An Additional Form of Rat Bcl-x, Bcl-xβ, Generated by an Unspliced RNA, Promotes Apoptosis in Promyeloid Cells*

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The bcl-2 oncogene product delays apoptotic cell death and prolongs the cell survival. We cloned two bcl-2-related cDNAs from a rat thymus cDNA library by low stringency hybridization with a rat bcl-2 fragment as a probe. One of these, designated bcl-α, was a counterpart of the human bcl-x, reported previously as a bcl-2-related gene (Boise, L. H., Gonzalez-Garcia, M., Postema, C. E., Ding, L., Lindsten, T., Turk, L. A., Mao, M., Nunez, G., and Thompson, C. B. (1993) Cell 74, 597-608). The other, designated bcl-xβ, was novel and found to be generated by an unspliced mRNA, whereas bcl-α was generated from a spliced transcript. The splice junction exactly corresponded to that found in the bcl-2 gene. bcl-xβ was specifically expressed in cerebellum, heart, and thymus. When bcl-xβ directed by a strong promoter was introduced into an interleukin-3-dependent promyeloid cell line, FDC-P1, DNA fragmentation was observed even in the growing state in the presence of interleukin-3 although not in the control transfectants. This finding suggests that the rat bcl-xβ gene product promotes apoptosis in the promyeloid cells.

Apoptosis is a process that is morphologically distinct from necrosis in cell death. Cells undergoing apoptosis shrink, the plasma membrane forms blebs, the nucleus condenses and necrosis in cell death. Cells undergoing apoptosis shrink, the nucleus condenses and necrosis in cell death. Cells undergoing apoptosis shrink, the nucleus condenses and necrosis in cell death. Cells undergoing apoptosis shrink, the nucleus condenses and necrosis in cell death. Cells undergoing apoptosis shrink, the nucleus condenses and necrosis in cell death. Cells undergoing apoptosis shrink, the nucleus condenses and necrosis in cell death. Cells undergoing apoptosis shrink, the nucleus condenses and necrosis in cell death. Cells undergoing apoptosis shrink, the nucleus condenses and necrosis in cell death. Cells undergoing apoptosis shrink, the nucleus condenses and necrosis in cell death. Cells undergoing apoptosis shrink, the nucleus condenses and necrosis in cell death.

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1 The abbreviations used are: IL-3, interleukin 3; PCR, polymerase chain reaction; GAPDH, glyceraldehyde-3-phosphate dehydrogenase bp, base pair(s); RT, reverse transcription.

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(Henderson et al., 1993). MCL-1 and A1, isolated from myeloid and murine hematopoietic cells, respectively, also has sequence similarity to bcl-2 (Koizum et al., 1993; Lin et al., 1993). Chicken bcl-x was cloned by low stringency hybridization with a bcl-2 probe as a bcl-2-related gene, and then two forms of bcl-x were isolated from a human cDNA library (Boise et al., 1993). The two forms, bcl-x-A and bcl-x-B, were considered to be generated by alternative splicing and overexpressed Bcl-x in an IL-3-dependent cell line delayed apoptosis on withdrawal of IL-3. In contrast, bcl-x-A reduced the survival activity of bcl-2 in apoptosis. However, the precise molecular mechanism is not clear, although Bcl-x-A interacts with Bcl-2 (Sato et al., 1994; Sedlak et al., 1995). An additional cDNA encoding Bcl-x lacking transmembrane tail has been reported (Fang et al., 1994). In addition, genes with proteins that regulate apoptosis by forming heterodimers have been cloned. Bax was isolated as a partner of Bcl-2 in forming a heterodimer, and overexpressed Bax prevents suppression activity of apoptosis by Bcl-2 (Oltvai et al., 1993). Recently, bad and bak cDNAs were isolated with gene products that form heterodimers with Bcl-x or Bcl-2 (Yang et al., 1995; Farrow et al., 1995; Chittenden et al., 1995; Kiefer et al., 1995). These findings suggest that several members of Bcl-2 family interact with one another for regulating apoptosis.

In the present study, we cloned an additional form of rat bcl-x. This form, designated bcl-xj, was generated from an unspliced mRNA and expressed specifically in heart, cerebralum, and thymus. The unspliced mRNA translation product, Bcl-xj, showed an ability to promote apoptosis.

MATERIALS AND METHODS

Construction of a cDNA Library—Poly(A)- RNA was isolated from a 3-week-old Wistar rat thymus through a cesium trifluoroacetate cushion (Henderson et al., 1987). The poly(A) RNA was primed with random hexamers for reverse transcription (Superscript II, Life Technologies, Inc.). The double-stranded cDNA was methylated and ligated with an EcoRI linker and inserted into an λgt10 phage vector (Huyhn et al., 1985).

Isolation of a Rat bcl-2 Fragment and Screening—A fragment of rat bcl-2 was amplified by polymerase chain reaction (PCR) (Saiki et al., 1987) using primers sequences that were common between mouse and human bcl-2s (5'-ATGGCCGACGCTGGAGAAA and 5'-CTGGATCCGAGGTGTCAG). The fragment was subcloned and confirmed to be derived from bcl-2 by sequencing. For plaque hybridization, the rat bcl-2 fragment was used as a probe, and the filters were finally washed with 0.1× SSC and 0.1% SDS at 65°C. Sequences of cDNA fragments from λgt10 phage DNA were isolated by EcoRI digestion and subcloned into BlueScript II SK+ (Stratagene, CA). The inserts were undirectionally digested with exonuclease III and mung bean nuclease. The both cDNA strands were sequenced by dideoxynucleotide chain termination using alkaline-denatured plasmids.

PCR Analysis of cDNA and Genomic DNA—For analyzing the gene structure, fragments were amplified by PCR using the cloned cDNA, pooled cDNA from rat thymus, and genomic DNA as templates. The PCR was performed using indicated primer sets by 30 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 45 s, and the PCR products were subjected to 1% agarose gel electrophoresis. To detect both bcl-xA and bcl-xB, a common sense primer A1 (5'-ATGGACGTGTTGAGCCCGCATCCT) and an antisense primer A3 (5'-GGCGATTGAGGAGACTGCTT) were used. To detect bcl-xj specifically, a sense primer A2 (5'-AAGGCTGAGACAGA) and an antisense primer A4 (5'-GGAGCTGACTGGA) were used. For bcl-xj, a sense primer A1 (5'-ATGGACGTGTTGAGCCCGCATCCT) and an antisense primer A4 (5'-GGAGCTGACTGGA) were used.

Total Southern Blot Analysis—Genomic DNA (3 μg) was digested with indicated restriction enzymes, separated by electrophoresis on a 1% agarose gel, and then transferred to a nylon membrane, Hybond N+ (Amersham Corp.). Hybridization was performed in a Rapid hyb buffer (Amersham Corp.) at 65°C with a BamHI-HindIII fragment (this HindIII site is derived from the vector) as a common probe for bcl-x and bcl-xj or with fragments amplified by PCR as specific probes for bcl-xA and bcl-xB. The hybridized filters were finally washed with 0.1× SSC and 0.1% SDS at 65°C. Autoradiography was performed using a Bioimaging analyzer, Fujix BAS 2000 (Fujji Photo Film, Co., Ltd., Tokyo, Japan).

Northern Blot Analysis—Total RNA was isolated from various tissues of 12-week-old adult Wistar rats by the acid guanidinium thiocyanate-phenol/chloroform method (Chomczynski and Sacchi, 1987). The poly(A) RNA was purified with a Pharmacia Biotech Inc. poly(A) RNA purification kit. The poly(A) RNA samples (3 μg) were electrophoresed in a formaldehyde/agarose gel. Hybridization was performed in the Rapid hyb buffer (Amersham Corp.) at 65°C with a BamHI-HindIII fragment of the rat bcl-2 or a BamHI-BamHI fragment of the rat bcl-x. Blots were then stripped by boiling and rehybridized with a GAPDH gene probe to verify the amount of mRNA loaded in all lanes. The GAPDH gene probe was obtained by PCR using the rat liver cDNA as a template (Tso et al., 1985).

Semi-quantitative Analysis of bcl-x mRNAs—To determine the expression level of bcl-x mRNAs, the relative amount of cloned cDNA was determined by semi-quantitative reverse transcription PCR (RT-PCR) (Kawasaki et al., 1988). Total cellular RNA (0.5 μg) or equivalent poly(A)+ RNA (25 ng) isolated from various tissues was reverse transcribed with oligo(dT)16 primer and RTase (HindIII fragment, Amersham Corp.) at 65°C with a GAPDH gene probe as a common probe. The PCR was performed using the primers 5'-ATGGACGTGTTGAGCCCGCATCCT and an antisense primer (Nishimura et al., 1990) (for bcl-xA or bcl-xB at 0.5× SSC and 0.1% SDS at 72°C × 45 s at 72°C × 30 cycles). To distinguish bcl-xA and bcl-xj, a common sense primer A1 and individual antisense primer A4 (5'-GTAAGCCGAAAGTCTGCAG) or A3 (5'-CTGGATCCGAGGTGTCAG) were used. To distinguish bcl-xA and bcl-xj, a common sense primer A1 and an antisense primer A3 were used for RT-PCR. Total RNA (0.5 μg) or poly(A)+ RNA without reverse transcription was also subjected to PCR under the same conditions. To quantify the relative expression level of bcl-xA and bcl-xj, the pooled cDNA (25 ng) was subjected to PCR containing the mixed primers A2 and A4 in the same manner. To assure that the PCR conditions were not biased towards one form, the plasminoids containing bcl-xA and bcl-xj or bcl-xA and bcl-xB were mixed in various ratios and subjected to PCR.

Regulation of bcl-x Expression in a FDC-P1 Cell Line—Fragments of bcl-x, bcl-xA, bcl-xj, and bcl-xB were subcloned into an Xba site of the plasmid pEF-BOS, which harbors an elongation factor 1α promoter (Mizushima and Nagata, 1990) (pEF-BOS was a kind gift from Dr. S. Nagata, Osaka Institute for Biosciences, Osaka, Japan). The plasmids (20 μg) were transfected into a promyeloid cell line (FDC-P1) with 1 μg of pST-neoB, which carries a neomycin-resistant gene (pST-neoB was a kind gift from Prof. S. Kondoh, Biotechnology center, Osaka University, Osaka, Japan). Transfection was performed as described by Potter et al. (1984) by electroporation with a Bio-Rad Gene pulser at 0.33 kV in a 0.4-cm cell. After selection with 0.5 mg/ml Geneticin G418 (Life Technologies, Inc.) in 96-well plates, transfected clones were maintained in RPMI 1640 medium containing 10% fetal bovine serum and 10% conditioned medium from WEHI-1, which secretes IL-3 (Nishimura et al., 1990) at 37°C in 5% CO2 (in this study, RPMI medium always contained 10% fetal bovine serum). Expression of transfected genes in individual clones was confirmed by Northern blotting.

Suppression and Promotion of Apoptosis—The growing transfected (approximately 2×106 colony-forming units) were washed twice with RPMI 1640 medium without the conditioned medium from WEHI-1. The cells were cultured at 2×106 cells/ml in the RPMI 1640 medium without IL-3 for indicated times. The surviving cells were concentrated by centrifugation and counted by a trypan blue exclusion method.

DNA Fragmentation—The transfected cells were kept at a density of 0.5×106 cells/ml for 3 days in medium containing 10% of the conditioned medium and harvested. DNA was isolated and subjected to 0.5% Triton X-100, 5% Dextran-HCI (pH 8.0), and 10 µM EDTA. The nuclei were pelleted by centrifugation and RNase A and proteinase K were added to the supernatants, and DNA fragments from the supernatants were precipitated with ethanol and subjected to 1.2% agarose gel electrophoresis.

RESULTS

Isolation of Rat bcl-x DNAs—Overexpression of the bcl-2 gene delays apoptotic cell death in some but not in all cultured cells. These findings suggest the presence of multiple pathways of apoptosis. Thus, we attempted to clone bcl-2-related genes by low stringency hybridization. We screened a rat thymus λgt10 cDNA library with a rat bcl-2 fragment obtained by PCR (Saiki et al., 1988). Of 1.5×106 plaques, one hundred with positive
Regulation of Apoptosis by bcl-x

signals were picked up, and 25 clones were finally purified by successive plaque hybridization. The clones were classified into four groups according to their restriction maps of their inserts. In the four groups, two gave strong hybridization signals, whereas the other two gave weak signals. The nucleotide sequence of each clone revealed that two clones corresponded to bcl-2a and bcl-2b (an unspliced form), and that the third one corresponded to bcl-x (Boise et al., 1993). The amino acid sequence of rat bcl-2 obtained was 95 and 98% identical to the human and mouse counterparts, respectively. The rat counterpart of human bcl-x, obtained showed 98% amino acid identity to the human clone (Boise et al., 1993). The fourth clone was novel; its 5′ region was same as that of rat counterpart of human bcl-x, but its 3′ region was completely different and diverges from bcl-x, at a position corresponding to the splice junction in the bcl-2 gene (Tsujimoto and Croce, 1986). Thus, we designated the two forms of rat bcl-x as bcl-xx and bcl-xi for rat counterpart of human bcl-x, and as bcl-xi for the novel form according to the designation of bcl-2. Fig. 1 shows the nucleotide and deduced amino acid sequences of rat bcl-xx and bcl-xi.

When fragments were amplified by PCR using pooled cDNA of rat thymus with a primer set (A1 and A3) for bcl-xx, a faint additional band was found as shown in Fig. 2 (lane 3; the position is indicated by bcl-xx). The position of the upper band corresponded to that of bcl-xx (lane 1). A DNA fragment from the lower and faint band was isolated and sequenced. The nucleotide sequence of this PCR product was almost identical to that of human bcl-x (Boise et al., 1993). The rat counterpart of the human bcl-x, was designated bcl-x.

Gene Structure of bcl-x—Total Southern blot in rat genome analysis showed a single band using a fragment corresponding to the 3′ region specific for bcl-xi as a probe (Fig. 3C). Probes for a 3′ region of bcl-xx and a common region for bcl-xx and -xi, however, gave two bands in the rat genome (Fig. 3, B and A, respectively). On the other hand, human genome gave a single band for bcl-x in total Southern blots by using the common probe (Fig. 3D). These findings suggest the presence of a pseudogene as well as a single bona fide gene in rat.

For showing that bcl-xx, -xi, and -xy are derived from a single gene by alternative RNA splicing, fragments were amplified by using rat genomic DNA as well as pooled cDNA and plasmids containing cloned cDNAs as PCR templates. A fragment amplified using the rat genomic DNA as a template with a primer set for bcl-xi was the same length as that from the cloned bcl-xi cDNA (primers A1 and B1) (Fig. 2, lanes 9 and 10), and its nucleotide sequence was identical to that of the cDNA of bcl-xi. Next, when a common primer set for bcl-xx and bcl-xy (primers A1 and A3) was used, genomic DNA template gave only one band that migrated to same extent as bcl-xy (Fig. 2, lane 4). The nucleotide sequence of this PCR product was identical to bcl-xy but showed no open reading frame by a deletion and insertion of nucleotides (data not shown). Thus, the shorter PCR fragment was considered to be derived from a processed pseudogene of a bcl-xy type in the rat genome. When a primer set specific to bcl-xx (primers A2 and A4 in Fig. 2) was used, no band was observed due to the presence of a long intron (Fig. 2, lane 7). These results suggest that the rat genome has a bona fide gene generating at least three alternatively spliced forms, a, b, and γ, and that bcl-xi is generated from an unspliced mRNA. Fig. 2 presents a model of the rat bcl-xx gene structure, in which the positions of PCR primers and hybridization probes are indicated. Because an additional cDNA from mouse lacking 70 nucleotides in the hatched exon in Fig. 2 has been recently reported (Fang et al., 1994), the exon may be divided into three exons.

The mouse bcl-2 gene has a short intron in the noncoding 5′-flanking region, which is often but not always spliced (Negrini et al., 1987). To test for the presence of a similar kind of intron in bcl-xx gene, the rat genomic DNA was amplified by a primer set spanning the 5′ noncoding region (F1 and F2). The length of the amplified genomic DNA was longer than that of the cloned cDNA (data not shown), with an additional stretch of approximately 85 bp. When cDNAs obtained from various rat tissues were used as PCR templates, we found a 235-bp band derived from the unspliced form and a 150-bp band derived from the spliced form. The ratio of the two bands was approximately 3:1 (data not shown). Therefore, the first intron located in the 5′ noncoding region was not always spliced. We have recently cloned a human genomic gene encoding bcl-x (GenBank accession number D30746). Its 5′ noncoding region resembled that of the rat bcl-xx cDNA. Comparing the nucleotide sequence of the human gene with that of the rat cDNA, the putative splice junction is shown in Fig. 1B by a closed arrowhead.

Expression of bcl-x—Poly (A) + RNA was isolated from various tissues from 12-week-old adult rats. As shown in Fig. 4 by Northern blotting, most tissues expressed bcl-x. As an internal control, GAPDH mRNA was quantified on the same membrane filter. Whereas bcl-2 was most highly expressed in thymus and thymus (Negrini et al., 1987), expression of bcl-x was found intensively in the thymus but not so high in the spleen.

Expression level of bcl-xi relative to bcl-xx and that of bcl-xy to bcl-xx were estimated by semi-quantitative analysis using RT-PCR. Total cellular RNA or poly (A) + RNA from the various rat tissues was used to synthesize cDNA with reverse transcriptase. The resulting cDNA was subjected to PCR amplification using a mixed primer set. For semi-quantification, a standard curve was obtained by using the mixture of two kinds of plasmids as described under “Materials and Methods.” Several combinations of forward and reverse primers were applied for the semi-quantitative analysis, and out of them, a primer set was selected to show more linearity under these conditions to eliminate the effect of bias during the amplification. To exclude the possibility of contamination by chromosomal DNA, PCR was performed using RNA without the reverse transcriptase reaction as control. When any bands corresponding to bcl-xi or bcl-xy were seen in the control experiments, the samples were excluded as RNA sources. As shown in Fig. 5B, no band was detected in the control experiments without the reverse transcriptase reaction. As shown in Fig. 5, bcl-xi was specifically expressed in the cerebellum, heart, and thymus. The level of bcl-xi expression relative to bcl-xx was less than 10%, and the relative expression did not change at the development from 6 days old to 12 weeks old as far as was examined in the thymus (Fig. 5), heart, and cerebellum (data not shown).

The ratio of bcl-xy to bcl-xx was also examined in the same way. Because the rat genome contained the processed pseudogene derived from bcl-xy, the lack of chromosomal DNA contamination was carefully confirmed as described above (Fig. 6B). As shown in Fig. 6, the ratio of bcl-xy was slightly varied depending on the tissues but was not as specific as bcl-xi. Boise et al. (1993) reported that the major form expressed in thymocytes was bcl-xx (bcl-xy). However, we found the major form was bcl-xx but not bcl-xy in the thymus. In addition, the expression of bcl-xy was not changed depending on the development from 6 days old to 12 weeks old as shown in Fig. 6.

Transfection of bcl-xi into a Promyeloid Cell Line—The cDNA construct with an elongation factor 1α promoter were introduced into a promyeloid cell line, FDC-P1, in which apoptosis is induced by depletion of IL-3. The stable transfectants, cotransfected with pStneo, were selected by G418, and the expressions of bcl-2, bcl-xx, or bcl-xi were confirmed by
Northern blotting as shown in Fig. 7. The expression of bcl-2, bcl-xα, bcl-xβ, or bcl-xγ was also confirmed by Western blotting (data not shown). The G418-resistant clones expressed bcl-xα in all the transfectant clones examined. On the other hand, in the transfectant-clones with bcl-xβ, bcl-xβ was expressed at lower levels than bcl-xα in those with bcl-xα (between 30 and 15% of bcl-xα) (lanes 4–6 in Fig. 7). Some G418-resistant transfectants did not express bcl-xβ (lanes 7 and 8 in Fig. 7). This result may indicate that the cells overexpressing bcl-xβ are incapable of surviving.

**Effects of Overexpressed Bcl-xα and Bcl-xβ on Apoptosis—** After depletion of IL-3, the surviving cellswere counted in two or three clones transfected with each cDNA. Representative results are shown in Fig. 8. All the transfectants of bcl-xα and bcl-2 showed marked delay of apoptosis; however, those transfected with bcl-xβ or bcl-xγ showed no significant increase or decrease in survival.
decrease in the surviving cell number as compared with the control transfectants, although more dead cells were found in the transfectants of bcl-x. Because dead cells were observed in the bcl-x expressing cells even in the growing state in the presence of IL-3, DNA fragmentation of the transfectants of bcl-x was examined in the presence of 10% of the conditioned medium from WEHI-3. As shown in Fig. 9, fragmented DNA was observed in FDC-β1 and FDC-β2 (transfectant clones of bcl-x), even in the presence of IL-3, but not in FDC-ST2 (control), FDC-α4 (bcl-xα), and FDC-b21 (bcl-2). This finding suggests that the bcl-xβ gene product promotes apoptosis.

DISCUSSION

Gene Structure of bcl-x—We isolated rat bcl-2-related cDNAs from a thymus cDNA library. One of them was a counterpart of the human bcl-x (Boise et al., 1993), and the second one was a novel one in which the 5' region was identical to that of bcl-xβ. In addition, the third type of cDNA (bcl-xyβ) was cloned by RT-PCR. Because the rat genome had a single copy of a bona fide gene as well as a processed pseudogene, the three different
forms are derived from the single gene by alternative splicing. Bcl-2 also has two different forms generated by alternative splicing (Tsujimoto and Croce, 1986). The splice junction of bcl-2 is exactly at the same site as that of bcl-x. This indicates that these two genes originated from a common ancestor accompanying the splice junctions. Because two forms of bcl-2 were designated bcl-2α and -2β, we propose that the three different forms generated by alternative splicing had better be designated bcl-xα, -xβ, and -xγ. bcl-xα and bcl-xβ correspond to the human bcl-2α (long type) and bcl-2β (short type), respectively, as designated by Boise et al. (1993). Gonzalez-Garcia et al. (1994) cloned mouse cDNAs of bcl-xβ as well as bcl-xα. The deduced sequence of mouse Bcl-xα is almost identical to that of rat except for one amino acid residue (alanine 43 to proline).

Presence of an Intron in the 5’ Noncoding Region—In the bcl-2 gene, the first intron was found in the 5’ noncoding region and not so spliced (Negrini et al., 1987). In the 5’ noncoding region of the bcl-x gene, the first intron was detected by PCR amplification. The physiological significance of the presence of the first intron is an open question. This short intron may affect the translation efficiency or stability of the mRNA. Nevertheless, the presence of a similar type of intron to that in the bcl-2 gene is worthy to be reported.

Expression of bcl-x—Rat bcl-x is expressed in various tissues, widespread judging from Northern blot. Whereas mouse bcl-2 is expressed exclusively and equally in thymus and spleen (Negrini et al., 1987; Eguchi et al., 1992), expression of rat bcl-x was found to be high in the thymus but not so high in the spleen. Boise et al. (1993) reported that chicken bcl-x is expressed significantly in kidney and eye and markedly in thymus. Although rat bcl-x was expressed not so intensively in thymus and kidney as in chicken, its expression in the thymus...
Promotion of apoptosis by introducing bcl-xL—Overexpression of bcl-xL, as well as bcl-2, delayed apoptosis induced by withdrawing IL-3 in the promyeloid cells. Bcl-xL gave no effect on cell number as reported (Boise et al., 1993). On the contrary, even in the presence of IL-3, overexpression of bcl-xL induced apoptosis as judged by detection of DNA fragmentation (Fig. 9) and release of DNA-histone complex (by a Cell Death Detection ELISA kit (Boehringer Mannheim)) (data not shown). On the other hand, Gonzalez-Garcia et al. (1995) injected the mouse bcl-xL cDNA into cultured sympathetic neurons and found that mouse Bcl-xL protect neurons from cell death. Because an additive effect of Bcl-xL and Bcl-xL was observed in inhibiting neuronal cell death, Bcl-xL may delay the cell death of the neurons by a different mechanism or by functioning at a different cellular site from that of Bcl-xL (Gonzalez-Garcia et al., 1995). In their experiments, the cDNA was injected into the neurons at a saturated level, whereas we isolated the stable transformants constitutively expressing Bcl-xL, and its expression level was much less than that of Bcl-xL. As another possibility, these apparent differences may be due to the cell types (promyeloid cells or neurons). We have, however, introduced bcl-xL into rat pheochromocytoma cells, PC-12, to obtain clones constitutively expressing bcl-xL. In PC12, apoptosis is known to be induced by depleting serum from culture medium. Apparent surviving cell number of bcl-xL transformants was decreased relative to that of the control cells after depleting serum.2 As for the other explanation, the different effect may be due to their species specificity because the length of Bcl-xL is different between rat and mouse one (rat and mouse Bcl-xL consist of 232 and 210 amino acid residues, respectively). It is now important to transplant the mouse bcl-xL cDNA into the promyeloid cells to understand the discrepancy.

Recently, several genes belonging to the bcl-2 family have been cloned. Some of these, BHRF1 and Bcl-xL, suppress apoptosis (Henderson et al., 1993; Boise et al., 1993). The other members of Bcl-2 family, Bax, Bad, Bcl-xL, and Bak may regulate cell death by interacting each other or sharing common functions (Oltvai et al., 1993; Yang et al., 1995; Boise et al., 1995; Farrow et al., 1995; Chittenden et al., 1995 and Kiefer et al., 1995).

The difference in physiological function of Bcl-2 and Bcl-xL is not understood, although Bcl-xL suppressed apoptosis induced by immunosuppressants but Bcl-2 did not (Gottschalk et al., 1994). One possible explanation is that these genes share common functions in different cell types and at different developmental stages.

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