Enforced Bcl-2 Expression Inhibits Antigen-mediated Clonal Elimination of Peripheral B Cells in an Antigen Dose-dependent Manner and Promotes Receptor Editing in Autoreactive, Immature B Cells

By Julie Lang,* B. Arnold,‡ G. Hammerling,‡ Alan W. Harris,§ Stanley Korsmeyer,‖ David R. Russell,* Andreas Strasser,§ and David Nemazee*¶

From the *Division of Basic Sciences, Department of Pediatrics, National Jewish Medical and Research Center, Denver, Colorado 80206; ‡Tumor Immunology Program, German Cancer Research Center, Im Neuenheimer Feld 280, 6900 Heidelberg, Germany; †Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria 3050 Australia; ‖Department of Medicine, Microbiology and Immunology, Howard Hughes Medical Institute, Washington University School of Medicine, St. Louis, Missouri 63198; and ¶Department of Immunology, University of Colorado Health Sciences Center, Denver, Colorado 80220

Summary

The mechanisms that establish immune tolerance in immature and mature B cells appear to be distinct. Membrane-bound autoantigen is thought to induce developmental arrest and receptor editing in immature B cells, whereas mature B cells have shortened lifespans when exposed to the same stimulus. In this study, we used Eμ-bcl-2-22 transgenic (Tg) mice to test the prediction that enforced expression of the Bcl-2 apoptotic inhibitor in B cells would rescue mature, but not immature, B cells from tolerance induction. To monitor tolerance to the natural membrane autoantigen H-2Kb, we bred 3–83md (anti-Kk,b) Ig Tg mice to H-2Kb mice or to mice expressing transgene-driven Kb in the periphery. In 3–83md/bcl-2 Tg mice, deletion of autoreactive B cells induced by peripheral Kb antigen expression in the liver (MT-Kb Tg) or epithelia (KerIV-Kb Tg), was partly or completely inhibited, respectively. Furthermore, Bcl-2 protected peritoneal B-2 B cells from deletion mediated by acute antigen exposure, but this protection could be overcome by higher antigen dose. In contrast to its ability to block peripheral self-tolerance, Bcl-2 overexpression failed to inhibit central tolerance induced by bone marrow antigen expression, but instead, enhanced the receptor editing process. These studies indicate that apoptosis plays distinct roles in central and peripheral B cell tolerance.

Apoptosis is an essential element in the development and homeostasis of many tissues. In the immune system, a number of important processes are regulated through the control of cell death and survival (1–4). Among these processes is immunological tolerance in which encounter with ligands that signal through antigen receptors affect the cell’s subsequent survival (5, 6). The lifespans of lymphocytes in vivo vary widely, from 1–2 d to many weeks (7). Self-antigen can shorten the lifespan of reactive B cells in a number of ways by promoting cell death through developmental arrest (8, 9), by increasing cell turnover (10, 11), by putting cells at a competitive disadvantage with nonautoreactive cells for unknown resources (12), by making the cells sensitive to Fas ligand–mediated killing by T cells (13), or apparently through direct induction of apoptosis (11, 14–18).

One regulator of lymphocyte survival is the Bcl-2 protein (1), which is highly expressed in long-lived lymphocytes and is poorly expressed in cells destined to turn over rapidly (19–26). Bcl-2, and the closely related Bcl-xL (27), are key protein regulators of apoptosis. Overexpression or inappropriate expression of these proteins can play a role in lymphoma development and can allow the continued survival of cells that would otherwise be lost through apoptosis (28–36). However, some forms of apoptosis, including Fas-mediated killing of certain lymphoid cells (37), are not inhibitable by Bcl-2. An important physiological...
role for Bcl-2 is evident from the phenotype of bd-2-deficient mice, which manifest a catastrophic apoptotic loss of mature lymphocytes (38, 39).

The ability of Bcl-2 to block B cell tolerance has been studied in several systems in which ligands that bind to the B cell receptor (BCR)1 can stimulate cell death in vivo or in vitro (9, 15, 33, 40–48). In some cases, these data have been contradictory, suggesting that the ability of Bcl-2 overexpression to block tolerance-mediated apoptosis may be contingent upon the precise quality of the BCR-mediated signal and perhaps other signals that may differ in certain experimental systems. In the well-characterized surface Ig+ B lymphoma WEHI-231, cross-linking of the BCR with antitimmunoglobulin antibody leads to cell death (for review see reference 18). In some studies (43, 44), but not others (40), transfection of bd-2 expression constructs protected the cells from BCR-mediated cell death. When transgenic (Tg) mice with enforced B cell overexpression of Bcl-2 were analyzed for B cell tolerance, bone marrow tolerance was perturbed in one study (9), but not in another (46), whereas peripheral tolerance was inhibited (15, 46). Similarly, germinal center B cells have been shown to be sensitive to killing induced by acute ligation of sIg, and this lethality has been perturbed in one study (9), but not in another (46), whereas peripheral tolerance was inhibited (15, 46). Similarly, germinal center B cells have been shown to be sensitive to killing induced by acute ligation of sIg, and this lethality has been perturbed in one study (9), but not in another (46), whereas peripheral tolerance was inhibited (15, 46). Similarly, germinal center B cells have been shown to be sensitive to killing induced by acute ligation of sIg, and this lethality has been perturbed in one study (9), but not in another (46), whereas peripheral tolerance was inhibited (15, 46). Similarly, germinal center B cells have been shown to be sensitive to killing induced by acute ligation of sIg, and this lethality has been perturbed in one study (9), but not in another (46), whereas peripheral tolerance was inhibited (15, 46). Similarly, germinal center B cells have been shown to be sensitive to killing induced by acute ligation of sIg, and this lethality has been perturbed in one study (9), but not in another (46), whereas peripheral tolerance was inhibited (15, 46). Similarly, germinal center B cells have been shown to be sensitive to killing induced by acute ligation of sIg, and this lethality has been perturbed in one study (9), but not in another (46), whereas peripheral tolerance was inhibited (15, 46).

Resting B cells encountering tissue-specific membrane-bound antigen in the periphery are eliminated, suggesting that in this case, peripheral tolerance is mediated by an apoptotic process (14). In contrast, it has been shown in several systems that immature B cells encountering membrane-bound or nuclear self-antigens in the bone marrow are blocked in their development (8, 9) and either undergo receptor editing (49, 50) or are eliminated (50–52). Our recent finding that in vitro tolerance induction of immature B cells stimulates intense receptor editing with little intermediate cell death (53, 54) suggests that antigen-induced programmed cell death makes only a minor contribution to central tolerance. Assuming that Bcl-2 is a key cell death regulator in B cells, this model predicts that enforced Bcl-2 expression can block clonal elimination of mature, but not immature, self-reactive B cells. To test this, peripheral and central tolerance in 3–83αδ (anti-H-2b) Ig Tg mice were compared to that occurring in 3–83αδ/Eμ-bd-2-22 double-Tg mice, in which Bcl-2 is constitutively expressed in B-lineage cells. (55), and Kε1V-Kb mice, in which Kb is expressed in epithelial cells under the control of the keratin IV promoter (56), were bred several generations onto the B10.D2 background (H-2b) before breeding with other Tg or H-2 congenic mice. 3–83αδ Tg mice, which express IgM and IgD forms of the 3–83 antibody, have been described (14). 3–83αδ mice were backcrossed a minimum of 10 times onto the B10.D2 background. C57BL/6 (H-2b), C3H–H-2Kb/Sfln (C3H.OH: Kd-Kb), and B10.D2/NsnJ (H-2) mice were purchased from Jackson Laboratory (Bar Harbor, ME). Mice overexpressing the human bd-2 gene in B-lineage cells were Tg lines Eμ–bd-2-22 (34) and bd-2-Ig N L (36). These mice were backcrossed a minimum of three generations to B10.D2 and subsequently bred with 3–83αδ Tg mice. The phenotypes of mice bearing either of the bd-2 transgenes were indistinguishable in our study. Unless otherwise stated, the experiments described made use of the Eμ–bd-2-22 line. The 3–83αδ/R AG-1 knockout mice (57) were bred with Eμ–bd-2-22 Tg mice. F2 crosses were set up to generate bd-2/3–83αδ/R AG-1 Tg mice with all transgenes hemizygous except where noted. All mice were bred and maintained under specific pathogen-free conditions in the animal care facility at the National Jewish Medical and Research Center (NJMRC, Denver, CO).

Mice. Tg mice, in which Kb antigen expression is directed to hepatocytes by the sheep metallothionein promoter

Abbreviations used in this paper: BCR, B cell receptor; BrdU, 5-bromo-2'-deoxyuridine; CD, central deletion; N.D., nondeleting; Tg, transgenic.

Materials and Methods

Mice. MT-Kb mice, in which Kb antigen expression is directed to hepatocytes by the sheep metallothionein promoter

Results

Enforced Bd-2 Expression Blocks B Cell Deletion Induced by A cute A ntigen A dministration at L ow D ose, but N ot at H igh D ose. The 3–83αδ Ig transgene encodes a BCR that is reactive to a number of MHC class I alloforms including...

Downloaded from
1515 Lang et al.

K$k$, D$k$, and K$b$, but fails to bind to H-2$d$; thus, 3–83 md/H-2$d$ mice contain a virtually monoclonal B cell population bearing the 3–83 BCR and are called nondeleting (ND)Tg mice (14). To probe the ability of enforced Bcl-2 expression to block tolerance to acute intraperitoneal antigen challenge, we injected NDTg and NDTg/bcl-2 mice with antigen-bearing cells, a protocol that stimulates rapid apoptosis of fully mature antigen-specific peritoneal B cells (15, 16).

Enforced Bcl-2 Expression Partially Blocks B Cell Deletion Induced by Hepatocyte-targeted Kb and Completely Blocks B Cell Deletion Induced by Epithelial Cell-targeted K$b$. To study peripheral B cell tolerance to natural chronic autoantigen exposure, we generated 3–83 md mice bearing MT-K$b$ or KerIV-K$b$ transgenes, which target cell surface expression of the K$b$ protein to hepatocytes or epithelia, respectively (55, 56). Relative to antigen-free mice, antigen-bearing 3–83 md mice had profoundly reduced B cell numbers in the lymph nodes (Fig. 2 A, top; Fig. 2 B, compare H-2$d$ to MT-K$b$ and KerIV-K$b$), and substantial, but on average, incomplete deletion of the B cells in the spleen (Fig. 2 C, top; Fig. 2 D and reference 14). Control mice bearing antigen on all tissues (Fig. 2, H-2$b$) exhibited the phenotype of central B cell tolerance in which antigen-reactive cells were absent from the spleen. In the mice that demonstrated peripheral B cell deletion (3–83 md/MT-K$b$ or 3–83 md/KerIV-K$b$), the remaining splenic cells manifested rapid turnover as assessed by their BrdU uptake over a 1-wk labeling period (Fig. 3). It is important to note that the MT-K$b$/3–83 md mice showed a more profound tolerance than KerIV-K$b$/3–83 md mice.
Furthermore, lymph node cells from B cells had incorporated BrdU over a 1-wk period (Fig. 3). B cell turnover was also normalized, and only Tg mice in which B cell deletion was effectively blocked, and

3–83

controls.

The ability of lymph node and spleen cells to secrete 3–83 antibodies in vitro and in vivo analysis of antibody secretion. Lymph node and spleen cells from mice of the indicated genotypes were cultured with antigen; data are presented as mean ± SEM with number of mice (n) analyzed shown. The table below the graph shows Tg genotypes (3–83, bd-2, MT-Kb, and/or KerIV-Kb) and H-2 haplotypes (d, no antigen; b, antigen). In C and D the RAG-1 genotype of analyzed mice (+/+ or −/−) is indicated.

Figure 4. In vitro and in vivo analysis of antibody secretion. Lymph node and spleen cells from mice of the indicated genotypes were cultured with antigen; data are presented as mean ± SEM with number of mice (n) analyzed shown. The table below the graph shows Tg genotypes (3–83, bd-2, MT-Kb, and/or KerIV-Kb) and H-2 haplotypes (d, no antigen; b, antigen). In C and D the RAG-1 genotype of analyzed mice (+/+ or −/−) is indicated.

Enforced Bcl-2 Expression Does Not Block Central B Cell Tolerance. The Eμ–bd-2–22 transgene drives expression of functionally active Bcl-2 in immature bone marrow B cells that normally lack Bcl-2 expression (46). To test the effect of this transgene on central B cell tolerance, we bred H-2b mice to 3–83μδ and bd-2/3–83μδ mice, generating central deleting (CD)Tg and CDTg/bd-2 mice, respectively. Like CDTg mice, CDTg/bd-2 mice lacked Id+ B cells in the peripheral lymphoid organs (Fig. 2, H-2b; Table 1) and Id+ antibodies in the sera (Fig. 4 C). In addition, no Id+ antibodies were found in the supernatants of LPS-stimulated spleen cells from CDTg or CDTg/bd-2 mice, whereas NDTg and NDTg/bd-2 controls had significant levels of Id+ antibodies in both the sera and LPS culture supernatants (Fig. 4 A;
Table 1. Effect of B-d-2 O verexpression on T tolerance Induction and R oceptor Editing in 3-83 Immunoglobulin T ransgenic M ices

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Lymph Node</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>54.1⁺</td>
<td>IgD⁺, λ⁺</td>
</tr>
<tr>
<td>NDTg +/+</td>
<td>82 ± 16 (12)</td>
<td>0.6 ± 0.2 (11)</td>
</tr>
<tr>
<td>NDTg/bd-2 ++/+</td>
<td>160 ± 40 (9)</td>
<td>1.6 ± 0.4 (9)</td>
</tr>
<tr>
<td>NDTg −/−</td>
<td>30 ± 2 (2)</td>
<td>0 (1)</td>
</tr>
<tr>
<td>NDTg/bd-2 −/−</td>
<td>580 (1)</td>
<td>2.1 (1)</td>
</tr>
<tr>
<td>CDTg +/+</td>
<td>0.3 ± 0.1 (19)</td>
<td>3.3 ± 0.6 (19)</td>
</tr>
<tr>
<td>CDTg/bd-2 ++/+</td>
<td>0.6 ± 0.2 (19)</td>
<td>14 ± 3 (18)</td>
</tr>
<tr>
<td>CDTg −/−</td>
<td>0.1 ± 0.1 (5)</td>
<td>0.1 ± 0.1 (3)</td>
</tr>
<tr>
<td>CDTg/bd-2 −/−</td>
<td>0.8 ± 0.4 (6)</td>
<td>0.1 ± 0.1 (4)</td>
</tr>
</tbody>
</table>

Absolute number of Idiotype-positive (54.1⁺), “edited” (IgD⁺, λ⁺), and immature (B220⁺, IgM⁻) B cells in 3-83 immunoglobulin transgenic mice in the presence (CDTg) or absence (NDTg) of antigen in the bone marrow. In the genotype column +/+ refers to normal levels of RAG-1 whereas −/− refers to RAG-1 homozygous knock-out mice. All numbers shown are averages ± SEM divided by 10⁵. The numbers in parenthesis refer to sample size.
indicative of immature B cells, these cells expressed RAG-2 messenger RNA transcripts (data not shown). These cells failed to secrete antibodies in LPS cultures and in vivo, as no antibodies were detected in the serum (Fig. 4 C). In CDTg/RAG-1−/− mice, no serum IgM was detected because RAG deficiency prevented development of Id− B cells (Fig. 4 D). These splenic immature B cells were short lived, as ~50% of the cells had incorporated BrdU + after 1 wk compared to only ~20% of B220+ cells from non-Tg or NDTg mice (data not shown). Thus, in contrast to its effect on peripheral tolerance, the bd-2 transgene appeared to have only a subtle effect on central B cell tolerance, allowing a subset of autoreactive nonfunctional, immature B cells to survive in the spleen for a short time.

Enforced Bcl-2 expression increases the number of Id− B cells. Bcl-2 overexpression increased the numbers of nonautoreactive, Id− B cells that developed in CDTg/ H-2b/ 3–83μ6 Tg mice by two- to fivefold (Fig. 7 B; Table 1). In contrast to the results with the mice bearing antigen targeted to peripheral tissues in which autoreactive B cells with enforced Bcl-2 expression were spared, the B cells appearing in the spleen and lymph nodes of CDTg/bd-2 mice were nonautoreactive and had undergone receptor editing (Figs. 2 and 5). One clear indication of receptor editing were nonautoreactive and had undergone receptor editing was the appearance of B cells bearing both the Tg heavy chain, as detected with anti-IgDa, and endogenously encoded light chains, detected with λ chain-specific antibody (Fig. 7 A, and reference 49). The increase in the percentages of "edited" cells was the result of an increase in the total number of these cells in the lymphoid organs (Table 1). Thus, Bcl-2 overexpression apparently enhanced receptor editing or allowed survival of cells that had undergone receptor editing, or both.

Altered Ig-κ/λ ratio suggests that Bcl-2 O overexpression promotes receptor editing. The elevated frequency of nonautoreactive B cells in the CDTg/bd-2 mice could theoretically have been the result of expansion or prolonged survival of B cells in the peripheral lymphoid organs. This simple explanation would predict that the relative frequencies of B cells bearing endogenous Ig-κ and Ig-λ light chains would be the same in both CDTg and CDTg/bd-2 mice.

To test this notion, we measured the frequencies of Id− B cells that expressed κ or λ light chains. Fig. 8 A shows that most of the increase in peripheral B cells of the CDTg/bd-2 mice could be accounted for by an increase in λ+ B cells, suggesting that the bd-2 transgene influences B cells undergoing light chain gene rearrangement. Interestingly, this elevated λ expression was also observed in bd-2 Tg mice lacking Ig transgenes (Fig. 8 B), which consistently had a significantly higher percentage (~17%) of λ+ B cells compared to non-Tg mice (~6% λ+).

Discussion

This study provides insight into the mechanisms by which Bcl-2 can perturb B cell tolerance. First, we have found that natural self-proteins expressed on epithelial cells can mediate B cell tolerance by accelerating cell death through a Bcl-2-inhibitable pathway. We have also found that foreign antigens, administered acutely into the perito-

Figure 7. Increased percentage of IgDα, λ− cells in CDTg mice with enforced Bcl-2 expression. 3–83μ6 Tg mice were bred with bd-2 Tg mice in the presence (CDTg) or absence (NDTg) of bone marrow antigen expression. To determine the extent of receptor editing, lymph node cells were double stained for Tg heavy chain (anti-IgDα) and endogenous light chain (anti-λ). (A) Each row illustrates FACSS® analysis from independent experiments. (B) Summary data showing percentage of IgDα-positive, Id− cells in both CDTg and CDTg mice with Bcl-2 overexpression. Data are presented as mean ± SEM.

Figure 8. Bcl-2 overexpression increases λ/κ ratio of B cells in both 3–83 CDTg mice and non-Ig Tg mice. (A) Lymph node cells from CDTg and CDTg/bd-2 mice were double stained with anti-IgDα and anti-κ antibodies. Percentage of lymph node cells staining positive for IgDα and negative for κ were scored as κ positive. (B) Increased percentage of λ expressing B cells in (non-Ig Tg) bd-2 Tg mice. The percentage of IgM+ cells that were also λ positive was determined. Data presented as mean ± SEM. Numbers above bars reflect number of mice examined.
neal cavity, can cause rapid B cell deletion that is rescued in an antigen dose-dependent manner by enforced Bcl-2 expression. Finally, we have made the novel observation that enforced Bcl-2 expression in immature B cells promotes receptor editing.

Consistent with the notion that tolerance mechanisms differ in immature and mature B cells, Bcl-2 overexpression was able to abrogate peripheral B cell tolerance, while only subtly altering central B cell tolerance. The ability of Bcl-2 to confer protection from deletion induced by peripherally expressed membrane self-antigen suggests that mature, auto-reactive B cells are tolerez by apoptosis induction. These results are partly in agreement with the studies of Honjo’s group (15, 46), who studied the effects of intraperitoneal injections of antitraimmunoglobulins and self-erythrocytes, rather than foreign antigens, on peritoneal B cells that were largely of the B-1 lineage. The signals regulating apoptosis and immune tolerance in B-1 and B-2 subsets are likely to differ (5, 6, 18, 60). In our 3-83 Tg mice, the Id B+ B cells, including those in the peritoneal cavity, are almost entirely B-2 B cells. Thus, our data are clear evidence for deletion of peritoneal B-2 B cells upon antigen encounter in vivo, which is inhibited by Bcl-2 overexpression in a dose-dependent manner. No such dose dependence was found in the study by Nisitani et al. (46), but analogous results have been obtained in bd-2 transfected WEHI-231 cells in which anti-IgM-mediated death could be blocked at low but not high antibody dose (43). These differences may reflect a difference in the density of tolerogenic antigen expression, in BCR affinity for antigen, or in the relative tolerance susceptibility of B-1 and B-2 B cells.

Similar to the results with acute antigen administration, peripheral B cells tolerated by chronic exposure to natural autoantigens were variably rescued by enforced Bcl-2 overexpression. Furthermore, the extent of rescue afforded by the Bcl-2 transgene appeared to be inversely correlated with the degree of deletion in its absence: in MT-Kb mice, which have a significant population of Kb expression in the density of tolerogenic antigen expression, in BCR affinity for antigen, or in the relative tolerance susceptibility of B-1 and B-2 B cells.

Consistent with the notion that tolerance mechanisms differ in immature and mature B cells, Bcl-2 overexpression was able to abrogate peripheral B cell tolerance, while only subtly altering central B cell tolerance. The ability of Bcl-2 to confer protection from deletion induced by peripherally expressed membrane self-antigen suggests that mature, auto-reactive B cells are tolerated by apoptosis induction. These results are partly in agreement with the studies of Honjo’s group (15, 46), who studied the effects of intraperitoneal injections of antitraimmunoglobulins and self-erythrocytes, rather than foreign antigens, on peritoneal B cells that were largely of the B-1 lineage. The signals regulating apoptosis and immune tolerance in B-1 and B-2 subsets are likely to differ (5, 6, 18, 60). In our 3-83 Tg mice, the Id B+ B cells, including those in the peritoneal cavity, are almost entirely B-2 B cells. Thus, our data are clear evidence for deletion of peritoneal B-2 B cells upon antigen encounter in vivo, which is inhibited by Bcl-2 overexpression in a dose-dependent manner. No such dose dependence was found in the study by Nisitani et al. (46), but analogous results have been obtained in bd-2 transfected WEHI-231 cells in which anti-IgM-mediated death could be blocked at low but not high antibody dose (43). These differences may reflect a difference in the density of tolerogenic antigen expression, in BCR affinity for antigen, or in the relative tolerance susceptibility of B-1 and B-2 B cells.

Similar to the results with acute antigen administration, peripheral B cells tolerated by chronic exposure to natural autoantigens were variably rescued by enforced Bcl-2 overexpression. Furthermore, the extent of rescue afforded by the Bcl-2 transgene appeared to be inversely correlated with the degree of deletion in its absence: in MT-Kb mice, which have a significant population of Kb expression in the density of tolerogenic antigen expression, in BCR affinity for antigen, or in the relative tolerance susceptibility of B-1 and B-2 B cells.
B cells. We propose that the bd-2 transgene expression allows autoreactive B cells more potential rearrangement attempts, promoting the development of more nonautoreactive B cells bearing "edited" BCRs. This is consistent with data concerning transgene-driven Bcl-2 overexpression in thymocytes in which positive selection is enhanced as a result of increased endogenous TCR rearrangements, presumably due to the increased lifespan of the double-positive thymocytes expressing the bd-2 transgene (1). The finding that Eu–bd-2-22 Tg mice have consistently high percentages of λ– B cells suggests that receptor editing is enhanced even in the absence of antibody transgenes. Data from R olink et al. have indicated that the Eu–bd-2-22 transgene prolongs in vitro survival of B cells undergoing light chain gene rearrangement resulting in high λ/κ ratios in cultured B cells (71).

One implication of our study is that altered or defective apoptosis in B cells encountering self-antigen in the periphery could provide a pool of potentially functional autoreactive B cells. In this study we have observed that the cells rescued from elimination by enforced Bcl-2 expression could sometimes also acquire functional reactivity. Further studies are needed to fully establish their functional capacity, but these results further suggest that although multiple independent levels of regulation are available to limit autoreactivity at each step in differentiation, tolerance to certain autoantigens may be definitively abrogated by a Bcl-2-inhibitable pathway.

The authors are grateful to Shirley Sobus and Bill Townsend for flow cytometry assistance, Leigh Landskroner for illustrations, R usell Y ager and Alicia Kuhl for technical assistance, and members of our laboratory for critical review of the manuscript.

This work was supported by a Howard Hughes Medical Institute Predoctoral Fellowship (to J. Lang), by the Arthritis Foundation (to D. Nemazee), the National Institutes of Health (R01 GM 44809, R01 AI 33608, and KO4 AI 01161; to D. Nemazee), and the National Health and Medical Research Council (Canberra, Australia; to A. Strasser). A. Strasser is a scholar of the Leukemia Society of America and a recipient of a Clinical Investigator Award from the Cancer Research Institute.

Address correspondence to Dr. Nemazee, Department of Pediatrics, National Jewish Medical and Research Center, 1400 Jackson St., Denver, CO 80206. Phone: 303-398-1623; FAX: 303-398-1225; E-mail: nemazee@njc.org

Received for publication 23 July 1997 and in revised form 4 September 1997.

References

20. Li, Y.S., K. Hayakawa, and R.R. Hardy. 1993. The regu-
lated expression of B lineage associated genes during B cell
differentiation in bone marrow and fetal liver. J. Exp. Med. 3:
951–960.
1995. Expression of Bcl-2, Bcl-x, and Bax after T cell activa-
thymocytes and in peripheral T lymphocytes. J. Immunol. 5:
2546–2554.
plays restricted distribution during T cell development and in-
hibits multiple forms of apoptosis but not clonal deletion in
24. Gonzalez-Garcia, M., R. Perez-Ballesteros, L. Ding, L. Duan,
the major bcl-x mRN A form expressed during murine devel-
opment and its product localizes to mitochondria. Development
(Camb.). 10:3033–3042.
Bcl-2 expression during T-cell development: early loss and late
return occur at specific stages of commitment to differentiation
and susceptibility to cell death in B lymphocytes. EMBO J.
5:1352–1588.
27. Boise, L.H., M. Gonzalez-Garcia, C.E. Postema, J. Ding, T.
1993. bcl-x, a bcl-2-related gene that functions as a dominant
28. Cory, S., A.W. Harris, and A. Strasser. 1994. Insights from
transgenic mice regarding the role of bcl-2 in normal and neo-
plastic lymphoid cells. Philos. Trans. R. Soc. Lond. Ser. B.
Bcl-2 expression promotes B- but not T-lymphoid develop-
ment in scid mice. Nat. (Lond.). 6470:457–460.
Differential effects of Bcl-2 on T and B cells in transgenic mice.
lation in scid mice.
32. Woodland, R.T., M.R. Schmidt, J.E. Ales-Martinez, L. Ding, M. Gonzalez-Gar-
by bcl-2-dependent and independent mechanisms in B lym-
phoma cells. EMBO J. 5:1901–1907.
33. Benhamou, L.E., T. Watanabe, D. Kitamura, P.A. Cazenave,
and P. Sarthou. 1994. Signaling properties of anti-immuno-
globulin-resistant variants of WEHI-231 B lymphoma cells.
34. Choi, M.S., L.H. Boise, A.R. Gottschalk, J. Quintans, C.B.
Thompson, and G.G. Klaus. 1995. The role of bcl-XL in
CD40-mediated rescue from anti-mu-induced apoptosis in
1357.
35. Fang, W., J.J. Rivard, J.A. Ganser, T.W. LeBien, K.A. Nath,
231 B lymphocytes from oxant-mediated death following
1994. Role of bcl-2 and IL-5 in the regulation of anti-IgM-
induced growth arrest and apoptosis in immature B cell lines.
A cooperative regulation model for B cell clonal deletion. J.
Immunol. 7:3294–3305.
37. Merino, R., D.A. Grillot, P.L. Simonian, S. Muthukkumar,
of anti-IgM-induced B cell apoptosis by Bcl-xL and CD40 in
WEHI-231 cells. Disassociation from cell cycle arrest and de-
pendence on the avidity of the antibody-IgM receptor inter-
Honjo. 1993. The bcl-2 gene product inhibits clonal deletion
of self-reactive B lymphocytes in the periphery but not in the
bone marrow. J. Exp. Med. 4:1247–1254.
39. Pulendran, B., G. Kannourakis, S. Nosiri, K.G. Smith, and
G.J. Nossal. 1995. Soluble antigen can cause enhanced apo-
B-cell death and elimination during germinal-centre immune
41. Lang et al. 1521

42. Choi, M.S., L.H. Boise, A.R. Gottschalk, J. Quintans, C.B.
Thompson, and G.G. Klaus. 1995. The role of bcl-XL in
CD40-mediated rescue from anti-mu-induced apoptosis in
1357.
43. Fang, W., J.J. Rivard, J.A. Ganser, T.W. LeBien, K.A. Nath,
231 B lymphocytes from oxant-mediated death following
44. Kamesaki, H., J.A. Zwiebel, J.C. Reed, and J. Cossman.
1994. Role of bcl-2 and IL-5 in the regulation of anti-IgM-
induced growth arrest and apoptosis in immature B cell lines.
A cooperative regulation model for B cell clonal deletion. J.
Immunol. 7:3294–3305.
45. Merino, R., D.A. Grillot, P.L. Simonian, S. Muthukkumar,
of anti-IgM-induced B cell apoptosis by Bcl-xL and CD40 in
WEHI-231 cells. Disassociation from cell cycle arrest and de-
pendence on the avidity of the antibody-IgM receptor inter-
Honjo. 1993. The bcl-2 gene product inhibits clonal deletion
of self-reactive B lymphocytes in the periphery but not in the
bone marrow. J. Exp. Med. 4:1247–1254.
47. Pulendran, B., G. Kannourakis, S. Nosiri, K.G. Smith, and
G.J. Nossal. 1995. Soluble antigen can cause enhanced apo-
B-cell death and elimination during germinal-centre immune
editing in self-reactive bone marrow B cells. J. Exp. Med. 4:
1009–1020.
Receptor editing: an approach by autoreactive B cells to escape tolerance. J. Exp. Med. 4:999–1008.


