Constitutive Expression of Bcl-2 in B Cells Causes a Lethal Form of Lupuslike Autoimmune Disease After Induction of Neonatal Tolerance to H-2^b Alloantigens

By Marcos López-Hoyos,* Roberto Carrió,* Ramón Merino,§ Luis Buelta,‡ Shozo Izui,‖ Gabriel Núñez,§ and Jesús Merino*

*From the Departments of *Immunology and ‡Pathology, Hospital Universitario M. Valdecilla, 39008 Santander, Spain; the §Department of Pathology, University of Michigan, Ann Arbor, Michigan 48109; and the ‖Department of Pathology, University of Geneva, 1211 Geneva 4, Switzerland*

Summary

The *bcl-2* protooncogene has been shown to provide a survival signal to self-reactive B cells, but it fails to override their developmental arrest after encounter with antigen. Furthermore, constitutive expression of *bcl-2* in B cells does not promote the development of autoimmune disease in most strains of mice, indicating that signals other than those conferred by *bcl-2* are required for long-term survival and differentiation of self-reactive B cells in vivo. To further examine the factors that are required for the pathogenesis of autoimmune disease, we have assessed the effect of *bcl-2* overexpression on the development of host-versus-graft disease, a self-limited model of systemic autoimmune disease. In this model, injection of spleen cells from (C57BL/6 × BALB/c)F_1 hybrid mice into BALB/c newborn parental mice induces immunological tolerance to donor tissues and activation of autoreactive F_1 donor B cells through interactions provided by allogeneic host CD4$^+$ T cells. BALB/c newborns injected with spleen cells from (C57BL/6 × BALB/c)F_1 mice expressing a *bcl-2* transgene in B cells developed high levels of anti–single-stranded DNA and a wide range of pathogenic autoantibodies that were not or barely detectable in mice injected with nontransgenic spleen cells. In mice injected with transgenic B cells, the levels of pathogenic autoantibodies remained high during the course of the study and were associated with long-term persistence of donor B cells, development of a severe autoimmune disease, and accelerated mortality. These results demonstrate that *bcl-2* can provide survival signals for the maintenance and differentiation of autoreactive B cells, and suggest that both increased B cell survival and T cell help play critical roles in the development of certain forms of systemic autoimmune disease.

The injection of semiallogeneic lymphoid cells into newborn parental mice induces immunological tolerance to donor tissues and establishment of a B and T cell chimerism (1, 2). In addition, the injection of spleen cells differing at MHC class II molecules, but not at MHC class I or non-MHC alloantigens, results in a lupuslike autoimmune syndrome also known as host-vs.-graft disease (HVGD)[1] (2–5). This syndrome is characterized by hypergammaglobulinemia, predominantly of the IgG1 and IgE isotypes (2), and the production of various autoantibodies (autoAbs), including anti–single-stranded (ss)DNA, antinuclear, and anticytoskeleton autoAbs (2, 6). The autoAb response appears secondary to polyclonal activation of donor B cells by tolerance-resistant alloreactive host Th2 CD4$^+$ T cells (7–10). However, autoAbs with known pathological consequences, such as anti–double-stranded (ds)DNA (11), anti-gp70 (12), antilaminin (13), or these against antineutrophil cytoplasm Ab components (ANCAs) (14, 15), are not found in mice injected at birth with F_1 cells. Another important feature of HVGD-associated autoimmune syndrome is that autoAb production is self-limited because of a rapid drop in B cell chimerism by 10 wk of age (2, 16). Indeed, reinjection of spleen cells induces a flare in the serological manifestations (16), emphasizing the central role of donor B cell chimerism in the development and self-limitation of the HVGD in tolerant mice. In addition to autoAb production, limited glomerular lesions are observed in these animals that totally disappear some weeks after the normalization of the serological parameters (16).

The proto-oncogene *bcl-2* was the first member of an ex-

[1] *Abbreviations used in this paper:* ANCA, antineutrophil cytoplasm antibody; anti-MPO, antimyeloperoxidase; anti-PR3, antiproteinase 3; autoAb, autoantibody; CB6F_1, (C57BL/6 × BALB/c)F_1; dsDNA, double-stranded DNA; gp70 IC: gp70–anti-gp70 immune complex; HVGD: host-vs.-graft disease; ssDNA, single-stranded DNA; Tg$^+$, Ig-*bcl-2* transgenic; Tg$^-$, Ig-*bcl-2* nontransgenic.

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panding family of genes that modulated the apoptotic pathway. A role for *bcl-2* in the selection of B lymphocytes was suggested by its tightly regulated expression pattern during B cell development (17, 18). Bcl-2 was highly expressed in pro-B and mature B cells but downregulated in pre-B/immature cells, stages of development associated with extensive cell death and clonal selection. Similarly, the expression of Bcl-2 declines in B cells of the germinal center, a site associated with selection of B cells bearing high-affinity Ig receptors for antigen (19, 20). Analysis of Bcl-2–deficient mice has demonstrated that Bcl-2 is essential for the maintenance of mature T and B lymphocytes (21, 22). In transgenic mice, overexpression of *bcl-2* protected B cells against many forms of cell death (23, 24) and, in some genetic backgrounds such as (C57BL/6 × SJL/J)F_1 mice, promoted the development of a lupuslike autoimmune syndrome (24). However, most strains of *bcl-2* transgenic mice failed to develop any sign of autoimmune disease (23, 25), indicating that factors other than *bcl-2* are necessary for the development of systemic autoimmune disease.

Here, we have evaluated the contribution of *bcl-2* to the development of autoimmune disease using the HVGD model, an established experimental system of a self-limited lupuslike syndrome secondary to a cognate allogeneic T–B cell interaction. Our results demonstrate that BALB/c mice injected with spleen cells from (C57BL/6 × BALB/c)F_1 ($CB6F_1$) mice overexpressing *bcl-2* in B cells, but not with spleen cells from nontransgenic $CB6F_1$ controls, developed a chronic and lethal form of systemic lupuslike disease. Development of autoimmunity correlated with the persistence of B cell chimerism and with the appearance of a broad spectrum of pathogenic autoAbs. These results indicate that disregulation of apoptotic mechanisms in B cells, together with appropriate T cell help, can be of paramount importance in the pathogenesis of certains forms of autoimmune disease.

Materials and Methods

Mice. BALB/c mice were purchased from CRIFFA (Barcelona, Spain). C57BL/6–Ig-*bcl-2* transgenic mice (Tg^+) were kindly provided by Dr. S.J. Korsmeyer (Washington University, St. Louis, MO; 23). Tg^+ or nontransgenic (Tg^-) $CB6F_1$ hybrid mice were obtained by crossing BALB/c and C57BL/6–Ig-*bcl-2* +/− heterozygous mice. All mice were maintained under pathogen-free conditions.

Induction of Neonatal Tolerance. Neonatal tolerance to $H\text{-}2^b$ alloantigens was induced by i.p. injection of 0.7×10^8 spleen cells from $CB6F_1$-Tg^+ hybrid mice or 10^8 spleen cells from $CB6F_1$-Tg^- into BALB/c mice within the first 24 h after birth, as described (2). These numbers were chosen in order to inject the same absolute number of B cells in both groups of mice, as determined by flow cytometric analysis. The percentage of plasma cells in donor spleen cells was calculated after Giemsa staining of spleen cell smears.

Flow Cytometric Analysis. $CB6F_1$-Tg^+ mice were identified by flow cytometry on PBLs using the 6C8 mAb (20) (hamster anti–human Bcl-2; PharMingen, San Diego, CA) and biotinylated $F(ab')_2$ goat anti–hamster IgG (Jackson ImmunoResearch, West Grove, PA), as previously described (17). To calculate the number of B cells in spleen cell suspensions from $CB6F_1$ mice, cells were stained with FITC-conjugated rat anti–mouse B220 mAb (clone RA3-6B2; PharMingen) and analyzed by flow cytometry. B cell chimerism in spleen and peripheral and mesenteric lymph nodes was assessed by flow cytometry using FITC-anti-B220 mAb and biotin-labeled mouse anti–mouse IA^b mAb (clone KH74; PharMingen), followed by streptavidin-PE (Tago, Inc., Burlingame, CA). Cells were analyzed in a FACScan® flow cytometer using Lysys II software (Becton Dickinson & Co., Mountain View, CA). Dead cells and debris were excluded based on forward and sideways light scatter.

Serological Studies. Serum levels of Ig bearing the donor Igh^b allotype in tolerant mice were assessed by ELISA as previously described (2). Results were expressed as milligram equivalent per milliliter of Igh^b-bearing Ig relative to that detected in the sera of age-matched normal C57BL/6 mice. Serum levels of IgG anti-dsDNA and anti-ssDNA autoAbs were determined by ELISA as described elsewhere (2, 26). IgG anticytoskeleton Abs were evaluated by ELISA as described previously (6). Results of anti-ssDNA, anti-dsDNA, and anti-cytoskeleton Abs were expressed in titration units referred to a standard curve obtained by serial dilutions of a serum pool obtained from 6–8-mo-old MRL *lpr/lpr* mice. The presence of ANCA was evaluated by indirect immunofluorescence on slides coated with neutrophils (Cormedica, Barcelona, Spain) using a 1/20 dilution of sera followed by FITC-conjugated rabbit anti–mouse IgG (DAKO, Glostrup, Denmark). IgG antiproteinase 3 (anti-PR3) or antimyeloperoxidase (anti-MPO) specifities of ANCA were determined by ELISA. Briefly, 100 μl of diluted serum (1:50) in 2% PBS/0.05% BSA/Tween 20 were incubated for 2 h at room temperature in microtiter wells previously coated with PR3 or MPO antigen (Shield Diagnostics, Dundee, England). After three washes, wells were incubated for 2 h at room temperature with 100 μl of alkaline phosphatase–conjugated goat anti–mouse γ chain–specific polyclonal Ab (Sigma Chemical Co., St. Louis, MO; 1 μg/ml). Results were expressed in units of absorbance at 405 nm. Serum levels of gp70–anti-gp70 immune complexes (gp70 IC) were determined by ELISA as described previously (27). Results were expressed as nanograms of Ab-bound gp70 per milliliter of serum. Serum RF activity was evaluated by ELISA on microtiter wells coated with mouse-purified mAb of the IgG1 or IgG2a subclasses, as described (27).

Histological Studies. Tissue samples from all major organs were fixed in 10% formaldehyde, imbedded in paraffin, and 3-μm sections were processed for hematoxylin-eosin staining, periodic acid-Schiff staining, and silver-metenamin impregnation, according to standard methods. Glomerular lesions were scored on a 0–4 scale based on the intensity and extent of histopathological changes as described previously (27). Deposition of IgM and IgG subclasses was assessed by direct immunofluorescence on kidney cryocut sections (4 μm) using FITC-rabbit anti–mouse IgM (Cappel Laboratories, Durham, NC) or rabbit anti–mouse IgG subclass-specific antiserum (Southern Biotechnology Associates, Birmingham, AL).

Statistical Analysis. Statistical analysis was performed using the Wilcoxon two-sample test. Probability values >5% were considered insignificant.

Results

Effects of a bcl-2 Transgene on the B Cell Chimerism Associated with Neonatal Injection of F_1 Spleen Cells. To analyze whether

Table 1. *Evolution of B Cell Chimerism in BALB/c Mice Neonatally Injected with Semiallogeneic Spleen Cells from CB6F$_1$-Tg$^+$ or CB6F$_1$-Tg$^-$ Mice*

Cells injected at birth	Age	Percent IA^{b+}, B220$^+$ cells/total B220$^+$ cells		
		Spleen	Peripheral lymph nodes	Mesenteric lymph nodes
CB6F$_1$-Tg$^-$	5 d	2.3 (0.1)/11.6	47.7 (0.6)/55.7	37.0 (1.4)/42.7
	3 wk	3.3 (1.5)/30.0	2.9 (0.4)/19.6	3.0 (0.5)/12.5
	20 wk	1.6 (0.0)/50.0	0.9 (0.5)/22.0	ND
CB6F$_1$-Tg$^+$	5 d	2.8 (1.1)/18.4	43.7 (13.1)/57.1	27.7 (17.5)/40.1
	3 wk	4.3 (1.4)/33.1	6.0 (1.5)/42.9	5.4 (1.3)/26.6
	20 wk	20.7 (15.0)/43.2	3.0 (2.4)/45.5	4.1 (1.6)/36.0

Spleen, peripheral, or mesenteric lymph node cells were stained with FITC anti-B220 mAb and biotin-anti-IA^b mAb, followed by streptavidin-PE. Results shown indicate the frequency of I-Ab$^+$ B200$^+$ cells in the total lymphoid population. Mean values and SD (in parentheses) are shown. The frequency of total B220$^+$ cells is also indicated. Results were calculated using five to eight mice in each group.

constitutive expression of *bcl-2* in donor B cells influences the development of HVGD in tolerant mice, newborn BALB/c mice (H-2^d) were injected intraperitoneally with 0.7×10^8 spleen cells from CB6F$_1$-Tg$^+$ or 10^8 spleen cells from CB6F$_1$-Tg$^-$ littermates (H-2$^{b/d}$) as controls. The number of spleen cells was chosen in order to inject the same absolute number of F$_1$ B cells in both groups of mice since donor B but not T cells are essential for HVGD development (2, 7–10). Flow cytometric studies indicated that >98% of the injected splenic B cells from CB6F$_1$-Tg$^+$ and CB6F$_1$-Tg$^-$ donors were small IgM$^+$B220$^+$ cells, and <0.5% of the spleen cells from both groups were plasma cells (data not shown). The establishment of tolerance to donor alloantigens was analyzed by measuring the percentage of donor I-A^{b+} cells in spleen and lymph nodes of injected mice. A similar percentage of donor I-A^{b+} cells was observed in spleen and lymph nodes of BALB/c recipients 5 d and 3 wk after injection of either CB6F$_1$-Tg$^+$ or CB6F$_1$-Tg$^-$ spleen cells (Table 1). By 20 wk after injection, however, donor I-A^{b+} cells were almost undetectable in BALB/c mice injected with CB6F$_1$-Tg$^-$ spleen cells. In contrast, the percentage of donor B cells in BALB/c mice injected with CB6F$_1$-Tg$^+$ spleen cells remained high at 20 wk (Table 1).

To further assess the chimerism and activation of donor B cells, the levels of Ig bearing donor Igh^b allotype were analyzed in tolerant mice (2). An increase in the amount of Ig bearing the donor Igh^b allotype was observed in both groups of BALB/c mice neonatally injected with CB6F$_1$ spleen cells (Fig. 1). However, the levels of Igs bearing donor Igh^b allotype were 5–10 times higher in BALB/c mice injected with CB6F$_1$-Tg$^+$ spleen cells than in the control group injected with CB6F$_1$-Tg$^-$ spleen cells. Moreover, BALB/c mice injected with CB6F$_1$-Tg$^+$ cells exhibited Igh^b allotype levels that remained elevated at least until week 12 which contrasted with a rapid decrease in serum Ig of donor Igh^b allotype in mice receiving nontransgenic spleen cells (Fig. 1).

Mice Injected with Splenic B Cells Overexpressing bcl-2 Develop High and Sustained Levels of a Wide Range of AutoAbs. We next examined the production of several autoAbs in BALB/c mice injected at birth with spleen cells from either CB6F$_1$-Tg$^-$ or CB6F$_1$-Tg$^+$ mice. As previously reported (2, 28), animals injected with CB6F$_1$-Tg$^-$ spleen cells produced increased levels of IgG anti-ssDNA autoAbs when compared with uninjected or CB6F$_1$-Tg$^+$ mice ($P < 0.001$), but low or undetectable levels of IgG anti-dsDNA autoAbs (Fig. 2 *A*). The kinetics of anti-ssDNA autoAb production in these animals was similar to that described for Igh^b production. The levels were maximum at 3–6 wk of age and returned to normal values by 12 wk of age. In contrast, the titers of anti-ssDNA autoAb were two- to threefold higher in BALB/c mice tolerized with CB6F$_1$-Tg$^+$ spleen cells than in the control group and remained elevated during the entire course of the study ($P < 0.005$ from week 6 to 20) (Fig. 2 *A*). In both groups of animals, IgG anti-ssDNA autoAbs were predominantly of the IgG1 isotype (data not shown), as previously described in this model (2, 8), sug-

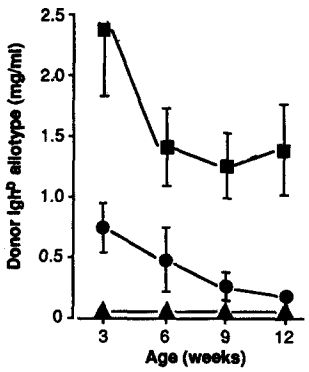


Figure 1. Serum levels of donor Ig of the Igh^b allotype. BALB/c mice were injected at birth with spleen cells from CB6F$_1$-Tg$^+$ mice (■) or from normal CB6F$_1$-Tg$^-$ mice (●). Uninjected BALB/c mice (▲) were used as controls. Results are expressed as milligram equivalent per milliliter of Igh^b bearing IgG relative to normal C57BL/6 serum. Results represent mean ± 1 SD of 8–19 mice per group. Significant differences were observed between BALB/c mice injected with spleen cells from CB6F$_1$-Tg$^+$ and CB6F$_1$-Tg$^-$ mice ($P < 0.001$ at 3 wk and $P < 0.05$ at 6, 9, and 12 wk).

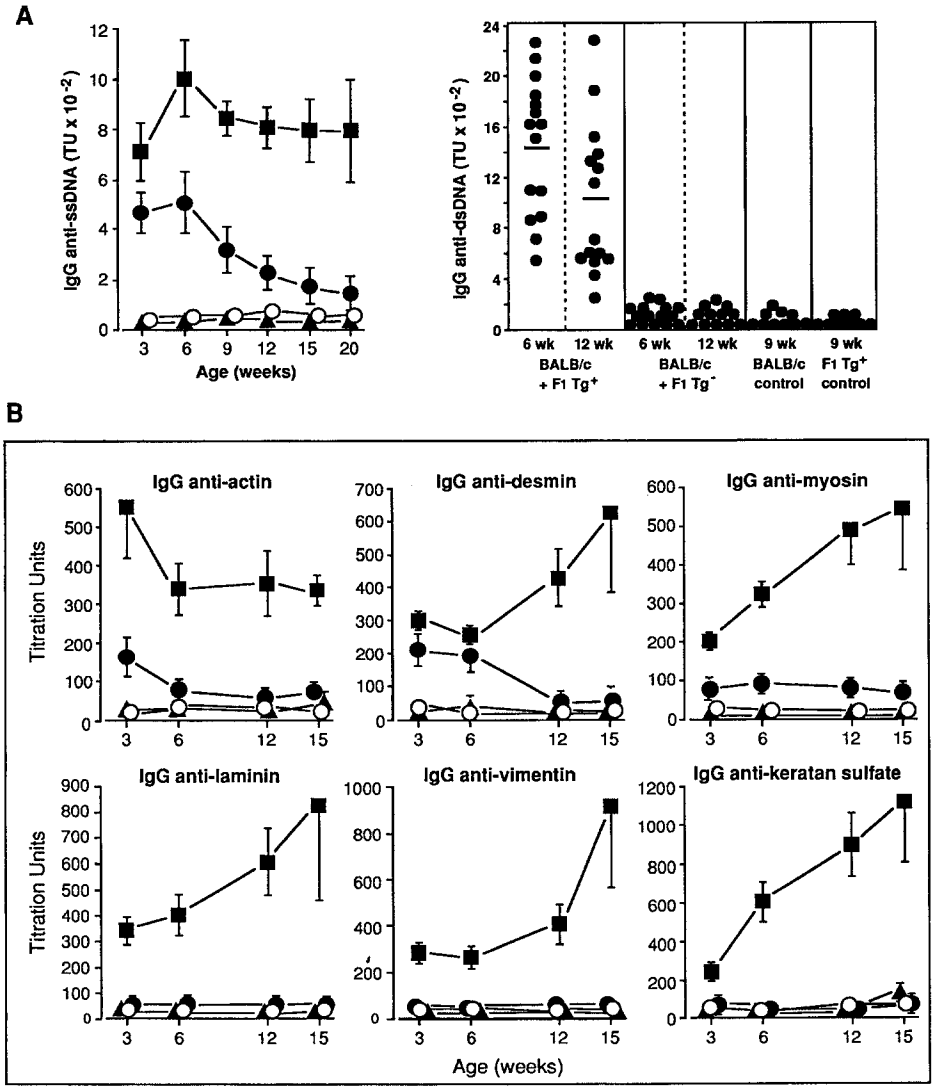


Figure 2. Serum levels of anti-ssDNA, anti-dsDNA (*A*), and anticytoskeleton (*B*) autoAbs. BALB/c mice were neonatally injected with spleen cells from $CB6F_1$-Tg^+ (■) or from normal $CB6F_1$-Tg^- mice (●). Uninjected BALB/c (▲) and $CB6F_1$-Tg^+ mice (○) were used as controls. Results are expressed in titration units by reference to a serum pool from MRL.*lpr*/*lpr* mice. Mean ± 1 SD of 15–20 mice per group are represented.

gesting that an interaction between host Th2 $CD4^+$ T and donor B cells is also responsible for autoAb production in mice injected with $CB6F_1$-Tg^+ spleen cells. Interestingly, the injection of spleen cells from $CB6F_1$-Tg^+ mice was accompanied by the production of high levels of IgG anti-dsDNA autoAbs when compared with mice injected with $CB6F_1$-Tg^- spleen cells ($P < 0.001$) (Fig. 2 *A*).

Since BALB/c mice injected with $CB6F_1$-Tg^+ spleen cells produced increased levels of autoAbs, which were undetectable in animals injected with $CB6F_1$-Tg^- spleen cells, the presence of a wide spectrum of autoAbs was analyzed in both groups of mice. Abnormal levels of antiaortic myosin, antismooth muscle actin, and antidesmin autoAbs were observed in both groups of tolerized mice, but the levels were much higher in mice injected with $CB6F_1$-Tg^+ cells ($P < 0.01$ except for IgG antidesmin autoAbs at weeks 3 and 6) (Fig. 2 *B*). Significantly, autoAbs reactive with vimentin, keratan sulfate, and laminin were found exclusively in BALB/c mice tolerized with $CB6F_1$-Tg^+ spleen cells ($P < 0.001$) (Fig. 2 *B*).

Production of ANCA and circulating gp70 IC is associated with accelerated autoimmune disease in humans and mice (12, 14, 15). Indirect immunofluorescence on fixed neutrophils with sera from mice injected with $CB6F_1$-Tg^+ spleen cells, but not with sera from animals injected with $CB6F_1$-Tg^- spleen cells, showed the typical cytoplasmic and perinuclear staining pattern compatible with the presence of ANCA (data not shown). Significantly, mice neonatally injected with $CB6F_1$-Tg^+ spleen cells produced high levels of anti-MPO and anti-PR3 ANCA, detectable by ELISA, at 6 and 12 wk of age (Fig. 3 *A*). In addition, these animals produced increased levels of gp70 IC (Fig. 3 *B*). In contrast, animals injected with $CB6F_1$-Tg^- spleen cells showed limited or undetectable levels of anti-MPO, anti-PR3 autoAbs and gp70 IC (Fig. 3, *A* and *B*). Importantly, uninjected BALB/c mice and donor $CB6F_1$-Tg^+ mice failed to produce significant levels of these autoAbs (Figs. 2 and 3), indicating that both the *bcl-2* transgene and allogeneic factors are required for the development of a wide spectrum of autoAbs. Finally, we evaluated the presence of anti-IgG1

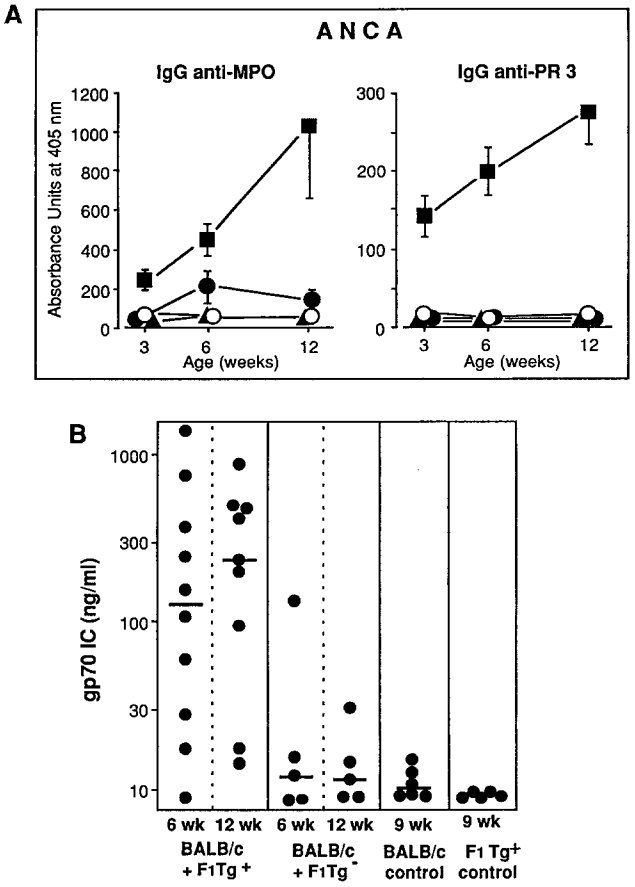


Figure 3. Serum levels of anti-MPO and anti-PR3 ANCA (*A*) and nephritogenic anti-gp70 IC (*B*). (*A*) BALB/c mice were neonatally injected with spleen cells from $CB6F_1$-Tg^+ (■) or from normal $CB6F_1$-Tg^- mice (●). Uninjected BALB/c (▲) and $CB6F_1$-Tg^+ mice (○) were used as controls. Results are expressed in absorbance units at 405 nm. The production of IgG anti-MPO ANCA between BALB/c mice injected with spleen cells from $CB6F_1$-Tg^+ and $CB6F_1$-Tg^- mice was statistically significant at 12 wk ($P < 0.001$). In contrast, significant differences were observed in the levels of IgG anti-PR3 ANCA between both groups of mice during the entire course of the study ($P < 0.01$). (*B*) gp70 IC levels were analyzed in 6- and 12-wk-old BALB/c mice neonatally injected with $CB6F_1$-Tg^+ or with $CB6F_1$-Tg^- spleen cells. 9-wk-old BALB/c and $CB6F_1$-Tg^+ mice were used as controls. Results are expressed in nanograms of Ab-bound gp70 per milliliter of serum. The differences between BALB/c mice injected with spleen cells from $CB6F_1$-Tg^+ and $CB6F_1$-Tg^- mice were statistically significant ($P < 0.01$ at 12 wk).

and anti-IgG2a RF in sera from mice injected with $CB6F_1$ spleen cells. There was no increase in RF activity in either group of mice injected with F_1 cells when compared to normal BALB/c mice (data not shown).

BALB/c Mice Injected with $CB6F_1$-Tg^+ Spleen Cells Develop Severe Autoimmune Disease and Accelerated Mortality. Unlike BALB/c mice injected at birth with $CB6F_1$-Tg^- spleen cells that remained healthy and survived, >50% of mice tolerized with $CB6F_1$-Tg^+ spleen cells died before 20 wk of age (Fig. 4). Significantly, the highest incidence of mortality coincided with the peak of ANCA and gp70 IC production (~12 wk of age; Figs. 3 and 4). Histopathological analysis revealed that BALB/c mice injected with

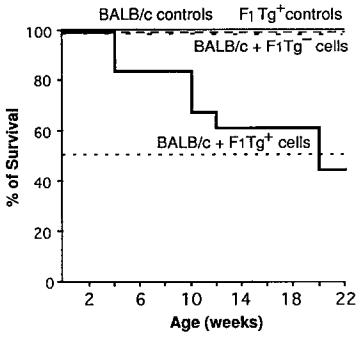


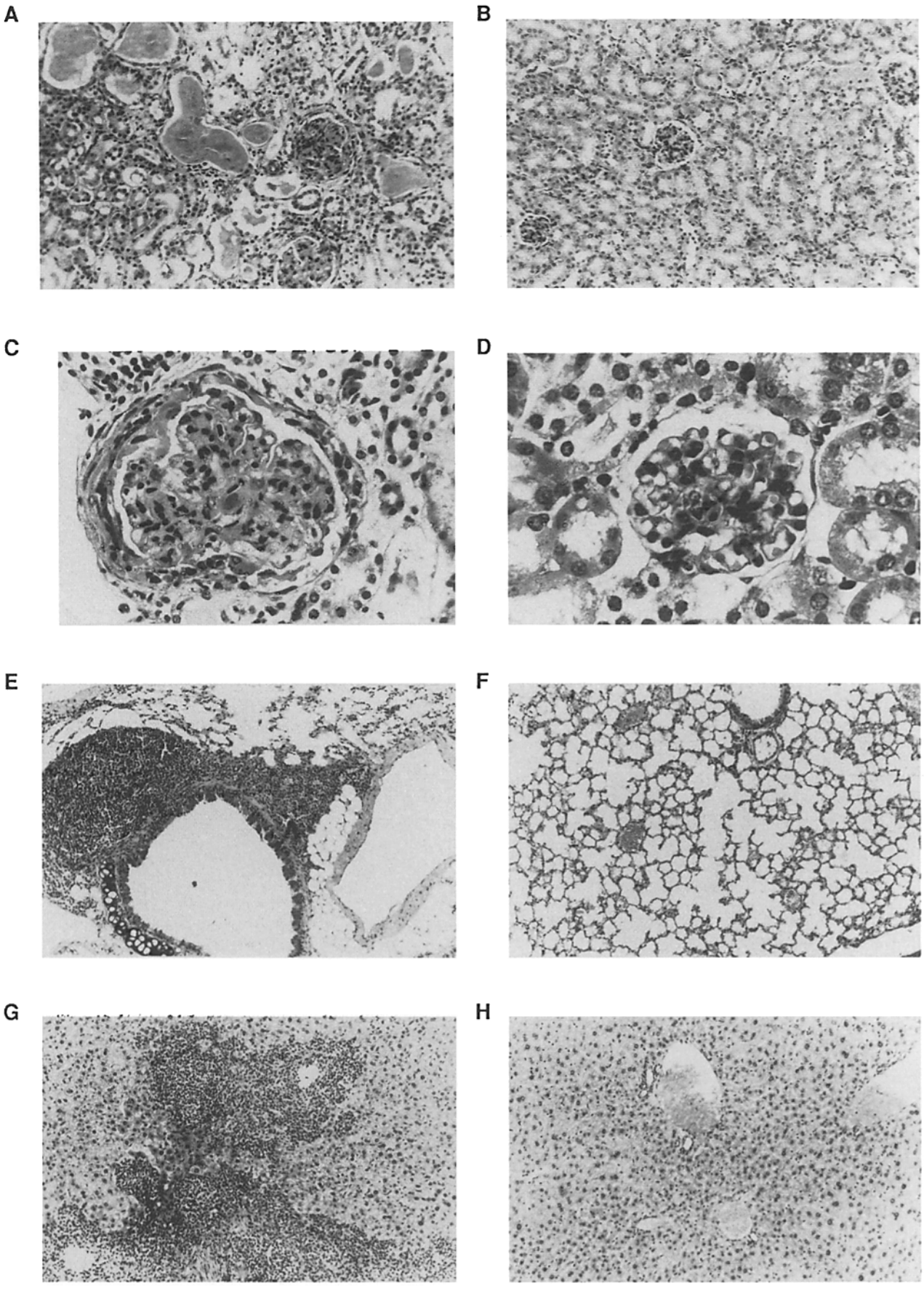
Figure 4. Mortality curve of BALB/c mice injected at birth with spleen cells from $CB6F_1$-Tg^+ mice (26 mice, *thick line*) or from normal $CB6F_1$-Tg^- mice (16 mice, *dashed line*). Uninjected BALB/c (12 mice, *thin line*) and $CB6F_1$-Tg^+ mice (8 mice, *dotted line*) served as controls.

$CB6F_1$-Tg^+ spleen cells developed a severe form of diffuse proliferative glomerulonephritis characterized by increased mesangial and glomerular cellularity, obliteration of glomerular architecture, and tubular cast formation (Fig. 5, *A* and *C*). Immunofluorescence studies demonstrated the presence of IgG and IgM deposits in the glomeruli of these mice (data not shown). In contrast, BALB/c mice injected with $CB6F_1$-Tg^- spleen cells exhibited minimal glomerular changes (Fig. 5, *B* and *D*). In addition, animals tolerized with $CB6F_1$-Tg^+ spleen cells developed interstitial lymphocytic pneumonitis characterized by prominent peribronchial (Fig. 5 *E*) and perivascular (data not shown) accumulation of mononuclear cells, and marked lymphoid infiltrates in the liver resembling chronic active hepatitis (Fig. 5 *G*). None of these histological abnormalities was observed in BALB/c mice injected with $CB6F_1$-Tg^- spleen cells (Fig. 5, *F* and *H*). Despite the production of high levels of anti-MPO and anti-PR3 ANCA, BALB/c mice injected with $CB6F_1$-Tg^+ spleen cells failed to develop typical lesions of vasculitis in small and medium vessels (data not shown).

Discussion

The HVGD that develops in mice tolerized at birth to alloantigens appears to reflect the activation of autoreactive F_1 donor B cells by tolerance-resistant host T cells (2, 7–10). We showed in the present report that injection of semiallogeneic splenic B cells expressing a *bcl-2* transgene into newborn parental mice induces sustained production of a wide range of autoAbs and early death. The death of these mice appears related to the development of a severe diffuse proliferative glomerulonephritis, although the contribution of liver and lung disease cannot be excluded. This severe form of systemic lupuslike disease markedly contrasts with the mild and self-limited autoimmune reaction that occurs after injection of spleen cells from nontransgenic mice (1, 2, 16).

Experimental work by several investigators has suggested that normal mice contain B cells with autoreactive potential in their peripheral lymphoid repertoire (29–31). However, it is thought that such autoreactive B cells are not efficiently stimulated to produce autoAbs because of the absence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of adequate antigen-specific T cell help (32). In the HVGD model, donor autoreactive B cells are thought to receive stimulatory signals from host helper T cells (7–10). For example, production of autoAbs bearing the allotypic markers of the F_1 donor are not observed in the absence of $CD4^+$ host T cells (7) or after treatment with anti-IL4 mAb (8). The mechanism responsible for the transient course of HVGD appears to be secondary to the progressive loss of B cell chimerism since reinjection of F_1 donor B cells into neonatally tolerized animals reactivates the autoimmune manifestations (16). Since *bcl-2* confers increased survival to B cells, our findings together with previous results (24, 33) suggest that *bcl-2* promotes enhanced and sustained autoAb production by prolonging the life span of autoreactive mature B cells and plasma cells. This conclusion is particularly relevant for B cells that produce certain autoAbs such as anti-DNA, antiactin, antidesmin, or antimyosin autoAbs since they were secreted, although at lower levels, by nontransgenic donor B cells. Thus, these results argue that the limited life span of potentially autoreactive B cells is a major safeguard against the development of certain types of autoAbs and perhaps autoimmune disease. These findings are in accord with those by Strasser et al. (24) who showed that (C57BL/6 × SJL/J)F_1 mice overexpressing *bcl-2* in the B cell compartment develop a lethal lupuslike syndrome. However, no such abnormalities were observed in other strains of *bcl-2* transgenic mice including C57BL/6, BALB/c, CB6F_1, (C57BL/6 × C3H)F_1, and (SWR/J × SJL/J)F_1 mice (23, 25, the present study, and Merino, R., and G. Núñez, unpublished results). Together, these observations support the conclusion that signals other than disregulated B cell survival are necessary for disease development. In the current report, we provide evidence that T cell help together with constitutive expression of *bcl-2* in B cells are necessary for the production of pathogenic autoAbs and the development of systemic autoimmune disease.

A major observation of the present study was that mice injected with semiallogeneic B cells from CB6F_1-Tg^+ mice produced high levels of certain pathogenic autoAbs. Indeed, several autoAbs such as those reactive with dsDNA, MPO, PR3, laminin, and gp70 self-antigens have been associated with the development of autoimmune kidney disease in humans and rodents (11–15). These potentially pathogenic autoAbs were almost exclusively found in chimeric mice injected with F_1 B cells expressing the *bcl-2* transgene. At least two different possibilities may explain these findings. First, it is possible that spleen cells from normal mice contain B cells or their precursors which, upon injection into parental mice, are capable of producing such pathogenic autoAbs. However, secretion of pathogenic autoAbs might be too low to be detected by the current methodology. In this scenario, *bcl-2* promotes the autoAb response and autoimmune disease by increasing the longevity of mature autoreactive B cell clones. A nonexclusive alternative explanation is that production of at least some of the pathogenic autoAbs may be suppressed in normal mice through censoring mechanisms that operate during B cell development. In this context, in vivo models of clonal B cell deletion using transgenic mice expressing Ig receptors specific for hen egg lysozyme (HEL) or surface erythrocyte antigens, have demonstrated that *bcl-2* can rescue self-reactive B cells from death during development or in peripheral tissues, respectively (34, 35). However, in the anti-HEL system, self-reactive B cells overexpressing *bcl-2* remained arrested and failed to differentiate into mature long-lived B cells (34). In the HVGD model, B cells that produce anti-dsDNA, anti-MPO, anti-PR3, antilaminin, or anti-gp70 autoAb might be rescued from death by *bcl-2* overexpression and allowed to expand and mature through signals provided by activated allospecific host $CD4^+$ T cells (7–10). Together, our results demonstrate that alteration of programmed cell death regulatory mechanisms in autoreactive B cells in the presence of appropriate T cell help can markedly potentiate the autoimmune process.

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Figure 5. Histological analysis of 20-wk-old BALB/c mice injected at birth with spleen cells from CB6F_1-Tg^+ or CB6F_1-Tg^- mice. Representative histological appearance of kidney from mice injected with CB6F_1-Tg^+ spleen cells showing the presence of a diffuse proliferative glomerulonephritis and tubular cast formation (×30) (*A*). High power view of glomerulus from mice injected with CB6F_1-Tg^+ spleen cells showing increased glomerular cellularity and obliteration of glomerular architecture (×300) (*C*). Peribronchial infiltration of plasma cells in the lung of mice injected with CB6F_1-Tg^+ spleen cells (×30) (*E*). Marked lymphoid infiltration of portal tracts extending into the hepatic parenchyma and bridging necrosis in the liver of mice injected with CB6F_1-Tg^+ spleen cells (×30) (*G*). Normal histological appearance of kidney (*B* and *D*), lung (*F*), and liver (*H*) of mice injected with CB6F_1-Tg^- spleen cells (×30 and ×300 in *D*).

Address correspondence to Dr. Jesús Merino, Dpt. de Inmunología, Hospital Universitario Marqués de Valdecilla, 39008 Santander, Spain.

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