Here Stan describes the research that identified the Bcl-2 gene and protein that revolutionized our understanding of how most malignant lymphomas come to be. That is the cells are not wildly dividing and growing like most cancers, rather the cells just don't die off as the should because high levels of the Bcl-2 protein are present within them and Bcl-2 prevents them from dying. Hence they grow in number and size and cause trouble. Stan was a leader- if not groundbreaking pioneer- in this research. This is what lead to worldwide acclaim within the community of medical scientists.

Bcl-2 is present in lots of different tissues. It is prominent where cell survival is frequent and necessary and nearly absent where cell death needs to occur. Hence the conclusion: Bcl-2 maintains cell function and life and its absence leads to cell death, known as apoptosis. Likewise, the same genes and proteins are present in all complex organisms be they worms, mice, or men!

REVIEW ARTICLE 1992

Bcl-2 Initiates a New Category of Oncogenes: Regulators of Cell Death

By Stanley J. Korsmeyer

SPECIFIC CHROMOSOMAL translocations are recurrently found in distinct types of malignancies.[1-4] Indeed, many of these interchromosomal translocations are essentially pathognomonic for a given tumor. The molecular cloning of chromosomal breakpoints has proven a rich source of novel proto-oncogenes. Experimental approaches indicate that deregulation of these genes represents a primary pathogenic event in the generation of tumors. Determining the normal developmental role of each gene promises to deliver insights into their oncogenic mechanism. The lessons provided from the study of one such oncogene, Bcl-2, argue that understanding the functional roles of genes found at breakpoints will have an enormous impact upon mammalian biology. Bcl-2 was discovered at the t(14;18)(q32;q21) breakpoint, the cytogenetic hallmark of human follicular lymphoma.[5-7] Bcl-2 is novel among proto-oncogenes in that it localizes to mitochondria.[8] Moreover, Bcl-2 shows the unique functional role of blocking programmed cell death independent of affecting proliferation.[8-10]

MULTIPLE ROADS TO NEOPLASIA

Studies of Bcl-2 emphasize the existence of multiple pathways in the generation of neoplasia (Fig 1). The increased cell number in neoplastic tissue can be viewed as a violation of normal homeostasis. The maintenance of homeostasis in normal tissue, in many respects, reflects a simple balanced equation of input (cellular proliferation and renewal) versus output (cell death). This is most easily envisioned for encapsulated organs such as the prostate, but is also true of the recirculating hematopoietic lineages. The maintenance of remarkably invariant cell numbers must reflect tightly regulated death pathways as well as controlled proliferation (Fig 1).

Programmed cell death represents a cell autonomous suicide pathway that helps restrict cell numbers. The well-defined loss of specific cells is crucial during embryonic development as part of organogenesis.[11] In mature tissues, genetically programmed demise regulates the volume of cells. Wyllie identified a morphologically distinct and highly ritualistic cell death entitled apoptosis.[12] Cells dying by apoptosis display marked plasma membrane blebbing, volume contraction, nuclear condensation, and the activation of an endonuclease that cleaves DNA into nucleosomal length fragments. Defining the precise genes and biochemical events that regulate this death program presents a major challenge.

REGULATORS OF PROGRAMMED CELL DEATH: A NEW CATEGORY OF ONCOGENES

Malignancies usually possess aberrations in more than a single pathway.[13] Either increased proliferation or decreased death might result in an expansion of cell numbers (Fig 1). To date, most of our knowledge concerning oncogenic events has concentrated on mechanisms of increased cell growth and proliferation. At the risk of oversimplification, Table 1 depicts two classes of oncogenes that regulate growth and proliferation. The first oncogenes discovered, category I, promote cell growth and proliferation.[14] Most of these can be classified as transcription factors or molecules involved in signal transduction. Classic examples would include Myc, a nuclear putative transcription factor; Ras, a G protein; and Abl, a tyrosine kinase. In general, these genes contribute to cancer after an alteration resulting in a gain of function. They usually display an autosomal dominant mechanism, in which a single altered allele is sufficient to confer the effect. Category II represents the classic tumor suppressor genes that in their wild-type form inhibit growth and proliferation.[15] A unique aspect of the two experimentally proven members, Rb and p53, is their frequent contribution to unchecked growth by loss of function. This reflects the autosomal recessive mechanism proposed by Knudson.[16] Of course, more complicated events also occur in this category in which one allele may be lost and the other mutated, a so-called "dominant negative."

The effects of deregulated Bcl-2 argue that it does not qualify as either a category I or II oncogene. Instead, Bcl-2 appears to constitute a member of a new category of oncogenes: regulators of programmed cell death (Table 1).

THE t(14;18) BREAKPOINT OF FOLLICULAR LYMPHOMA PROVIDES THE Bcl-2 GENE

The t(14;18) (q32;q21) constitutes the most common chromosomal translocation in human lymphoid malignancies. Approximately 85% of follicular and 20% of diffuse B-cell lymphomas possess this translocation.[17,18] As a disease, follicular lymphoma provided many clues concerning the ultimate function of the Bcl-2 molecule. Follicular lymphoma often presents as a low-grade malignancy composed of small resting IgM/IgD B cells. Over time, conversion to an aggressive high-grade lymphoma with a diffuse large-cell architecture frequently occurs in these patients.[19] The location of the Ig heavy chain locus at 14q32 and the B-cell phenotype of this lymphoma provided the rationale for cloning the chromosomal breakpoint. Aberrant Ig heavy chain gene rearrangements in t(14;18) lymphomas proved to be the chromosomal breakpoint and delivered a candidate oncogene, Bcl-2, at 18q21.[5-7] While follicular lymphoma possesses a mature B-cell phenotype, the molecular archeology of the breakpoint argues that the translocation

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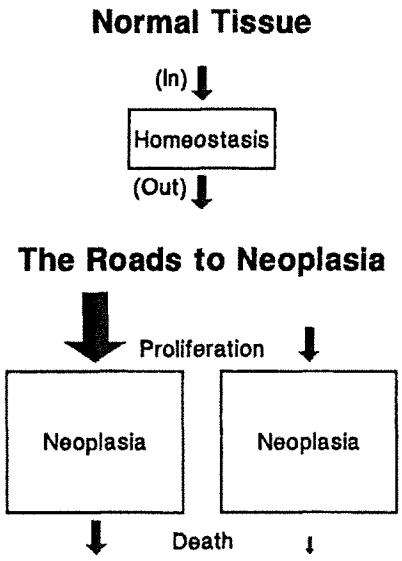

Fig 1. Schematic representation of normal tissue homeostasis with balanced input and output reactions. Alternate roads to neoplasia are depicted as either increased proliferation (In) or decreased death (Out).

occurs earlier in development at a pre-B–cell stage (Fig 2). DNA sequence analysis of both chromosomal breakpoints showed that the derivative der(14) juncture was located at a joining (J_H) region, while the der(18) involved a diversity (D_H) segment.[5,20,21] Moreover, extra nucleotides, "N" segments, were found at each chromosomal junction, typical of normal Ig joints. Ig recombinase produces endonucleolytic cleavages at Ig heavy chain diversity (D) and joining (J) segments during pre-B–cell development. Within the progenitor cells of follicular lymphoma these vulnerable sites of double-stranded (ds) DNA breaks are recombined with a fractured Bcl-2 gene from chromosome segment 18q21. Approximately 70% of the breakpoints on 18 are clustered within a major breakpoint region (MBR) in the 3' untranslated region of the Bcl-2 gene (Fig 2). Curiously, neither the MBR or the more distant minor cluster region (mcr)[22] of Bcl-2 possess the typical heptamer-spacer-nonamer motif recognized by Ig recombinase. Thus, the precise mechanism for localized DNA breakage of Bcl-2 remains uncertain. Of note, 8-bp χ-like consensus sequences have been noted adjacent to Bcl-2 breakpoints, raising the possibility that such variable tandem repeats may participate.[23]

Table 1. Oncogene Categories

Category I:	Growth and proliferation genes
	Transcription factors or signal transduction
	Myc, Ras, Abl
	Gain of function
Category II:	Tumor suppressor genes
	Inhibit growth and proliferation
	Rb, p53
	Loss of function
Category III:	Programmed cell death genes
	A. Antidotes to programmed cell death
	Bcl-2
	Gain of function
	B. Death pathway genes
	p53
	Loss of function

Bcl-2 is normally located on chromosome segment 18q21.3 in a telomere to centromere orientation (Fig 3). The Bcl-2 gene possesses 3 exons, the first of which is untranslated. Two potential promoter regions exist. P1 is GC rich with multiple SP1 sites and is used predominantly. While P2 has a classic TATA and CAAT-box and an SV40 decamer/Ig octamer motif, its use is minimal.[24,25] Bcl-2 is an enormous gene in which a 225-kb intron II divides the protein encoding exons II and III.[26] A molecular consequence of the translocation is the movement of the Bcl-2 gene to the der(14) chromosome (Fig 3). This places Bcl-2 in the same transcriptional orientation as the Ig heavy chain locus giving rise to chimeric RNAs. However, translocation does not interrupt the protein encoding region so that normal and translocated alleles produce the same sized, 25-Kd protein. Hematopoietic progenitors, including pre-B cells, possess high levels of Bcl-2.[27] Some mature B cells and, especially, B-cell lines have low levels of Bcl-2 RNA. In contrast, t(14;18)-bearing B cells have inappropriately elevated levels of the Bcl-2–Ig fusion RNA.[24,25,28] This increased steady-state RNA reflects both increased transcription as well as a processing advantage for the Bcl-2–Ig fusion allele.

Bcl-2 BLOCKS PROGRAMMED CELL DEATH

Whether the newly discovered genes found at chromosomal breakpoints could be shown to be transforming remained a major question. We and others placed deregulated Bcl-2 into Epstein-Barr virus-induced lymphoblastoid B-cell lines (LCLs) and assessed the classic measures of oncogenesis: clonogenicity and tumorigenicity.[29,30] Bcl-2 improved the clonogenicity of LCLs in soft agar, but was insufficient as a single agent to confer tumorigenicity to LCL. Consequently, we examined Bcl-2's ability to complement other cellular oncogenes, especially *myc*. Retroviruses overexpressing Bcl-2 were introduced into LCLs that possessed a deregulated *myc* gene. Bcl-2 complemented *myc*, increasing the frequency and shortening the latency of tumor formation by these LCLs in immunodeficient nude mice.[29] Bcl-2 was also shown to cooperate with c-*myc* in B-cell precursors, some of which became tumorigenic.[9]

These observations raised questions as to whether Bcl-2 might be directly involved in a growth factor pathway. To assess this, retroviral or plasmid-based vectors overproducing Bcl-2 were introduced into a variety of interleukin (IL)-dependent cell lines. Such lines were examined to determine if Bcl-2 would spare the need for a specific ligand/receptor interaction. However, no long-term growth factor-independent cell lines emerged after overexpression of Bcl-2 in IL-2, IL-3, IL-4, or IL-6 requiring lines. However, a more novel effect was noted that helped define a physiologic role for Bcl-2. Bcl-2 conferred a death-sparing effect to certain hematopoietic cell lines after growth factor withdrawal.[8-10] This was noted in the IL-3–dependent early hematopoietic cell lines FDCP1, FL5.12, and 32D. This

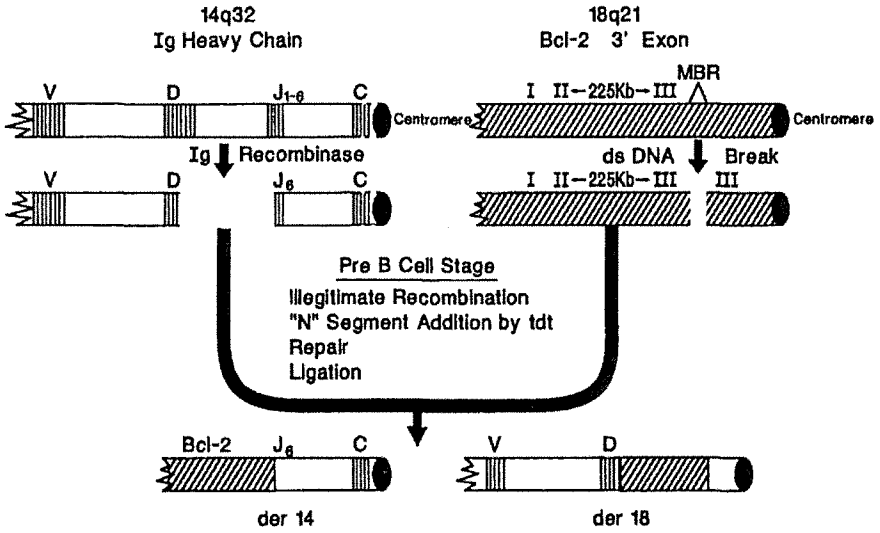

Fig 2. Mechanism of recombination at the t(14;18) breakpoint. The breakpoint on the derivative (der) 14 chromosome occurs at a J segment, whereas the der(18) involves a D segment. This finding argues that the translocation occurs at a pre-B–cell stage when Ig recombinase has generated endonucleolytic cleavage at a D and J segment. In the progenitors of lymphoma, these sites illegitimately recombine with a ds DNA break in the MBR of Bcl-2 exon III at 18q21.

effect was not restricted to the IL-3/IL-3 receptor signal transduction pathway in that granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-4–deprived cells displayed a similar response. Yet, Bcl-2–enhanced cell survival was not universal as neither IL-2–dependent T-cell lines nor an IL-6–dependent myeloma line showed a consistent effect upon factor withdrawal. Thus, even these early studies indicated a selectivity to Bcl-2's interference with cell death.

These observations prompted a detailed examination of the interference of Bcl-2 with cell death. Bcl-2 does not obviously influence cell cycle progression, nor does it alter the dose response to limiting concentrations of IL-3.[10] Instead, Bcl-2 blocked the plasma membrane blebbing, volume contraction, nuclear condensation, and endonucleolytic cleavage of DNA known as apoptosis.[8] Factor-deprived cells return to Go, but do not die. However, they can be rescued after 30 days of deprivation by the readdition of IL-3, indicating they are not terminally differentiated or permanently arrested.

Bcl-2's LOCALIZATION TO MITOCHONDRIA: A CLUE TO ITS FUNCTION?

Bcl-2 lacks a characteristic signal peptide but has a C-terminal hydrophobic region that serves as an integral membrane anchor.[8,31] Monoclonal antibodies to Bcl-2 protein detected a punctate cytoplasmic distribution of Bcl-2, first suggesting Bcl-2 was localized to an organelle.[8] Dual-fluorescence staining of cells when examined with a laser scanning confocal microscope indicated that Bcl-2 was coincident with the distribution of mitochondria. Subsequent subcellular fractionations showed that Bcl-2 was an integral membrane protein present in the heavy membrane fraction. A series of density gradients indicated that Bcl-2 colocalized with mitochondrial membranes. When mitochondria were disrupted into inner and outer mitochondrial membranes by hypotonic lysis, Bcl-2 was present in the mitoplast fraction possessing the inner membrane with the succinate dehydrogenase activity.[8] The location of Bcl-2 in mitochondria provides a novel vantage point from which to address the regulation of programmed death. Bcl-2's interrelationship with a major function of mitochondria such as oxidative phosphorylation, electron transport, the transport of proteins, metabolites or ions is now under close scrutiny. A remaining challenge is to determine whether Bcl-2 represents a metabolic checkpoint that helps elect cell survival or death.

TOPOGRAPHIC DISTRIBUTION OF Bcl-2 PROTEIN IN TISSUES DEMONSTRATING APOPTOSIS

The spatial distribution of Bcl-2 within organized tissues was examined to obtain clues concerning its normal physiologic roles. Bcl-2 protein displays a remarkably restricted topographic distribution within mature tissues character-

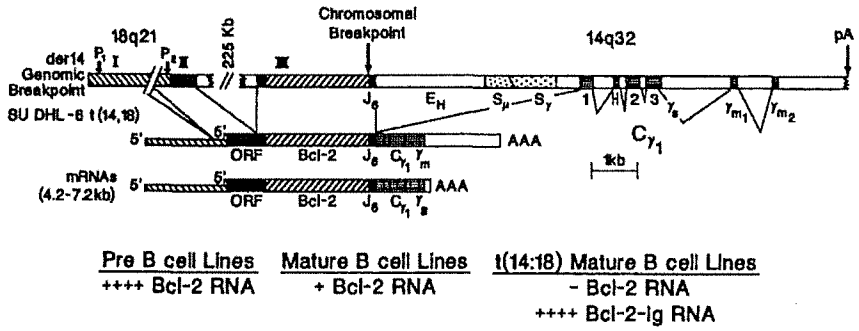

Fig 3. Bcl-2–Ig fusion gene. The t(14;18) introduces Bcl-2 into the Ig locus, placing it in the same transcriptional orientation as Ig. This generates a series of chimeric RNAs. Relative levels of steady-state RNA within cell lines are indicated.

ized by apoptotic cell death.[27] One of the most dramatic examples is provided by 2° germinal centers (Fig 4). Immunohistochemical assessment showed that the follicular mantle, composed of long-lived recirculating IgM/IgD B cells, possessed an abundant amount of Bcl-2.[27,32] Of note, Bcl-2 protein was essentially absent from the dark zone of proliferating centroblasts and from the basal portion of the light zone where centrocytes are dying by apoptosis. Yet, Bcl-2 expression returns in B cells in the more apical portion of the light zone, where it has been proposed that a subset of B cells are selected for survival by their affinity for residual antigen present on follicular dendritic cells (Fig 4).

An initial survey of tissues argued strongly that Bcl-2 had roles beyond B cells.[27] The thymus has distinct cortical and medullary regions that possess thymocytes at serial stages of maturation. Bcl-2 was present in the surviving mature thymocytes of the medulla. The majority of cortical thymocytes, most of which die by apoptosis, displayed no Bcl-2. All hematopoietic lineages that derive from a renewing stem cell also display Bcl-2. While present in the precursor cells, it is absent in their most differentiated and terminal progeny. Another category of tissues that express Bcl-2 is glandular epithelium that undergoes hyperplasia or involution, usually in response to hormonal stimuli or growth factors. In addition, Bcl-2 is also present in complex differentiating epithelium. In organized epithelium, Bcl-2 is restricted to stem cell and proliferation zones. Bcl-2 is present in the lower crypts of the intestine, but not along the upper crypt-villus axis. Similarly, the basal layer of epidermis shows Bcl-2, while suprabasalar layers do not. In all of these normal tissues, apoptosis molds developing structures or helps maintain tissue homeostasis. Bcl-2 may be required to save the progenitor and long-lived cells in such lineages.

B CELL MEMORY: A PHYSIOLOGIC ROLE FOR Bcl-2

The restriction of Bcl-2 to the zones of surviving B cells within germinal centers first suggested that this antidote to apoptosis might be involved in immune responsiveness (Fig 4). To assess this directly required an in vivo model.

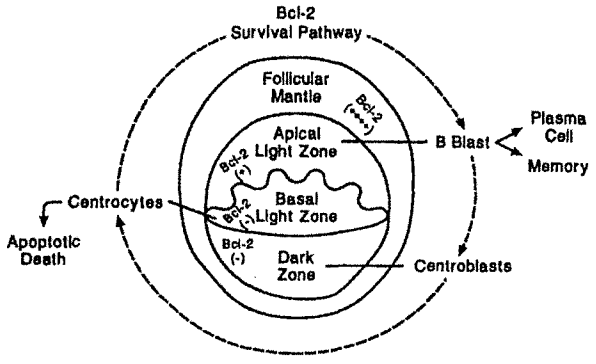

Fig 4. A schematic representation of the distribution of Bcl-2 protein within 2° germinal centers. The return of Bcl-2 within B cells in the more apical portion of the light zone coupled with the extended secondary response of Bcl-2–Ig transgenic mice argues that Bcl-2 is involved in a survival pathway within germinal centers.

Consequently, we turned to transgenic mice bearing a Bcl-2–Ig minigene that recapitulated the molecular consequence of the human translocation.[33] Bcl-2 of transgenic origin was overexpressed relative to the endogenous gene in a B-cell–predominant pattern.[34] However, the topographic distribution of Bcl-2 was maintained within germinal centers of these transgenic mice. The primary antibody response of Bcl-2–Ig transgenics to a T-dependent antigen was relatively normal. Moreover, the number of antigen-stimulated cells that committed to a memory response was unaltered. However, the length of the secondary immune response in Bcl-2 transgenics was markedly protracted.[35] Bcl-2–Ig transgenic mice displayed large numbers of antibody-secreting plasma cells 75 days after a secondary immunization, a time at which normal mice had returned to a baseline response. Moreover, adoptive transfer studies documented an extended lifetime for the memory B cells of transgenic mice overexpressing Bcl-2. Memory B cells from transgenics persisted, even in the absence of antigen, when moved to an antigen-naive recipient. An independent set of transgenic mice in which Bcl-2 expression was enforced by an SV40 promoter and Ig enhancer was analyzed by Strasser et al and also demonstrated markedly protracted antibody responses.[36] Moreover, these mice bred on an SJL background exhibited autoantibodies and kidney abnormalities, raising the possibility that Bcl-2–mediated B-cell survival could contribute to autoimmunity. These functional studies are in concert with the restriction of Bcl-2 in germinal centers to portions of the light zone implicated in the selection and maintenance of plasma cells and memory B cells (Fig 4). Of note, centrocytes treated with anti-Ig plus anti-CD40 are rescued from apoptosis[37] and are induced to express Bcl-2.[38] Moreover, the crosslinking of surface Ig plus IL-4 or B-cell growth factor (BCGF) induces transcription of Bcl-2.[28] From this combination of direct and indirect data it appears that one physiologic role for Bcl-2 involves the generation and maintenance of B-cell memory.

PROGRESSION TO HIGH-GRADE LYMPHOMA: A COST OF LIVING LONGER

A stringent test of a gene's oncogenic capacity is to place it into the germline of mice to observe its effects during the development of an entire organism. Transgenic mice bearing a Bcl-2–Ig minigene initially displayed a polyclonal follicular lymphoproliferation that selectively expanded a small resting IgM/IgD B-cell population. Cell cycle analysis confirmed that approximately 97% of the expanded B cells reside in Go/G1. These recirculating B cells accumulate because of an extended survival rather than an increased proliferation. While resting, these cells can readily enter the cell cycle and display protracted proliferation after activation.[33,34] Despite a fourfold increment in resting B cells, Bcl-2–Ig mice are initially quite healthy. However, over time these transgenics progress from indolent follicular hyperplasia to diffuse large-cell immunoblastic lymphoma.[39] A long latency period and progression from polyclonal hyperplasia to monoclonal high-grade malignancy is an indictment of secondary genetic abnormalities. Approximately half of the high-grade tumors possess a

This is it! The cells do not die like they should.

c-*myc* translocation involving an Ig H chain locus. These tumor cells have complemented an inherent survival advantage (Bcl-2) with a gene that promotes proliferation (*myc*). When Bcl-2 transgenic mice were mated to *myc* transgenic mice, a rapidly emerging undifferentiated hematopoietic leukemia occurred, providing further testimony for the potent synergy of this particular oncogene combination.[40] In addition to promoting cell cycle progression, *myc* has recently been shown to promote apoptosis.[41] Thus, the overexpression of *myc* may specifically benefit from Bcl-2's ability to block apoptosis. Finally, the Bcl-2–Ig mice document the prospective importance of the t(14;18) in setting the stage for tumor progression and lymphomagenesis.

BEYOND B CELLS: Bcl-2 IN T-CELL DEVELOPMENT

The regional distribution of Bcl-2 within the thymus (medullary thymocytes were positive and most cortical thymocytes were negative) suggested that Bcl-2 is differentially regulated during T-cell maturation and involved in the salvation of T cells. The medulla is composed of $CD4^+8^-$ and $CD4^-8^+$ mature thymocytes that have survived thymic education, whereas the cortex is predominantly populated by $CD4^+8^+$ immature thymocytes, the vast majority of which (95% to 98%) are destined to die. The high rate of death in the thymus appears to result from the molding of an initial T-cell receptor (TCR) repertoire by both positive and negative forces.[42] T-cell maturation requires an interaction between TCR and major histocompatibility complex (MHC) molecules—positive selection. In contrast, thymocytes bearing TCRs reactive against self antigens are deleted during T-cell development—negative selection. However, the precise molecular events that determine the positive or negative outcome for each T cell remain uncertain.

To assess the role of Bcl-2 in T-cell development, transgenic mice were generated in which the proximal lck promoter was used to redirect Bcl-2 to the immature T cells in the cortex of the thymus.[43] The idea was to place this particular antidote to programmed death proximal to positive and negative selection. A separate transgenic model dissected by Strasser et al also overexpressed Bcl-2 in the thymus and displayed similar effects.[44] Curiously, total thymocyte numbers in Lck^{Pr}–Bcl-2 transgenics were normal, providing the first indication that a substantial amount of cell death was still occurring. However, the introduction of Bcl-2 into the normally vulnerable cortical thymocytes protected them from a wide variety of apoptotic stimuli, including glucocorticoids, radiation, and anti-CD3 treatment (Fig 5). Moreover, Bcl-2 altered T-cell maturation, increasing a distinct subpopulation of cells that had intermediate levels of TCR, so called $TCR/CD3^{Med}$ cells. This subset of cells was at a transition point to becoming single positive cells as they displayed $CD4^+8^{lo}$ or $CD4^{lo}8^+$ phenotypes. This population of thymocytes has been proposed as an intermediate stage of thymocyte development after positive selection.[45,46] Despite these profound alterations by Bcl-2, negative selection still occurred. lck^{Pr}–Bcl-2 mice eliminated their self-reactive T cells. Examination of Vβ repertoires indicated that this loss occurred by clonal deletion within the thymus (Fig 5).[43] One interpretation of these

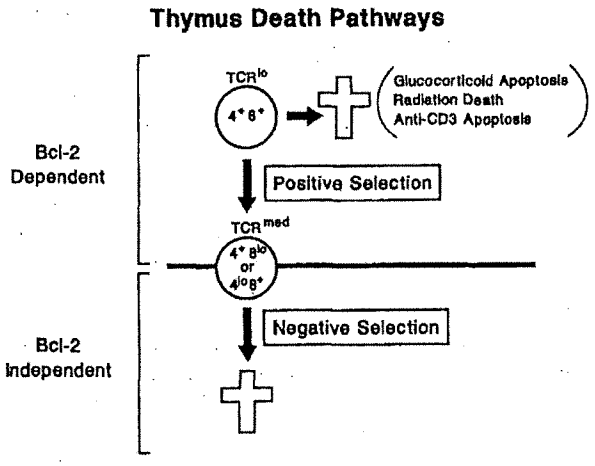

Fig 5. A schematic summary of the findings in Lck^{Pr}–Bcl-2 transgenic mice. Transgenic mice indicate that Bcl-2–dependent and –independent pathways exist within the thymus.

transgenic models is that the thymus has multiple death pathways. Bcl-2 represents a tool to begin distinguishing death pathways. These mice argue that negative selection appears to be Bcl-2–independent and provide a model to explore the effects of Bcl-2 upon positive selection.

CLINICAL APPLICATIONS OF Bcl-2

The discreet topographic restriction of Bcl-2 in normal lymphoid organs prompted the evaluation of Bcl-2 protein within hematologic malignancies. All B-cell lymphomas with Bcl-2 rearrangements show high levels of Bcl-2 protein.[32,47,48] An initial survey suggested that non-Hodgkin's lymphoma (NHL) patients with Bcl-2 rearrangements had a poorer response to therapy than patients without a Bcl-2 rearrangement.[49] However, a larger study showed no relationship between the presence of a t(14;18) translocation and the prognosis in follicular lymphoma.[50] Consistent with this finding, most follicular and diffuse lymphomas without Bcl-2 rearrangement also displayed intense Bcl-2 staining.[48] Perhaps mechanisms beyond translocations deregulate Bcl-2 within these tumors. Alternatively, these malignancies may reflect transformation of B-cell subsets with normally high levels of endogenous Bcl-2.

The detection of a single clonogenic cell has constituted the Holy Grail in the search for minimal residual disease. Unique chromosomal translocations, each associated with a distinct type of neoplasm, provide a molecular hallmark for tumor cells. The focused nature of the t(14;18) breakpoints has enabled generic amplification procedures using polymerase chain reaction (PCR).[22,51,52] This enables the detection of one malignant cell within approximately 10^5 normal cells. Such PCR approaches hold tremendous promise in improving the ability to (1) detect residual clones, (2) define the natural history of disease, (3) quickly evaluate therapies, and (4) stratify patients into appropriate treatment groups. However, after attaining the elusive goal of sensitivity, does it really matter? The current challenge is to place the assessment of minimal residual disease into the setting of clinical trials to determine the "decision making" con-

tent of such information. Caveats do exist. In previously untreated low-grade advanced-stage NHL patients, all patients with an amplifiable breakpoint remained positive in their bone marrow after treatment.[53] Moreover, follicular lymphoma patients who have remained in remission for years after chemotherapy still possess cells bearing the t(14;18).[54] In this setting, the presence of PCR-detectable clones does not signify imminent relapse. This may well reflect the biology of Bcl-2, which blocks cell death rather than promoting proliferation. It is even conceivable that lymphoma patients possess some cells that bear a t(14;18) but lack all the genetic alterations of malignancy. The therapeutic approach to low-grade lymphoma remains controversial, but their proclivity for clinical progression[19] has prompted trials of intensive regimens. Gribbon et al have provided exciting evidence that residual lymphoma cells in transplanted bone marrow affect the length of disease-free survival.[55] In vitro purging with a cocktail of monoclonal antibodies converted half of the autologous bone marrows to PCR negativity for the t(14;18). At a median follow-up of 23 months after ablative therapy and autologous bone marrow transplantation (ABMT), 39% of patients reinfused with PCR-positive marrow relapsed, while only 5% relapsed with PCR-negative marrow. The majority of patients had low-grade histology and longer time points will determine if this improves overall survival. This prognostic marker is not simply a reflection of tumor burden. The correlation held true for patients in complete or partial remission and those with or without histologic evidence for bone marrow involvement at the time of ABMT. But, which cells are the culprits? Is the response to in vitro purging a parallel measure of a lymphoma's resistance to ablative therapy? Alternatively, the investigators cogently argue that reinfused tumor cells may be the risk. Either way, this study argues that minimal residual disease matters, and provides an important intermediate end point. Detection of minimal residual disease appears to predict response and identifies a subset of patients for modified therapy.

Bcl-2 immunolocalization can be of diagnostic utility in distinguishing malignant lymphoma from reactive follicular hyperplasia. In reactive lymph nodes, Bcl-2 is strongly positive in the mantle zone, but predominantly absent from the follicular centers. Follicular lymphomas display an inverse pattern often with a nodular distribution of Bcl-2 in which the malignant follicle is intensely positive, surrounded by a rim of weakly positive cells. Of note, Bcl-2 protein is also found in plasma cell dyscrasias, but intensity can vary with the cell morphology. Chronic myelogenous leukemia is strongly positive for Bcl-2 consistent with the presence of Bcl-2 in normal myeloid progenitors. Bcl-2 protein in T-cell neoplasms appears to parallel their corresponding stage of T-cell development. Most lymphoblastic lymphomas with an immature phenotype ($CD4^+8^+$) fail to express Bcl-2. Whereas, T-cell malignancies of mature phenotype ($CD4^-8^+$ or $CD4^+8^-$) show intermediate levels of Bcl-2, consistent with the level expected in the interfollicular T-cell compartment and recirculating T cells. Thus, the spectrum of hematopoietic neoplasms in which Bcl-2 may confer a survival advantage may extend beyond t(14;18)-bearing tumors.

Variant translocations occur in about 15% of Burkitt's lymphomas and have been noted to juxtapose immunoglobulin κ or λ light chain locus instead of a heavy chain locus with the *myc* gene.[56] In contrast, the Bcl-2 translocation in follicular lymphomas is almost entirely between the Ig heavy chain loci and Bcl-2. Curiously, investigators have noted that the Bcl-2 rearrangements in chronic lymphocytic lymphoma (CLL) (approximately 10%) rather preferentially involve κ or λ light chain loci with the 5′ flank of Bcl-2. Of interest, some regional variation may exist in the incidence of Bcl-2 rearrangement. For example, follicular B-cell lymphomas in Japan appear to have a lower incidence of Bcl-2 rearrangement and rearrangements with Ig κ or λ light chain loci have been noted.[57]

The identity of the cellular origin of Hodgkin's disease remains uncertain. No clonal rearrangements of Bcl-2 were noted in Hodgkin's disease tissue at the sensitivity of a Southern blot (>2% to 5% of all cells). However, Bcl-2/Ig junctures have been detected by some investigators exploiting the sensitivity of a PCR.[58] However, this finding has remained controversial in that others have failed to detect Bcl-2/Ig amplification products.[59] In addition, little to no Bcl-2 is usually present in Reed-Sternberg cells.

An additional note of caution has been voiced by investigators examining benign lymph nodes and tonsils displaying follicular hyperplasia. PCR assays showed Bcl-2/Ig junctures in 13 of 24 (54%) cases examined. The frequency of Bcl-2/Ig–bearing cells within such lesions was apparently quite rare, but each Bcl-2/Ig juncture possessed an unique N segment arguing for its authenticity. Thus, not all cells that possess a Bcl-2/Ig juncture need be fully malignant. It is even possible that reactive follicular hyperplasia provides a favorable sanctuary for such cells.

PROGRAMMED CELL DEATH AND NEOPLASIA

Transgenic mice that overexpress Bcl-2 develop tumors documenting the prospective oncogenic importance of interfering with programmed cell death. Because multiple death pathways exist, there may be additional members of this novel category of oncogenes: regulators of programmed cell death (Table 1). Moreover, elegant genetic studies within the nematode, *Caenorhabditis elegans,* have identified both effectors (ced3 and ced4) and a repressor (ced9) in a single cell death pathway.[60,61] Bcl-2 represents one mammalian subset of death pathway genes, antidotes to cell death (Table 1). This subset, like Bcl-2, would contribute to neoplasia by a gain of function mechanism, essentially an autosomal dominant mode. Moreover, many forms of programmed death in mammalian cells can be inhibited or delayed by blocking transcription or translation. This suggests that distinct death pathway genes exist. Once isolated, mammalian death pathway genes may prove to contribute to neoplasia, but by a loss of function mechanism. A classic autosomal recessive mode might be envisioned in which eliminating death genes would result in extended cell survival. Recent studies by Yonish-Rovach et al[62] indicate that this prophesy may already be fulfilled. They noted that

overexpression of wild-type p53 induces apoptosis in a myeloid leukemia cell line.[62] This also emphasizes that single proteins might serve multiple oncogenic roles. The lessons from Bcl-2 provide a hypothesis for further testing over the coming years. Perhaps alteration of a cell death gene (category III) is a frequent primary aberration in neoplasia (Table 1). Extended cell survival may prove to be a key event that increases the opportunity to acquire additional genetic defects in growth and proliferation genes (category I) or tumor suppressor genes (category II).

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