Bcl-xL and Bcl-2 Repress a Common Pathway of Cell Death

By Debra T. Chao,* Gerald P. Linette,* Lawrence H. Boise,‡ Lynn S. White,* Craig B. Thompson,‡ and Stanley J. Korsmeyer*

From the *Howard Hughes Medical Institute, Division of Molecular Oncology, Departments of Medicine and Pathology, Washington University School of Medicine, St. Louis, Missouri 63110; and the ‡Howard Hughes Medical Institute, Departments of Medicine, Molecular Genetics, and Cell Biology, The University of Chicago, Chicago, Illinois 60637

Summary

The effect of Bcl-xL upon the developmental death of T cells was assessed by generating transgenic mice that expressed Bcl-xL within all thymocyte subsets. Bcl-xL protected thymocytes from a variety of apoptotic stimuli, including γ irradiation, glucocorticoids, and anti-CD3 treatment. Bcl-xL altered thymocyte maturation, resulting in increased numbers of CD3<sup>int/hi</sup> and CD4<sup>-8</sup> thymocytes. Overall, the phenotype of Bcl-xL transgenics was essentially indistinguishable from a Bcl-2 transgenic model. Overexpression of Bcl-xL or Bcl-2 resulted in the down-regulation of the other molecule, providing further evidence of their reciprocal regulation. In a genetic test of redundancy, the Bcl-xL transgene rescued mature T cells in Bcl-2 null mice. Immunoprecipitation indicated that Bcl-xL, like Bcl-2, heterodimerized with the death-promoting molecule Bax in thymocytes. This in vivo model argues that Bcl-xL, like Bcl-2, functions in a common pathway to repress cell death.

Regulated cell death is indispensable for the proper differentiation and maintenance of homeostasis in multiple lineages. The creation of a functional immune system is particularly dependent upon the selective survival and apoptotic demise of specific cells during lymphocyte maturation (1, 2). The susceptibility to death for a developing lymphocyte after the same stimulus varies markedly at different stages of development. Bcl-2 is one molecule that helps to determine the cell-autonomous susceptibility to apoptosis. For example, mature CD4 or CD8 single-positive (SP)<sup>+</sup> medullary thymocytes are resistant to apoptotic signals and possess high levels of the death repressor molecule, Bcl-2. In contrast, the CD4<sup>-8</sup> double-positive (DP) cortical thymocytes lack Bcl-2 and are highly vulnerable to a wide variety of apoptotic stimuli (3, 4).

Recently, an expanding family of Bcl-2 homologues has been identified. They share homology that is principally, but not exclusively, clustered within two conserved domains, entitled Bcl-2 homology 1 and 2 (BH1 and BH2) (5, 6). This includes Bax, which heterodimerizes with Bcl-2 and counters its activity. The ratio of Bcl-2/Bax can determine whether a given cell will execute or ignore an apoptotic stimulus (7). Single–amino acid substitutions within the BH1 or BH2 domain of Bcl-2 disrupted Bcl-2/Bax heterodimers, but not Bcl-2/Bcl-2 homodimers. Bcl-2 mutants that were unable to heterodimerize with Bax no longer inhibited apoptosis (6). This indicates that this family possesses both positive and negative regulators of death and that these proteins function at least in part through protein–protein interactions.

Given the existence of one regulatory pair, Bcl-2 and Bax, that can promote or repress apoptosis, an important question arises as to the rationale for further family members. An additional member, Bcl-x, has also been shown to regulate apoptosis in cell lines (8). A long form, Bcl-xL, which possesses BH1 and BH2, will repress cell death. A short RNA species noted in humans, Bcl-xS, lacks the BH1 and BH2 domains and has been noted to counter the protective effect of Bcl-2. It is of interest that species specificity in the alternatively spliced product, Bcl-xS, has been noted. Bcl-xS is readily detected in human but not in murine thymocytes, while Bcl-xL is present in the mouse thymus (9, 10).

Prior studies of gain-of-function Bcl-2 transgenic mice noted effects of this death repressor upon both thymocyte survival and maturation. When Bcl-2 was uniformly expressed within all thymocytes including DP cells, it conferred resistance to glucocorticoids, γ irradiation, and T cell–specific stimuli such as anti-CD3 treatment. However, the levels of Bcl-2 were unable to block the negative selection deletion of thymocytes (11, 12). Subsequently, a Bcl-2 transgene was introduced into genetic models in which thymocyte maturation was blocked.
Bcl-2 rescued CD8+ thymocytes in MHC class I −/− and α/β TCR mice but had no effect on CD4 lineage maturation. Moreover, α/β TCR transgenic mice revealed that Bcl-2 was up-regulated at the CD4+8+ stage during positive selection (13). Studies with Bcl-2-deficient mice have indicated that Bcl-2 is not solely required as a single member for the completion of T cell development. Instead, Bcl-2 −/− mice are unable to maintain homeostasis demonstrating apoptotic loss of both lymphocyte lineages postnatally (14).

To test whether Bcl-xL would repress cell death in normal development and affect maturation, we generated a Bcl-xL transgenic model. We wished to explore whether Bcl-xL functions through a separate pathway or impacts a common pathway shared with Bcl-2. To compare the effects of Bcl-xL with those of Bcl-2, we generated mice using the same lck proximal promoter to express transgenic Bcl-xL. Despite the alternating normal pattern of expression of Bcl-2 and Bcl-xL in the thymus (3, 10, 15), the phenotype of gain-of-function Bcl-xL transgenics was remarkably similar to Bcl-2 transgenics. Moreover, both repressor molecules heterodimerized with Bax in vivo. These in vivo data favor a model in which Bcl-xL and Bcl-2 function in a common pathway to regulate cell death.

Materials and Methods

Mice. A 0.8-kb cDNA of human Bcl-xL (8) was inserted into the lck-hGH vector (16) to generate the transgenic animals as previously described (11). lck−/−bcl-xL transgenic mice bred on the C57BL6/J background were maintained in a pathogen-free animal facility at Washington University (St. Louis, MO). Bcl-2-deficient animals (14) were bred to lckpr−/−bcl-xL transgenic mice in the same facility.

Cell Preparations. Single-cell suspensions were made from lymphoid organs after lysing red blood cells with Tris-NH4Cl buffer and counted on a hemocytometer by trypan blue exclusion. Splenic T cells were purified by immunomagnetic negative selection with anti-HSA, anti-B220, and anti-–Mac-1 as previously described (13). The purity of splenic T cells was usually 85–90%, determined by anti-CD3 staining.

Western Blot Analysis. Single-cell suspensions were lysed in 150 mM NaCl, 10 mM Tris, pH 7.4, and 1% Triton X-100 buffer. After quantitation of the amount of protein using the Bradford method (17), 35 μg of protein was run in each lane of a 12.5% SDS-polyacrylamide gel. Blots were developed with enhanced chemiluminescence (Amersham Corp., Arlington Heights, IL) or diaminobenzidine.

Antibodies. The mAbs 3F11 (hamster IgG anti-murine Bcl-2) and 4D2 (hamster IgG anti-murine Bax) were used as background antibody prepared by protein A chromatography. 13.6 is a polyclonal antisera raised against human Bcl-x that cross-reacts with murine Bcl-x. 7B2 is a mAb recognizing human and murine Bcl-x (murine IgG). The details of antibody production are described elsewhere (18). All other mAbs used in this study were purchased from Pharmingen (San Diego, CA). Secondary antibodies were biotinylated goat anti-hamster or anti-rabbit antibodies obtained from Caltag Laboratories (San Francisco, CA).

Viability Assay. Cells were plated at 10⁶ cells/ml in DME supplemented with 5% FCS (GIBCO BRL, Gaithersburg, MD). At each time point, the cells were collected and viability was determined by propidium iodide exclusion.

In Vivo Anti-CD3 Injection. 50 μg of affinity-purified anti-CD3 (clone 2C11; Pharmingen Inc.) or vehicle control (PBS) was injected intraperitoneally into 8–12-wk-old transgenic and control littermates. Thymi were removed 48 h after treatment. Single-cell suspensions were made in cold medium and stained for surface CD4 and CD8 within 2 h.

Immunoprecipitation. Fresh thymocytes were washed twice in serum-free, methionine-free DME (GIBCO BRL). Metabolic labeling was then performed with 40 μCi/ml of Trans3S-label (ICN Biomedicals, Inc., Costa Mesa, CA) for 3–4 h. Cells were harvested and lysed in an NP-40 isotonic lysis buffer with protease inhibitors (142.5 mM KCl, 5 mM MgCl2, 10 mM Hepes, pH 7.2, 0.25% NP-40) by nutation for 30 min in the cold room. Immunoprecipitation was performed as previously described (7). 35S-labeled bands were quantitated by PhosphorImager scanning (Molecular Dynamics, Inc., Sunnyvale, CA).

Results

Generation of Transgenic Mice. A transgenic construct was created by inserting the human Bcl-xL cDNA downstream of the lck proximal promoter (16). The 3′ untranslated portion of the construct possessed hGH exons, introns, and a poly(A) addition site to ensure proper processing of the transcript (Fig. 1 A). lckpr−/−bcl-xL lines 12 and 24, which expressed high levels of Bcl-xL, were propagated. The majority of experiments were done with the highest expressing line 12 and confirmed with line 24.

Transgene expression was analyzed by Western blot and flow cytometry. Bcl-xL was highly expressed in both thymus and spleen. The purified splenic T cell subpopulation showed a marked increase in Bcl-xL (Fig. 1 B). Flow cytometry using permeabilized thymocytes indicated that Bcl-xL levels were uniformly greater in transgenic than in normal thymocytes (Fig. 1 C). Three-color flow cytometric analysis revealed comparable levels of Bcl-xL in double-negative (DN) (CD4−8−), DP (CD4+8+), and CD4 SP or CD8 SP thymocytes (data not shown).

Bcl-xL Alters Thymocyte Maturation. All transgenic animals had an increase in CD3−/CD4−/CD8− and a reciprocal decrease in CD3+ thymocytes compared with control littermates (Fig. 2 A). Parallel changes were seen in the α/β T cell subset of transgenic animals, but no alteration was found in γ/δ T cells (data not shown). An increase in intermediate CD4+8+ and CD4+CD8+ thymocytes, which represent a transitional stage in thymic maturation, was also consistently noted in transgenic animals (Fig. 2 B). Moreover, all lckpr−/−bcl-xL mice had an increase in CD8 SP thymocytes, while some also displayed an increase in the CD4+ cells (Fig. 2 B, Table 1). Although overexpression of Bcl-xL did not substantially increase the total number of thymocytes, it altered the distribution of T cell subsets (Table 1). The ratio of CD4 to CD8 SP thymocytes was skewed to 1.9 in transgenic versus 3.5 in control mice. In addition, the total number of splenic lymphocytes was uniformly higher in Bcl-xL transgenic mice. The absolute number of B cells was unchanged. Flow cytometric analysis demonstrated an increase in T cells as well as an increased percentage of CD8 SP cells in the spleen of the transgenics (Fig. 2 C). Thus, constitutive overexpression...
of Bcl-xL altered thymocyte maturation in a pattern similar to that of Bcl-2 transgenic mice (11), resulting in a marked excess of CD8 SP cells.

**Prolonged Survival of Transgenic Thymocytes.** We next asked whether Bcl-xL altered the survival and susceptibility of thymocytes to apoptosis. Since we had previously generated Bcl-2 transgenic mice using the same promoter, we were able to compare the effects of Bcl-xL and Bcl-2 on thymocyte cell death. Thymocyte suspensions from lck<sup>+/−</sup>-bcl-x<sup>L</sup>, lck<sup>+/−</sup>-bcl-2, and wild-type littermates were plated in 5% FCS (Fig. 3 A). After 100 h, 80% of both Bcl-xL and Bcl-2 transgenic thymocytes were viable, whereas <10% of the normal thymocytes survived. Similarly, splenic T cells from both transgenics also revealed improved survival in vitro (data not shown). The survival between Bcl-xL- and Bcl-2-expressing thymocytes or splenic T cells was similar.

**Bcl-xL Protects Thymocytes from Glucocorticoid and Radiation-induced Apoptosis.** The protective ability of overexpression of Bcl-xL to several different apoptotic stimuli in thymocytes was tested. Both glucocorticoid treatment and low-dose irradiation are known to cause a rapid depletion of immature thymocytes by apoptosis. Thus, thymocytes were treated either with 100 nM dexamethasone (Fig. 3 B) or 225 rad of γ irradiation (Fig. 3 C). By 48 h, most of the normal thymocytes were dead, whereas 50−60% of either lck<sup>−/−</sup>-bcl-x<sup>L</sup> or lck<sup>−/−</sup>-bcl-2 thymocytes were still viable (Fig. 3 C). The transgenic thymocytes were resistant to anti-Fas Ab treatment in a similar matter (data not shown).

**lck<sup>−/−</sup>-bcl-x<sup>L</sup> Thymocytes Are Resistant to Anti-CD3-induced Apoptosis In Vivo.** To assess whether Bcl-xL was able to...
Table 1. *Bcl-xL Affects T Cell Maturation*

|                     | Transgenic | Wild type |
|---------------------|------------|-----------|
| Total thymocytes    | 93.5 ± 35.4| 77.4 ± 35.1|
| (× 10^6)            |            |           |
| Splenic T cells     | 27.1 ± 8.8 | 9.8 ± 2.5 |
| (× 10^6)            | CD4/CD8 ratio |CD4/CD8 ratio |
| Percentage of total | CD4         | 3.4 ± 1.2 | 3.5 ± 1.8 |
| thymocytes          | 16.1 ± 5.7 |           |
| Percentage of total | CD8         | 21.7 ± 6.4| 8.9 ± 2.8 |
| splenic cells       | 26.3 ± 7.4 | 15.4 ± 4.3| 2.5 ± 0.3 |

A In Vitro Survival

![Graph A](image1)

B Dex (100 nM)

![Graph B](image2)

C Radiation (225 Rad)

![Graph C](image3)

Figure 3. *Bcl-xL* prolongs thymocyte survival in vitro and protects from glucocorticoid and γ irradiation-induced apoptosis. Thymocytes from wild-type (■), *kk−* *bcl-xL* (□), and *kk−* *bcl-2* (○) mice were plated at 106 cells/ml in DMEM with 5% FCS (A), with 100 nM dexamethasone (B), or with 225 rad γ irradiation (C). Cell viability was determined by propidium iodide exclusion.

Figure 4. Anti-CD3 induced apoptosis. Total thymocytes from 12-wk-old mice were analyzed 48 h after in vivo injection of (A) PBS or (B) anti-CD3 mAb for cell number (in parenthesis) and thymocyte subsets by surface CD4 and CD8. (C) Combined data from a total of six independent experiments and >10 mice in each category (ages 8–12 wk old). Each experiment compares transgenics with littermate controls as wild type (Wt). Plotted values represent the percentages of thymocytes remaining after in vivo treatment with anti-CD3 Ab, in which 100% represents the average thymocyte number after treatment with PBS.
block a T cell–specific apoptotic signal, an anti-CD3 mAb was injected intraperitoneally into transgenic mice and control littermates. 48 h after treatment, thymi were harvested and compared with mice treated with PBS alone (Fig. 4 A). After anti-CD3 administration, the number of DP thymocytes was decreased substantially (90%) in wild-type animals. However, lck<sup>−</sup>-<bcl>-<sup>−</sup> mice were resistant to in vivo anti-CD3 treatment in the DP population (Fig. 4 B). While the extent of protection varied, all transgenic mice had substantially more thymocytes after in vivo anti-CD3 treatment compared with their wild-type littermates in six independent experiments (Fig. 4 C).

**Reciprocal Down-regulation of Endogenous Bcl-x<sub>L</sub> and Bcl-2.** A previous report had shown that endogenous Bcl-2 was down-regulated in the B cells of animals bearing the Bcl-2-Ig transgene (19). A similar down-regulation of endogenous Bcl-2 was also observed in lck<sup>−</sup>-<bcl>-<sup>−</sup> transgenic mice using 3F11, an mAb specific for mouse Bcl-2 (Fig. 5). It is noteworthy that, in both the thymocytes and splenic T cells of lck<sup>−</sup>-<bcl>-<sup>−</sup> mice, the endogenous Bcl-2 (3F11 mAb) protein expression was down-regulated. In parallel, endogenous Bcl-x<sub>L</sub> (13.6 Ab) expression was also decreased in the thymocytes of lck<sup>−</sup>-<bcl>-<sup>−</sup> animals (Fig. 5). The minimal amount of Bcl-x<sub>L</sub> in splenic T cells was also suppressed in the lck<sup>−</sup>-<bcl>-<sup>−</sup> animals. We were unable to determine if endogenous Bcl-x<sub>L</sub> was down-regulated in Bcl-x<sub>L</sub>-transgenic mice since the polyclonal Ab 13.6 recognizes both human and murine Bcl-x<sub>L</sub>. The reciprocal relationship of Bcl-2 and Bcl-x<sub>L</sub> protein levels suggests that their regulation may be coordinated in an inverse fashion.

**Bcl-x<sub>L</sub> Rescues Bcl-2-deficient Cells.** The phenotype of the Bcl-x<sub>L</sub> gain-of-function transgenics was similar to that of comparable Bcl-2 transgenics. Consequently, we tested whether Bcl-x<sub>L</sub> would substitute for Bcl-2 in vivo. lck<sup>−</sup>-<bcl>-<sup>−</sup> transgenics were mated to Bcl-2-deficient mice to determine whether selective expression of Bcl-x<sub>L</sub> would reverse the apoptotic loss of T cells in Bcl-2 null mice. Analysis of peripheral lymphocyte populations in healthy-appearing Bcl-2<sup>-/-</sup> mice demonstrated considerable heterodimerizing Bcl-x<sub>L</sub> (Fig. 8 B). Phosphorlmager analysis of Bcl-x<sub>L</sub> (13.6 Ab) and murine Bcl-2 (3F11 mAb) in thymocytes and splenic T cells from lck<sup>−</sup>-<bcl>-<sup>−</sup>, wild-type (wt), and lck<sup>−</sup>-<bcl>-<sup>−</sup> mice. The 13.6 polyclonal antibody recognizes both human and murine Bcl-x<sub>L</sub>. The 3F11 mAb recognizes the endogenous murine Bcl-2 but not the transgene origin human Bcl-2.

**Bcl-x<sub>L</sub> Also Heterodimerizes with Bax In Vivo**. Since Bcl-x<sub>L</sub> would substitute for Bcl-2 in countering cell death, we explored whether they both function in a common pathway. Bcl-2 heterodimerizes with a homologue Bax, which promotes cell death (7). Consequently, we examined thymocytes from normal and lck<sup>−</sup>-<bcl>-<sup>−</sup> transgenic mice to determine whether Bcl-x<sub>L</sub> associates with Bax (Fig. 8). Normal thymocytes, when radiolabeled and immunoprecipitated with an anti-Bcl-x antibody, revealed moderate amounts of Bcl-x<sub>L</sub> and some associated Bax (Fig. 8 A). It is of potential interest that several other bands were also seen (Fig. 8 A). However, only a minority of the total amount of Bax in normal thymocytes was heterodimerized with Bcl-x<sub>L</sub>. In Bcl-x<sub>L</sub>-transgenic mice, however, immunoprecipitation of Bcl-x<sub>L</sub> coprecipitated substantial portions of endogenous Bax (Fig. 8 A). Moreover, immunoprecipitation of Bax demonstrates considerable heterodimerizing Bcl-x<sub>L</sub> (Fig. 8 B). Phosphorlmager analysis of primary and secondary immunoprecipitates indicates that, in thymocytes from normal control mice, only 30% of Bax is heterodimerized with Bcl-x<sub>L</sub> (Fig. 8 A, upper panel), while the supernatant of that immunodepletion reveals that 70% of Bax is unbound (Fig. 8 A, lower panel). In contrast, in the presence of the Bcl-x<sub>L</sub> transgene, a substantial portion of Bax (77%) is heterodimerized with Bcl-x<sub>L</sub>, while only 23% of Bax is unbound (Fig. 8 B). The heterodimerization

![Figure 5](image-url) Down-regulation of endogenous Bcl-x<sub>L</sub> and Bcl-2. Western blot analysis of Bcl-x<sub>L</sub> (13.6 Ab) and murine Bcl-2 (3F11 mAb) in thymocytes and splenic T cells from lck<sup>−</sup>-<bcl>-<sup>−</sup>, wild-type (wt), and lck<sup>−</sup>-<bcl>-<sup>−</sup> mice. The 13.6 polyclonal antibody recognizes both human and murine Bcl-x<sub>L</sub>. The 3F11 mAb recognizes the endogenous murine Bcl-2 but not the transgene origin human Bcl-2.

![Figure 6](image-url) 8 A, lower panel). In contrast, in the presence of the Bcl-x<sub>L</sub> transgene, a substantial portion of Bax (77%) is heterodimerized with Bcl-x<sub>L</sub>, while only 23% of Bax is unbound (Fig. 8 B). The heterodimerization
of >50% of Bax with either Bcl-2 or Bcl-xL resulted in repression of cell death in a cell line system (20). Thus, transgenic Bcl-xL as well as Bcl-2 displays substantial heterodimerization in vivo with the death-promoting molecule Bax.

Discussion

We generated a gain-of-function transgenic model to determine whether Bcl-xL, which has been shown to block apoptosis within certain cell lines (8), would alter normal developmental cell death within an animal model. Overexpressed Bcl-xL in normal thymocytes inhibited cell death after dexamethasone, γ irradiation, and anti-CD3 treatment. Moreover, lineage-specific expression of Bcl-xL altered T cell maturation. Increased populations of CD3\(^{\text{int/hi}}\) thymocytes and intermediate CD4\(^{+}\)CD8\(^{+}\) cells in transition to becoming SP cells were noted. In addition, a skew to CD8 cells was noted both in the thymus and the periphery. The overall phenotype of the \(lck^{-}\)-bcl-xL transgenic mice was nearly indistinguishable from that of \(lck^{-}\)-bcl-2 transgenics generated with the same promoter (11). Detailed studies of Bcl-2 transgenics indicated that Bcl-2 was able to promote maturation in the absence of critical signals (13). This Bcl-xL model strengthens the argument that repressing apoptosis may enable differentiation irrespective of the specific molecule that inhibits the death pathway.

Recently, there has been a marked expansion in the number of Bcl-2 family members. This has raised important questions as to whether each molecule has an entirely distinct function or whether they all regulate a common pathway. The similarity of Bcl-2 and Bcl-xL transgenics suggests that these two molecules, which both repress cell death, might be interchangeable. The selective introduction of Bcl-xL into the T cells of Bcl-2-deficient mice restored resistance to apoptotic stimuli and rescued mature T cell populations. This rigorously established that Bcl-xL could substitute for Bcl-2 in maintaining T cell homeostasis. Since the Bcl-xL transgene was only expressed in T cells, the capacity for Bcl-xL to replace Bcl-2 in B cells was not assessed. However, the normal physiologic roles of Bcl-2 and Bcl-xL could prove to be distinct. Bcl-2 displays an on-off-on pattern of expression during T cell development. The most immature DN thymocytes possess Bcl-2, which is down-regulated at the DP stage. Bcl-2 is subsequently reexpressed during positive selection, and the protein remains high in mature CD4 and CD8 thymocytes (3, 13). In contrast, Bcl-x is principally expressed in the cortex and not the medulla (15). Only minimal amounts of Bcl-xL are found in DN thymocytes, but DP cells have high amounts of Bcl-xL. Bcl-xL is no longer expressed in SP thymocytes or peripheral T cells (10). However, it is Bcl-xL, but not Bcl-2, that responds to activation being reexpressed in mature T cells after TCR engagement (18). It is noteworthy that both Bcl-2 and Bcl-xL transgenic mice demonstrated a reciprocal down-regulation of the other member. This pattern of regulation is consistent with a sensing mechanism that would help coordinate the alternating patterns of Bcl-2 and Bcl-xL expression during development. This example emphasizes that homologues that display functional redundancy in genetic complementation tests may normally have slightly different physiologic roles, thereby accounting for their comaintenance during evolution.

The serial, alternating pattern of Bcl-2 and Bcl-xL expression and the fact that each gain-of-function model appeared essentially identical suggested that both Bcl-2 and Bcl-xL im-

Figure 6. Bcl-xL substitutes for Bcl-2 function in vivo. (A) Flow cytometric analysis of spleen mononuclear cells from representative, healthy 7-wk-old mice. Total spleen cell numbers of each animal are shown in parenthesis under each panel. Numbers indicating the percentages of total spleen cells that were B (B220+) or T (CD3+) cells are shown next to the boxed populations. (B) Graphic presentation of the absolute number (in millions) of splenic T (closed bar) and B cells (open bar) from representative littermate mice at 7 wk of age.
Figure 7. Bcl-xL restores resistance to apoptotic stimuli in Bcl-2-deficient thymocytes. Fresh thymocytes from Bcl-2-/- (■) and kk-bcl-xL/Bcl-2-/- mice (○) were plated at 10^6 cells/ml in DMEM with 5% FCS (A). In B, thymocytes were treated with 100 nM dexamethasone, and, in C, with 225 rad γ irradiation. A representative of four independent experiments is shown. Animals used in this experiment were 6-wk-old littermates.

Impact a common pathway. The emerging Bcl-2 gene family shares its principal homology within two conserved domains entitled BH1 and BH2 (5). Bcl-2 is known to heterodimerize with a death-promoting family member, Bax (7). Mutagenesis analysis of Bcl-2 indicated that BH1 and BH2 are novel domains that regulate heterodimerization. Point mutations within BH1 and BH2 argue that Bcl-2 must heterodimerize with Bax to repress death (6). Consequently, we assessed whether Bcl-xL would heterodimerize with Bax in vivo as part of their common effects. In normal thymocytes, only a minority of Bax (30%) was heterodimerized with the modest levels of Bcl-xL. However, transgenic levels of Bcl-xL resulted in the majority of Bax (77%) being heterodimerized with Bcl-xL. In a separate study using a cell line, various levels of Bcl-2, Bcl-xL, Bad, and Bax were rigorously quantitated and correlated with the susceptibility to apoptosis. When ~50% or more of the Bax in FL5.12 cells was complexed in heterodimers, apoptosis was suppressed after IL-3 deprivation (20). Thus, the difference in the percentage of Bax found in heterodimers with Bcl-xL within these transgenics is predicted to be functionally significant. The capacity of Bcl-xL to mimic Bcl-2 and substitute for it in repressing T cell death may relate to its ability to heterodimerize with a substantial portion of Bax. However, only a minority of native Bax (30%) is associated with Bcl-xL in normal thymocytes. It is possible that other bands noted in the anti-Bcl-xL immunoprecipitate might represent independent partners for Bcl-xL that may also prove biologically significant in normal development.

The alternative death repressor molecules of Bcl-2 and Bcl-xL appear to sequentially regulate cell death at serial stages of T cell development. These studies of transgenic mice indicate that Bcl-xL and Bcl-2 can repress cell death through a common pathway in which their interaction with Bax is one shared parameter.

We thank K. Haag, P. Goda for expert animal husbandry, and Mary Pichler for excellent secretarial assistance. We are grateful to members of our lab for helpful discussion, advice, and support.

G. P. Linette is supported by the National Institutes of Health grant T32 HL-07088. L.H. Boise is a fellow of the Leukemia Society of America. This work was supported by National Institutes of Health grant CA-49712.
Address correspondence to Dr. Stanley J. Korsmeyer, Division of Molecular Oncology, Department of Medicine and Pathology, Howard Hughes Medical Institute Research Laboratories, Washington University School of Medicine, 660 South Euclid Avenue, Box 8022, Saint Louis, MO 63110.

Received for publication 7 April 1995 and in revised form 5 May 1995.

References

1. Cohen, J.J., and K.C. Duke. 1992. Apoptosis and programmed cell death in immunity. Annu. Rev. Immunol. 10:267–293.
2. Shortman, K. 1992. Cellular aspects of early T cell development. Curr. Opin. Immunol. 4:140–146.
3. Veis, D., C.L. Sentman, E.A. Bach, and S.J. Korsmeyer. 1993. Expression of the Bcl-2 protein in murine and human thymocytes and in peripheral T lymphocytes. J. Immunol. 151:2546–2554.
4. Gratiot-Deans, J., L. Ding, L.A. Turka, and G. Núñez. 1993. Bcl-2 proto-oncogene expression during human T cell development. J. Immunol. 151:3–91.
5. Williams, G.T., and C.A. Smith. 1993. Molecular regulation of apoptosis: genetic controls on cell death. Cell. 74:777–779.
6. Yin, X.-M., Z.N. Oltvai, and S.J. Korsmeyer. 1994. BH1 and BH2 domains of Bcl-2 are required for inhibition of apoptosis and heterodimerization with Bax. Nature (Lond.) 369:321–323.
7. Oltvai, Z.N., C.L. Milliman, and S.J. Korsmeyer. 1993. Bcl-2 heterodimerizes in vivo with a conserved homolog, bax, that accelerates programmed cell death. Cell. 74:609–619.
8. Boise, L.H., M. González-García, C.E. Postema, L. Ding, T. Lindsten, L.A. Turka, X. Mao, G. Núñez, and C.B. Thompson. 1993. bcl-x, a bcl-2-related gene that functions as a dominant regulator of apoptotic cell death. Cell. 74:597–608.
9. González-García, M., R. Pérez-Ballestero, L. Ding, L. Duan, L.H. Boise, C.B. Thompson, and G. Núñez. 1994. Bcl-xL is the major Bcl-x mRNA form expressed during murine development and its product localizes to the mitochondria. Development (Camb.). 120:3033–3042.
10. Ma, A., J.C. Pena, B. Chang, E. Margosian, L. Davidson, F.W. Alt, and C.B. Thompson. 1995. Bcl-x regulates the survival of double positive thymocytes. Proc. Natl. Acad. Sci. USA. 92:4763–4767.
11. Sentman, C.L., J.R. Shutter, D. Hockenbery, O. Kanagawa, and S.J. Korsmeyer. 1991. bcl-2 inhibits multiple forms of apoptosis but not negative selection in thymocytes. Cell. 67:879–888.
12. Strasser, A., A.W. Harris, and S. Cory. 1991. bcl-2 transgene inhibits T cell death and perturbs thymic self-censorship. Cell. 67:889–899.
13. Linette, G.P., M.J. Grusby, S.M. Hedrick, T.H. Hansen, L.H. Glimcher, and S.J. Korsmeyer. 1994. Bcl-2 is upregulated at the CD4+ CD8+ stage during positive selection and promotes thymocyte differentiation at several control points. Immunity. 1:197–205.
14. Veis, D., C.M. Sorenson, J.R. Shutter, and S.J. Korsmeyer. 1993. Bcl-2-deficient mice demonstrate fulminant lymphoid apoptosis, polycystic kidneys, and hypopigmented hair. Cell. 75:229–240.
15. Krajewski, S., M. Krajewska, A. Shabaik, H. Wang, S. Irie, L. Fong, and J.C. Reed. 1994. Immunohistochemical analysis of in vivo pattern of Bcl-x expression. Cancer Res. 54:5501–5507.
16. Chaffin, K.E., C.R. Beals, T.M. Wilkie, K.A. Forbush, M.I. Simon, and R.M. Perlmuter. 1990. Dissection of thymocyte signaling pathways by in vivo expression of pertussis toxin ADP-ribosyl-transferase. EMBO (Eur. Mol. Biol. Organ.) J. 9:3821–3829.
17. Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248–254.
18. Boise, L.H., A. Minn, P.J. Noel, C.H. June, M. Accavitti, T. Lindsten, and C.B. Thompson. 1995. CD28 costimulation can promote T cell survival by enhancing the expression of Bcl-xL. Immunity. 3:87–98.
19. McDonnell, T.J., G. Núñez, F. Platt, D. Hockenbery, L. London, J.P. McKearn, and S.J. Korsmeyer. 1990. Deregulated Bcl-2-immunoglobulin transgene expands a resting but responsive immunoglobulin M and D-expressing B-cell population. Mol. Cell. Biol. 10:1901–1907.
20. Yang, E., J. Zha, J. Jockel, L.H. Boise, C.B. Thompson, and S.J. Korsmeyer. 1995. Bad, a heterodimeric partner for Bcl-xL and Bcl-2, displaces Bax and promotes cell death. Cell. 80:285–291.