blood 1996 88: 386-401

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Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 900, Washington DC 20036.

REVIEW ARTICLE

Molecular Thanatopsis: A Discourse on the BCL2 Family and Cell Death

By Elizabeth Yang and Stanley J. Korsmeyer

BCL2 PREVENTS MULTIPLE FORMS OF CELL DEATH AND DEFINES A NEW CLASS OF ONCOGENES

EIGHTY-FIVE PERCENT of follicular lymphomas and 20% of diffuse B-cell lymphomas have a characteristic t(14;18) translocation.[1,2] In this translocation, the proto-oncogene *BCL2* at chromosome segment 18q21 is juxtaposed with the Ig heavy chain locus at 14q32, resulting in deregulated expression of *BCL2*.[3-6] The discovery that *BCL2*, unlike oncogenes studied previously, functions in preventing programmed cell death (PCD) instead of promoting proliferation established a new class of oncogenes.[7-9]

BCL2 prolongs cell survival. The initial observation of BCL2's ability to enhance cell survival was that overexpression of BCL2 increased the viability of certain cytokine-dependent cells upon cytokine withdrawal. In interleukin-3 (IL-3)–dependent pro-B–cell lines and promyeloid cell lines, BCL2 overexpression prolonged cell survival upon IL-3 withdrawal and maintained the cells in G_0.[7,8] The observation was extended to IL-4– and granulocyte-macrophage colony-stimulating factor (GM-CSF)–dependent cells[8] and in certain IL-2–dependent[11] and IL-6–dependent[12] cells. BCL2 was also capable of protecting T cells against a variety of apoptotic signals, including glucocorticoids, γ-irradiation, phorbol esters, ionomycin, and cross-linking of cell surface molecules by anti-CD3 antibody. The protective effects were observed in T-cell hybridomas transfected with *BCL2* and in thymocytes and peripheral T cells from transgenic mice with expression of *BCL2* under the control of the proximal promoter of *lck* (*lck*^{pr})[13] or the Ig heavy chain enhancer (E_μ).[14]

Overexpression of BCL2 alters lymphoid development and leads to neoplasia. The in vivo effects of *BCL2* were initially investigated using transgenic mice with *BCL2* overexpression targeted to B cells or to T cells. Transgenic mice bearing a *BCL2-Ig* minigene harbor expanded B-cell compartments. Mice in which the *BCL2* transgene expression is targeted to T cells by the *lck* proximal promoter exhibit increased $CD3^{med}$ and increased $CD4^-CD8^+$ single-positive thymocytes compared with littermate controls.[13] When the *BCL2* transgene is expressed in B lymphocytes, the mice develop follicular hyperplasia, some of which progress to high-grade monoclonal lymphomas[15-18] (Fig 1). When expression is directed to T cells, fully one third of the mice develop peripheral T-cell lymphomas[19] (Fig 1). A long latency and progression from polyclonal hyperplasia to monoclonal malignancy are consistent with the hypothesis that oncogenic events in addition to *BCL2* overexpression are necessary for tumor formation. In lymphomas arising in *BCL2-Ig* transgenic mice, a common second hit is translocation of the *Myc* oncogene.[16] (The interaction between *Myc* and *BCL2* will be specifically discussed in a later section.) These transgenic mice experiments illustrated that cell death is normally a well-regulated process in lymphoid development and that lack of cell death is tumorigenic. Deleterious mutations that would have resulted in cell death can be retained when apoptosis is inhibited. The progression to lymphoma in these *BCL2* transgenic mice constitutes in vivo evidence that the t(14;18) and *BCL2* overexpression play a primary role in oncogenesis.

BCL2 protects against neuronal cell deaths. Prompted by these studies, BCL2 has been found to protect against death in a variety of cell types. Notably, BCL2 protects against neuronal cell death induced by various apoptotic stimuli. BCL2 inhibited apoptosis in PC12 pheochromocytoma cells after nerve growth factor (NGF) withdrawal.[20,21] Microinjection of a *BCL2* construct driven by the neuron-specific enolase promoter into cultured rat sympathetic neurons also resulted in the prevention of programmed death after NGF deprivation.[22] Other experiments suggested that not all neuronal cell deaths are inhibitable by BCL2. For example, BCL2 rescued embryonic chick sensory neurons dependent on nerve growth factor, brain-derived neurotrophic factor, and neurotrophin-3, but not ciliary neurons dependent on ciliary neurotrophic factor.[23] In addition to growth factor dependency, BCL2 has been shown to counter death in a neuronal cell line after serum and glucose withdrawal, membrane peroxidation, and treatment with calcium ionophore and menadione, an inducer of free radical formation.[24] In animal models, overexpression of *BCL2* under the neuron-specific enolase or phosphoglycerate kinase promoter led to neuronal hypertrophy by decreasing naturally occurring cell death. The brains from transgenic animals were larger than wild-type littermates by 12%.[25] The number of motoneurons in the facial nucleus and the ganglion cell layer of the retina was increased by 40% to 50%.[25] Overexpression of *BCL2* in these animals also protected against experimental cell death. This is evidenced by a 50% reduction in the volume of brain infarction in transgenic mice after occlusion of the middle cerebral artery[25] and by continued survival of facial motoneurons after axotomy in transgenic animals, whereas those in wild-type mice degenerate.[26] The role of *BCL2* in normal neuronal physiology has been explored in *Bcl2*-deficient mice. Superior cervial ganglion cells from *Bcl2*$^{-/-}$ mice died more rapidly after NGF deprivation than those from wild-type mice,[27] suggesting that *BCL2* is an important regulator of sympathetic neuron survival during the period of naturally occurring programmed neuronal death.

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Submitted August 9, 1995; accepted February 16, 1996.

E.Y. is supported by a Pfizer fellowship.

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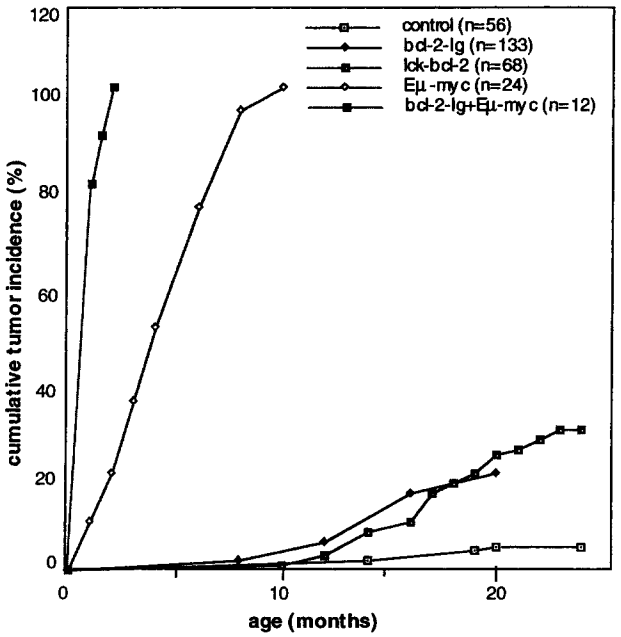

Fig 1. *BCL2* overexpression leads to neoplasia, which is synergized by *c-MYC* overexpression. Cumulative tumor incidence in *BCL2-Ig*, *lck^{pr}-BCL2*, and *E$_\mu$-MYC* transgenic mice and *BCL2-Ig* + *E$_\mu$-MYC* double transgenic mice compared with littermate controls.[16-19,116]

Virus-induced cell death can be blocked by BCL2. Upon infection of host cells, adenovirus expresses several virally encoded genes. Expression of the adenovirus E1A oncogene alone stimulates host cell proliferation accompanied by apoptosis, which can be p53 dependent.[28,29] Simultaneous expression of the E1B 19-kD protein suppresses E1A-induced apoptosis,[28] allowing foci formation after adenovirus infection. BCL2 shares limited homology with E1B 19-kD protein and can substitute for its ability to inhibit E1A-induced cell death.[30,31] This is an example of BCL2's ability to repress cell death due to abnormal proliferation. BCL2 can also block apoptosis in serum-deprived cells expressing the Tax protein of human T-cell leukemia virus human T-cell lymphotropic virus type 1 (HTLV-1).[32] In another system, BCL2 is able to inhibit programmed cell death induced by lytic infection of the alphavirus, Sindbis, allowing the establishment of persistent viral infection.[33]

BCL2 functions in multiple cell death systems. Numerous examples now exist in which apoptosis due to external toxic stimuli can be rescued by BCL2 (Table 1). An interesting system is the "apoptosis" of nuclei in cell-free Xenopus oocyte extracts, which can be inhibited by BCL2.[34] This in vitro system offers the potential for dissecting individual components of apoptosis. Other examples in which BCL2 plays a role include transforming growth factor β (TGFβ)-induced growth arrest and cell death in M1 cells[35,36] and chemotherapeutic drug-induced apoptosis in cancer cells.[37-44]

BCL2 does not repress all cell deaths. Despite numerous positive examples, BCL2 does not prevent every cell death. BCL2 does not have a substantial effect on negative selection of thymocytes.[13] Also, it does not easily prevent apoptosis in targets of cytotoxic T-cell killing.[45] However, observations that BCL2 can occasionally affect outcomes by these stimuli suggest that results can be dose-related. Because BCL2 is able to inhibit apoptosis resulting from so many different signals and intracellular pathways, it must act after the convergence of many signals in the apoptotic pathway. Because overexpression of BCL2 does not protect every example of cell death, it is theorectically possible that more than one distal pathway of cell death exists. Alternatively, individual BCL2 family members may prove more effective in certain contexts than others.

BIOCHEMICAL AND CELL BIOLOGICAL STUDIES OF BCL2 ACTIVITY

BCL2's full activity requires an integral membrane position. The carboxy terminus of BCL2 contains a hydrophobic 19-amino acid stretch reminiscent of a membrane spanning domain. Subcellular fractionation, immunofluorescence, and confocal microscopy studies using anti-

Table 1. Cell Deaths Repressed by BCL-2

Lymphoid
Factor withdrawal—IL-2, IL-3, IL-4, IL-6, GM-CSF
Glucocorticoid
γ Irradiation
Phorbol esters
Calcium
Cross-linking by anti-CD3
Neuronal
Factor withdrawal—NGF, BDNF, Neurotrophin-3
Serum withdrawal
Calcium
Infarction
Axotomy
Naturally occurring cell death
Fibroblasts
Serum deprivation and MYC induction
Oncogene-related
MYC-induced
E1A-induced
p53-mediated
Viral infections
Adenovirus
Sindbis virus
HTLV-1
Chemotherapeutic drugs
DNA synthesis inhibitors
Alkylating agents
Topoisomerase inhibitors
Microtubule inhibitors
Antimetabolites
Oxidant stress
H_2O_2
Menadione
Membrane peroxidation
Others
TGF-β
Staurosporine
Loss of extracellular matrix

BCL2 antibodies indicated that BCL2 is an intracellular membrane protein whose distribution varies somewhat depending on cell type. BCL2 has been most convincingly localized to mitochondria, its predominant site in hematopoietic cells, as well as smooth endoplasmic reticulum and perinuclear membrane.[10,46-49] Targeting studies using purified mitochondria and in vitro-translated BCL2 protein showed that the carboxy terminus functions as a signal anchor sequence responsible for targeting and insertion into the mitochondrial outer membrane. This exposes most of the polypeptide to the cytosol, in which it remains sensitive to protease digestion.[50] BCL2 devoid of the signal anchor sequence is only partially functional in protection against apoptosis. However, a portion of the truncated BCL2 is still bound to its membrane-associated heterodimerizing partner, BAX.[51] Substitution of the BCL2 mitochondrial anchor sequence with the yeast outer membrane protein Mas70p signal anchor sequence retargets the protein into the mitochondrial outer membrane and fully restores BCL2's activity, as measured by the ability to inhibit E1A-induced cytotoxicity.[52] A fusion protein of BCL2β/IL-2 receptor transmembrane domain produced similar results.[53] These studies argue that BCL2's full function depends on its subcellular membrane localization. Most of the amino portion of BCL2 is exposed, in which it may interact with proteins in the cytosol or other BCL2-like molecules similarly anchored in the mitochondria. BCL2 function is not dependent on an intact electron transport/oxidative phosphorylation chain, as is shown by BCL2's ability to block apoptosis in cells lacking mitochondrial DNA and unable to carry out electron transport.[54]

BCL2 can inhibit oxidant induced apoptosis. The mitochondrial outer membrane, the endoplasmic reticulum, and the nuclear envelope are all sites implicated in the production of reactive oxygen species (ROS). The localization of BCL2 to these sites prompted investigation into the role of ROS in programmed cell death. BCL2 can protect cells against H_2O_2 and t-butyl hydroperoxide or menadione, which generate O_2^-.[51,55] At low concentrations, these oxidant stresses kill cells by an apoptotic process. Agents that decrease reactive oxygen species, such as N-acetylcysteine, glutathione peroxidase, and desferrioxamine, can partially protect against apoptosis.[51] Furthermore, BCL2 can protect against death induced by agents that decrease intracellular glutathione (GSH), such as buthionine sulfoximine and ethacrynic acid.[55] This suggests that reactive oxygen species may be involved in apoptotic pathways rescuable by BCL2. The endogenous production of intracellular peroxides, as measured by the conversion of the oxidation-sensitive fluorescent dye DCFH to DCF, is not significantly changed in the presence of BCL2. BCL2 also does not have a significant effect on the generation of superoxide, O_2^-. BCL2 does inhibit lipid peroxidation, a downstream event in oxidative damage and a frequent accompaniment of apoptosis.[51] However, subsequent reports of BCL2's ability to rescue cells from programmed cell death occurring under hypoxic conditions in which the generation of ROS is greatly reduced suggest that ROS are not essential for PCD.[56,57] Thus, BCL2's death repressor function does not solely depend on the protection of cellular constituents from oxidative damage. Although BCL2 can block oxidant-induced apoptosis, in the absence of a proven biochemical activity, it remains an open question whether BCL2 has a direct or indirect role on the oxidant pathway.

BCL2 and intracellular calcium fluxes. Another area of investigation into BCL2 function that relates to BCL2's localization to the endoplasmic reticulum is intracellular calcium homeostasis. Ca^{2+} has been implicated in apoptosis because of the Ca^{2+} dependence of certain internucleosomal DNA fragmentations and the ability of Ca ionophores A23187 and ionomycin to induce lymphocyte apoptosis. Although the total cellular Ca^{2+} content has not been consistently shown to change with the induction of cell death, a redistribution of intracellular Ca^{2+} can result.[58] Studies using thapsigargin, an inhibitor of the ER-associated Ca^{2+} pump, indicated that apoptosis is associated with an efflux of Ca^{2+} from the ER into the cytosol and that BCL2 can block this flux of Ca^{2+} across the ER membrane.[59,60] Although intriguing, mobilization of intracellular Ca^{2+} stores is but one step in the complex cell death pathway. Whether BCL2's effect on calcium homeostasis is direct or indirect is still uncertain.

BCL2 FOUNDS A FAMILY OF CELL DEATH REGULATORS

BAX, a heterodimerizing partner of BCL2, is a death promoter. Identification of a number of BCL2 homologs, some of which bind to BCL2, suggests that BCL2 functions, at least in part, through protein-protein interactions. The first of these homologs, BAX, was identified by coimmunoprecipitation with BCL2 protein. BAX is a 21-kD protein that shares homology with BCL2 principally clustered in two conserved regions, BH1 and BH2 (Fig 2). In addition, an exon juncture in BH2 is conserved. BAX heterodimerizes with BCL2 and homodimerizes with itself.[61] Site-directed mutagenesis of BH1 and BH2 in BCL2 showed that these two domains were important for binding to BAX. When binding was disrupted, BCL2's protective function was also eliminated, suggesting that BCL2 must bind BAX to exert its effect. Most noteworthy are the substitutions of a single amino acid Gly145 in BH1 with either alanine or glutamic acid and Trp188 with alanine in BH2, which completely disrupted binding to BAX and abrogated BCL2's death-repressor effect.[62] When BAX was overexpressed in cells, apoptotic death in response to a death signal was accelerated, earning its designation as a death promoter. When BCL2 was overexpressed, it heterodimerized with BAX and death was repressed.[61] Thus, the ratio of BCL2 to BAX determines the amount of BCL2/BAX heterodimers versus BAX/BAX homodimers and is important in determining susceptibility to apoptosis (Fig 3). BAX protein contains a hydrophobic carboxy terminus like BCL2 and has been colocalized to mitochondria with BCL2 (unpublished observations). BAX is widely expressed in tissues, including a number of sites in which cells die during normal maturation.[61,63] Moreover, the BCL2 to BAX ratio varies during the developmental history of a given lineage, such as T lymphocytes. For example, BCL2 is present in the immature, double-negative thymocytes and in the mature, single-positive T cells. However, it is absent at the double-positive stage when selection oc-

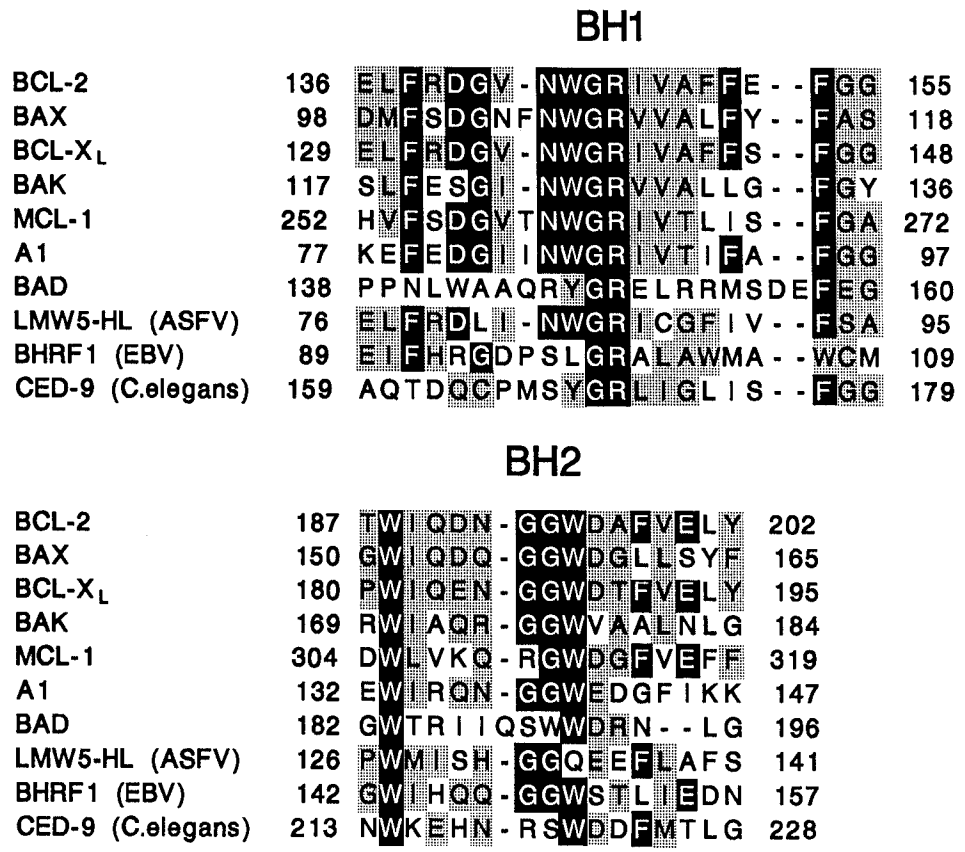

Fig 2. The BCL2 family. Alignment of BCL2 homologs in BH1 and BH2 domains. Numbers denote amino acid positions. Identical amino acids are outlined in black and conserved residues are shaded. Dashes denote gaps in the sequence to maximize alignment.

curs.[64,65] The lack of BCL2 may enable thymocytes to die if they fail to receive an appropriate signal.

BCL-X is similar to BCL2 but shows different lineage specificity. Another homolog that is functionally similar to *BCL2* is *BCL-X,* which was cloned by low stringency hybridization using *BCL2* as a probe. BCL-X displays 44% amino acid identity to BCL2. The gene product exists in two forms, BCL-X_L, which encodes 233 amino acids and contains the highly conserved BH1 and BH2 domains (Fig 2), and an alternatively spliced form, BCL-X_S, which lacks a 63 amino acid stretch encompassing BH1 and BH2.[66,67] BCL-X_L, similar to BCL2, inhibits apoptosis in many assay systems. BCL-X_S, on the other hand, counters the protective effect of BCL2 or BCL-X_L. Overexpression of BCL-X_L in FL5.12 cells protects them from apoptosis upon IL-3 withdrawal just as BCL2 does.[66] In vivo, thymocytes from mice expressing the *BCL-X* transgene under the *lck* proximal promoter show increased survival and are protected against glucocorticoid-, γ-irradiation–, and anti-CD3–induced death.[68] Moreover, *BCL-X_L* overexpression altered thymocyte maturation in a pattern essentially identical to *BCL2* overexpression, ie, *lck^{pr}-BCL-X_L* mice had increased $CD3^{int/hi}$ thymocytes and an excess of CD8 single-positive thymocytes, just as were found in *lck^{pr}-BCL2* transgenic mice.[13,68,69] BCL-X_L can heterodimerize with BAX in mammalian cells, and single amino acid substitutions in BH1 abolished binding to BAX and abrogated the death-repressor effect.[70] In addition, the introduction of a *BCL-X_L* transgene that was expressed in T-cell development rescued T-cell survival in *$bcl2^{-/-}$* animals,[68] showing the genetic capacity of BCL-X_L to substitute for BCL2. Like BCL2, BCL-X has a hydrophobic carboxy terminal transmembrane domain and its subcellular distribution is similar to BCL2.[71]

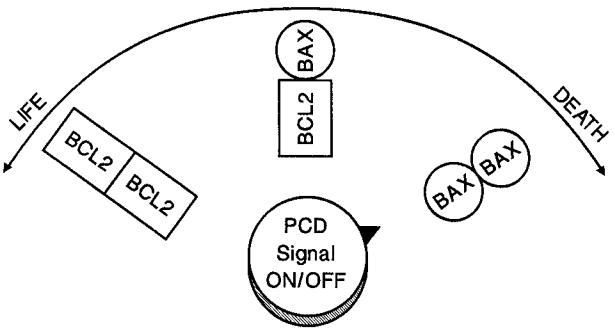

Fig 3. Susceptibility to PCD. The relative ratios of BCL2 and BAX heterodimers to homodimers determine the susceptibility to PCD.

Despite their similarities, functional differences do exist between BCL-X and BCL2. WEHI-231.7 cells undergo programmed cell death upon cross-linking of IgM and upon exposure to immunosuppressants CsA, FK506, and rapamycin, all of which can be suppressed by BCL-X_L but not by BCL2.[72] Activation of peripheral T cells leads to rapid induction of BCL-X_L but not BCL2.[66] Most recently, experi-

Table 2. Lineage-Specific Roles for BCL-2 and BCL-XL

BCL-2	BCL-XL
B-cell memory	B-cell maturation
Mature B-cell survival	Double-positive thymocyte survival
Mature T-cell survival	T-cell activation
Kidney development	Brain development
Melanocyte survival	

ments of human peripheral T-cell activation using anti-CD3 and anti-CD28 in the presence of IL-2 showed that costimulation by these two molecules significantly enhances BCL-X_L, but not BCL2, expression.[73] Differences in expression patterns also exist. During thymocyte development, BCL2 is expressed in immature $CD4^-CD8^-$ double-negative cells, but not in $CD4^+CD8^+$ double-positives, and again in mature $CD4^+$ or $CD8^+$ single-positives.[64,65] BCL-X_L expression, on the contrary, has a reciprocal pattern. Expression is present during the double-positive stage, but is lost as thymocytes mature to single-positive T cells.[71,74] The patterns of expression suggest that BCL2 may be most important in maintaining the homeostasis of resting T cells, whereas BCL-X_L may be more important in postactivation survival decisions. BCL-X_L expression is also notably higher than that of BCL2 in the adult brain.[71,75,76] Therefore, BCL-X_L and BCL2 display differences in cell-type specificity and perhaps their physiologic roles provide an explanation for their comaintenance (Table 2).

MCL1 and A1 are inducible genes. Other *BCL2*-like genes are expressed in response to definable signals. *MCL1* was cloned from a myeloid leukemia line after induction by phorbol ester. It shares homology with BCL2 mostly at the carboxy region, including BH1 and BH2 (Fig 2), but differs from BCL2 towards the amino terminus at which two PEST sequences reside.[77] *A1,* another *BCL2* family member (Fig 2), is a hematopoietic-specific early response gene induced by GM-CSF and lipopolysaccharide (LPS).[78] Both MCL1 and A1 show strong binding to BAX in the yeast two-hybrid system,[70,79] suggesting that heterodimers of MCL1/BAX and A1/BAX may exist. However, attempts to coimmunoprecipitate MCL1 and BAX in mammalian cells have been unsuccessful to date.[80] MCL1 exhibits minimal or no effect on cell death in the limited systems examined thus far.[80,81] Therefore, the precise roles of MCL1 and A1 in cell death paradigms are still under exploration.

BAK antagonizes BCL2 activity. A new player in the cell death death pathway, *BAK* (*BCL2* homologous *a*ntagonist/*k*iller), was found by three independent groups through interaction with E1B-19k protein or degenerate polymerase chain reaction (PCR) cloning.[82-84] BAK is a BCL2 family member with BH1 and BH2 domains (Fig 2) and is functionally similar to BAX. BAK interacts with BCL2 and BCL-X_L, in addition to E1B-19k, and opposes their death-repressor activity when coexpressed in IL-3–dependent FL5.12 cells[83,84] and NGF-dependent rat sympathetic neurons.[82] One experimental exception did exist in an Epstein-Barr virus–transformed lymphoblastoid cell line WI-L2, in which BAK actually enhanced survival after serum deprivation and menadione treatment.[84] This may reflect a context dependence of this protein's effect. BAK also has the capacity to activate a cell death pathway when induced in Rat-1 fibroblasts.[83] Thus, there are multiple death repressors (BCL2, BCL-X_L, and E1B-19K) and multiple death promoters (BAX, BCL-X_S, and BAK; Table 3). BAK seems to differ from BAX in its preference for heterodimerizing partners. For example, BAK appears to prefer BCL-X_L over BCL2.[82] BAK and BAX may also have different cell-type specificities, as has been shown for BCL2 and BCL-X_L.

BCL2 homology extends to DNA viruses. Conservation of the *BCL2* family of genes is remarkable in that *BCL2* has homologs in the DNA viruses. In addition to the adenovirus *E1B-19k* gene already mentioned, the *BHRF1* gene of EBV that is expressed early in lytic and some latent infections is homologous to *BCL2* in the BH1 and BH2 domains[6,85] (Fig 2). Recently, an open reading frame (ORF16) in the T-lymphotropic herpesvirus saimiri (HVS) was reported as a novel member of the *BCL2* family.[86] The African swine fever virus encodes a homologous gene, *LMW5-HL* (Fig 2), which is also expressed early in infection of mononuclear phagocytes.[87] The function of these viral homologs may be to maintain host cell viability while infection is being established.

Yeast two-hybrid assays show specificity of heterodimer formation among BCL2 family members. The increasing number of BCL2 homologs prompted the use of the yeast two-hybrid system to assess which members could dimerize. Each family member was fused to the DNA binding domain and the transcription activation domain of the yeast GAL4 gene, and all the possible combinations were scored for *lacZ* activation in yeast[70,79] (Table 4). BAX was found to strongly heterodimerize with BCL-X_L, MCL1, and A1, in addition to BCL2, suggesting that it may be a common partner in the regulation of cell death. In contrast, BCL-X_S, which opposes BCL-X_L and BCL2, only heterodimerizes with BCL-X_L and BCL2, suggesting that this alternatively spliced form that reverses protection by BCL-X_L and BCL2 may do so by sequestering these molecules. Similarly, BAK heterodimerizes more strongly with BCL-X_L than with BCL2.[82] Homodimers of BAX and BCL2 were also recapitulated in this system. The results from yeast two-hybrid assays showed that there is selectivity in heterodimer formation within the BCL2 family of proteins and that there is a hierarchy to the strength of binding between the various partners. Therefore, within a given mammalian cell, the presence and the concentration of each member might determine the predominant dimer species. Although BH1 and BH2 domains in BCL2 are essential for heterodimer formation, deletion mapping in yeast

Table 3. BCL-2 Family Death Repressors and Death Promoters

Death Repressors	Death Promoters
BCL-2	BAX
BCL-X_L	BCL-X_S
E1B-19K	BAK
CED-9	BAD

Only molecules with established cell death functions in mammalian cells are shown.

Table 4. Dimer Formation in Yeast Two-Hybrid Assay

GAL4-DNA Binding Domain	GAL 4-Activating Domain						
	BCL-2	BAX	BCL-X_L	BCL-X_S	MCL-1	A1	BAD
BCL-2	+	+	−/+*	+	−/+*	+	+
BAX	+	+	+	−	+	+	−
BCL-X_L	−	+	−/+*	+	−/+*	−	+
MCL-1	−	+	−	−	−	−	−
BAD	+	−	+	−	−	−	−

* Differences between findings in two reports.[70,79]

two hybrid indicates that other regions of molecules in this family also regulate dimer formation.

ADDITIONAL BCL2- AND BCL-X_L–INTERACTING PROTEINS MODULATE CELL DEATH

BAD negatively regulates BCL2 and BCL-X_L and displaces BAX. Efforts to further examine BCL2's ability to interact with other proteins in the cell death pathway uncovered additional BCL2-interacting proteins that modulate BCL2's activity. Yeast two-hybrid screening and λ expression cloning both showed a new heterodimerizing partner of BCL2 and BCL-X_L, called BAD (*BCL2/BCL-X_L–associated death promoter*).[88] This player in the cell death pathway differs from other family members with homology limited to the most conserved amino acids in the BH1 and BH2 domains (Fig 2). BAD also lacks the typical carboxy terminal transmembrane domain, suggesting that it is not an integral membrane protein. BAD's interaction with BCL2 and BCL-X_L was verified in mammalian cells. Although BAD was discovered by virtue of its interaction with BCL2, it binds BCL-X_L more strongly. When expressed in FL5.12 cells, BAD countered the death-repressor effect of BCL-X_L efficiently and that of BCL2 to a lesser extent. The strong interaction between BAD and BCL-X_L sequesters BCL-X_L, resulting in freed BAX, and cell death is restored. BAD displaces BAX from BCL-X_L/BAX or BCL2/BAX heterodimers in a concentration-dependent manner. In one cell line examined, when approximately 50% of all cellular BAX is heterodimerized with BCL-X_L or BCL2, the cell is resistant to apoptosis. Conversely, in cells in which 80% of BAX is found in homodimers, an apoptotic signal results in cell death.[88] This finding suggests that BAD negatively regulates cell death by modulating the amount of BAX in homodimers versus heterodimers (Fig 4). Formally, it is not certain whether the active moiety in regulating cell death is the BAX/BAX homodimer or each BCL-X_L/BAX or BCL2/BAX heterodimer. Alternatively, both may be active, and the ratio of the heterodimers to the homodimers may be the critical determinant. The discovery of BAD showed that the cell death regulators BCL2 and BCL-X_L themselves are regulated by protein-protein interactions.

BAG1 can positively modulate BCL2 activity. Another protein found by interactive cloning, BAG1, positively modulates BCL2's death-repressor activity.[89] BAG1 has been shown to interact with BCL2 in vitro. BAG1 is not homologous to BCL2 family members and contains an ubiquitin-like domain, suggesting that its mechanism might involve effects on protein stability. Curiously, coexpression of BAG1 assisted BCL2 in protecting Jurkat T cells against anti-Fas antibody and cytotoxic T-cell killing. This finding suggests that death signals that appear to be BCL2-independent may be repressed by BCL2 if the appropriate modulatory proteins are present.

CAENORHABDITIS ELEGANS SHARES CONSERVED CELL DEATH GENES WITH MAMMALS

CED-9 is a homolog of BCL2 in C elegans. Important contributions to the understanding of programmed cell death have come from the genetic studies of the nematode *C elegans*. In the development of the hermaphrodite worm, 131 of the 1,090 somatic cells undergo programmed cell death in a genetic pathway defined by 14 genes. Two of these genes,

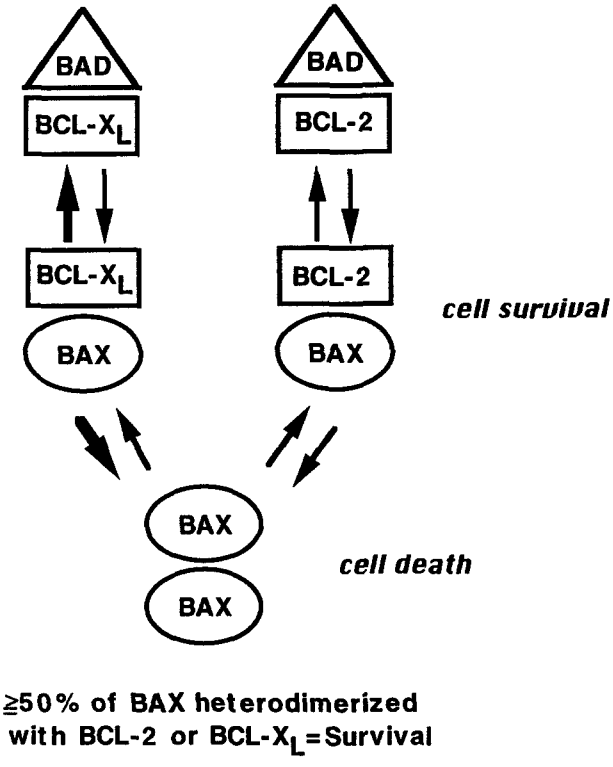

Fig 4. BAD is a negative regulator of apoptosis. BAD displaces BAX from BCL2/BAX or BCL-X_L/BAX heterodimers, allowing more BAX/BAX homodimer formation, which promotes death.

ced-3 and *ced-4,* are required for cell death to occur. In *ced-3* or *ced-4* mutants, all 131 cells that are normally destined to die live. A third gene, *ced-9,* represses the death pathway and protects cells that are destined to live.[90,91] In *C elegans,* the phenotype of *ced-3, ced-9* double mutants is the same as *ced-3* single mutants, ie, cells live. This indicates that *ced-9* is not downstream of *ced-3,* but *ced-9* could be an upstream negative regulator of *ced-3* and *ced-4.* Epistasis mapping has also established that *ced-4* is upstream of *ced-3. ced-9* is a regulator of cell death, whereas *ced-3,* and maybe *ced-4,* encode effector molecules of cell death. In a gain-of-function (gf) *ced-9* mutant, all 131 cells live, whereas loss-of-function (lf) mutations cause cells that normally should live to die.[92,93] CED-9 shows significant structural and functional homology to BCL2. CED-9 and BCL2 amino acid sequences share 24% identity and 49% similarity. They have similar hydrophobicity profiles, including the carboxy terminal signal anchor sequence. CED-9 has the highly conserved amino acids of BH1 and BH2 domains and the conserved exon junction in BH2[94] (Fig 2). Functionally, transgenic *BCL2* can block some cell deaths in *C elegans* and can partially substitute for *ced-9* by preventing ectopic deaths in *ced-9(lf)* mutants.[94,95] Interestingly, the same amino acid mutation of Gly145Glu in BCL2 results in loss of function, but is a gain-of-function mutation in CED-9. This confirms the critical functional role of the BH1 domain, but also indicates differences over this evolutionary gap. The sequence and functional conservation between *ced-9* and *BCL2* suggests that, in its basic tenets, the genetic pathway of cell death may be common to all multicellular organisms.

CED3/ICE FAMILY OF PROTEINS ACT AS EFFECTOR MOLECULES OF CELL DEATH

CED-3 is homologous to IL-1β–converting enzyme (ICE). Cloning of the *C elegans ced-3* gene showed that the protein is homologous to the mammalian enzyme ICE.[96] ICE is a cysteine protease that cleaves the 33-kD pro–IL-1β at an aspartic acid residue into the biologically active 17.5kD IL-1β. Active ICE is composed of two subunits, p10 and p20, which associate to form a heterotetramer. The homology between CED-3 and ICE suggested that ICE may function as a mammalian cell death gene. Indeed, overexpression of ICE causes Rat-1 fibroblasts to undergo apoptosis, which can be inhibited by BCL2 and crmA,[97] a cowpox virus protein that inhibits ICE-like cysteine proteases. ICE itself has not proven to be directly affected by BCL2, and many cells that undergo apoptosis do not express IL-1β. Mice deficient in ICE can not synthesize mature IL-1β, but their thymocytes are able to undergo apoptosis induced by dexamethasone and γ-irradiation, suggesting that ICE is not essential for these cell death processes.[98,99] *Ice−/−* thymocytes show some improved survival after treatment with high doses of anti-Fas in vitro.[99] However, there is no evidence that the Fas pathway is normally used in thymocyte selection. Overexpression of ICE can also accelerate anti-Fas–induced apoptosis in tissue culture cells expressing Fas, which can be inhibited by crmA.[100,101] Thus, ICE or an ICE-like molecule is involved in Fas-mediated apoptosis.

Differentially processed ICH-1 can induce or inhibit apoptosis. A homolog of ICE, NEDD2,[102] also called ICH-1,[103] contains the cysteine protease active site motif QACRG and comes in two differentially processed forms, ICH-1_L and ICH-1_S. ICH-1_S diverges from ICH-1_L immediately after the conserved QACRG motif and truncates shortly downstream. Initially identified as a highly expressed gene in early embryonic brain development,[102] ICH-1_L induces apoptosis when overexpressed in fibroblasts and neuroblastoma cells, whereas ICH-1_S has the opposite effect of inhibiting death due to serum starvation.[103] If ICH-1 is similar to ICE in subunit structure, then ICH-1_S may act as a dominant negative by binding to ICH-1_L and preventing functional tetramer formation. This programmed cell death gene is reminiscent of *BCL-X,* which also encodes for two differentially processed gene products that have opposite effects on cell death. ICH-1_L–induced death can be inhibited by BCL2, but minimally inhibited by crmA.[103] The natural substrates of ICH-1 are not yet known.

Table 5. CED-3/ICE Homologs

Death-Repressing	Death-Promoting
ICH-1_S	CED-3
	ICE
	ICE-1_L/NEDD 2
	CPP32/Yama/Apopain

CPP32/Yama cleaves PARP and promotes cell death. Another ICE-like enzyme found through homology searches is the human 32-kD cysteine protease CPP32, also called Yama or apopain.[104-106] Proteolytic cleavage of the 32-kD inactive zymogen gives two subunits p18 and p12, the equivalents of the p20 and p10 subunits of ICE, which appear to associate to form the CPP32 complex. When coexpressed in Sf9 cells, these two CPP32 subunits induce apoptosis, whereas each subunit alone is ineffective.[104] Extracts from cells committed to apoptosis or purified CPP32, but not ICE, can cleave poly(ADP-ribose) polymerase (PARP) at an Asp site to generate the signature 85-kD fragment, a proteolytic event known to occur early in many forms of PCD.[106,107] In addition, crmA, which can block apoptosis, can block the cleavage of PARP by CPP32/Yama in vitro.[105] CrmA also inhibits PARP cleavage when transfected into lymphoma cells treated with anti-Fas and breast carcinoma cells treated with TNF.[105] CPP32/Yama may prove to be a physiologic mediator of apoptosis in hematopoietic cells, in which its expression is high and ICE expression is low.

A mammalian *ICE/ced-3* family of programmed cell death genes are being identified whose products function as effectors of cell death[108] (Table 5). These CED-3/ICE effector molecules and those yet to be cloned are likely to interact with the BCL2 pathway directly or indirectly to execute apoptosis (Fig 5). The physiologic substrates of these cysteine proteases have not all been identified, but PARP serves at least as a molecular marker of this process. Different physiologic roles may be fulfilled by different ICE proteases; for example, ICE itself is likely to be mainly involved in the inflammatory response. The existence of multiple ICE proteases may also reflect different lineage specificity or different substrate specificity.

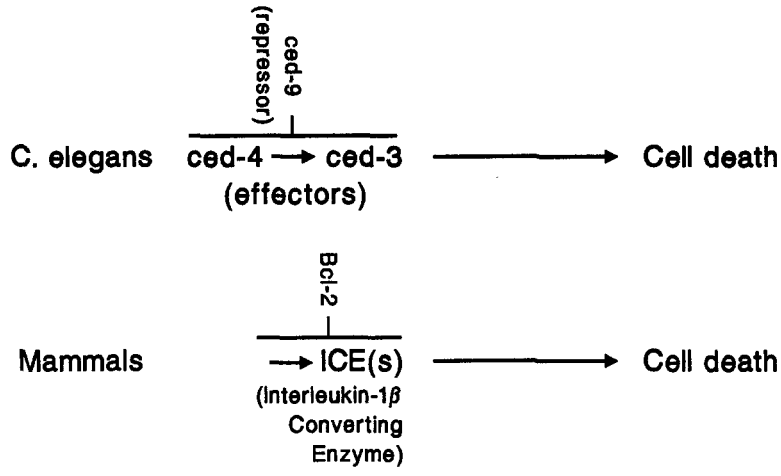

Fig 5. ***C elegans*** **and mammals share a common genetic cell death pathway. CED-9 represses cell death and CED-4 and CED-3 are required for the execution of cell death. Similarly, BCL2 protects cells from apoptosis, whereas the ICE-like proteases (mammalian homologs of CED-3) are cell death effectors.**

GENETIC KNOCKOUT STUDIES SHOW LINEAGE-SPECIFIC ROLES FOR BCL2, BCL-X, AND BAX

Bcl2-deficient mice develop polycystic kidneys, immunodeficiency, and hair hypopigmentation. The normal developmental role of the BCL2 family members can be addressed by gene disruption animal models. BCL2 is initially widely expressed during embryogenesis. However, in the nervous system, eg, BCL2 expression decreases and becomes much more restricted postnatally.[109] Newborn *Bcl2*$^{-/-}$ knockout mice are viable, but within 1 week of life become distinguishable from *Bcl2*$^{+/+}$ littermates in appearance. The majority of the *Bcl2*$^{-/-}$ mice then become ill and die at a few weeks of age. They develop polycystic kidney disease with marked dilatation of proximal and distal tubules and collecting ducts, resulting in renal failure.[110-112] In the normal fetal kidney, detection of strong BCL2 expression in the developing subcapsular condensations of mesenchymal cells destined to differentiate into proximal nephrons suggested that BCL2 may be important in maintaining cell survival during inductive interactions between epithelium and mesenchyme.[113] Comparisons of embryonic kidneys from *Bcl2*$^{+/+}$ and *Bcl2*$^{-/-}$ mice showed that *Bcl2*$^{-/-}$ kidneys contain many fewer nephrons and greatly increased apoptosis within metanephric blastemas of metanephroi at embryonic day 12. Growth and development of *Bcl2*$^{-/-}$ embryonic metanephroi are also reduced in culture, indicating that the abnormality in *Bcl2*$^{-/-}$ kidneys is cell autonomous.[114] The lymphoid organs, thymus and spleen, are initially normal in *Bcl2*$^{-/-}$ mice. Thymocyte development is normal, and B and T cells undergo selection successfully. However, at 4 to 8 weeks of age, the lymphoid organs undergo massive cell death and involution, showing a failure to maintain homeostasis in both the B- and T-cell populations in the absence of BCL2.[110-112] The *Bcl2*$^{-/-}$ mice also turn gray at 5 to 6 weeks of age, at the time of the second hair follicle cycle.[110-112] The hypopigmentation in the *Bcl2*$^{-/-}$ mice may reflect increased sensitivity to free radicals generated during melanin synthesis or decreased melanocyte survival at a time when endogenous growth factors for melanocytes, such as MSH, are limiting. The phenotype of the knockout mice proves that embryonic development can proceed in most lineages in the absence of BCL2. However, BCL2 is required for normal embryonic metanephrogenesis. Postnatally, it is critical for the maintenance of lymphocytes and melanocytes.

Bcl-x–deficient mice exhibit massive cell death in the central nervous system (CNS) and reduced lymphoid maturation. While the absence of BCL2 allows viable pups to be born, the absence of BCL-X results in embryonic lethality. *Bcl-x*$^{-/-}$ mice are dead around embryonic day 13 (E13).[115] There is extensive cell death throughout the brain and spinal cord in regions of postmitotic, differentiating neurons, in which BCL-X is normally highly expressed. In contrast to BCL2, BCL-X appears to be essential for brain development. In the hematopoietic system, massive cell death is observed in the developing liver. In chimeric mice derived from *Bcl-x*$^{-/-}$ ES cells injected into *Rag-2*–deficient blastocysts, immature B cells are dramatically reduced, but the maturation of T cells is not affected.[74] The survival of *Bcl-x*$^{-/-}$ immature T and B cells are both decreased. *Bcl-x*$^{-/-}$ thymocytes died more rapidly than wild-type or *Bcl-x*$^{+/-}$ thymocytes in response to dexamethasone, γ-irradiation, or anti-CD3. Decreased survival is found in the double-positive thymocytes in which BCL-X is normally highly expressed, whereas the single-positives and peripheral T cells showed comparable survival to *Bcl-x*$^{+/-}$ heterozygotes.[74,115] Thus, BCL-X seems to be important in immature double-positive thymocytes, whereas BCL2 is more important in the maintenance of mature single-positive lymphoid cells. Offspring from matings between transgenic *lck*^{pr}*-BCL-X*$_L$ mice and *Bcl2*$^{-/-}$ mice showed that BCL-X$_L$ can rescue the apoptotic loss of peripheral T cells in *Bcl2*$^{-/-}$ mice.[68] Even though transgenic *BCL-X*$_L$ can functionally substitute for *BCL2*, the reciprocal pattern of expression of *BCL2* and *BCL-X*$_L$ suggests that the two genes differ in their physiologic roles. Consistent with the dramatic difference in the phenotypes of the *Bcl2* and *Bcl-x* knockout mice, these two highly homologous genes are not simply redundant, but rather exhibit clear differences in lineage specificity (Table 2).

Bax-deficiency results in lymphoid hyperplasia and male germ cell hypoplasia. Several cell death repressors, including BCL2, BCL-X$_L$, and E1B-19K, have been shown to function through heterodimerization with BAX. Yeast two-

hybrid assays also showed that BAX interacts widely with other family members. These findings suggested that BAX may have a central role in the regulation of apoptosis. One prediction is that BAX may be necessary for cell death; alternatively, heterodimers of BAX may be required for death repression. Interestingly, whether BAX deficiency results in hyperplasia or hypoplasia appears to be tissue specific. $Bax^{-/-}$ mice appear to be healthy, indicating that BAX is not essential for development of the organism. Thymocyte numbers of $Bax^{-/-}$ mice are increased 1.6-fold over wild-type controls, and the splenic B cells are similarly increased 1.8-fold. On the other hand, male $Bax^{-/-}$ mice are infertile, and $Bax^{-/-}$ testes exhibited a marked increase in cell death clustered in the germ cells. The seminiferous tubules were abnormal, and multinucleated giant cells and pyknotic cells were present. The complete cessation of mature sperm cell production was accompanied by an expansion of the premeiotic 2N cell population, suggesting a role for BAX in meiosis. However, $Bax^{-/-}$ ovaries display an accumulation of atrophic granulosa cells that presumably failed to undergo apoptosis. Thus, the phenotypic abnormalities of $Bax^{-/-}$ can be either hyperplasia or hypoplasia, depending on the cell type. Because the affected tissues of $Bax^{-/-}$ mice are not identical to the affected tissues of $Bcl2^{-/-}$ mice, BCL2 may not always act through interaction with BAX. The $Bax^{-/-}$ mice dramatically illustrated that not only is there lineage specificity in the BCL2 family members, but that, depending on cell type, the same molecule can have a positive or a negative effect on cell death.[127]

BCL2 FAMILY COOPERATES WITH OTHER CANCER GENES

BCL2 cooperates with MYC by inhibiting MYC-induced apoptosis. An early functional study of *BCL2* showed that it can cooperate with the oncogene *c-Myc* to immortalize pre-B cells.[7] Subsequently, it was found that inappropriate *c-Myc* expression under conditions such as heat shock in Chinese hamster ovary (CHO) cells or serum deprivation of Rat-1 fibroblasts lead to rapid onset of apoptosis.[117,118] Constitutive expression of BCL2 inhibited MYC-induced apoptosis,[117,119] allowing immortalization by MYC to occur. Most recently, cell culture experiments using inducible *MYC*-constructs in serum-deprived fibroblasts showed that expression of MYC activated both proliferation and apoptosis and that the survival of the cell was dependent on survival factors.[120] Factors such as IGF-1 suppress the inherent genetic apoptotic program. The induction of apoptosis and its inhibition by specific cytokines were not dependent on new protein synthesis. MYC-induced apoptosis may be a consequence of the imbalance of proliferative pathways, ie, a conflict of signals. Alternatively, it may be the result of the lack of a survival factor, such as a cytokine or BCL2, when MYC has induced a dual signal, proliferation and apoptosis[120] (Fig 6). The dual signal hypothesis predicts that accumulation of mitogenic mutations alone may result in cell death when paracrine factors are depleted, but simultaneous or additional acquisition of events suppressing cell death, such as upregulation of BCL2, will lead to carcinogenesis. Double transgenic mice expressing both *BCL2* and *Myc* exhibited hyperproliferation of pre-B and B cells and developed tumors of a hematolymphoid cell type at a markedly increased rate[18,116] (Fig 1). Synergy between these oncogenes of two different classes results in more potent transformation than by either oncogene alone.

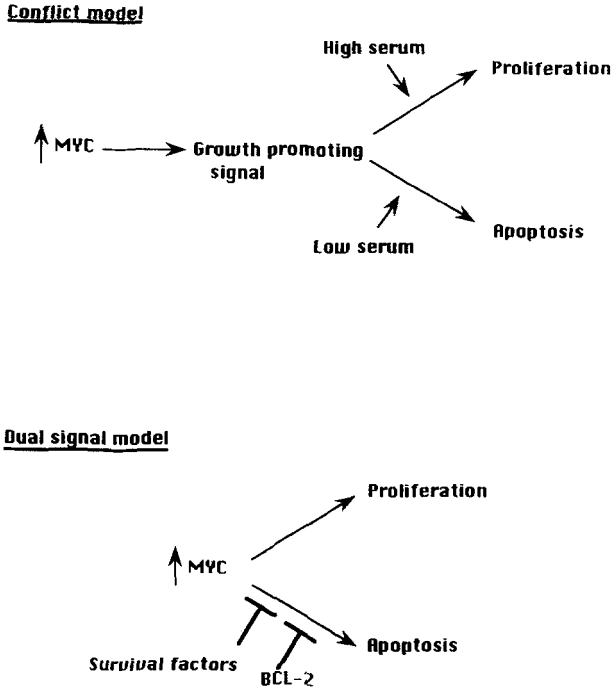

Fig 6. MYC-induced apoptosis. Conflict model: MYC induces a growth signal that results in proliferation in high serum conditions, but in serum starvation, cells are unable to proliferate and apoptosis results. Dual signal model: MYC induces both a proliferative and an apoptotic program. The apoptotic response can be suppressed by survival factors in serum or by death repressor molecules such as BCL2.

BCL2 can inhibit p53-dependent and p53-independent cell death pathways. The tumor-suppressor gene *p53* can induce apoptosis. For many genotoxic death pathways, wild-type *p53* activity is required. The first direct evidence of this is the inability of $p53^{-/-}$ thymocytes to undergo apoptosis in response to γ-irradiation or etoposide, although they remain susceptible to killing by glucocorticoid and calcium.[121,122] However, the dependence on *p53* can be overcome at high doses of the toxic agents, suggesting a threshold effect,[123] or if the cells are cycling, as in the case of activated T cells.[124] Thus, there are *p53*-dependent and *p53*-independent mechanisms of cell death, and both can be inhibited by BCL2. Recently, it has been shown that *BAX* expression is modulated at the transcription level during *p53*-mediated apoptosis in selected cells.[36,125] In M1 myeloid leukemia cells, expression of *p53* induces apoptosis. This is correlated with upregulation of BAX, resulting in increased BAX/BCL2 ratio. In a survey of cell lines with wild-type, mutant, and deficient *p53* status, BAX was induced in response to γ-irradiation in cell lines that are both apoptosis competent and have wild-type *p53*.[126] However, there is no evidence that *bax* is required for p53 induced deaths.[127] In an in vivo model of

choroid plexus tumor progression comparing $p53^{+/-}$ with $p53^{-/-}$ mice, it was found that aggressive tumor progression occurred in the absence of *p53* function attributable to decreased apoptosis.[128] The function of *p53* as a tumor-suppressor gene may be largely explained by its role in promoting cell death.

CLINICAL ASPECTS OF BCL2

Studies of t(14;18) in lymphomas support the multi-hit oncogenesis model. *BCL2* was first described as the deregulated oncogene in t(14;18) lymphomas. One initial study of 20 patients with follicular lymphoma possessing a large-cell component correlated the presence of the t(14;18) with a poor response to therapy.[129] Subsequent larger studies composed of both large-cell and small-cleaved cell follicular lymphomas have not shown a prognostic significance of having a t(14;18).[130-132] However, many B-cell lymphomas that lack the t(14;18) also have high levels of BCL2 protein.[133,134] Clones harboring the t(14;18) translocation are commonly found in normal individuals. A large percentage of normal tonsils were found to contain cells positive by PCR for t(14;18),[135,136] and many healthy individuals harbored t(14;18)-containing B cells in their peripheral blood.[137] Another study found peripheral blood lymphocytes from 55% of normal individuals and 35% of autopsied spleens contained cells with PCR-detectable t(14;18).[138] These findings confirm the conclusion from transgenic mice experiments that translocation involving *BCL2* alone is not sufficient to cause cancer, ie, additional events are necessary for malignant transformation to occur. Moreover, the frequency of translocations increased significantly with age, being 40 times greater in the spleen and 13 times greater in the peripheral blood in the oldest age group (>60 years) compared with the youngest age group (<20 years).[138] The increase in the frequency of t(14;18) cells with age parallels the increase in lymphoma incidence with age. It is likely that both t(14;18)-bearing cells and secondary hits increase over time. These epidemiologic correlates illustrate the importance of extended cell survival as a primary event in a multihit oncogenesis model.

The presence of t(14;18) provides a convenient way to observe patients after therapy. It has been shown that cells positive for t(14;18) persist in patients in prolonged complete remission,[139,140] but whether this predicts imminent relapse remains uncertain. More recently, investigators have looked for the disappearance of translocation-bearing cells from bone marrows after myeloablative therapy or in vitro purging.[141,142] Detection of the translocation by PCR provides a means to assess the success of the elimination of the lymphoma clone upon myeloablation or purging. The literature suggests that detection of cells with translocation may correlate with shorter remission.

BCL2 expression is found in tumors of some hormonally responsive epithelium. BCL2 expression has been investigated in nonlymphoid tumors. It is well established that some breast carcinomas, prostate cancers, and non–small-cell lung cancers express BCL2. In breast carcinoma, BCL2 expression is positively correlated with estrogen receptor and progesterone receptor positivity. Conversely, loss of BCL2 expression is associated with known poor prognostic indicators, such as estrogen receptor negativity, epidermal growth factor receptor positivity, *p53* mutation, and high histologic grade.[143-145] The normal epithelium from which breast carcinoma arises expresses BCL2, suggesting that BCL2 expression allows cells to live longer and accumulate genetic alterations. Loss of BCL2 is likely to be a late event accompanied by additional genetic changes. In multivariate analysis, it appears that the prognostic role of BCL2 is related to *p53* status, which itself has independent prognostic significance.[144]

BCL2 expression is also found in cancers of another hormonally responsive tissue, the prostate. High levels of BCL2 are observed in androgen-independent tumors,[146] in particular those tumors that persist after androgen ablation therapy,[147] leading to the speculation that BCL2 function allows the neoplastic prostate cells to survive in a hormonally deprived environment.

High BCL2 expression is correlated with poor response to chemotherapy. Programmed cell death is not only an important normal physiological process, but it is also how cancer cells die when treated with a variety of chemotherapeutic drugs, including inhibitors of DNA synthesis, alkylating agents, topoisomerase inhibitors, microtubule inhibitors, and antimetabolites. The ability of BCL2 to inhibit cell death induced by many of these agents with different mechanisms of action is consistent with BCL2 being a downstream molecule in the apoptotic pathway.[37-44] Cell lines transfected with *BCL2* show increased resistance to nitrogen mustard, camptothecin, VP-16, platinum compounds, methotrexate, Ara-C, adriamycin, and cyclophosphamide.[40,43,44] These observations are borne out in the clinical arena. High BCL2 expression is associated with low remission rate in acute myeloid leukemia.[148] In an analysis of acute lymphocytic leukemia (ALL) and acute myeloid leukemia (AML) patients at diagnosis and relapse, it was found that both the percentage of BCL2 expressing cells and the intensity of BCL2 staining were higher at relapse than at presentation. In de novo AML and ALL, the intensity of BCL2 staining and the number of positive cells were lower in cases that responded to chemotherapy than in nonresponders; therefore, high BCL2 expression is an indicator of poor response in acute leukemia.[149]

In a cell line model, cells selected for acquired resistance to cytotoxic drugs associated with overexpression of the *MDR1* gene showed induction of BCL-X_L. These cells were also resistant to γ-irradiation induced apoptosis.[150] Thus, induction of BCL-X_L may play a role in the etiology of chemotherapy and radiation-resistant tumors and may prove to have prognostic significance as well.

Apoptosis as a therapeutic target. Given that inappropriate survival can be a primary event in tumorigenesis and that cells undergo apoptosis in response to chemotherapy, the outcome of cancer may be affected by changing the setpoint at which cells undergo apoptosis in response to a signal (Fig 7). In cancers that overexpress BCL2, decreasing BCL2 expression may allow a cell that contains otherwise intolerable genetic alterations to die. Altering the threshold for cell death, one may render the cancer cell more sensitive to chemotherapeutic agents. This might be approached by

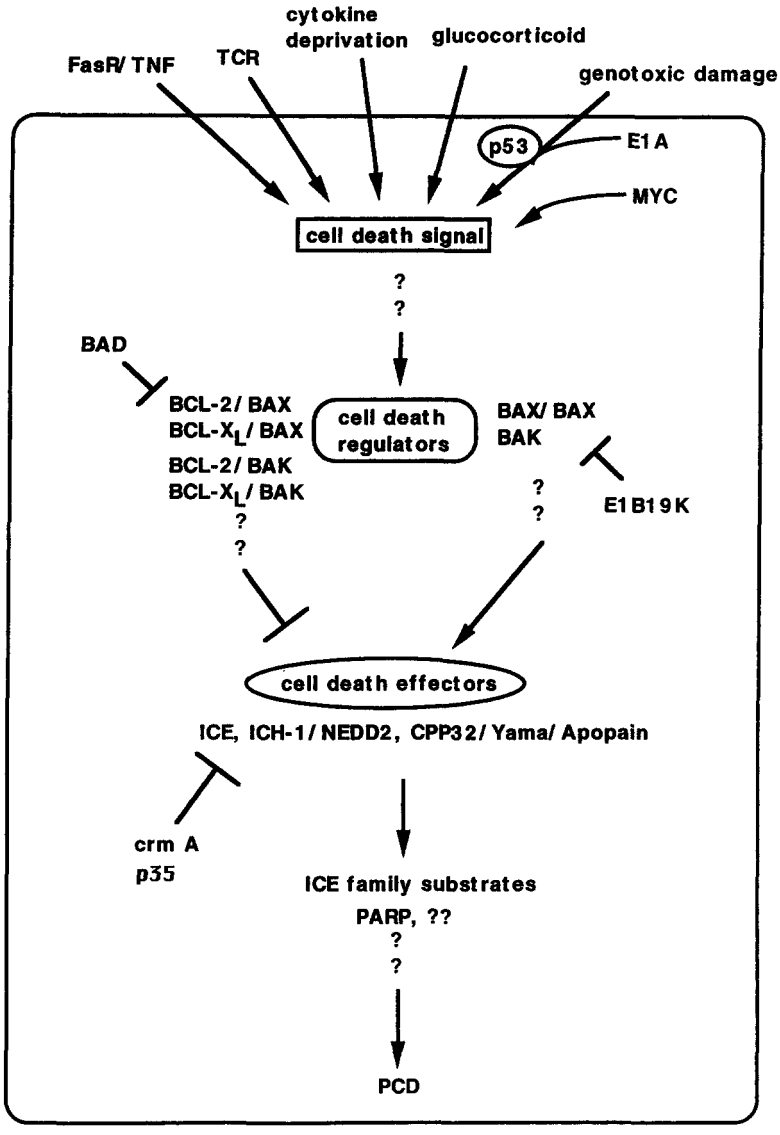

Fig 7. Schematic of the cell death pathway. Various stimuli generate a cell death signal(s), the ratio of heterodimers of the cell death regulators determine the susceptibility to death, and cell death effectors execute PCD. The precise intermediate steps and the critical protease substrates are not known.

downregulating BCL2 expression in cancer cells, either by targeting BCL2 directly or indirectly through an upstream regulator of BCL2. Because the susceptibility to cell death can be determined by competing positive versus negative regulators in the BCL2 family, the threshold of death could be altered by changing the ratio of these members. For example, small molecules that selectively disrupt certain dimerized pairs might accelerate tumor cell death in response to therapy. As with all therapy, one needs to maximize targeting to the cancer cell and minimize systemic toxicity. The lineage-specific expression of some members of the BCL2 family provides hope that cell-type–specific therapies might be possible. The various members of the ICE family of enzymes or their substrates may also have cell-type specificity that may be exploited as targets of drug therapy. Small molecules, such as the chloromethylketone tetrapeptides, already exist that can inhibit ICE activity in vitro and in cell culture systems. Small molecules that activate select cysteine proteases but not others might also provide the desired specificity of a useful therapeutic.

CONCLUSION

Since its discovery in the mid-1980s, the proto-oncogene *BCL2* has been proven to be a central player in mammalian cell death pathways. The extension of cell death research to include organisms such as *C elegans* has shown remarkable conservation of the basic priniciples of apoptosis across evolution, arguing that the genetic pathway of cell death is common to all multicellular organisms. The last few years have witnessed an expansion of molecules involved in cell death, in both BCL2 homologs and other classes of proteins, including new proteins of the ICE family and known proteins such as p53. The ever-increasing number of BCL2 homologs can be categorized into those that extend cell survival, such as BCL2, BCL-X_L, E1B-19k, BHRF1, and CED-9, or those that promote death, such as BAX, BCL-X_S, BAD, and BAK.

Knockout mice argue that each member of the BCL2 family will serve a pivotal role in select tissues. In addition, the identification of new proteins, such as BAD, which modulate BCL2 and BCL-X_L activity, suggests that the multiple cell death proteins must be tightly regulated. Although the precise biochemical activity of BCL2 remains uncertain, genetic studies of the *BCL2* family members have established the importance of these genes in the normal development and maintenance of the organism. Inappropriate cell survival resulting from the deregulation of cell death genes can be a first step in oncogenesis. Once a tumor is established, its response to therapy can also be affected by its propensity to undergo programmed cell death. A remaining challenge is to define the intermediate steps that connect the sets of signals, regulators, and effectors that comprise apoptosis (Fig 7). Perhaps this knowledge will provide the basis for designing effective cancer therapies that target the cell death pathway.

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