Brief Definitive Report

B Cell–specific Transgenic Expression of Bcl2 Rescues Early B Lymphopoiesis but Not B Cell Responses in BOB.1/OBF.1-deficient Mice

Cornelia Brunner,[1] Dragan Marinkovic,[1] Jörg Klein,[2] Tatjana Samardzic,[1] Lars Nitschke,[2] and Thomas Wirth[1]

[1]*Department of Physiological Chemistry, University of Ulm, D-89081 Ulm, Germany*
[2]*Institute of Virology and Immunobiology, University of Wuerzburg, D-97078 Wuerzburg, Germany*

Abstract

Mice deficient for the transcriptional coactivator BOB.1/OBF.1 show several defects in B cell differentiation. Numbers of immature transitional B cells in the bone marrow are reduced and fewer B cells reach the periphery. Furthermore, germinal center B cells are absent and marginal zone (MZ) B lymphocytes are markedly reduced. Increased levels of B cell apoptosis in these mice prompted us to analyze expression and function of antiapoptotic proteins. Bcl2 expression is strongly reduced in BOB.1/OBF.1-deficient pre–B cells. When BOB.1/OBF.1-deficient mice were crossed with Bcl2-transgenic mice, B cell development in the bone marrow and numbers of B cells in peripheral lymphoid organs were normalized. However, neither germinal center B cells nor MZ B cells were rescued. Additionally, Bcl2 did not rescue the defects in signaling and affinity maturation found in BOB.1/OBF.1-deficient mice. Interestingly, Bcl2-transgenic mice by themselves show an MZ B cell defect. Virtually no functional MZ B cells were detected in these mice. In contrast, mice deficient for Bcl2 show a relative increase in MZ B cell numbers, indicating a previously undetected function of Bcl2 for this B cell compartment.

Key words: B cell differentiation • germinal center reaction • affinity maturation • marginal zone • transcription factor

Introduction

BOB.1/OBF.1 (Bob.1, OBF-1, or OCA-B; references 1–4) is a B cell–specific transcriptional coactivator. It interacts with the Oct1 and Oct2 transcription factors and augments their function. BOB.1/OBF.1 is expressed at all stages of B cell development, albeit at different expression levels. Highest BOB.1/OBF.1 expression was found in germinal center B cells and germinal center–derived B cell lymphomas (5, 6). Expression levels are regulated both by transcription as well as protein stability (7, 8). In T cells, expression of functional BOB.1/OBF.1 is inducible by costimulation (9). A myristylated BOB.1/OBF.1 protein isoform exists that is localized in the membrane fraction (10).

Inactivation of BOB.1/OBF.1 by gene targeting revealed its critical role for B lymphopoiesis. BOB.1/OBF.1-deficient mice completely lack germinal centers upon immunization with thymus-dependent antigens. Consequently, these mice fail to mount a strong immune response with secondary Ig-isotypes (11–13). In addition, a virtually identical phenotype was observed in mice lacking the Oct2 transcription factor (14). Although early B lymphopoiesis still occurs, BOB.1/OBF.1 is also critical for the formation of immature transitional B cells in the bone marrow (15). BOB.1/OBF.1-deficient mice lack marginal zone (MZ) B lymphocytes, whereas the B1 B cell compartment is apparently unaffected (16). The observed reduction of B cell numbers in bone marrow and spleen of BOB.1/OBF.1-deficient mice could have two reasons: the generation of B cells could be affected or alternatively, the B cells generated might undergo apoptosis. Indeed, BOB.1/OBF.1-deficient B cells show increased levels of apoptosis (15).

Here, we show that expression levels of Bcl2 in pre–B cells from BOB.1/OBF.1-deficient mice were reduced compared with wild-type mice. When BOB.1/OBF.1$^{-/-}$ mice were crossed with Bcl2-transgenic mice, several defects such as absolute B cell numbers and the maturation state in bone marrow and spleen were rescued. However, Bcl2 failed to rescue MZ B cells, cell signaling, and the germinal center reaction.

Address correspondence to Thomas Wirth, Dept. of Physiological Chemistry, Albert-Einstein-Allee 11, University of Ulm, Ulm, D-89081 Germany. Phone: 49-73-15-02-3270; Fax: 49-73-15-02-2892; E-mail: thomas.wirth@medizin.uni-ulm.de

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Materials and Methods

Mice. Wild-type mice, BOB.1/OBF.1$^{-/-}$, Bcl2-22 transgenic (Bcl2 tg), Bcl2$^{-/-}$ (17), or Bcl tg × BOB.1/OBF.1$^{-/-}$ mice (C57BL/6 background) were obtained from our breeding facility. Mice were analyzed at 8–12 wk.

Protein Immunoblots and RNase Protection Assay. Western blot analyses were performed as described previously (16) with monoclonal mouse Bcl2- or RelA-specific polyclonal antibodies (Santa Cruz Biotechnology, Inc.). RNase protection assays were performed with the kit provided by Becton Dickinson with the mApo2 multi-probe template set.

FACS® Analysis. FACS® analyses (16) were performed with the following antibodies: anti–B220-biotin, anti–CD21-FITC, anti–CD23-PE, anti–IgM-PE, anti–IgD-biotin, anti–CD1-FITC, anti–B220-PerCP, and anti–TNP-biotin (BD Biosciences). Biotin-labeled antibodies were revealed by streptavidin–Cy-Chrome or streptavidin-APC (BD Biosciences). Surface marker expression was analyzed with a four-color FACSCalibur™ and CELLQuest™ software (Becton Dickinson). Calcium influx into B220^{+} cells was analyzed as described previously (16). B220^{+} cells were sorted with FACsort™ plus cytometer (Becton Dickinson).

Determination of Serum Antibodies and Immunizations. Serum Ig titers were determined in serial dilutions by ELISA with isotype-specific goat anti-Ig–coated plates (Southern Biotechnology Associates, Inc.), goat anti-Ig alkaline phosphatase–linked antibodies, and the substrate *p*-nitrophenyl phosphate (Sigma-Aldrich). Mouse Igs of various isotypes (Southern Biotechnology Associates, Inc.) were used as internal standards.

Mice were immunized intraperitoneally with 100 μg TNP_{13}-OVA (Biosearch Technologies), preincubated in Alu-Gel-S (Serva) at day 0, boosted at day 14, and the ELISA with TNP_{14}-BSA was performed as described previously (18).

For affinity measurements, sera of each day and each group of mice were pooled and ELISAs with TNP_3-BSA– and TNP_{14}-BSA–coated plates were done. Ratios of TNP_3-BSA–binding and TNP_{14}-BSA–binding antibodies were calculated for each time point.

Mice were injected intravenously with 100 μg TNP-Ficoll (Biosearch Technologies). After 30 min, spleens were taken and used for histology and FACS® analysis.

Histological Analyses. Spleen sections were stained as described previously (16) with anti–IgM-PE (BD Biosciences) and MOMA-1–FITC (Serotec) or IgM-PE and IgD-FITC antibodies (BD Biosciences). Sections from TNP-Ficoll–treated animals were stained with a combination of anti–TNP-biotin (BD Biosciences) and MOMA-1–FITC antibodies. Biotinylated antibodies were revealed with streptavidin–Cy-Chrome (BD Biosciences). Nuclei were stained with DAPI (Roche). Germinal centers were visualized using PNA-biotin (Vector Laboratories; reference 19). OpenLab software (Improvision) was used for analysis.

Results and Discussion

Reduced Bcl2 Expression in BOB.1/OBF.1-deficient pre–B Cells. We noted previously a higher level of B cell apoptosis in BOB.1/OBF.1-deficient mice (15). Therefore, we analyzed the expression of pro- and antiapoptotic genes in BOB.1/OBF.1-deficient B cells. Bone marrow pre–B cell lines continuously growing in the presence of IL-7 plus stroma cells were established from wild-type and mutant mice to analyze cells of the same differentiation stage for the two genotypes (20; unpublished data). RNA was analyzed for expression of apoptosis-related genes. Interestingly, expression levels for Bcl2 were reduced in BOB.1/OBF.1-deficient pre–B cells (Fig. 1 A). This result was confirmed in protein immunoblots (Fig. 1 B). To analyze Bcl2 expression in mature peripheral B cells, B220^{+} cells were sorted from spleens of either wild-type or BOB.1/OBF.1-deficient mice. This analysis did not reveal significant differences in the expression levels of Bcl2 (Fig. 1 C). Therefore, the regulation of Bcl2 by BOB.1/OBF.1 is stage-dependent and most likely indirect.

B Cell–specific Expression of Bcl2 Rescues B Cell Numbers in Bone Marrow and Spleen. In the bone marrow, BOB.1/OBF.1-deficient mice show reduced numbers of transitional B cells and recirculating B cells. In the spleen, numbers of B cells are reduced, especially in the long-lived IgM^{low}/IgD^{high} population. We asked whether these defects could be corrected by transgenic expression of Bcl2. BOB.1/OBF.1$^{-/-}$ mice were crossed with mice expressing the Bcl2 transgene in B cells (21). Expression of Bcl2 by itself resulted in an increase of B cell numbers in the bone marrow and spleen of wild-type mice (Table I). Importantly, the reductions of B cell numbers observed in BOB.1/OBF.1-deficient mice were rescued both in the bone marrow as well as in the spleen (Table I). Numbers of all affected B cell populations were higher than in wild-type mice and approached numbers observed in the Bcl2 single transgenic mice. The increased ratio of apoptotic B lymphocytes was also corrected (Table I). These results demonstrate that forced expression of Bcl2 is sufficient to regenerate both normal numbers as

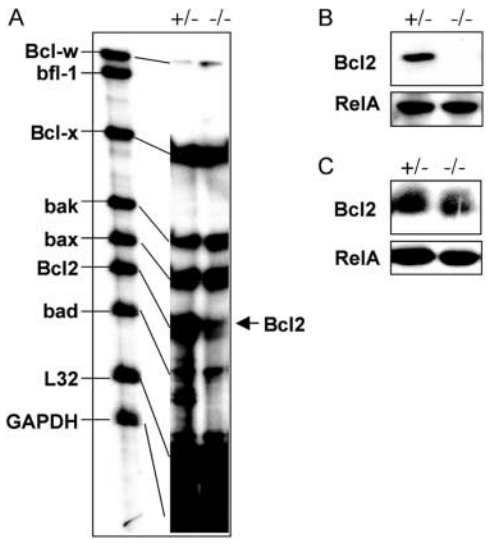

Figure 1. Impaired Bcl2 expression in bone marrow–derived IL7-dependent pre–B cells of BOB.1/OBF.1-deficient mice. (A) RPA was performed with 20 μg of total RNA prepared from pre–B cell lines of the indicated genotype and the murine Apo2 multi-probe template set. (B and C) Western blots were performed with antibodies specific for Bcl2 and, as a control, RelA. (B) 50 μg of protein extracts from IL-7–dependent bone marrow pre–B cell lines established from BOB.1/OBF.1$^{+/-}$ or $^{-/-}$ mice. (C) Protein extracts from 10^6 B220^{+} sorted splenocytes.

Table I. *B cell–specific Transgenic Expression of Bcl2 Rescues B Cell Numbers and Maturation Phenotypes in Bone Marrow and Spleen of BOB.1/OBF.1-deficient Mice*

	Wild type	Bcl2 tg	BOB.1/OBF.1 ko	Bcl2 tg × BOB.1/OBF.1 ko
			Absolute cell number × 10^7	
Spleen				
Total cellularity	9.0 ± 0.7	22.3 ± 2.2	6.2 ± 1.2	21.7 ± 1.6
$B220^+$	5.5 ± 0.9	16.8 ± 1.3	2.3 ± 0.3	14.5 ± 2.5
T1	1.8 ± 0.3	4.6 ± 0.5	1.1 ± 0.1	6.1 ± 0.6
T2	1.9 ± 0.3	5.6 ± 0.9	0.6 ± 0.1	3.7 ± 1.3
M	1.8 ± 0.3	5.7 ± 0.7	0.6 ± 0.1	3.8 ± 1.1
Annexin V^+/B220	0.46 ± 0.24	0.89 ± 0.29	0.25 ± 0.08	1.22 ± 0.59
Bone marrow				
Total cellularity	2.3 ± 0.4	3.3 ± 1.1	2.0 ± 0.7	2.7 ± 0.9
Pro/Pre B	0.54 ± 0.06	0.61 ± 0.08	0.64 ± 0.15	0.64 ± 0.15
Immature	0.32 ± 0.08	0.89 ± 0.14	0.33 ± 0.14	0.92 ± 0.09
Transitional	0.12 ± 0.02	0.31 ± 0.11	0.04 ± 0.01	0.16 ± 0.05
Recirculating	0.23 ± 0.06	0.57 ± 0.16	0.06 ± 0.04	0.26 ± 0.06
	%	%	%	%
Spleen				
$B220^+$	61.9 ± 9.7	75.3 ± 5.7	36.7 ± 5.2	66.6 ± 11.4
T1	20.4 ± 2.9	20.8 ± 2.2	16.9 ± 1.6	27.9 ± 2.7
T2	21.0 ± 5.8	24.9 ± 3.9	10.0 ± 1.1	17.1 ± 6.1
M	19.4 ± 3.6	25.4 ± 3.3	9.1 ± 2.0	17.6 ± 4.9
Annexin V^+/B220	8.3 ± 4.3	5.3 ± 1.7	10.7 ± 3.4	8.4 ± 4.1
Bone marrow				
Pro/Pre B	23.3 ± 2.5	18.6 ± 2.5	32.2 ± 7.3	23.7 ± 5.6
Immature	14.0 ± 3.4	26.9 ± 4.3	16.4 ± 7.2	34.1 ± 3.2
Transitional	5.1 ± 0.6	9.5 ± 3.4	1.8 ± 0.5	5.8 ± 1.8
Recirculating	10.0 ± 2.7	17.2 ± 5.0	3.2 ± 2.1	9.5 ± 2.2

The total cell numbers per spleen or femur from animals at 8–9 wk of age of each genotype were determined. Before analyses splenic cells were purified by Ficoll gradient. Bone marrow cells were stained with a combination of anti-B220 and anti-IgM antibodies to discriminate between pro/pre–B cells, immature, transitional and recirculating B cells. Splenocytes were stained with a mixture of anti-B220, anti-IgM, and anti-IgD antibodies to distinguish between the $IgM^{high}IgD^{low}$ (T1), $IgM^{high}IgD^{high}$ (T2), and $IgM^{low}IgD^{high}$ (M) populations and to determine the overall B cell numbers. Additionally, splenocytes were stained with a mixture of anti-B220 and anti–Annexin V antibodies. Mean percentages as well as absolute numbers for B cells of each compartment with standard deviation out of six to eight animals of each genotype are given.

well as a normal cell surface phenotype of B lymphoid cells in BOB.1/OBF.1-deficient mice.

Bcl2 Does Not Rescue Defective Immune Responses and Germinal Center Reactions. BOB.1/OBF.1 is important for antigen-dependent B cell responses (11–13). To analyze whether antibody responses were rescued by Bcl2 expression, we determined levels of serum immunoglobulins. Bcl2-transgenic wild-type mice showed increased serum immunoglobulin levels of all isotypes compared with controls. Although immunoglobulin levels in Bcl2 tg × BOB.1/OBF.$1^{-/-}$ mice were increased compared to knockout mice, they failed to reach wild-type levels (Fig. 2 A). This indicates that increased numbers of peripheral B cells resulted in only a partial rescue of immunoglobulin production.

We examined the antigen-specific T cell–dependent immune responses against TNP-OVA. Immunization of wild-type and Bcl2-transgenic mice led to a strong anti-TNP IgG1 production. In contrast, BOB.1/OBF.1-deficient mice showed only weak primary and secondary IgG1 responses, which were only partially corrected in tgBcl2/BOB.1/OBF.$1^{-/-}$ mice (Fig. 2 B). IgG1 production after 21 d and secondary restimulation in the tgBcl2/BOB.1/OBF.$1^{-/-}$ animals was significantly increased, but still ~10-fold lower than in wild-type mice. Given the two- to threefold higher numbers of B cells in tgBcl2/BOB.1/OBF.$1^{-/-}$ compared with wild-type mice, this indicates a profound defect in immunoglobulin production.

We wondered whether these mice did perform affinity maturation or whether increased Ig-levels represented an enhanced primary response. ELISAs were performed with sera of TNP-OVA–immunized mice with TNP_3-BSA– and TNP_{14}-BSA–coated plates. The ratio of low epitope

(TNP_3) to high epitope (TNP_{14}) binding of antibodies is proportional to their relative affinities, and affinity maturation can be measured in this way (22). Both wild-type and Bcl2-transgenic mice showed affinity maturation during primary and secondary responses (Fig. 2 C). Affinity maturation was completely absent in BOB.1/OBF.1-deficient mice and present at only low levels after secondary immunization in Bcl2 tg × BOB.1/OBF.$1^{-/-}$ mice. These results show for the first time a complete lack of affinity maturation in BOB.1/OBF.$1^{-/-}$ mice, a defect that cannot be rescued by expression of Bcl2.

After immunization with TNP-OVA, mice were analyzed for the formation of germinal centers. Staining of spleen sections with PNA revealed germinal centers in wild-type and Bcl2 tg mice. However, no germinal centers were detectable in BOB.1/OBF.1-deficient mice and Bcl2 tg × BOB.1/OBF.1-deficient mice (Fig. 2 D).

We had shown previously that Ca^{2+} influx after IgM stimulation is reduced in BOB.1/OBF.1-deficient B cells (16, 18). This defect is barely corrected by the Bcl2 transgene (Fig. 3). On a wild-type background, Bcl2 expression also resulted in slightly increased BCR signaling. These results indicate that transgenic Bcl2 does not efficiently rescue B cell functions, such as BCR signaling, Ig production, and affinity maturation. They require the presence of BOB.1/OBF.1. For the progression of pre–B cells to immature and mature B cells, competent signaling of the BCR is needed (23). In BOB.1/OBF.$1^{-/-}$ mice, transition from pro– to pre–B cells is not affected (15). Therefore, pre-BCR signaling might not be affected in BOB.1/OBF.1-deficient cells. In adult mice, Bcl2 is found in the pro- and pre–B cell stages, then at lower levels throughout the immature B cell stage, and finally at higher levels in the mature IgD^+ B cells (24). Bcl2 expression is diminished or absent in germinal centers (25, 26). Down-regulation of Bcl2 occurs at stages and sites associated with extensive apoptosis combined with powerful selection processes of B cells undergoing productive assembly of IgH and IgL chains or somatic hypermutations of their V region genes (26). Expression of Bcl2 in BOB.1/OBF.1-deficient B cells may result in impaired positive or negative selection processes during B cell maturation and consequently in an accumulation of B cells bearing either nonreactive or autospecific antigen receptors.

Bcl2 Does Not Rescue MZ B Cell Compartment in BOB.1/OBF.$1^{-/-}$ *Mice.* MZ B cells represent a distinct subset of effector B lymphocytes. They are exclusively located in the spleen, at the border between white and red pulp (27). In addition to the requirement of BOB.1/OBF.1 for germinal center formation, the MZ B cell compartment is also affected in BOB.1/OBF.1-deficient mice (16). We asked whether transgenic Bcl2 expression could rescue this de-

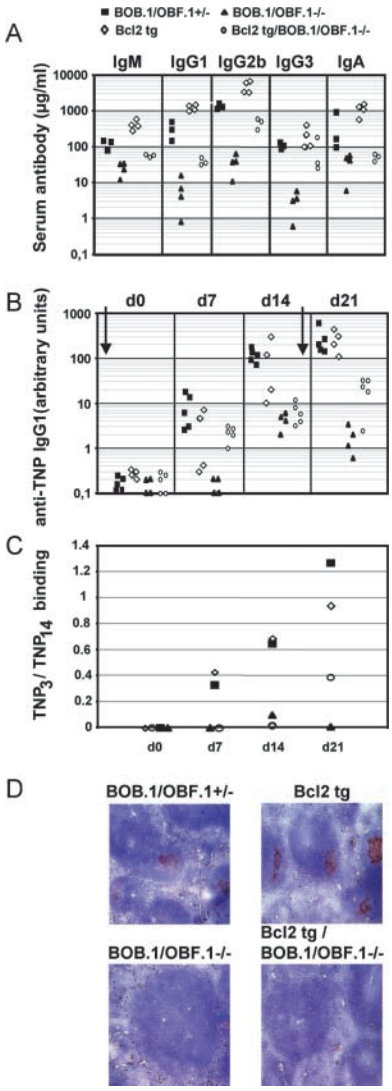

Figure 2. Bcl2 fails to rescue defective T cell–dependent immune responses in BOB.1/OBF.1-deficient mice. (A) Mice (age 10–12 wk) were bled and Ig levels determined by ELISA. Each symbol represents one individual mouse. (B) Analyses of IgG1 responses in TNP-OVA–immunized mice (age 10–12 wk). Each symbol represents one individual mouse. Arrows indicate days of TNP-OVA injection. (C) Sera of immunized mice (B) were pooled and assayed for TNP_3-BSA–binding and TNP_{14}-BSA–binding. The ratio of TNP_3- to TNP_{14}-binding is proportional to the affinity of the TNP-specific antibodies. (D) Spleen sections from TNP-OVA–immunized animals (day 14) stained with biotinylated PNA. Binding of PNA to germinal centers is visualized using streptavidin-HRP and AEC compound as a substrate. Sections were counterstained with hematoxylin.

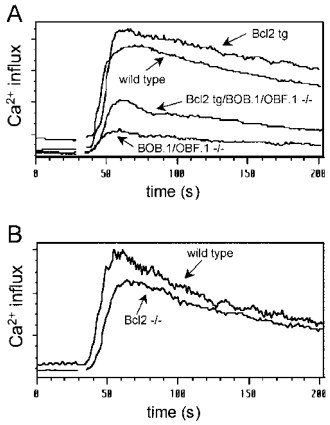

Figure 3. Bcl2 does not rescue impaired Ca^{2+} influx in response to IgM cross-linking. Calcium influx upon BCR stimulation into splenic B cells ($B220^+$) from wild-type, BOB.1/OBF.$1^{-/-}$, Bcl2 tg, and Bcl2 tg × BOB.1/OBF.$1^{-/-}$ (A) or wild-type and Bcl$2^{-/-}$ mice (B) was monitored by flow cytometry. Data are representative for four independent experiments. IgM levels per cell were comparable between all analyzed genotypes (not depicted).

fect. B220^{+} splenocytes were stained for CD21 and CD23 to discriminate between newly formed (transitional; CD21^{low}CD23^{low}), follicular (CD21^{int}CD23^{high}), and MZ B cells (CD21^{high}CD23^{low}). The MZ B cell population was not only absent in BOB.1/OBF.1-deficient mice (16) but also in wild-type mice expressing the Bcl2 transgene (Fig. 4 A). Consequently, this population was also absent in Bcl2 tg × BOB.1/OBF.1$^{-/-}$ mice (Fig. 4 A). Identical results were obtained when we checked for CD1^{high} cells among IgM^{high}/IgD^{low} cells, which are also markers of MZ B cells (unpublished data).

To exclude the possibility that absent MZ B cells in Bcl2-transgenic mice might be a consequence of misexpression of cell surface markers, we performed immunohistochemical examinations of spleens. The MOMA-1 antibody, specific for metallophilic macrophages, demarcates the border between MZ and follicle (27). The characteristic rim of IgM-positive MZ B cells was lacking in sections from BOB.1/OBF.1$^{-/-}$ mice (Fig. 4 B). Although IgM-positive cells could be detected in Bcl2 tg as well as in Bcl2 tg × BOB.1/OBF.1$^{-/-}$ mice, these cells also stained positive for IgD (Fig. 4 C). Because MZ B cells show only low levels of IgD and a high level of IgM (27, 28), this result suggests that cells outside of the MOMA-1 rim in the spleens of Bcl2-transgenic and Bcl2 tg × BOB.1/OBF.1$^{-/-}$ mice are not typical MZ B cells.

MZ B cells will rapidly capture intravenously injected antigens such as TNP-Ficoll. We injected mice with TNP-Ficoll and analyzed binding by FACS® and immunohistochemical analysis. In wild-type mice, TNP-Ficoll was exclusively bound by CD21^{high}CD23^{low} MZ B cells (16; unpublished data), which were exclusively localized outside the metallophilic macrophage rim (Fig. 4 D). No TNP-Ficoll–capturing B cells were seen in BOB.1/OBF.1$^{-/-}$ mice, and only low numbers in Bcl2-transgenic or Bcl2 tg × BOB.1/OBF.1$^{-/-}$ mice were seen by FACS® (unpublished data). In the latter cases, no TNP-Ficoll–capturing cells outside the rim of MOMA-staining were detected (Fig. 4 D). Therefore, these B cells cannot be considered functional MZ B cells.

The MZ is a complex structure consisting of a stromal cell framework in which specialized B cells, macrophages, and APCs interact. We performed a detailed analysis of the MZ structure to investigate whether the observed alterations of MZ B cells correlated with changes in the general MZ structure. Splenic follicles were properly developed in all mouse lines as judged by analyses of reticular fibroblasts and MZ macrophages, recognized by the ER-TR7 and

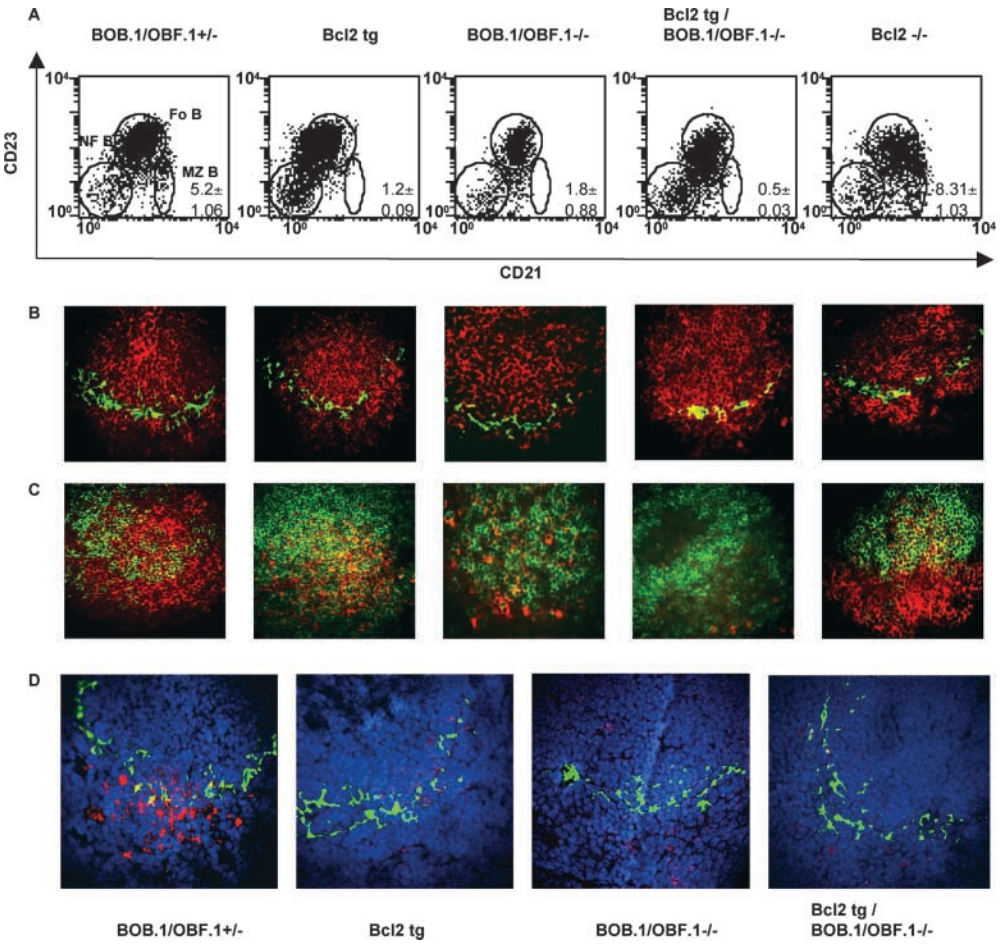

Figure 4. Bcl2 does not rescue marginal zone (MZ) B cell compartment in BOB1/OBF.1$^{-/-}$ mice. (A) Splenocytes from mice of the indicated genotypes were stained with a combination of antibodies specific for B220, CD21, and CD23. Newly formed (NF), follicular (FO), and MZ B cells are indicated. Mean percentages for MZ B cells with standard deviation (four animals each) are given. (B) Spleen sections from indicated genotypes were stained with antibodies recognizing IgM (PE/red) and MOMA-1 (FITC/green) or (C) IgM (PE/red) and IgD (FITC/green). (D) Spleen sections from TNP-Ficoll–immunized animals were stained with biotin-labeled anti-TNP (red) and FITC-labeled anti–MOMA-1 (green) antibodies. Nuclei were stained with DAPI (blue).

ER-TR9 antibodies (16; unpublished data). Therefore, the observed alterations of the MZ B cell compartment in BOB.1/OBF.1$^{-/-}$ and Bcl2-transgenic mice are not due to general changes in MZ architecture.

The MZ B Cell Population of Bcl2-deficient Mice Is Enlarged. The finding that Bcl2-transgenic mice have a reduced MZ B cell population could indicate that overexpression of Bcl2 is incompatible with a MZ B cell phenotype. Therefore, we investigated the MZ B cell population in Bcl2-deficient mice (17). We found a twofold increase in the number of MZ B cells, indicating that expression levels of Bcl2 affect the MZ B cell compartment (Figs. 4, A and B). Staining of histological sections of Bcl2-deficient mice revealed an enlarged IgM$^+$ B cell population outside the follicles. Several models are entertained regarding the generation of MZ B cells. Recent findings suggested that strong B cell receptor–derived signals block MZ B cell development (19, 29), whereas weak signals favor their differentiation. Consistent with this hypothesis, BCR cross-linking–induced Ca^{2+} influx was slightly decreased in Bcl2-deficient B cells compared with wild-type cells, whereas the signal was higher in Bcl2-transgenic B cells (Fig. 3 B). These observations could explain the altered MZ B cell populations in Bcl2-deficient and Bcl2-transgenic animals. Alternatively, the lineage decision of transitional B cells into MZ or follicular B cells may depend on the survival rate. Slower B cell turnover in Bcl2-transgenics may favor survival of follicular versus MZ B cells. Alternatively, due to the increased B cell numbers in the spleen, B cells with follicular phenotype (IgM^{low}IgD^{high}) may fill up other spaces than usual, displacing the MZ B cells from their usual location, which indeed was detected in spleen histology (Fig. 4 C). MZ B cells require the expression of LFA-1 and $\alpha4\beta1$ integrins for their specific localization (30). Bcl2 might influence the presence and/or function of integrins or chemokines, which are necessary for compartmentalization of MZ B cells. Together, these findings indicate an important role of Bcl2 during B cell maturation.

In summary, we have demonstrated a dependence of Bcl2 expression on BOB.1/OBF.1 in pre–B cells. This could explain the higher rate of apoptosis of early BOB.1/OBF.1-deficient B cells. The expression of Bcl2 in BOB.1/OBF.1-deficient B cells rescued their numbers and the maturation phenotype but not their function. This indicates a fundamental and complex role of BOB.1/OBF.1 in B cells independent of cell survival.

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