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Development of murine systemic lupus erythematosus in the absence of BAFF

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Abstract

Objective: To determine whether SLE can develop in the absence of BAFF in a SLE-prone host.

Methods: Starting with C57BL/6 mice that express a human *Bcl2* transgene (Tg) in their B cells (thereby rendering B cell survival largely independent of BAFF-triggered signals), we introgressed this Tg into NZM 2328 mice genetically deficient in BAFF (NZM.*Baff*^{-/-}) to generate NZM.*Baff*^{-/-}.*Bcl2*^{Tg} mice. Expression of human BCL2 and lymphocyte profiles were assessed by FACS; serologic profiles were assessed by ELISA; renal immunopathology was assessed by immunofluorescence and histology; and clinical disease was assessed by development of severe proteinuria and death.

Results: In comparison to their non-Tg NZM.*Baff*^{-/-} littermates, NZM.*Baff*^{-/-}.*Bcl2*^{Tg} mice over-expressed BCL2 in their B cells and developed increased percentages and numbers of B cells and plasma cells, increased serum levels of IgG autoantibodies, increased glomerular deposition of IgG and C3, and increased glomerular and tubulointerstitial pathology, culminating in severe proteinuria and death. The time course for development of SLE features in NZM.*Baff*^{-/-}.*Bcl2*^{Tg} mice was more rapid than that previously observed in NZM 2328 wild-type mice (median mortality 4.5 months vs 7.5 months). NZM.*Baff*^{-/-}.*Bcl2*^{Tg} mice remained responsive to BAFF, since re-introduction of the *Baff* gene into these mice further accelerated the course of disease (median mortality 3 months).

Conclusions: BAFF may be dispensable to development of disease as long as B cell survival is preserved via a BAFF-independent pathway. This may help explain the limited and variable clinical success with BAFF antagonists in human SLE. In addition, NZM.*Baff*^{-/-}.*Bcl2*^{Tg} mice may serve as a powerful murine model for the study of BAFF-independent SLE.

Introduction

One of the classic hallmarks of systemic lupus erythematosus (SLE) is B cell hyperactivity. Studies in murine SLE have conclusively demonstrated that B cells are indispensable, in that otherwise SLE-prone MRL.*lpr/lpr* or NZM 2328 (NZM) mice fail to develop SLE when genetically rendered B cell-deficient (1, 2).

BAFF (also known as BLyS) is a vital survival and differentiation factor for B cells (3–6). In non-SLE-prone mice, antagonism of BAFF results in considerable, albeit sub-total, reduction in B cells (7), and genetic disruption of the *Baff* gene results in even greater, albeit still sub-total, reduction in B cells (8). Comparable observations have been made in SLE-prone mice, with pharmacologic neutralization with either of two BAFF antagonists or genetic elimination of BAFF leading to substantial (sub-total) reduction in B cells and concurrent clinical efficacy (9–11).

In human SLE, treatment with any of four different BAFF antagonists also results in sub-total reduction in circulating B cells (12–16). However, clinical efficacy has not always been achieved. Whereas each of the four phase-III clinical trials in SLE with the anti-BAFF mAb, belimumab, met its primary endpoint (17–20), the phase-II trials with either blisibimod or atacicept have not (13, 21), and only one of the two phase-III trials with tabalumab met its primary endpoint (15, 16). Even treatment with belimumab, which is approved by the FDA for the treatment of SLE (reviewed in (22)), failed to elicit a clinical response in >40% of subjects in its phase-II or phase-III SLE clinical trials (17, 18, 20, 23).

The heterogeneity in clinical response to BAFF antagonism raises the possibility that incomplete B cell depletion may be a contributing factor to the clinical resistance of some patients to BAFF-antagonist therapy. That is, pathogenic B cells that survive pharmacologic neutralization of BAFF may continue to promote disease in some patients. Accordingly, the degree of (pathogenic) B cell depletion induced by a BAFF antagonist may be sufficient to ameliorate disease activity in some SLE patients, whereas the degree of (pathogenic) B cell depletion induced by the same BAFF antagonist may be insufficient to ameliorate disease activity in other SLE patients.

To test whether a SLE-prone host otherwise clinically responsive to BAFF elimination would remain clinically responsive if B cells were preserved in the face of elimination of BAFF, we turned to mice in which the B cells harbor a *Bcl2* transgene (Tg). In such mice, survival of B cells, which constitutively over-express BCL2, largely does not depend upon BAFF-triggered signals (24, 25).

Accordingly, we introgressed the *Bcl2* Tg into NZM mice genetically deficient in BAFF (NZM.*Baff*^{-/-}) (11) to generate NZM.*Baff*^{-/-}.*Bcl2*^{Tg} mice. We demonstrate that these mice, despite the life-long absence of BAFF, develop the immunologic, pathologic, and clinical features of SLE over a time course even more rapid than that of NZM wild-type (WT) mice. Thus, BAFF is dispensable to development of disease in NZM mice under conditions in which B cell survival is preserved via a BAFF-independent pathway. By inference, some SLE patients may be inherently resistant to BAFF-directed therapies by virtue of pathogenic

B cells whose survival is independent of BAFF. NZM.*Baff*^{-/-}.*Bcl2*^{Tg} mice may serve as a powerful murine model in the study of this BAFF-independent component of SLE.

Materials and methods

General.

All reported studies were approved by the University of Southern California (USC) Institutional Animal Care and Use Committee (IACUC).

Mice.

NZM WT and NZM.*Baff*^{-/-} mice (11) were maintained in a single specific pathogen-free room. B6.Cg-Tg(BCL2)22Wehi/J (C57BL/6 background bearing a human *Bcl2* Tg expressed only in B lineage cells; B6.*Bcl2*^{Tg}) mice (26) were obtained from Jackson Laboratories and housed in the same specific pathogen-free room. NZM.*Baff*^{-/-}.*Bcl2*^{Tg} mice were generated by introgressing the *Bcl2*^{Tg} genotype from B6.*Bcl2*^{Tg} mice into NZM.*Baff*^{-/-} mice by the marker-assisted selection protocol (27, 28) using 47 microsatellite markers. These markers were chosen to include those regions identified as susceptibility loci in NZM mice (29). The N7 backcross generation was fully congenic. NZM.*Baff*^{+/+}.*Bcl2*^{Tg} and non-Tg NZM.*Baff*^{+/+} littermate mice were generated by mating NZM.*Baff*^{-/-}.*Bcl2*^{Tg} mice with NZM WT (*Baff*^{+/+}) mice. Results are reported for female mice only.

For the purpose of identifying Foxp3⁺ T regulatory (Treg) cells in NZM WT mice, NZM^{*foxp3-gfp*} mice were generated by introgressing the *Foxp3-gfp* knock-in genotype from B6^{*foxp3-gfp*} mice (obtained from Dr. Song Guo Zheng) into NZM WT mice. For the purpose of identifying Foxp3⁺ cells in NZM.*Baff*^{-/-}.*Bcl2*^{Tg} mice, NZM^{*foxp3-gfp*} mice were first crossed with NZM.*Baff*^{-/-} mice to generate NZM^{*foxp3-gfp*}.*Baff*^{-/-} mice. The *Bcl2*^{Tg} genotype was then introgressed from B6.*Bcl2*^{Tg} mice into NZM^{*foxp3-gfp*}.*Baff*^{-/-} mice to yield NZM^{*foxp3-gfp*}.*Baff*^{-/-}.*Bcl2*^{Tg} mice.

Cell surface staining.

Spleen mononuclear cells were stained with combinations of fluorochrome-conjugated mAb specific for CD1d, CD3, CD4, CD8, CD19, CD44, CD62L, CD69, CD138, sIgD, or sIgM (BioLegend or BD Bioscience) to identify total B (CD19⁺) cells, follicular (FO) B (CD3⁻CD19⁺IgM^{lo}IgD^{hi}) cells, marginal zone (MZ) B (CD3⁻CD19⁺IgM^{hi}IgD^{lo}CD1d^{hi}) cells, plasma (CD3⁻CD138⁺CD19^{lo/-}) cells, total CD4⁺ cells, total CD8⁺ cells, activated memory T (CD4⁺CD44^{hi}CD62L^{lo}) cells, and recently-activated CD4⁺ T (CD4⁺CD69⁺) cells. Data were analyzed with FlowJo 7.6.1 software (Treestar).

Intracellular staining for BCL2.

Spleen mononuclear cells were first stained for surface CD3 and CD19 as above. Following treatment with Fix/Perm buffer (BioLegend), the cells were stained for intracellular BCL2 with phycoerythrin-conjugated anti-human BCL2 or isotype control mAb (BioLegend) and analyzed as above.

Serum total IgG and IgG autoantibody levels.

Serum levels of total IgG and IgG autoantibodies were determined by ELISA (2). Autoantibody OD values were normalized to the mean OD of serum from 5-month-old MRL-*lpr* mice, the latter arbitrarily assigned a value of 100 U/ml.

Kidney histology.

Sections of formalin-fixed kidneys were stained with hematoxylin and eosin and assessed by light microscopy (30).

Kidney immunofluorescence.

Sections of snap-frozen kidneys were stained for IgG or C3 deposition using FITC-conjugated goat F(ab')₂ fragment anti-mouse IgG or C3 antibodies (MP Biomedicals) (2).

Assessment of clinical disease.

Mice were monitored for development of severe proteinuria or death, and development of each was recorded separately. To assess proteinuria, reagent strips for urinary protein (Albustix, Bayer) were dipped in mouse urine and were assigned a score (0–4) by visual color comparison to the supplied standard color key. Severe proteinuria was defined as 3+ on two consecutive examinations prior to natural death or 3+ at the time of euthanasia. Since the death of some NZM.*Baff*^{+/−}.*Bcl2*^{Tg} mice was very precipitous and resulted in the absence at the moribund state of assessment of proteinuria (which, presumably would have been severe), severe clinical disease in these mice was taken as development of either severe proteinuria or death.

In vitro Treg cell function.

NZM^{*foxp3-gfp*} WT T responder (Tresp; CD4⁺Foxp3[−]) cells and Treg (CD4⁺Foxp3⁺) cells from NZM^{*foxp3-gfp*} WT or NZM^{*foxp3-gfp*}.*Baff*^{+/−}.*Bcl2*^{Tg} mice were isolated by FACS-sorting spleen CD4⁺ T cells. The Tresp cells were stained with proliferation dye eFluor 670 (eBioscience) and were stimulated with anti-CD3 mAb (0.025 mg/ml, BioLegend) and irradiated B6 WT non-T cells (30 Gy, 1:1 ratio) in the presence of graded ratios of Treg cells. At day 3, cells were harvested, and the division index (DI; total number of Tresp cell divisions/original number of Tresp cells), the proliferation index (PI; total number of Tresp cell divisions/number of Tresp cells undergoing at least one division), and percentage of dividing cells (number of Tresp cells undergoing at least one division/original number of Tresp cells) were evaluated by FACSCalibur (BD Biosciences). Results are expressed as % suppression, defined as ((the value obtained in cultures with Tresp cells only) — (the value obtained in cultures with Tresp cells + Treg cells)) / (the value obtained in cultures with Tresp cells only).

In vitro responsiveness of B cells to BAFF.

Spleen B cells were isolated by autoMACS (Miltenyi) with anti-mouse CD19 microbeads (Miltenyi). To assess B cell survival, B cells were cultured (2×10^5 /0.2 ml) in triplicate in 96-well flat-bottomed plates in RPMI 1640 + 10% FCS, with or without exogenous

recombinant BAFF (100 ng/ml; R&D). Viability of the cells was determined at 48 hr by staining with annexin-V and propidium iodide (BioLegend).

Statistical analysis.

All analyses were performed using SigmaStat software (SPSS). Parametric testing between two groups was performed by the unpaired t test and among three or more groups by one-way ANOVA. When the data were not normally distributed or the equal variance test was not satisfied, non-parametric testing was performed by the Mann-Whitney rank sum test between two groups and by Kruskal-Wallis one-way ANOVA on ranks among three or more groups. Survival data were analyzed by the log rank test.

Results

Selective over-expression of BCL2 in NZM.*Baff*^{-/-}.*Bcl2*^{Tg} mouse B cells.

To confirm at the protein level that BCL2 is selectively over-expressed in the B cells of NZM.*Baff*^{-/-}.*Bcl2*^{Tg} mice, B (CD19⁺) and T (CD3⁺) cells from NZM WT, NZM.*Baff*^{-/-}, and NZM.*Baff*^{-/-}.*Bcl2*^{Tg} mice were analyzed for intracellular expression of BCL2. Expression of BCL2 in NZM WT B cells was similar to that in NZM WT T cells (Figure 1). Expression of BCL2 in B cells and T cells from NZM.*Baff*^{-/-} mice was lower than that in the corresponding cells from NZM WT mice, the flip side of the previously reported increase in BCL2 expression in C57BL/6 mice bearing a *Baff* transgene (31). In contrast, whereas expression of BCL2 in T cells from NZM.*Baff*^{-/-}.*Bcl2*^{Tg} mice was similar to that in NZM WT T cells, expression of BCL2 in B cells from NZM.*Baff*^{-/-}.*Bcl2*^{Tg} mice was greatly increased.

B-lineage cell profiles.

In non-SLE-prone mice, BAFF deficiency results in dramatic reductions in B cells at or beyond the T2 stage, including FO B cells and MZ B cells (7, 8). Introduction of a *Bcl2*^{Tg} into the B cells of such BAFF-deficient mice restores B cells other than MZ B cells (24). We observed a similar pattern with NZM mice. Consistent with our previous observations (11), NZM.*Baff*^{-/-} mice at 2 months of age harbored decreased percentages and numbers of total B (CD19⁺) cells, FO B (CD19⁺IgM^{lo}IgD^{hi}) cells, and MZ B (CD19⁺IgM^{hi}IgD^{lo}CD1d^{hi}) cells as compared to those in age-matched NZM WT mice. In NZM.*Baff*^{-/-}.*Bcl2*^{Tg} littermates of the NZM.*Baff*^{-/-} mice, percentages and numbers of total B cells and FO B cell were restored to at least WT levels, whereas essentially no restorative effect on the MZ B cell population was observed (Figure 2a–f).

In line with the reductions in mature B cells, we previously documented that NZM.*Baff*^{-/-} mice harbor fewer plasma cells than do NZM WT mice (30). Consistent with these findings, 2-month-old NZM.*Baff*^{-/-} mice harbored decreased percentages and numbers of plasma cells than did age-matched NZM WT mice. Strikingly, the percentages and absolute numbers of CD3⁻CD138⁺CD19^{lo/-} plasma cells in the NZM.*Baff*^{-/-}.*Bcl2*^{Tg} littermates of the NZM.*Baff*^{-/-} mice surpassed WT levels (Figure 2g–h).

T cell profiles.

As opposed to the stark differences across the three mouse cohorts in spleen B cell-lineage cells, no differences were noted in percentages of CD4⁺ and CD8⁺ cells within the spleen total T cell population (Figure 2i–j). Nevertheless, differences in activated CD4⁺ T cell subsets were noted. Within the CD4⁺ cell subset, the percentages of activated memory (CD4⁺CD44^{hi}CD62L^{lo}) cells were reduced in NZM.*Baff*^{−/−} mice but were restored in their NZM.*Baff*^{−/−}.*Bcl2*^{Tg} mice littermates (Figure 2k). Moreover, the percentages within the CD4⁺ cell subset of recently-activated (CD4⁺CD69⁺) cells were similar in NZM WT and NZM.*Baff*^{−/−} mice, whereas they were increased in NZM.*Baff*^{−/−}.*Bcl2*^{Tg} mice (Figure 2l).

Serologic profiles.

BAFF deficiency in non-SLE-prone mice not only leads to marked reductions in mature B cells but also leads to marked reductions in baseline serum Ig levels and Ig responses to T cell-dependent and T cell-independent antigens (7, 8). We previously demonstrated that, compared to NZM WT mice, the serum levels of total IgG and some IgG autoantibodies in NZM.*Baff*^{−/−} mice are substantially reduced through 6 months of age, although the levels “catch up” by 7–9 months of age (11). Consistent with the increases in B cells, plasma cells, and activated CD4⁺ T cells, serum levels of total IgG and IgG autoantibodies were greater in 2-month-old NZM.*Baff*^{−/−}.*Bcl2*^{Tg} mice than in their littermate NZM.*Baff*^{−/−} mice (Figure 3). Serum levels of IgG anti-chromatin and IgG anti-dsDNA continued to rise through 4 months of age, and the declines in serum total IgG and IgG autoantibody levels at 5–6 months of age reflect the premature death (see below) of those mice that harbored high serum levels of total IgG and IgG autoantibodies at earlier time points.

Glomerular IgG and C3 deposition.

NZM WT mice develop considerable glomerular deposition of IgG and C3 by 6 months of age, with such deposition being substantially reduced in NZM.*Baff*^{−/−} mice (11). To assess the time of onset of glomerular IgG and C3 deposition, we first examined mice 2 months of age. At this young age, glomerular deposition of IgG was already evident in each of the eight NZM.*Baff*^{−/−}.*Bcl2*^{Tg} mice examined. In contrast, glomerular IgG deposition was detected in none of the seven NZM.*Baff*^{−/−} littermate mice examined and in only one of the four age-matched control NZM WT mice examined (Figure 4a–c).

Glomerular deposition of C3 lagged behind glomerular deposition of IgG, with unequivocal deposition at 2 months of age observed in only one of the eight NZM.*Baff*^{−/−}.*Bcl2*^{Tg} mice examined and in none of the seven NZM.*Baff*^{−/−} littermate mice or four age-matched control NZM WT mice examined (Figure 4d–f).

In older mice, the disparity between NZM.*Baff*^{−/−}.*Bcl2*^{Tg} and NZM.*Baff*^{−/−} mice was more apparent. Glomerular deposition of both IgG and C3 in each of the five NZM.*Baff*^{−/−}.*Bcl2*^{Tg} mice examined at 5–7 months of age was at least as robust as that in age-matched NZM WT mice. In contrast, glomerular deposition of IgG in NZM.*Baff*^{−/−} mice was considerably weaker, with such deposition not being appreciated in four of the 12 mice examined at 5.5–8 months of age and being only weakly detected in six of these mice. Moreover, no glomerular deposition of C3 was detected in any of these 12 NZM.*Baff*^{−/−} mice (Figure 4g–i).

Renal pathology.

At 2 months of age, occasional interstitial/perivascular infiltrates were observed in a minority of NZM.*Baff*^{-/-}.*Bcl2*^{Tg} mice. No such infiltrates were appreciated in NZM.*Baff*^{-/-} littermate mice or in age-matched control NZM WT mice. Glomeruli appeared normal in all 2-month-old mice studied, including NZM.*Baff*^{-/-}.*Bcl2*^{Tg} mice (Figure 4m–r).

In contrast, marked glomerular swelling and hypercellularity, along with widespread interstitial/perivascular infiltrates were the rule in NZM.*Baff*^{-/-}.*Bcl2*^{Tg} mice by 5 months of age. These pathological changes were at least as severe as those in 7-month-old NZM WT mice. Conversely, evaluation of kidney sections from NZM.*Baff*^{-/-} mice as old as 8 months revealed no appreciable histological disease (Figure 4s–x).

Clinical disease.

We have previously shown that NZM WT mice start to develop severe proteinuria at 5–6 months of age, with 50% of the mice being affected by 7 months and 90+% being affected by 10 months. In contrast, development of severe proteinuria in NZM.*Baff*^{-/-} mice is markedly attenuated, with no mice being affected at 8 months of age, and only 20% being affected by 12 months (11). Consistent with this latter observation, NZM.*Baff*^{-/-} mice in the present study failed to manifest severe proteinuria by 8 months of age (the oldest age at which the mice were euthanized). In sharp contrast, development of severe proteinuria among NZM.*Baff*^{-/-}.*Bcl2*^{Tg} mice was appreciated as early as 3 months of age, with 50% of the mice being affected by 4 months ($p < 0.001$ in comparison to NZM.*Baff*^{-/-} mice; Figure 5 left).

Mortality patterns paralleled the proteinuria patterns. We previously reported that NZM WT mice began dying at 6 months of age, with 50% mortality at 7–8 months and 90% mortality at 10–11 months, whereas all NZM.*Baff*^{-/-} mice remained alive at 9 months of age with only 10% mortality at 12 months (11). In the present study, all NZM.*Baff*^{-/-} mice remained alive through 8 months of age, whereas NZM.*Baff*^{-/-}.*Bcl2*^{Tg} mice died as early as 3 months of age, with 50% mortality at 4.5 months (Figure 5 right). That is, full-blown (accelerated) SLE clinical disease developed even in the life-long absence of BAFF.

Relative decrease of Treg cells in NZM.*Baff*^{-/-}.*Bcl2*^{Tg} mice.

Given the importance of Treg cells to immune homeostasis and the prevention of autoimmunity in general (32) and the suppression of SLE in specific (33), the accelerated onset of disease in NZM.*Baff*^{-/-}.*Bcl2*^{Tg} mice raised the possibility that Treg cell number and/or function was altered in NZM.*Baff*^{-/-}.*Bcl2*^{Tg} mice relative to that in NZM WT mice. To address this possibility, we generated NZM WT and NZM.*Baff*^{-/-}.*Bcl2*^{Tg} mice that bore a *Foxp3-gfp* knock-in genotype (NZM^{*foxp3-gfp*} and NZM^{*foxp3-gfp*}.*Baff*^{-/-}.*Bcl2*^{Tg} mice, respectively), thereby permitting not only identification of Foxp3⁺ (Treg) cells but also permitting their functional capacities.

As observed with NZM WT and NZM.*Baff*^{-/-}.*Bcl2*^{Tg} mice not bearing the *Foxp3-gfp* knock-in genotype, B (CD19⁺) cells were greatly expanded in NZM^{*foxp3-gfp*}.*Baff*^{-/-}.*Bcl2*^{Tg} mice relative to that in NZM^{*foxp3-gfp*} mice (Supplemental Figure 1A). In contrast, these mice

harbored similar numbers of CD3⁺ cells, CD4⁺, and Foxp3⁺ cells. Whereas the slopes of the regression lines for Foxp3⁺ cells vs CD3⁺ cells and for Foxp3⁺ cells vs CD4⁺ cells were close in NZM^{foxp3-gfp} and NZM^{foxp3-gfp.Baff^{-/-}.Bcl2^{Tg}} mice (0.057 vs 0.116 and 0.086 vs 0.110), the slope for Foxp3⁺ cells vs CD19⁺ cells was much shallower in NZM^{foxp3-gfp.Baff^{-/-}.Bcl2^{Tg}} mice (0.0185) than in NZM^{foxp3-gfp} mice (0.0779; Supplemental Figure 1B), pointing to a relative inability of NZM^{foxp3-gfp.Baff^{-/-}.Bcl2^{Tg}} mice to generate Treg cells in response to the degree of B cell expansion.

To determine whether Treg cell function was also altered in NZM^{foxp3-gfp.Baff^{-/-}.Bcl2^{Tg}} mice, we tested Treg cells from NZM^{foxp3-gfp} and NZM^{foxp3-gfp.Baff^{-/-}.Bcl2^{Tg}} mice for *in vitro* suppressor activity against NZM^{foxp3-gfp} WT Tresp cells. Regardless of whether suppression was assessed by DI, PI, or percentages of cells that underwent division, *in vitro* suppressor activity of NZM^{foxp3-gfp.Baff^{-/-}.Bcl2^{Tg}} Treg cells was virtually identical to that exhibited by NZM^{foxp3-gfp} WT Treg cells (Supplemental Figure 1C).

Sensitivity of NZM.Baff^{-/-}.Bcl2^{Tg} mice to restoration of BAFF.

The fact that full-blown (accelerated) SLE clinical disease developed in BAFF-deficient NZM.Baff^{-/-}.Bcl2^{Tg} mice raised the possibility that disease in these mice might be insensitive to BAFF. That is, the constitutive over-expression of BCL2 in the B cells of these mice may have maximally stimulated their inherent autoimmune diathesis and rendered them unresponsive to further BAFF-generated “autoimmunogenic” signals.

To determine whether B cells from NZM.Baff^{-/-}.Bcl2^{Tg} mice remained responsive to BAFF, we cultured B cells from NZM WT, NZM.Baff^{-/-}, and NZM.Baff^{-/-}.Bcl2^{Tg} mice in the absence or presence of exogenous BAFF. In all cases, survival of B cells was enhanced when BAFF was added to the cultures (Figure 6A). Not surprisingly, survival of B cells from NZM.Baff^{-/-}.Bcl2^{Tg} mice, by virtue of their over-expression of BCL2, was greater than that of B cells from either NZM WT or NZM.Baff^{-/-} mice in either the absence or presence of exogenous BAFF.

To establish that restoration of BAFF to NZM.Baff^{-/-}.Bcl2^{Tg} mice would also have an *in vivo* effect, these mice were mated to BAFF-sufficient NZM WT (Baff^{+/+}) mice. All resulting offspring were genetically Baff^{+/+}, with each pup having a 50% chance of bearing the Bcl2^{Tg}.

Ten NZM.Baff^{+/+}.Bcl2^{Tg} mice and nine littermate non-Tg NZM.Baff^{+/+} mice were generated. In stark contrast to non-Tg NZM.Baff^{+/+} mice that remained clinically healthy for their entire 8-month observation period, NZM.Baff^{+/+}.Bcl2^{Tg} mice developed clinical disease very quickly. Eight NZM.Baff^{+/+}.Bcl2^{Tg} mice were either dead or moribund by 3 months of age, and the other two mice succumbed by 4 months and 5 months of age, respectively (p < 0.001 in comparison to non-Tg NZM.Baff^{+/+} mice). Importantly, development of clinical disease in NZM.Baff^{+/+}.Bcl2^{Tg} mice was more robust than that in NZM.Baff^{-/-}.Bcl2^{Tg} mice, in that only 40% of the latter had developed severe clinical disease by 3 months of age, and 13% of them continued to remain free of severe proteinuria even at 6.5 months of age (p = 0.020; Figure 6B).

Discussion

Not only does pharmacologic neutralization of BAFF or its genetic elimination effectively treat “natural” SLE in SLE-prone mice (9–11), but life-long deficiency of BAFF also protects against development of IFN α -accelerated disease (2), and short-term neutralization of BAFF with TACI-Ig at the time of IFN α challenge delays IFN α -accelerated disease (34). Thus, even the explosive disease promoted by IFN α is highly sensitive to BAFF elimination/neutralization. This profound sensitivity of SLE disease to loss of BAFF notwithstanding, we now document that full-blown SLE can develop in a BAFF-deficient SLE-prone host in which B survival is not critically dependent upon BAFF.

Given that NZM.*Baff*^{-/-}.*Bcl2*^{Tg} mice harbor more B cells, plasma cells, and activated CD4⁺ T cells than do NZM WT mice, it may not be surprising that development of serological autoimmunity, renal immunopathology, and clinical disease is accelerated in these mice relative to that in BAFF-sufficient NZM WT mice. The blunted expansion of Treg cells for the degree of B cell expansion in NZM.*Baff*^{-/-}.*Bcl2*^{Tg} mice relative to that in NZM WT mice, consistent with a role for BAFF in promoting Treg cells (35), may be a contributing factor. B cell expansion may be more limited (and, hence, disease onset may be delayed) in NZM WT mice than in NZM.*Baff*^{-/-}.*Bcl2*^{Tg} mice due to the relative abundance of Treg cells in the former. In any case, the early expansions of B cells, recently-activated CD4⁺ T cells, and activated memory CD4⁺ T cells in NZM.*Baff*^{-/-}.*Bcl2*^{Tg} mice parallel similar expansions in other genetically-modified NZM mice that develop accelerated disease, including NZM mice doubly-deficient in both TNF receptors, NZM mice doubly-deficient in the BAFF receptors, BCMA and TACI, and NZM mice deficient in NCF2 (36–38).

Although our studies do not address the critical quantitative threshold for B cells, plasma cells, and/or activated CD4⁺ T cells needed for BAFF-independent disease, our studies do raise the plausibility of SLE patients maintaining such threshold levels despite treatment with a BAFF antagonist and, thereby, remaining resistant to BAFF-antagonist therapy. BAFF is certainly not the only clinically relevant B cell survival factor, as evidenced by the persistence of substantial numbers of circulating B cells in SLE patients treated with the BAFF antagonists belimumab (12), blisibimod (13), atacicept (14), or tabalumab (15, 16). Activities of such non-BAFF B cell survival factors (not neutralized by belimumab) may help explain why 39% of SLE patients failed to achieve the pre-defined clinical response in each of the successful phase-III belimumab trials (17–20).

One factor that may importantly contribute to B cell survival in SLE is BCL2. BCL2 is up-regulated in B cells (and T cells) that infiltrate the tubulointerstitium in human SLE nephritis, and the BCL2 inhibitor, venetoclax (previously known as ABT-199), prolonged survival and prevented proteinuria and development of tubulointerstitial inflammation in the (NZBxNZW)F1 SLE model (39). The relationship between the degree of up-regulation of BCL2 and clinical responsiveness to a BAFF antagonist (i.e., belimumab) in human SLE remains unknown and should be assessed. Of note, acceptable safety and tolerability of venetoclax has been documented in a phase-I trial in human SLE (40). Given that restoration of BAFF to NZM.*Baff*^{-/-}.*Bcl2*^{Tg} mice results in further aggravation of disease, the addition

of a BCL2 inhibitor might be a useful therapeutic option especially in SLE patients clinically unresponsive to BAFF antagonism.

It must be stressed that the clinical efficacy seen with BAFF antagonists may not solely due to their effects on B cells. In both murine and human SLE, the reduction in B cells following treatment with anti-CD20 mAb is much greater than that following treatment with BAFF antagonists (41–45). However, despite good clinical efficacy with this approach in murine SLE, the two phase-II/III SLE trials with the anti-CD20 mAb, rituximab, failed to reach their primary endpoints (44, 45) as did the phase-III SLE trial with the anti-CD20 mAb, ocrelizumab (46).

Given that greater levels of B cell depletion do not necessarily translate into better clinical responses, it is possible that the (variable) success of BAFF antagonists in their clinical trials versus the failure of anti-CD20 mAb in their clinical trials may, at least in part, be related to non-B cells. Whereas CD20 (the target of anti-CD20 mAb) is expressed only on B cells (47), BAFF receptors (the targets of BAFF) are expressed not only on B cells but (weakly) on certain non-B cells as well (48–52). Indeed, we recently demonstrated that BAFF has a B cell-independent effect in experimental autoimmune encephalomyelitis (53), so BAFF may be contributing to SLE not only through its agonist effects on B cells but through effects on non-B cells as well. In any case, the present study demonstrates in the NZM murine SLE model that, regardless of the putative non-B cell-mediated contribution of BAFF to SLE, BAFF can be dispensable to development of disease as long as B cell survival is preserved via a BAFF-independent pathway. Importantly, the BAFF-independent disease that develops in NZM.*Baff*^{-/-}.*Bcl2*^{Tg} mice is aggravated by re-introduction of BAFF, proving that SLE disease can be effected via BAFF-independent and BAFF-dependent pathways in the same host.

To our knowledge, NZM.*Baff*^{-/-}.*Bcl2*^{Tg} mice represent the first and only mouse model of SLE that is fully independent of BAFF. Whereas a large percentage of SLE patients does not clinically respond to BAFF-antagonist therapy (13, 15–19, 21, 23), thereby pointing to a substantial BAFF-independent component to human SLE, the clinical responses to BAFF neutralization or elimination is highly robust among commonly utilized murine SLE models (9–11), thereby pointing to a very limited (if any) BAFF-independent component in these models. NZM.*Baff*^{-/-}.*Bcl2*^{Tg} mice can now serve as a valuable model in the investigation of the *in vivo* BAFF-independent component to SLE.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Shlomchik MJ, Madaio MP, Ni DH, Trounstein M, Huszar D. The role of B cells in *lpr/lpr*-induced autoimmunity. *Journal of Experimental Medicine*. 1994;180(4):1295–306. [PubMed: 7931063]
2. Jacob N, Guo SH, Mathian A, Koss MN, Gindea S, Putterman C, et al. B cell and BAFF dependence of IFN- α -exaggerated disease in systemic lupus erythematosus-prone NZM 2328 mice. *Journal of Immunology*. 2011;186(8):4984–93.
3. Thompson JS, Schneider P, Kalled SL, Wang L, Lefevre EA, Cachero TG, et al. BAFF binds to the tumor necrosis factor receptor-like molecule B cell maturation antigen and is important for maintaining the peripheral B cell population. *Journal of Experimental Medicine*. 2000;192(1):129–35. [PubMed: 10880534]
4. Do RKG, Hatada E, Lee H, Tourigny MR, Hilbert D, Chen-Kiang S. Attenuation of apoptosis underlies B lymphocyte stimulator enhancement of humoral immune response. *Journal of Experimental Medicine*. 2000;192(7):953–64. [PubMed: 11015437]
5. Rolink AG, Tschopp J, Schneider P, Melchers F. BAFF is a survival and maturation factor for mouse B cells. *European Journal of Immunology*. 2002;32(7):2004–10. [PubMed: 12115621]
6. Litinskiy MB, Nardelli B, Hilbert DM, He B, Schaffer A, Casali P, et al. DCs induce CD40-independent immunoglobulin class switching through BLyS and APRIL. *Nature Immunology*. 2002;3(9):822–9. [PubMed: 12154359]
7. Gross JA, Dillon SR, Mudri S, Johnston J, Littau A, Roque R, et al. TACI-Ig neutralizes molecules critical for B cell development and autoimmune disease: Impaired B cell maturation in mice lacking BLyS. *Immunity*. 2001;15(2):289–302. [PubMed: 11520463]
8. Schiemann B, Gommerman JL, Vora K, Cachero TG, Shulga-Morskaya S, Dobles M, et al. An essential role for BAFF in the normal development of B cells through a BCMA-independent pathway. *Science*. 2001;293(5537):2111–4. [PubMed: 11509691]
9. Gross JA, Johnston J, Mudri S, Enselman R, Dillon SR, Madden K, et al. TACI and BCMA are receptors for a TNF homologue implicated in B-cell autoimmune disease. *Nature*. 2000;404(6781):995–9. [PubMed: 10801128]
10. Ramanujam M, Wang XB, Huang WQ, Liu Z, Schiffer L, Tao HO, et al. Similarities and differences between selective and nonselective BAFF blockade in murine SLE. *Journal of Clinical Investigation*. 2006;116(3):724–34. [PubMed: 16485042]
11. Jacob CO, Pricop L, Putterman C, Koss MN, Liu Y, Kollaros M, et al. Paucity of clinical disease despite serological autoimmunity and kidney pathology in lupus-prone New Zealand Mixed 2328 mice deficient in BAFF. *Journal of Immunology*. 2006;177(4):2671–80.
12. Stohl W, Hiepe F, Latinis KM, Thomas M, Scheinberg MA, Clarke A, et al. Belimumab reduces autoantibodies, normalizes low complement levels, and reduces select B cell populations in patients with systemic lupus erythematosus. *Arthritis and Rheumatism*. 2012;64(7):2328–37. [PubMed: 22275291]
13. Furie RA, Leon G, Thomas M, Petri MA, Chu AD, Hislop C, et al. A phase 2, randomised, placebo-controlled clinical trial of blisibimod, an inhibitor of B cell activating factor, in patients with moderate-to-severe systemic lupus erythematosus, the PEARL-SC study. *Annals of the Rheumatic Diseases*. 2015;74(9):1667–75. [PubMed: 24748629]
14. Dall'Era M, Chakravarty E, Wallace D, Genovese M, Weisman M, Kavanaugh A, et al. Reduced B lymphocyte and immunoglobulin levels after atacicept treatment in patients with systemic lupus erythematosus. *Arthritis and Rheumatism*. 2007;56(12):4142–50. [PubMed: 18050206]
15. Isenberg DA, Petri M, Kalunian K, Tanaka Y, Urowitz MB, Hoffman RW, et al. Efficacy and safety of subcutaneous tabalumab in patients with systemic lupus erythematosus: results from ILLUMINATE-1, a 52-week, phase III, multicentre, randomised, double-blind, placebo-controlled study. *Annals of the Rheumatic Diseases*. 2016;75(2):323–31. [PubMed: 26338095]
16. Merrill JT, van Vollenhoven RF, Buyon JP, Furie RA, Stohl W, Morgan-Cox M, et al. Efficacy and safety of subcutaneous tabalumab, a monoclonal antibody to B-cell activating factor, in patients with systemic lupus erythematosus: results from ILLUMINATE-2, a 52-week, phase III, multicentre, randomised, double-blind, placebo-controlled study. *Annals of the Rheumatic Diseases*. 2016;75(2):332–40. [PubMed: 26293163]

17. Navarra SV, Guzman RM, Gallacher AE, Hall S, Levy RA, Jimenez RE, et al. Efficacy and safety of belimumab in patients with active systemic lupus erythematosus: a randomised, placebo-controlled, phase 3 trial. *Lancet*. 2011;377(9767):721–31. [PubMed: 21296403]
18. Furie R, Petri M, Zamani O, Cervera R, Wallace DJ, Tegzova D, et al. A phase III, randomized, placebo-controlled study of belimumab, a monoclonal antibody that inhibits B lymphocyte stimulator, in patients with systemic lupus erythematosus. *Arthritis and Rheumatism*. 2011;63(12):3918–30. [PubMed: 22127708]
19. Stohl W, Schwarting A, Okada M, Scheinberg M, Doria A, Hammer AE, et al. Efficacy and safety of subcutaneous belimumab in systemic lupus erythematosus: a fifty-two-week randomized, double-blind, placebo-controlled study. *Arthritis & Rheumatology*. 2017;69(5):1016–27. [PubMed: 28118533]
20. Zhang FC, Bae SC, Bass D, Chu M, Egginton S, Gordon D, et al. A pivotal phase III, randomised, placebo-controlled study of belimumab in patients with systemic lupus erythematosus located in China, Japan and South Korea. *Annals of the Rheumatic Diseases*. 2018;77(3).
21. Isenberg D, Gordon C, Licu D, Copt S, Rossi CP, Wofsy D. Efficacy and safety of atacicept for prevention of flares in patients with moderate-to-severe systemic lupus erythematosus (SLE): 52-week data (APRIL-SLE randomised trial). *Annals of the Rheumatic Diseases*. 2015;74(11):2006–15. [PubMed: 24951103]
22. Stohl W, Hilbert DM. The discovery and development of belimumab: the anti-BLyS–lupus connection. *Nature Biotechnology*. 2012;30(1):69–77.
23. Wallace DJ, Stohl W, Furie RA, Lisse JR, McKay JD, Merrill JT, et al. A phase II, randomized, double-blind, placebo-controlled, dose-ranging study of belimumab in patients with active systemic lupus erythematosus. *Arthritis & Rheumatism-Arthritis Care & Research*. 2009;61(9):1168–78. [PubMed: 19714604]
24. Tardivel A, Tinel A, Lens S, Steiner QG, Sauberli E, Wilson A, et al. The anti-apoptotic factor Bcl-2 can functionally substitute for the B cell survival but not for the marginal zone B cell differentiation activity of BAFF. *European Journal of Immunology*. 2004;34(2):509–18. [PubMed: 14768056]
25. Sasaki Y, Casola S, Kutok JL, Rajewsky K, Schmidt-Supprian M. TNF family member B cell-activating factor (BAFF) receptor-dependent and -independent roles for BAFF in B cell physiology. *Journal of Immunology*. 2004;173(4):2245–52.
26. Strasser A, Whittingham S, Vaux DL, Bath ML, Adams JM, Cory S, et al. Enforced BCL2 expression in B-lymphoid cells prolongs antibody responses and elicits autoimmune disease. *Proceedings of the National Academy of Sciences of the United States of America*. 1991;88(19):8661–5. [PubMed: 1924327]
27. Morel L, Yu Y, Blenman KR, Caldwell RA, Wakeland EK. Production of congenic mouse strains carrying genomic intervals containing SLE-susceptibility genes derived from the SLE-prone NZM2410 strain. *Mammalian Genome*. 1996;7(5):335–9. [PubMed: 8661718]
28. Markel P, Shu P, Ebeling C, Carlson GA, Nagle DL, Smutko JS, et al. Theoretical and empirical issues for marker-assisted breeding of congenic mouse strains. *Nature Genetics*. 1997;17(3):280–4. [PubMed: 9354790]
29. Waters ST, Fu SM, Gaskin F, Deshmukh US, Sung SSJ, Kannapell CC, et al. NZM2328: A new mouse model of systemic lupus erythematosus with unique genetic susceptibility loci. *Clinical Immunology*. 2001;100(3):372–83. [PubMed: 11513551]
30. Jacob CO, Guo SH, Jacob N, Pawar RD, Putterman C, Quinn WJ, et al. Dispensability of APRIL to the development of systemic lupus erythematosus in NZM 2328 mice. *Arthritis and Rheumatism*. 2012;64(5):1610–9. [PubMed: 22127792]
31. Mackay F, Woodcock SA, Lawton P, Ambrose C, Baetscher M, Schneider P, et al. Mice transgenic for BAFF develop lymphocytic disorders along with autoimmune manifestations. *Journal of Experimental Medicine*. 1999;190(11):1697–710. [PubMed: 10587360]
32. Kim JM, Rasmussen JP, Rudensky AY. Regulatory T cells prevent catastrophic autoimmunity throughout the lifespan of mice. *Nature Immunology*. 2007;8(2):191–7. [PubMed: 17136045]

33. Scalapino KJ, Tang QZ, Bluestone JA, Bonyhadi ML, Daikh DI. Suppression of disease in New Zealand Black/New Zealand White lupus-prone mice by adoptive transfer of ex vivo expanded regulatory T cells. *Journal of Immunology*. 2006;177(3):1451–9.
34. Liu Z, Bethunaickan R, Huang WQ, Ramanujam M, Madaio MP, Davidson A. IFN- α confers resistance of systemic lupus erythematosus nephritis to therapy in NZB/W F1 mice. *Journal of Immunology*. 2011;187(3):1506–13.
35. Walters S, Webster KE, Sutherland A, Gardam S, Groom J, Liuwantara D, et al. Increased CD4⁺Foxp3⁺ T cells in BAFF-transgenic mice suppress T cell effector responses. *Journal of Immunology*. 2009;182(2):793–801.
36. Jacob N, Yang HT, Pricop L, Liu Y, Gao XN, Zheng SG, et al. Accelerated pathological and clinical nephritis in systemic lupus erythematosus-prone New Zealand Mixed 2328 mice doubly deficient in TNF receptor 1 and TNF receptor 2 via a Th17-associated pathway. *Journal of Immunology*. 2009;182(4):2532–41.
37. Jacob CO, Yu N, Sindhava V, Cancro MP, Pawar RD, Putterman C, et al. Differential development of systemic lupus erythematosus in NZM 2328 mice deficient in discrete pairs of BAFF receptors. *Arthritis & Rheumatology*. 2015;67(9):2523–35. [PubMed: 25989238]
38. Jacob CO, Yu N, Yoo DG, Perez-Zapata LJ, Barbu EA, Kaplan MJ, et al. Haploinsufficiency of NADPH oxidase subunit neutrophil cytosolic factor 2 is sufficient to accelerate full-blown lupus in NZM 2328 mice. *Arthritis & Rheumatology*. 2017;69(8):1647–60. [PubMed: 28471497]
39. Ko K, Wang J, Perper S, Jiang YL, Yanez D, Kaverina N, et al. Bcl-2 as a therapeutic target in human tubulointerstitial inflammation. *Arthritis & Rheumatology*. 2016;68(11):2740–51. [PubMed: 27159593]
40. Lu P, Fleischmann R, Curtis C, Ignatenko S, Clarke SH, Desai M, et al. Safety and pharmacodynamics of venetoclax (ABT-199) in a randomized single and multiple ascending dose study in women with systemic lupus erythematosus. *Lupus*. 2018;27(2):290–302. [PubMed: 28691866]
41. Ahuja A, Shupe J, Dunn R, Kashgarian M, Kehry MR, Shlomchik MJ. Depletion of B cells in murine lupus: Efficacy and resistance. *Journal of Immunology*. 2007;179(5):3351–61.
42. Bekar KW, Owen T, Dunn R, Ichikawa T, Wang W, Wang R, et al. Prolonged effects of short-term anti-CD20 B Cell depletion therapy in murine systemic lupus erythematosus. *Arthritis and Rheumatism*. 2010;62(8):2443–57. [PubMed: 20506300]
43. Lin W, Seshasayee D, Lee WP, Caplazi P, McVay S, Suto E, et al. Dual B cell immunotherapy is superior to individual anti-CD20 depletion or BAFF blockade in murine models of spontaneous or accelerated lupus. *Arthritis & Rheumatology*. 2015;67(1):215–24. [PubMed: 25303150]
44. Merrill JT, Neuwelt CM, Wallace DJ, Shanahan JC, Latinis KM, Oates JC, et al. Efficacy and safety of rituximab in moderately-to-severely active systemic lupus erythematosus: The randomized, double-blind, phase II/III systemic lupus erythematosus evaluation of rituximab trial. *Arthritis and Rheumatism*. 2010;62(1):222–33. [PubMed: 20039413]
45. Rovin BH, Furie R, Latinis K, Looney RJ, Fervenza FC, Sanchez-Guerrero J, et al. Efficacy and safety of rituximab in patients with active proliferative lupus nephritis: The Lupus Nephritis Assessment with Rituximab study. *Arthritis and Rheumatism*. 2012;64(4):1215–26. [PubMed: 22231479]
46. Mysler EF, Spindler AJ, Guzman R, Bijl M, Jayne D, Furie RA, et al. Efficacy and safety of ocrelizumab in active proliferative lupus nephritis: Results from a randomized, double-blind, phase III study. *Arthritis and Rheumatism*. 2013;65(9):2368–79. [PubMed: 23740801]
47. Reff ME, Carner K, Chambers KS, Chinn PC, Leonard JE, Raab R, et al. Depletion of B cells in vivo by a chimeric mouse human monoclonal antibody to CD20. *Blood*. 1994;83(2):435–45. [PubMed: 7506951]
48. Laabi Y, Gras MP, Brouet JC, Berger R, Larsen CJ, Tsapis A. The BCMA gene, preferentially expressed during B lymphoid maturation, is bidirectionally transcribed. *Nucleic Acids Research*. 1994;22(7):1147–54. [PubMed: 8165126]
49. von Bülow GU, Bram RJ. NF-AT activation induced by a CAML-interacting member of the tumor necrosis factor receptor superfamily. *Science*. 1997;278(5335):138–41. [PubMed: 9311921]

50. Thompson JS, Bixler SA, Qian F, Vora K, Scott ML, Cachero TG, et al. BAFF-R, a newly identified TNF receptor that specifically interacts with BAFF. *Science*. 2001;293(5537):2108–11. [PubMed: 11509692]
51. Chang SK, Mihalcik SA, Jelinek DF. B lymphocyte stimulator regulates adaptive immune responses by directly promoting dendritic cell maturation. *Journal of Immunology*. 2008;180(11):7394–403.
52. Chang SK, Arendt BK, Darce JR, Wu X, Jelinek DF. A role for BLyS in the activation of innate immune cells. *Blood*. 2006;108(8):2687–94. [PubMed: 16825497]
53. Stohl W, Banfalvi A. B cell-independent contribution of BAFF to murine autoimmune disease. *Clinical Immunology*. 2016;172:111–6. [PubMed: 27430521]

Figure 1. Selective over-expression of BCL2 in B cells from NZM.Baff^{-/-}.Bcl2^{Tg} mice.

Left: Spleen cells from the indicated mice were stained for surface CD19 and CD3 and for intracellular BCL2. The dot plots illustrate CD19 vs CD3 staining, and the numbers in the panels indicate the percentages of CD19⁺ cells and CD3⁺ cells. Center and right: The histograms illustrate intracellular BCL2 staining in CD19⁺ (center) and CD3⁺ (right) cells. The black lines indicate staining with anti-BCL2, and the red filled areas indicate staining with the isotype control.

Figure 2. Spleen lymphocyte profiles.

Spleen total B (CD19⁺) cells (a, b), FO B (CD19⁺IgM^{hi}IgD^{lo}) cells (c, d), MZ B (CD19⁺IgM^{lo}IgD^{hi}CD1d^{hi}) cells (e, f), plasma (CD3⁺CD138⁺CD19^{lo}) cells (g, h), CD4⁺ cells (i), CD8⁺ cells (j), CD4⁺ activated memory (CD4⁺CD44^{hi}CD62L^{lo}) cells (k), and CD4⁺ recently-activated (CD4⁺CD69⁺) cells from NZM WT (WT; n = 4), NZM.*Baff*^{-/-} (BAFFKO; n = 7), and NZM.*Baff*^{-/-}.*Bcl2*^{Tg} (BAFFKO.BCL2; n = 8) mice (2 months old) are plotted. Results are presented as percentages of total spleen mononuclear cells (a, g) or as percentages of total B cells (c, e), percentages of total T cells (i, j), or percentages of total CD4⁺ cells (k, l) and as absolute numbers (b, d, f, h). Each circle represents an individual mouse. The composite results are plotted as box plots. The lines inside the boxes indicate the medians, and the outer borders of the boxes indicate the 25th and 75th percentiles. “*” indicates p < 0.05; “**” indicates p < 0.01; “***” indicates p < 0.005; and “****” indicates p < 0.001 in comparison to WT mice.

Figure 3. Serologic profiles.

NZM.*Baff*^{-/-}.*Bcl2*^{Tg} (N = 18) and littermate NZM.*Baff*^{-/-} (N=12) mice were longitudinally bled at the indicated ages. Serum levels of total IgG (top), IgG anti-chromatin (middle), and IgG anti-dsDNA (bottom) are plotted as mean $\pm$ SD. “***” indicates $p < 0.01$; “****” indicates $p < 0.005$; and “*****” indicates $p < 0.001$. Due to accelerated mortality among NZM.*Baff*^{-/-}.*Bcl2*^{Tg} mice, blood was collected from 9 NZM.*Baff*^{-/-}.*Bcl2*^{Tg} mice at 4 months, 7 NZM.*Baff*^{-/-}.*Bcl2*^{Tg} mice at 5 months, and 4 NZM.*Baff*^{-/-}.*Bcl2*^{Tg} mice at 6 months.

Figure 4. Renal immunopathology.

Kidney sections from NZM WT (a, d, g, j, m, p, s, v), NZM.*Baff*^{-/-}.*Bcl2*^{Tg} (b, e, h, k, n, q, t, w), and NZM.*Baff*^{-/-} (c, f, i, l, o, r, u, x) mice at the indicated ages were stained for IgG immunofluorescence (a-c, g-i) or C3 immunofluorescence (d-f, j-l) or with hematoxylin and eosin (H&E; m-x) for histologic evaluation. The blue arrows point to interstitial/perivascular infiltrates, and the black arrows points to swollen/hypercellular glomeruli. Original magnification 100X for immunofluorescence micrographs and 200X (m-o, s-u) and 400X (p-r, v-x) for H&E micrographs.

Figure 5. Clinical disease.

NZM.*Baff*^{-/-} (n = 22, black lines) and NZM.*Baff*^{-/-}.*Bcl2*^{Tg} (n = 23, red lines) mice were monitored for 8 months for development of severe proteinuria (left) and survival (right). Data are plotted as the fraction of mice over time that did not develop severe proteinuria (left) or remained alive (right). Censored data are indicated as black diamonds (NZM.*Baff*^{-/-}) and red squares (NZM.*Baff*^{-/-}.*Bcl2*^{Tg}).

Figure 6. Responsiveness of NZM.*Baff*^{-/-}.*Bcl2*^{Tg} mice to BAFF.

Panel A: Spleen B cells from the indicated mice were cultured in medium with or without exogenous recombinant BAFF (100 ng/ml), and viability was determined at 48 hours by staining with annexin-V + propidium iodide. Viable cells were taken as those staining with neither annexin-V nor propidium iodide. Results from a representative experiment are presented as mean $\pm$ SD. “**” denotes $p < 0.01$; “***” denotes $p < 0.005$. Panel B: NZM.*Baff*^{-/-}.*Bcl2*^{Tg} (n = 23, red lines), NZM.*Baff*^{+/-}.*Bcl2*^{Tg} (n = 10, blue lines), and NZM.*Baff*^{+/-} (n = 9, green lines) mice were monitored for 8 months for development of severe clinical disease (severe proteinuria or death). Data are plotted as the fraction of mice over time that did not develop severe clinical disease. Censored data are indicated as red (NZM.*Baff*^{-/-}.*Bcl2*^{Tg}) and green (NZM.*Baff*^{+/-}) circles.