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Deregulated Bcl-2-Immunoglobulin Transgene Expands a Resting but Responsive Immunoglobulin M and D-Expressing B-Cell Population

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We characterized the basis for the follicular lymphoproliferation in transgenic mice bearing a Bcl-2-immunoglobulin (Bcl-2-Ig) minigene representing the t(14;18) of human follicular lymphoma. Discriminatory S1 nuclease protection assays revealed that the Bcl-2-Ig transgene was overexpressed relative to endogenous mouse *Bcl-2* in spleen and thymus. Western (immunoblot) analysis demonstrated the overproduction of the human 25-kilodalton Bcl-2 protein, which arose from the transgene, in spleen, thymus, and the expanded B-cell subset. Despite the generalized lymphoid pattern of deregulation, two-color flow cytometry and density gradient centrifugation indicated that the expanded lymphocytes were predominantly small, resting B cells coexpressing B220, immunoglobulin M (IgM), IgD, Ia, and κ. Cell cycle analysis confirmed that about 97% of these expanded B cells reside in G_0/G_1. An extensive characterization of transgenic lines revealed a fourfold excess of IgM-IgD-expressing B cells in spleen and dramatically increased numbers in bone marrow. While resting, these cells proliferated in response to lipopolysaccharide and anti-IgM and demonstrated normal B-cell colony formation in soft agar. Moreover, these B cells, which demonstrated an extended survival in vitro even in the absence of stroma, were also resting in G_0, yet were capable of proliferative responses. These findings provide consistent evidence that the accumulation of B cells after Bcl-2 overproduction is secondary to prolonged cell survival and not increased cell cycling. This suggests a unique role for *Bcl-2* as a proto-oncogene that enhances cell survival independent of promoting cell division.

Specific types of chromosomal translocations are highly associated with distinct malignant diseases. Previously identified proto-oncogenes were shown to flank several of these breakpoints and were either fused to other genes, e.g., *bcr-abl* (26), or deregulated as with *myc* (2, 7, 27). These models suggested that examination of other recurrent translocations would reveal new proto-oncogenes at their breakpoints. The t(14;18)(q32;q21) of follicular B-cell lymphoma has provided one such opportunity. The molecular cloning of the derivative 14 breakpoint (1, 3, 28) led to the identification of the putative proto-oncogene *Bcl-2* at 18q21. The translocation juxtaposed *Bcl-2* with the immunoglobulin locus, resulting in inappropriately elevated levels of a Bcl-2-immunoglobulin (Bcl-2-Ig) fusion RNA for the mature stage of these follicular B-cell tumors (4, 10, 25).

To assess the prospective importance of the t(14;18), we created a transgenic mouse model bearing a Bcl-2-Ig minigene. These mice initially develop an indolent polyclonal follicular lymphoproliferation rather than a life-threatening monoclonal malignant disease (19). These observations raised important questions as to whether the Bcl-2-Ig minigene construct was deregulated in its expression, resulting in inappropriate amounts of RNA and overproduction of the 25-kilodalton (kDa) Bcl-2 protein. We demonstrated that despite the overexpression of Bcl-2 in both lymphoid lineages, the dramatic effects were on B cells, while T-cell numbers were not increased and T-cell subsets were not altered. The expanded polyclonal B cells proved to be a distinct subset of high-density, resting yet responsive immunoglobulin M (IgM)-IgD-expressing B cells.

MATERIALS AND METHODS

Flow immunofluorescence analysis of single-cell suspensions. The techniques and monoclonal antibody reagents used for single-color flow cytometry have been presented previously (19).

For two-color analysis, single-cell suspensions were stained with a primary biotin-conjugated rat monoclonal antibody (MAb). The second incubation contained an avidin-phycoerythrin conjugate (Biomeda Corp., Foster City, Calif.) and fluorescein-conjugated rat anti-mouse MAb. Contour plots from the analysis of 10^4 viable cells were plotted on a 4-decade $\log_{10}$ scale of increasing green fluorescence on the x axis and red fluorescence on the y axis.

Density gradient centrifugation and B-cell purification. Purified splenic B cells were prepared by incubation with AT83A (anti-Thy1.2 [24]) antibody or J1j (19) for 30 min on ice. Cells were washed once with Hanks balanced salt solution and suspended in Iscove's modified Dulbecco medium containing Low-Tox-M rabbit complement (Cedarlane Laboratories, Hornby, Ontario, Canada) for 1 h at 37°C. Cells were washed three times in Hanks balanced salt solution before layering onto Percoll (Pharmacia, Uppsala, Sweden) density gradients and then spun at 1,400 × g for 30 min. Cells at the density layer interfaces were harvested, washed three times in phosphate-buffered saline, stained, and analyzed by flow cytometry.

S1 nuclease protection assay. A segment of the mouse β-actin gene containing exon 1, intron 1, and a portion of

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exon 2 was cloned into the *Bam*HI-*Eco*RI sites of M13mp10. An [α-^{32}P]dCTP uniformly labeled antisense single-stranded DNA probe was prepared by primer extension. The DNA was cut with *Ava*II, and a single-stranded probe was prepared from an alkaline agarose gel. The 600-base-pair murine Bcl-2 probe extended from 50 base pairs upstream of the ATG start site to the 3′ *Bam*HI site of exon 2 (20) and was cloned into the *Hin*dIII-*Bam*HI restriction sites in pBluescript (Strategene, San Diego, Calif.). The human Bcl-2 probe consisted of a 350-bp *Pst*I-*Bam*HI portion of the exon 2-coding sequence cloned into pBluescript. The 5′ end of the antisense strand of the human and mouse Bcl-2 probes was end labeled at the *Bam*HI site with [γ-^{32}P]dATP and resulted in approximately equivalent specific activities (10,000 and 7,500 cpm/ng of DNA, respectively). The probes (100,000 cpm) were hybridized for 16 h at 55°C with the appropriate RNA samples and then digested with 200 U of nuclease S1 for 1 h at 37°C (16). Protected DNA fragments were size separated on a 6% sequencing gel, fixed, dried, and autoradiographed.

Cell culture and CFU-B assay. Primary spleen cell cultures were established with Dulbecco modified Eagle medium supplemented with 5% fetal calf serum as described previously (19). Spleen cell suspensions for the B-cell colony (CFU-B) assay were prepared at a density of 2.5×10^5 mononuclear cells per ml in McCoy medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 15% fetal calf serum (Hyclone Laboratories, Logan, Utah), 2 mM L-glutamine, 16 μg of L-asparagine per ml, 8 μg of L-serine per ml, and 5×10^{-5} M beta-mercaptoethanol, similar to that described by Kincade et al. (13). Mononuclear cells were plated at a density of 5×10^4/ml by mixing with prewarmed Bacto-Agar (Difco Laboratories, Detroit, Mich.) and additional medium or medium supplemented with lipopolysaccharide (LPS) (Difco). Samples of 1 ml were added to 35-mm culture dishes (Nunc, Inc., Naperville, Ill.). Triplicate cultures were maintained at 37°C in a humidified atmosphere of 5% CO_2. Colonies were defined as clusters of greater than 20 cells and were counted on day 5 of culture.

Cell cycle analysis. Single-cell suspensions from fresh tissue were washed in phosphate-buffered saline, fixed in 70% ethanol, and stained with propidium iodide (10 μg/ml) and RNase (62 μg/ml) in phosphate-buffered saline in a manner similar to that presented by Crissman and Steinkamp (6). Relative DNA content (red fluorescence) was assessed by flow cytometry with a FACSCAN analyzer (Becton Dickinson and Co., Paramus, N.J.).

DNA and RNA levels of cultured splenic mononuclear cells were simultaneously determined by acridine orange staining (17) and analyzed by flow cytometry.

Western blot (immunoblot) analysis. Single-cell suspensions were lysed in 1% Triton X-100–0.15 M NaCl–10 mM Tris (pH 7.4) with 50 μg of phenylmethylsulfonyl fluoride per ml and 2 μg of aprotinin per ml at 4°C for 30 min. Cleared lysates from 1×10^6 cells were boiled in sodium dodecyl sulfate sample buffer (2% sodium dodecyl sulfate, 200 mM β-mercaptoethanol) for 4 min before being run on a sodium dodecyl sulfate-12.5% polyacrylamide gel. Gels were transferred overnight (12 to 16 h) to nitrocellulose (Nitroplus 2000; Micron Separations, Inc., Westboro, Mass.). Blots were blocked in 3% nonfat dry milk (Carnation) for 2 h. Rabbit polyclonal antiserum to purified Bcl-2 from a procaryotic expression vector (D. Hockenberry and S. J. Korsmeyer, unpublished data) was used at a 1:100 dilution in phosphate-buffered saline–0.05% Tween 20 for 2 h. Hamster anti-Bcl-2 MAb (D. Hockenberry et al., unpublished data) was used as undiluted hybridoma supernatant for 2 h. Horseradish peroxidase-conjugated affinity-isolated goat anti-rabbit IgG antibody (Tago, Burlingame, Calif.) was incubated with the blot for 2 h. The secondary reagent for the hamster antibody was a biotinylated goat anti-hamster IgG antibody (gift of R. Schrieber) incubated for 2 h followed by horseradish peroxidase-conjugated streptavidin (Zymed) at 1:1,000 dilution for 1 h. The blot was developed with diazobenzidine and enhanced with nickel chloride (0.03%).

RESULTS

Deregulated expression of Bcl-2-Ig transgene. To assess the relative levels of endogenous mouse Bcl-2 RNA and human Bcl-2-Ig transgene RNA, we developed species-specific S1 nuclease protection assays. Endogenous mouse RNA protected a 600-nucleotide (nt) fragment of murine Bcl-2 exon 2 probe, while transcripts arising from the Bcl-2-Ig transgene protected 350 nt of the human Bcl-2 exon 2 probe (Fig. 1). A quantitative S1 assessment of mouse β-actin RNA ensured that equivalent amounts of intact spleen and thymus RNA were being compared from transgenic mice (+) and nontransgenic controls (−). The end-labeled human and murine Bcl-2 probes had roughly comparable specific activities. The human pre-B-cell line, REH, and the murine pro-B-cell line, FL5.12, both known to highly express Bcl-2, provided standards of comparison (Fig. 1). The majority of Bcl-2 RNA in transgenic spleen originated from the Bcl-2-Ig transgene. The level of human Bcl-2 RNA in transgenic spleen approached the level found in pre-B cells (REH) and t(14;18)-bearing lymphomas. The thymus consistently demonstrated transgene RNA at levels less than those in spleen. Consistent with the prior observation that Bcl-2 transcription is associated with lymphocyte activation, only low levels of endogenous mouse Bcl-2 were present within control (−) spleen and thymus. Transgenic spleen and thymus (+) possessed somewhat less endogenous murine Bcl-2 than their normal (−) counterparts.

Overproduced Bcl-2 25-kDa protein. We had prepared a hamster MAb, 6C8, that specifically detects human but not mouse Bcl-2 and a rabbit heteroantibody that recognizes both human and mouse Bcl-2 (Hockenberry and Korsmeyer, unpublished data). Cell lysates of 1×10^6 normal (−) or transgenic (+) spleen and thymus cells were compared with the t(14;18)-bearing human lymphoma cell line, SU-DHL-6. The amount of 25-kDa protein in transgenic spleen approached that found in human t(14;18) cell lines and was greater than levels in transgenic thymus (Fig. 2). Studies presented below documented that a small, resting B cell is the expanded population of lymphocytes in Bcl-2-Ig transgenics. Purified populations of these cells (B^+) demonstrated levels of Bcl-2 protein comparable to those in total spleen (+) (Fig. 2). Consistent with the low levels of endogenous Bcl-2 RNA in normal (−) spleen and thymus, the heteroantibody detected no 25-kDa protein in spleen, thymus, or resting B cells from normal animals. However, 25-kDa Bcl-2 protein was easily detected by the heteroantibody within the comparable transgenic (+) samples. Thus, the abundant Bcl-2-Ig RNA results in overproduced Bcl-2 protein in splenic B cells and thymocytes.

Excess B cells in spleen, lymph node, and bone marrow. Given the overexpression and overproduction of transgenic Bcl-2 in spleen and thymus, we wished to quantitate the precise subsets of lymphocytes affected and examine their organ distribution. Table 1 summarizes the flow cytometry immunophenotype data compiled on spleen, bone marrow,

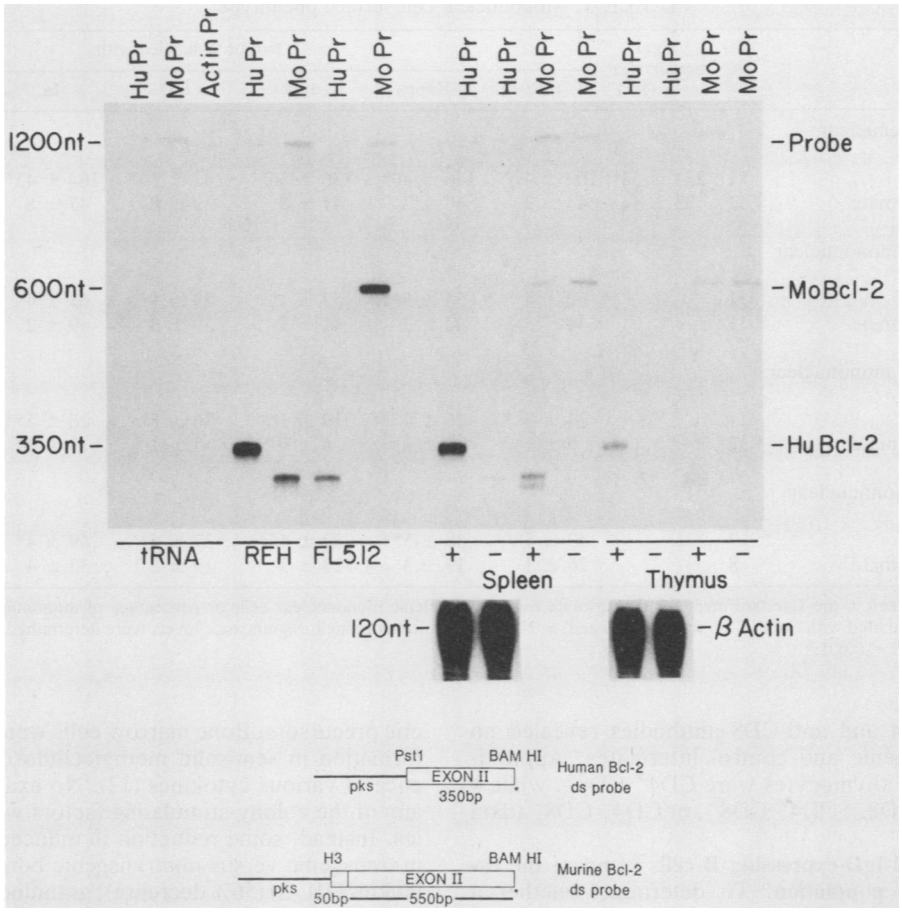

FIG. 1. S1 nuclease protection assay. Total cellular RNA(s) (20 μg) from transgenic (+) and control littermate (−) spleen and thymus was hybridized with either mouse (MoPr) or human (HuPr) Bcl-2 end-labeled probes as indicated (*) and protected 350- and 600-nt fragments, respectively. A uniformly labeled single-stranded antisense murine β-actin probe served as an internal standard and protected a 120-nt fragment. The smaller (~300-nt) protected fragments observed reflect cross hybridization of either murine probe with human message or the converse. ds, Double stranded; bp, base pairs.

and lymph node from four transgenic founder lines. No individual transgenic line varied significantly from other transgenics in its percentage or absolute numbers of cells, so that data for the transgenics were combined and compared with data from the normal littermates (Table 1). The spleen, as an encapsulated organ, allowed comparison of total numbers of cells. Splenic mononuclear cells were significantly expanded in transgenics [(234 ± 58) × 10^6] versus normals [(93 ± 14) × 10^6] when examined at age 8 to 20 weeks ($P < 0.05$). A three- to fourfold increment in mononuclear cells expressing any of the B-cell surface markers B220, IgM, IgD, kappa, and Ia was noted (Table 1). Highly significant increases in the percentage of B cells were also observed in transgenic bone marrow and lymph node. Bone marrow was striking, with the transgenics displaying 19% IgM-expressing and 26% IgD-expressing B cells. A trend of reciprocally decreased T-cell percentages (Thy1, L3T4) was noted in the spleen and achieved statistical significance in the lymph nodes of transgenics (Table 1).

Spleen, lymph node, and marrow showed no significant increase in the percentage of T cells, while the absolute numbers of thymocytes were reduced in Bcl-2-Ig transgenics. Transgenic mice at 8 weeks of age possessed (91.5 ± 10) × 10^6 cells per thymus in contrast to (160 ± 33.8) × 10^6 in normal littermates ($P < 0.001$). Two-color flow cytometry

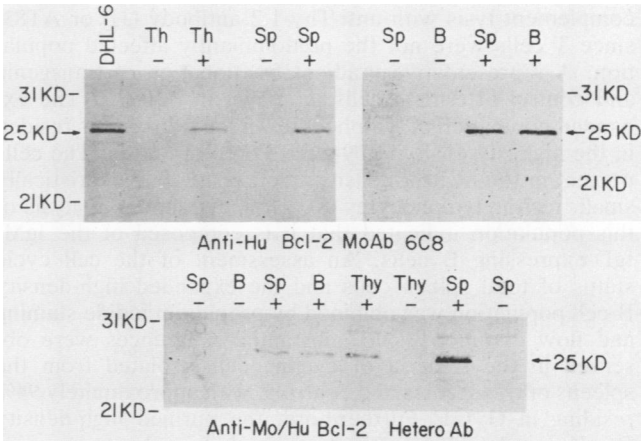

FIG. 2. Western blot analysis of 25-kDa (KD) Bcl-2 protein. Lysates from 1 × 10^6 thymus (Th), spleen (Sp), or high-density B cells (B) from transgenics (+) or nontransgenics (−) were assessed with the anti-human (Hu) Bcl-2 hamster MAb (MoAb) 6C8 or a heteroantibody (Hetero Ab) that recognized both mouse (Mo) and human Bcl-2.

TABLE 1. Mononuclear cell surface phenotype[a]

Cell	n	Total mononuclear cells (10^6)	No. or % labeled with: B220	Kappa	IgM	IgD	Ia	Thy1	L3T4
Absolute splenic mononuclear cells ($\times 10^6$)									
Transgenic	11	234 ± 58*	169 ± 47*	146 ± 40*	136 ± 36*	142 ± 39*	164 ± 45*	42 ± 9	47 ± 13
Nontransgenic littermate	11	93 ± 14	43 ± 9	40 ± 7	41 ± 8	39 ± 8	47 ± 8	27 ± 5	23 ± 4
Percentage of splenic mononuclear cells									
Transgenic	11		62 ± 4**	55 ± 5**	53 ± 4*	53 ± 5*	63 ± 4*	21 ± 3	19 ± 2
Nontransgenic littermate	11		44 ± 3	42 ± 3	41 ± 3	39 ± 3	49 ± 2	29 ± 2	24 ± 2
Percentage of marrow mononuclear cells									
Transgenic	11		24 ± 3**	26 ± 4***	19 ± 3***	26 ± 5**	26 ± 5**	3 ± 0.5	6 ± 1
Nontransgenic littermate	12		9 ± 1	9 ± 1	4 ± 0.5	5 ± 1	13 ± 2	3 ± 0.5	4 ± 1
Percentage of nodal mononuclear cells									
Transgenic	7		49 ± 5***	38 ± 5**	32 ± 4**	37 ± 5**	49 ± 4*	44 ± 5	40 ± 6
Nontransgenic littermate	8		26 ± 3	19 ± 3	17 ± 3	19 ± 2	32 ± 4	67 ± 3***	56 ± 3**

[a] Data represent the mean ± the standard error for the absolute number of splenic mononuclear cells or percentage of mononuclear cells from the spleen, marrow, or lymph node labeled with each of the antibodies listed. *n*, Number of mice examined. Significance levels were determined by Student's *t* test: *, $P < 0.05$, **, $P < 0.01$; ***, $P < 0.001$.

comparing anti-CD4 and anti-CD8 antibodies revealed no difference in transgenic and control littermates. Approximately 80 to 85% of thymocytes were $CD4^+$ $CD8^+$, while 4 to 7% were $CD4^+$ $CD8^-$, $CD4^-$ $CD8^+$, or $CD4^-$ $CD8^-$ (data not shown).

Small resting IgM-IgD-expressing B cells compose the expanded lymphocyte population. To determine whether a precise subpopulation of B cells was expanded, we performed two-color flow cytometry on splenic mononuclear cells. Representative contour plots of transgenic and normal mice in Fig. 3 indicate that the expanded lymphocyte population was coexpressing IgM, IgD, and Ia. In addition, simultaneous staining for B220 and kappa was also observed (data not shown).

A discontinuous density gradient centrifugation was performed to further examine the distribution of the expanded B cells. T cells were eliminated from the spleen cells by complement lysis with anti-Thy-1.2 antibody (J1j or AT83) since T cells were not the predominantly affected population. A representative gradient fractionation of transgenic and control littermate cells is shown in Table 2. The expanded population of lymphocytes in the transgenics resided in the highest-density (1.09-g/ml) Percoll fraction. The cells present in the highest-density fraction are characteristically small, resting lymphocytes (8). Flow cytometric analysis of this population indicated that it is composed of the IgM-IgD-expressing B cells. An assessment of the cell cycle status of total spleen cells and the expanded high-density B-cell population was obtained by propidium iodide staining and flow cytometry. No substantial differences were observed in the fraction of cycling cells isolated from the spleens of transgenics and controls, with approximately 94% residing in $G_0/G1$. Furthermore, the purified high-density B-cell population selectively expanded in the transgenics showed no increase in the fraction of cycling cells (3%) compared to control littermates (5%).

Hematopoietic progenitor cell assays. While the Bcl-2-Ig transgene expands a mature B-cell population, we also wished to assess its potential influence on earlier hematopoietic precursors. Bone marrow cells were assessed for colony formation in semisolid methylcellulose assays in the presence of various cytokines (12). No exaggerated response to any of the colony-stimulating factors was noted in transgenics. Instead, some reduction in induced colonies was noted in transgenic versus nontransgenic bone marrow with interleukin-3 (IL-3) (56% decrease), granulocyte-macrophage colony-stimulating factor (45% decrease), macrophage colony-stimulating factor (42% decrease), and granulocyte colony-stimulating factor (26% decrease). A similar reduction in CFU was noted in the spleen cells of transgenic versus normal animals (data not shown). A portion of this decrease no doubt reflects the increased number of mature B cells present in transgenic marrow.

Transgenic B cells are responsive to LPS and anti-IgM. To determine whether the expanded B cells in Bcl-2-Ig transgenic mice were capable of forming B-cell colonies (CFU-B), we used a soft-agar assay utilizing LPS. Simultaneous immunophenotypes of freshly isolated spleen cells permitted the data to be normalized as CFU-B/B cells and CFU-B/spleen. No substantial difference in the ability of transgenics to form CFU-B on a per-B-cell basis was noted when compared to normals (Table 3). A second measure of B-cell responsiveness was performed by assessing the incorporation of [^{3}H]thymidine after LPS or anti-IgM cross-linking. The proliferative responses of transgenic (+) and normal (−) splenocytes were similar when assessed at day 3 after LPS or anti-IgM activation, although a trend of increased responsiveness at 8 months noted in the transgenics may reflect their expanded B cells (Table 4). Similarly, transgenic spleen cells demonstrated normal responsiveness to IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, and IL-7 compared to control littermates (data not shown). The proliferative response of control spleen cells markedly diminished by day 7 after either LPS or anti-IgM. However, the day 7 proliferative response to LPS or anti-IgM was significantly better ($P < 0.02$) in Bcl-2-Ig transgenic cells (Table 4). This observation was examined in more detail by determining the time course of LPS responsiveness in normals and transgenics. Both

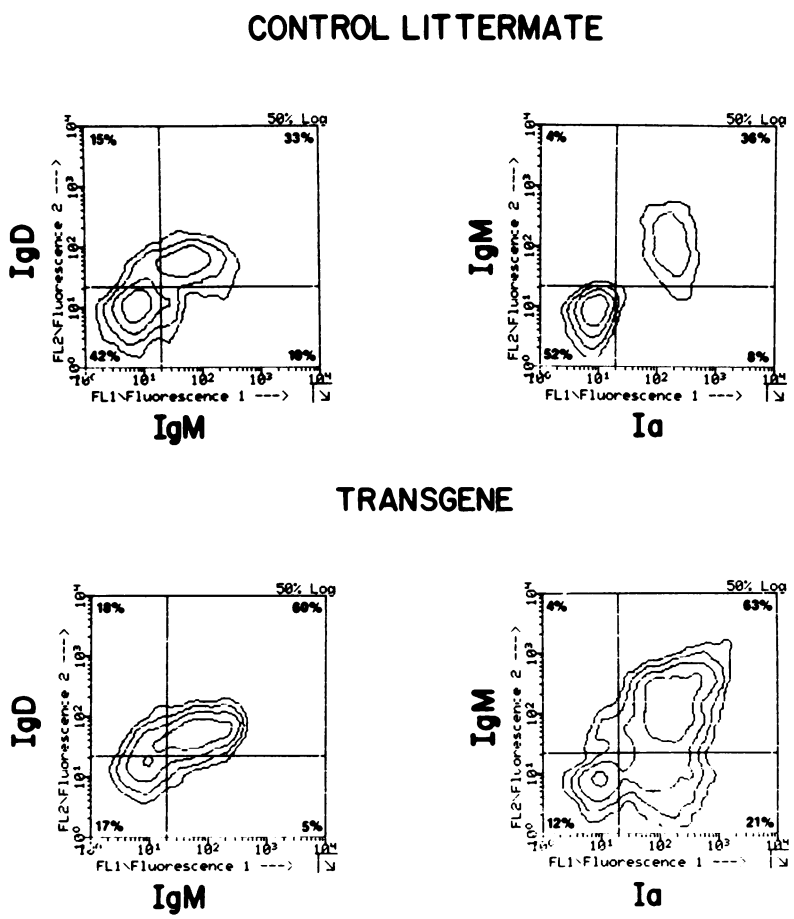

FIG. 3. Two-color immunofluorescence contour plots of spleen cells. Cell suspensions and staining with fluorescein-conjugated or biotinylated MAbs followed by incubation with avidin-phycoerythrin conjugates were done as described in the Materials and Methods. Cursors were positioned based on staining with secondary antibody alone and isotype-matched negative control antibodies. The percentages given represent the proportion of cells present within each quadrant.

sources of cells show equivalent proliferative responses at day 3 post-LPS. The normal response declines by day 5, whereas the transgenic response is prolonged to day 5 and falls thereafter (data not shown).

Long-term surviving transgenic B cells in vitro are in G_0 but are responsive to mitogens. The prolonged proliferation of freshly isolated transgenic spleen cells prompted a further examination of the phenomenon that transgenic B cells displayed extended survival in vitro. Cell cycle analysis of surviving spleen cells (IgM-IgD-expressing B cells) at day 17 of culture in 5% fetal calf serum was performed by flow cytometry after staining with acridine orange. Virtually all the surviving cells from lines M-23 and M-62 (98.8 and 99.5%, respectively) were in G_0. Consistent with this observation, these cultures never resulted in long-term self-renewing proliferating cell lines. This extended B-cell survival

TABLE 2. Density gradient centrifugation of transgenic spleen cells[a]

Density (g/ml)	No. of cells	
	Transgenic	Control littermate
Hanks balanced salt solution + cells	4×10^5	4×10^5
1.065	4×10^5	4×10^5
1.075	1×10^6	1×10^6
1.08	3.2×10^6	2.5×10^6
1.085	5.2×10^6	5×10^6
1.09	7×10^7	1.4×10^7
Total	8.2×10^7	2.33×10^7

[a] T-depleted splenocytes were fractionated over discontinuous Percoll gradients. Cells from each Percoll fraction were harvested and analyzed.

TABLE 3. Spleen CFU-B assay[a]

Mouse	Transgene	Colonies/dish		Colonies/10^4 B cells		10^4 Colonies/ spleen	
		Medium	LPS	Medium	LPS	Medium	LPS
23/11/35/2/6	−	0.3	53	0.1	19	0.1	21
43/101/4/3	−	0.7	149	0.3	53	0.1	20
23/11/62*	−	7.0	338	2.3	109	3	157
23/11/66*	−	17.3	588	4.5	153	19	659
23/11/35/2/5	+	1.7	227	0.5	65	1.4	189
43/101/4/6	+	0	101	0	30	0	86
23/11/61*	+	6.7	690	1.8	180	8	784

[a] Data are presented as the mean number of colonies for triplicate cultures and normalized for the number of B cells per culture dish (i.e., colonies per 10^4 B cells) and the number of colonies per spleen. Mice were 7 weeks old with the exception of those indicated by an asterisk (*), which were 38 weeks old.

TABLE 4. Splenocyte proliferative response to LPS and αIgM[a]

Transgene	Time of culture (days)	[3H]-thymidine incorporation (cpm)				
		8-Week-old mice		8-Month-old mice		
		Medium	LPS	Medium	LPS	αIgM
−	3	890 ± 360	45,108 ± 35,736	6,199 ± 2,214	72,537 ± 7,249	65,499 ± 2,556
+		1,977 ± 1,325	37,503 ± 14,556	7,038 ± 2,433	101,776 ± 21,058	130,784 ± 16,621
−	7	486 ± 220	2,667 ± 359	2,192 ± 1,222	5,104 ± 2,681	3,055 ± 646
+		564 ± 400	13,682 ± 4,413	4,858 ± 3,340	15,269 ± 7,666	27,191 ± 4,754

[a] Proliferation was assessed by [3H]-thymidine incorporation during the last 8 h of culture at day 3 or 7. Each data point represents the mean ± standard deviation of proliferative responses from three animals.

in vitro was still manifest when transgenic B cells from the spleen were depleted of stromal cells by Percoll gradient density centrifugation before in vitro culture. While resting, these surviving B cells were capable of responding to LPS. They demonstrated a proliferative response that continued for at least 10 days and were shown to be entering the cell cycle as early as 48 h post-LPS addition.

DISCUSSION

We previously generated mice possessing a Bcl-2-Ig transgene that was expressed in a predominantly lymphoid pattern (19). In this study, we documented that the Bcl-2-Ig transgene is actually deregulated, adding evidence for its causative role in polyclonal follicular lymphoproliferation. Discriminatory S1 nuclease protection assays clearly demonstrated that the bulk of RNA was derived from the transgene. Prior studies of t(14;18)-bearing human lymphomas indicated that the translocated allele was overexpressed, while the normal *Bcl-2* allele was quiescent (10, 25). S1 analysis indicated that steady-state levels of endogenous mouse Bcl-2 RNA were somewhat less in transgenics, compatible with a partial down-regulation of endogenous murine Bcl-2. In addition, the amount of transgene-originated 25-kDa Bcl-2 protein was increased in spleen, thymocytes, and small, resting B cells compared with endogenous mouse Bcl-2 protein.

Inclusion of the immunoglobulin heavy-chain enhancer should enable the Bcl-2-Ig transgene to be expressed throughout B-cell development from pre-B to plasma cell stages as well as within T cells. Despite this, an expanded B-cell population resulted, predominantly composed of small, resting IgM-IgD-expressing B cells, while thymocyte subsets were unaltered. In a separate experimental system, a retroviral vector introduced overexpressed Bcl-2 into IL-dependent cell lines. Deregulated *Bcl-2* resulted in prolonged survival after factor deprivation for IL-3-dependent pro-B lymphocytes and promyelocytes (29; G. Nunez, L. London, D. Hockenberry, M. Alexander, J. P. McKearn, and S. J. Korsmeyer, J. Immunol., in press) but not for an IL-2-dependent T-cell line (Nunez et al., in press).

Bcl-2-Ig mice demonstrated an approximately fourfold excess of B cells that displayed simultaneous B220, IgM, IgD, Ia, and κ staining. Prior studies of B-cell kinetics have documented that normal mice have relatively constant numbers of peripheral B cells (2.0×10^8) (18). In addition, the production rate of B cells is not altered appreciably after B-cell depletion (9, 21). The excess of B cells in the transgenics indicates a marked abnormality of normal homeostatic mechanisms. Perhaps the most dramatically different organ was the bone marrow. In normal bone marrow, B cells are reported to be mostly IgM^+ IgD^- (22, 23). The approximate fivefold increment in IgD-bearing B cells in Bcl-2-Ig transgenics would be compatible with a recirculating IgM-IgD-expressing population (11). These findings in transgenics are parallel to findings in t(14;18)-bearing follicular lymphoma patients who routinely display disseminated disease with clonal cells in blood and bone marrow (5, 15). The data imply that a relatively normal subpopulation of IgM-IgD-expressing B cells accumulates because of an extended life span.

We showed that the expanded mature B-cell compartment is not a consequence of an increase in the percentage of cycling cells. These findings are in marked contrast to results reported for the prelymphomatous Eμ-*myc* transgenic mice (14). Greater than twofold more splenic B-lineage lymphocytes were actively cycling in transgenic Eμ-*myc* mice compared with normal littermates (22% versus 8%).

The characteristics of these transgenic B cells are similar to human follicular lymphoma cells. The t(14;18) and the consequent deregulation of *Bcl-2* may be an early event in B-cell tumorigenesis ensuring an extended survival to a distinct B cell that increases its chance of acquiring additional genetic abnormalities. Transgenic mice provide a model to examine the physiologic role of this B-cell subset selected by Bcl-2 overproduction.

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