a pressure cooker at 115°C for 3 minutes (decloaking chamber; Biocare Medical, Concord, CA), transferred to boiling deionized water, and allowed to cool for 20 minutes. After 5 minutes of treatment in 3% H_2O_2, slides were incubated with rabbit polyclonal activated caspase-3 (Cell Signaling, Nottingham, UK) antibodies diluted 1:100 in CAS-Block (Zymed Lab, San Francisco, CA) for 3 hours at room temperature or overnight at 4°C, washed 3 times with Optimax (Biogenex HK583 Lab, San Ramon, CA), incubated for 30 minutes with anti-rabbit Envision$^+$ (DAKO K4007, Dako, Glostrup, Denmark), and developed with 3′3′ diaminobenzidine or −3-amino 9-ethyl carbazole.

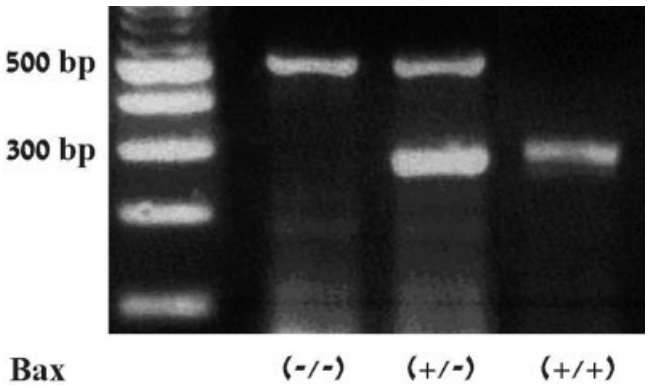

Figure 1. Genetic identity of the various mice tested. One band of Bax gene (300 bp) can be seen in the Bax$^{+/+}$ mice, and 2 bands (300 and 500 bp) in the hemizygotic Bax$^{+/-}$ mice. The Bax$^{-/-}$ mice have only 1 band (500 bp) in the neo gene.

Western Blot

Liver tissue samples (20 mg) were homogenized in lysis buffer and quantified for protein levels using a commercial assay (Bio-Rad Laboratories, Redmond, WA). Proteins (60 μg/sample) were separated using sodium dodecylsulfate (20 μg/sample) polyacrylamide gels (12.5%) under denaturing conditions and electrotransferred onto nitrocellulose (Bio-Rad) for 1 hour at 100 V. Membranes were blocked with 5% nonfat milk in Tris-buffered saline containing 0.1% Tween 20 overnight at 4°C. Primary antibodies [anti-Bcl-2 polyclonal; Oncogene, Cambridge, MA; anti-Bax (Cell Signaling); and anti-α-actin (Sigma, St. Louis, MO)] were used at 1:1,000 dilution in Tris-buffered saline containing 0.1% Tween 20 with 5% nonfat milk. After the samples were incubated overnight with the primary antibodies at 4°C, horseradish peroxidase-conjugated secondary antibodies were added (1:4,000 dilution) for 1 hour at room temperature. Films were developed using enhanced chemiluminescence methods and exposed to X-ray films (Amersham, Arlington Heights, IL).[15,16]

Statistics

Data are given as mean ± standard error of the mean. To analyze statistically significant differences in mean continuous parameters among the 3 groups and the 3 time points, we performed group analysis of variance, with Dunnett's multiple comparisons option for comparison with the control group and Duncan's multiple comparison option for comparison among all groups. Statistical significance was defined as $P < 0.05$.

RESULTS

Genetic Identity by polymerase Chain Reaction

Figure 1 shows the genetic identity of the various mice tested. One band of Bax gene (300 bp) was seen in the Bax$^{+/+}$ mice, and 2 bands (300 and 500 bp) were seen

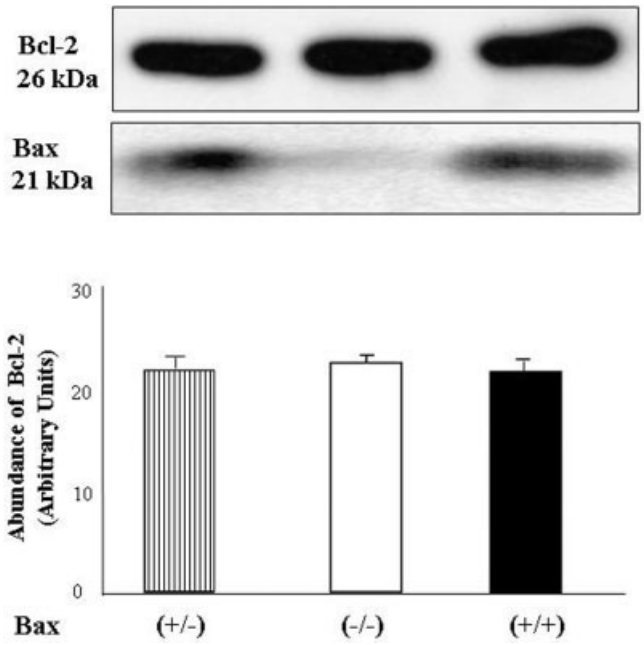

Figure 2. Bcl-2 and Bax content on Western blot. Note the similar levels of Bcl-2 protein expression in the 3 groups of ischemic livers. As expected, Bax protein was not expressed in Bax-deficient livers and was detected in normal $Bax^{+/+}$ livers.

in the heterozygote $Bax^{+/-}$ mice. In the $Bax^{-/-}$ mice, only 1 band (500 bp) was seen in the neo gene.

Bcl-2 and Bax Content

Similar levels of Bcl-2 protein expression were detected in the 3 groups of livers by Western blot (Fig. 2). As expected, Bax protein was not expressed in Bax-deficient livers, and it was detected in $Bax^{+/+}$ normal livers (Fig. 2).

Liver Enzymes (Fig. 3)

A statistically significant difference was noted in mean alanine transaminase/aspartate transaminase/dehydrogenase levels among the different time points in all 3 ischemic groups (overall p between groups: 0.005/0.002/0.0001).

A statistically significant difference was noted in mean alanine transaminase/aspartate transaminase/dehydrogenase levels at 1 minute of reperfusion in all 3 ischemic groups overall p between groups: 0.005/0.002/0.001. There was a statistically significant difference in mean alanine transaminase/aspartate transaminase/dehydrogenase within 1 minute of reperfusion between the ischemic $Bax^{+/+}$ and $Bax^{-/-}$ groups ($P \leq 0.05$). There was no statistically significant difference in mean alanine transaminase/aspartate transaminase/dehydrogenase levels among the groups within 15 minutes of reperfusion.

In the ischemic $Bax^{+/-}$ group, liver enzyme levels were intermediate between the levels in the ischemic $Bax^{+/+}$ and $Bax^{-/-}$ groups (Fig. 3).

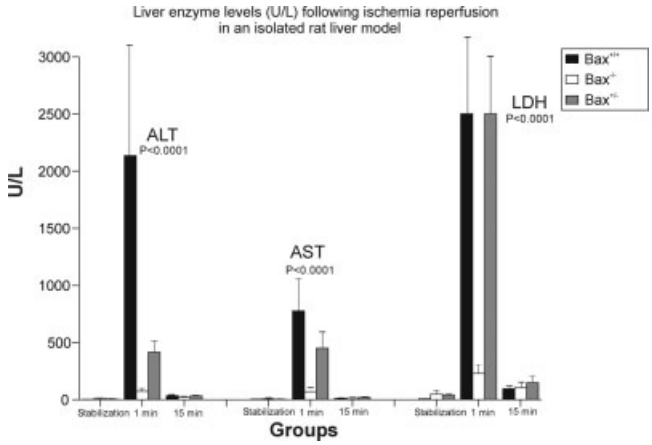

Figure 3. Liver enzymes. A statistically significant difference was noted in mean alanine transaminase/aspartate transaminase/dehydrogenase levels at 1 minute of reperfusion in all 3 ischemic groups (overall p between groups: 0.005/0.002/0.0001). There was a statistically significant difference in mean alanine transaminase/aspartate transaminase/dehydrogenase within 1 minute of reperfusion between the ischemic $Bax^{+/+}$ and $Bax^{-/-}$ groups ($P \leq 0.05$). There was no statistically significant difference in mean alanine transaminase/aspartate transaminase/dehydrogenase levels among the groups within 15 minutes of reperfusion

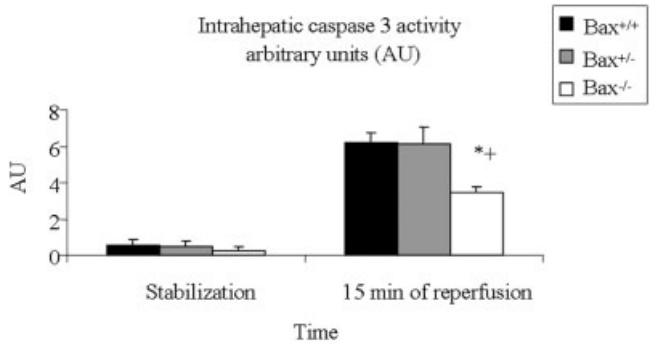

Figure 4. Intrahepatic caspase-3 activity. A statistically significant difference was noted in mean caspase-3 activity between groups ($^{+}P < 0.0001$). Caspase-3 activity increased significantly in the ischemic $Bax^{+/+}$ group (6.1 ± 1.6 arbitrary units) compared with the ischemic $Bax^{-/-}$ group (2.7 ± 0.6; $^{*}P < 0.05$).

Intrahepatic Caspase-3 Activity

A statistically significant difference was noted in mean caspase-3 activity between groups ($P < 0.0001$). Caspase-3 activity increased significantly in the ischemic $Bax^{+/+}$ group (6.1 ± 1.6 arbitrary units) compared with the $Bax^{-/-}$ group (2.7 ± 0.6; $P < 0.05$) (Fig. 4). In the ischemic $Bax^{+/-}$ group, caspase-3 activity was intermediate (6.0 ± 1.2) between the levels in the ischemic $Bax^{+/+}$ and $Bax^{-/-}$ groups.

Histologic Studies

Histologic examination of the liver tissue sections using hematoxylin-eosin staining showed liver damage in the ischemic $Bax^{+/+}$ group, manifested by marked diffuse

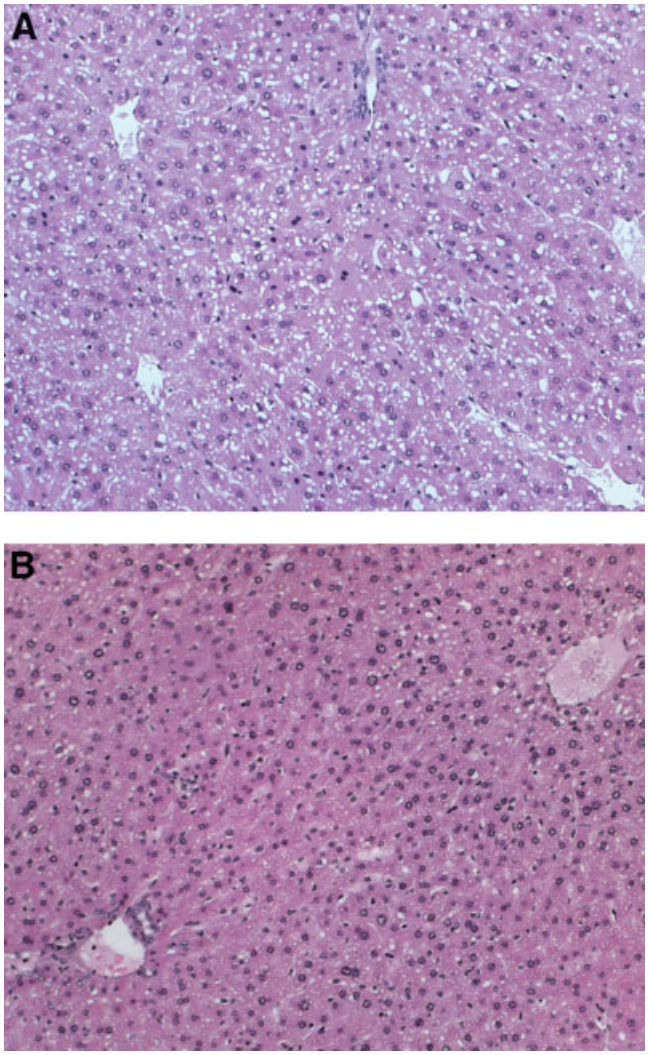

Figure 5. Histologic hematoxylin-eosin study of liver sections. (A) The ischemic Bax$^{+/+}$ livers show marked diffused micro- and macrovesicular steatosis and several apoptotic bodies. (B) The ischemic Bax$^{-/-}$ livers show mild hydropic degeneration, with no steatosis or apoptosis.

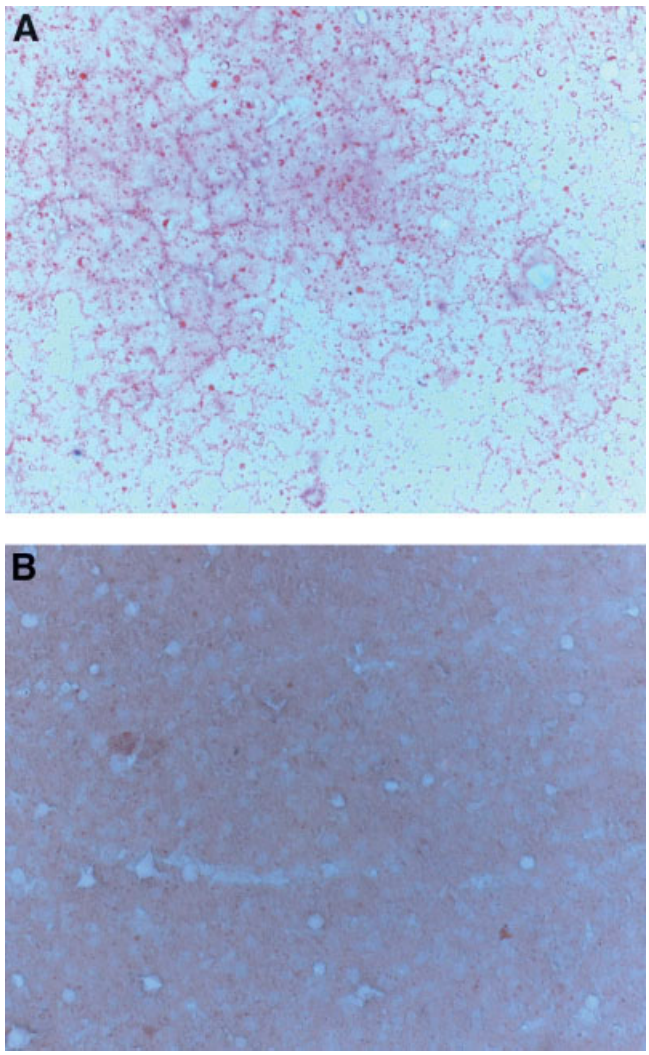

Figure 6. ORO staining, showing in the Bax$^{+/+}$ group diffused microvesicular fat droplets in hepatocytes (A). In the Bax$^{-/-}$ group no fatty changes were noted in hepatocytes (B).

micro- and macrovesicular steatosis and several apoptotic bodies (Fig. 5A). In the Bax$^{-/-}$ group, the liver structure was better preserved, with mild hydropic degeneration and no steatosis or apoptosis (Fig. 5B). These findings were confirmed by staining for fat using ORO, showing in the Bax$^{+/+}$ group diffused microvesicular fat droplets in hepatocytes (Fig. 6A). In the Bax$^{-/-}$ group no fatty changes were noted in hepatocytes (Fig. 6B). There was a statistically significant difference in mean number of apoptotic cells detected among the groups (by hematoxylin-eosin, immunohistochemistry for caspase-3, or the TUNEL assay, $P = 0.0001$ for all) (Fig. 7). Hematoxylin-eosin staining yielded a mean of 54.2 ± 15.2 positively-stained cells that were detected in the ischemic Bax$^{+/+}$ group compared with 5.6 ± 2.9 in the ischemic Bax$^{-/-}$ group (Fig. 7) ($P < 0.05$). In the ischemic Bax$^{+/-}$ group, the mean number of apoptotic cells detected was intermediate between the levels in the ischemic Bax$^{+/+}$ and Bax$^{-/-}$ groups.

TUNEL Assay

Apoptosis in the ischemic liver was confirmed by TUNEL assay.

Liver sections from the ischemic Bax$^{+/+}$ group showed a highly fluorescent signal with intensely stained, scattered diffuse nuclei (Fig. 8A) and a significantly greater increase in mean number of apoptotic cells (38.2 ± 11.9) compared with the ischemic Bax$^{-/-}$ group (6.3 ± 6.6; $P < 0.05$) (Fig. 7). By contrast, the intensity and quantity of fluorescence was significantly decreased in the Bax$^{-/-}$ ischemic group (Fig. 8B), and only rare nuclei stained positively.

Immunohistochemistry for Caspase-3

Liver sections from the ischemic Bax$^{+/+}$ group were highly positively stained for the activated form of

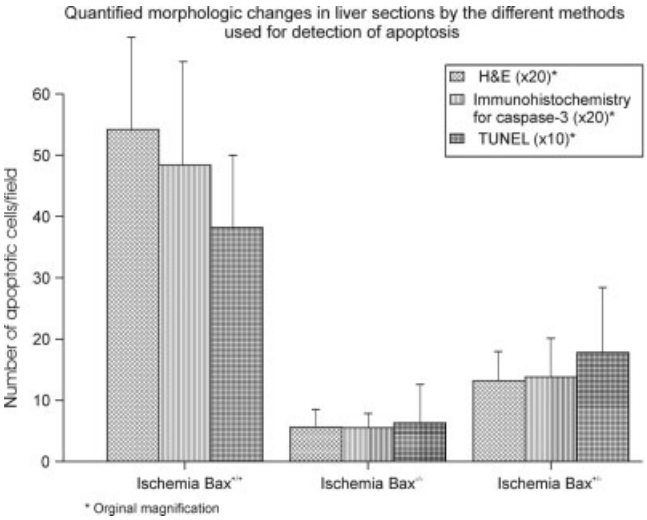

Figure 7. There was a statistically significant difference in mean number of apoptotic cells detected among the groups (by hematoxylin-eosin, immunohistochemistry for caspase-3, or the TUNEL assay, $P = 0.0001$ for all).

caspase-3. Scattered positive nuclei and cytoplasmic staining were noted in hepatocytes as well as sinusoidal cells (Fig. 9A). The $Bax^{+/+}$ group showed a significant increase in the mean number of apoptotic cells (48.4 ± 17.0) compared with the $Bax^{-/-}$ group (5.5 ± 2.4, $P < 0.05$) (Fig. 7).

However, in livers of the $Bax^{-/-}$ ischemic group, only rare cells stained for caspase-3 activity (Fig. 9B).

DISCUSSION

The major finding of the present study in Bax knockout mice is that decreased expression of the proapoptotic Bax gene attenuates I/R-induced apoptotic liver injury via inhibition of caspase-3 activity. Bax ablation led to significantly reduced liver enzyme levels after reperfusion in transgenic mice compared to nontransgenic mice, significantly reduced microvesicular steatosis as determined by ORO staining and apoptotic activity (quantitatively), as determined by the 3 histologic methods (hematoxylin-eosin stain, TUNEL, and immunohistochemistry for caspase-3). In addition, we show for the first time that Bax ablation is associated with decreased baseline caspase-3 activity. Our results confirm the notion that apoptosis is a key mechanism of ischemic injury, and that strategies inhibiting apoptosis confer protection. Bax ablation had overwhelming protective effects, with almost complete prevention of apoptosis. Baseline caspase-3 activity level was lower in the $Bax^{-/-}$ transgenic mice. However, after reperfusion, caspase-3 activation was prevented in the transgenic mice, whereas a significant (3-fold) increase was detected in the nontransgenic mice. In the ischemic $Bax^{+/-}$ group, the level of hepatic injury (as measured by liver enzymes, intrahepatic caspase-3 activity, and apoptosis markers) was intermediate between the levels in the ischemic $Bax^{+/+}$ and $Bax^{-/-}$ groups.

Our isolated liver model is a nonrecirculating in vitro

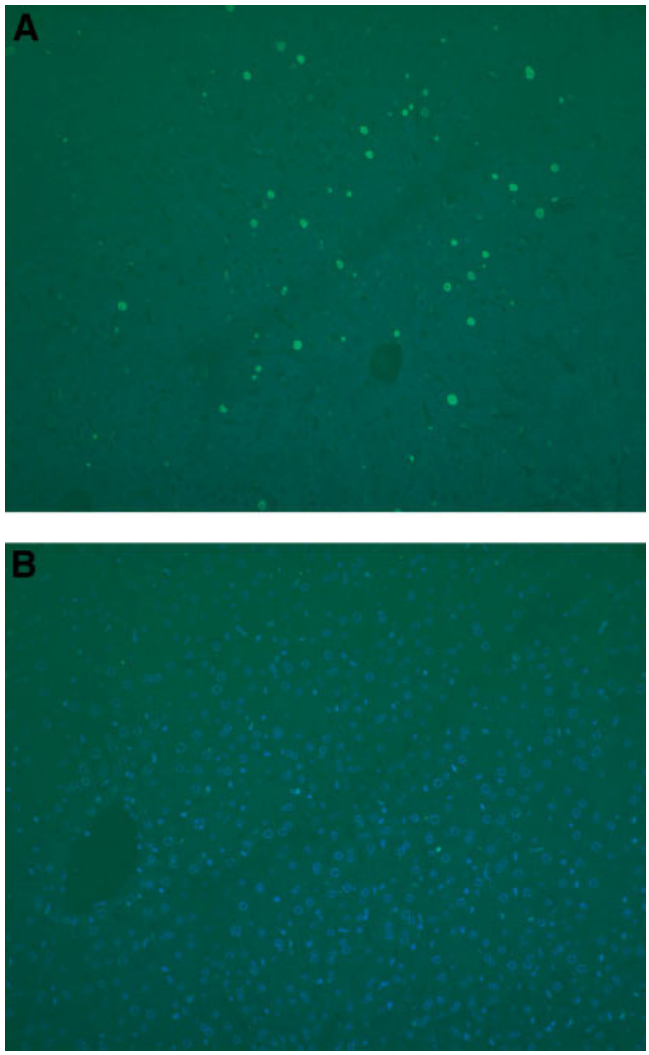

Figure 8. TUNEL assay (immunofluorescent labeling). (A) The $Bax^{+/+}$ livers show a highly fluorescent signal that highlights intensely stained scattered diffuse nuclei. (B) In the $Bax^{-/-}$ livers, the intensity and quantity of fluorescence is significantly decreased.

system. Following 90 minutes of ischemia and 1 minute of reperfusion, the levels of liver enzymes were found to be very high, representing injury to the ischemic liver. At 15 minutes of reperfusion, there was no longer massive leakage of the liver enzymes, so that decreased levels were detected in the efferent perfusate samples. By contrast, caspase activity was measured intrahepatically as a marker of apoptosis, and at 15 minutes of reperfusion, the apoptotic process continued to be active. However, further in vivo transplant models are needed to determine the significance of the findings reported in this study.

Poor initial graft function remains a major clinical problem following liver transplantation. Its incidence appears to depend on the length of cold ischemia and has thus been ascribed to injury from harvesting, cold storage, and warm reperfusion after blood vessel reconnection. We have investigated in our study the effect of

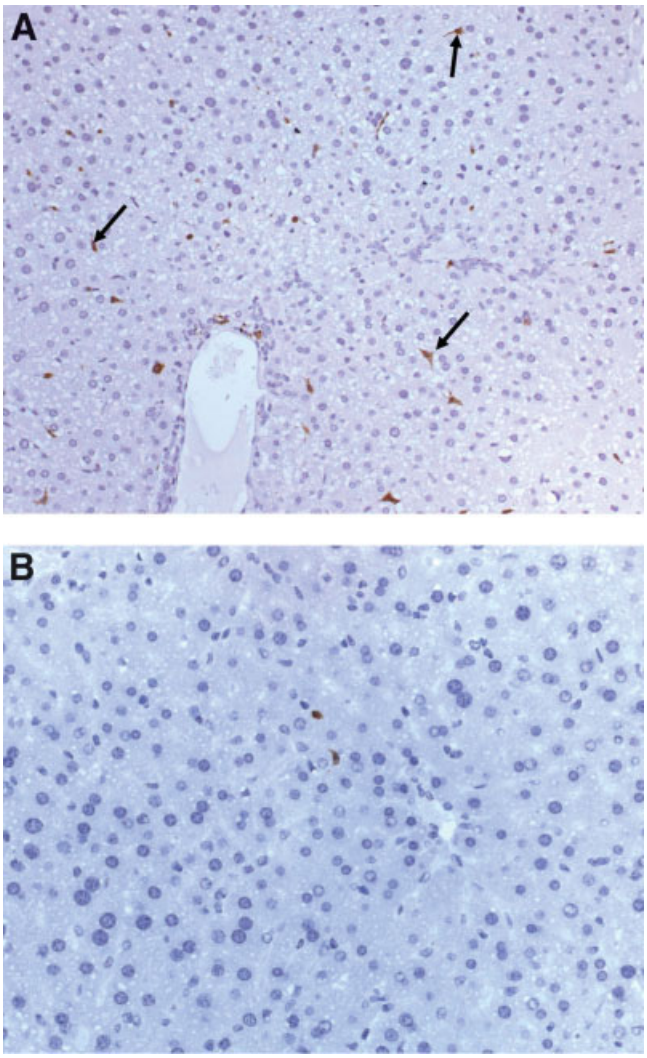

Figure 9. Immunohistochemistry for caspase-3. (A) The Bax$^{+/+}$ livers show scattered positive nuclear and cytoplasmic stains for the activated form of caspase-3. Hepatocytes and sinusoidal endothelial cells stained (arrows) positively for caspase-3. (B) In the Bax$^{-/-}$ livers, only rare cells stain for caspase-3 activity.

warm I/R injury in a model of isolated mouse liver. Pagliari et al.,[17] recently reported that the proapoptotic molecules Bax and Bak are directly activated by heat and induce the release of cytochrome c, while blocking the antiapoptotic Bcl-2 proteins prevent this event.

Bax and Bak, promote activation of the mitochondrial apoptotic pathway. Wei et al.[18] found that deletion of both Bax and Bak (but not 1 of them) renders the cells completely resistant to all major mitochondrial death stimuli. Both Bax and Bak could be activated by Bid following tumor-necrosis factor alpha stimulation.[18,19] However, others found that Bax is apparently localized in the cytosol and translocates to the mitochondria in response to death stimuli, undergoing a conformational change,[20] whereas Bak seems to constitutively reside in the mitochondria, indicating 2 distinct activation pathways.

Von Ahsen et al.[21] reported that Bid and Bax induce apoptosis by causing translocation of the cytochrome c from the mitochondria into the cytoplasm, thereby triggering Apaf-1-mediated caspase activation. Bax was found to directly induce the release of cytochrome c from isolated mitochondria by indirectly inhibiting the activation of caspases, particularly, caspase-3.[9] Accordingly, in the present study, in our isolated mouse liver model, ablation of the Bax gene was associated with the inhibition of intrahepatic caspase-3 activity, which attenuated the apoptotic injury caused by I/R.

Bailly-Maitre et al.[22] supplied further evidence of the role of the Bax gene in apoptotic hepatic injury by showing that absence of the antiapoptotic protein, Bax Inhibitor-1, in Bax Inhibitor-1 knockout mice protected the liver from endoplasmic reticulum stress and I/R injury.

Although in warm and cold hepatic ischemia, necrotic cell death is also the underlying mechanism of injury,[1] in this study we did not evaluate necrosis formation.

We also noted in our study, using immunohistochemistry, that sinusoidal cells stained positively for caspase-3 (Fig. 9A). Sinusoidal endothelial cells are a cellular target of I/R injury.[23] Sinusoidal endothelial cell injury causes microcirculatory disturbances, including leukocyte migration, platelet activation, and ultimately, secondary injury to hepatocytes, leading to organ dysfunction and nonfunction.[23] The sinusoidal endothelial cell injury in this process is apparently caused by apoptotic cell death,[24] as indicated by the classic morphological features of apoptosis in sinusoidal endothelial cells after I/R injury.

In conclusion, decreased expression of the proapoptotic Bax gene in Bax knockout mice attenuated the apoptotic injury induced by I/R in liver through inhibition of caspase-3 activity. The superior tolerance of Bax knockout livers to I/R injury suggests that this gene may serve as a potential target for therapeutic intervention in hepatic I/R injury.

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