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Development of systemic lupus erythematosus in NZM 2328 mice in the absence of any single BAFF receptor

Chaim O. Jacob[1], **Ning Yu**[1], **Shunhua Guo**[2], **Noam Jacob**[2], **William J. Quinn III**[3], **Vishal Sindhava**[3], **Michael P. Cancro**[3], **Beatrice Goilav**[4], **Chaim Putterman**[5], **Thi-Sau Migone**[6], and **William Stohl**[2]

[1]Division of Gastrointestinal and Liver Diseases, Department of Medicine, University of Southern California Keck School of Medicine, Los Angeles, CA 90033

[2]Division of Rheumatology, Department of Medicine, University of Southern California Keck School of Medicine, Los Angeles, CA 90033

[3]Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA 19104

[4]Division of Pediatric Nephrology, Children's Hospital at Montefiore, Bronx, NY 10467

[5]Division of Rheumatology, Albert Einstein College of Medicine, Bronx, NY 10461

[6]Human Genome Sciences, Inc., Rockville, MD 20850

Abstract

Objective—To determine the necessity for any individual BAFF receptor in the development of SLE.

Methods—*Bcma*, *Taci*, and *Br3* null mutations were introgressed into NZM 2328 mice. NZM.*Bcma*$^{-/-}$, NZM.*Taci*$^{-/-}$, and NZM.*Br3*$^{-/-}$ mice were evaluated for lymphocyte phenotype and BAFF receptor expression by flow cytometry, B cell responsiveness to BAFF by *in vitro* culture, serum BAFF and total IgG and IgG anti-dsDNA levels by ELISA, renal immunopathology by immunofluorescence and histopathology, and clinical disease.

Results—NZM.*Bcma*$^{-/-}$, NZM.*Taci*$^{-/-}$, and NZM.*Br3*$^{-/-}$ mice failed to surface-express BCMA, TACI, and BR3, respectively. Transitional and follicular B cells from NZM.*Br3*$^{-/-}$ mice were much less responsive to BAFF than the corresponding cells from wild-type (WT), NZM.*Bcma*$^{-/-}$, or NZM.*Taci*$^{-/-}$ mice. In comparison to WT mice, NZM.*Bcma*$^{-/-}$ and NZM.*Taci*$^{-/-}$ mice harbored increased spleen B cells, T cells, and plasma cells (PC), whereas serum total IgG and IgG anti-dsDNA levels were similar. Despite their paucity of B cells, NZM.*Br3*$^{-/-}$ mice harbored increased T cells and WT-like numbers of PC and levels of IgG anti-dsDNA. Serum BAFF levels were increased in NZM.*Taci*$^{-/-}$ and NZM.*Br3*$^{-/-}$ mice but were decreased in NZM.*Bcma*$^{-/-}$ mice. Despite their phenotypic differences, renal immunopathology and clinical disease in NZM.*Bcma*$^{-/-}$, NZM.*Taci*$^{-/-}$, and NZM.*Br3*$^{-/-}$ mice were at least as severe as in WT mice.

Conclusions—Any single BAFF receptor, including BR3, is dispensable to development of SLE in NZM mice. Development of disease in NZM.*Br3*$^{-/-}$ mice demonstrates that BAFF/BCMA and/or BAFF/TACI interactions contribute to SLE and that profound, life-long reduction in B cells does not guarantee protection from SLE.

Address correspondence to William Stohl, MD, PhD, Division of Rheumatology, University of Southern California, 2011 Zonal Ave. HMR 711, Los Angeles, CA 90033, 323-442-1959 (office), 323-442-2874 (FAX), stohl@usc.edu; or to Chaim O Jacob, MD, PhD, University of Southern California, 2011 Zonal Ave. HMR 705, Los Angeles, CA 90033, jacob@usc.edu.

Introduction

One of the characteristic features of SLE is B cell hyperactivity. Accordingly, any factor that positively affects B cells has an *a priori* likelihood of playing a pathogenetic role in SLE. One such factor is BAFF (BLyS), a 285-amino acid type-II transmembrane protein member of the TNF ligand superfamily (1, 2). *In vitro* and *in vivo* studies have demonstrated BAFF to be a vital B cell survival factor (3–5) and to promote differentiation of immature B cells to mature B cells (6) and Ig class switching and production (7). Indeed, BAFF-deficient mice display marked global reductions in mature B cells and in baseline and antigen-driven serum Ig levels (8, 9).

The connection between BAFF and SLE is very strong. Constitutive over-expression of BAFF in non-autoimmune mice leads to SLE-like features, including elevated circulating titers of multiple autoantibodies and immune complex-mediated glomerulonephritis (GN) (10, 11). In human SLE, circulating BAFF levels are elevated in as many as 50% of patients (12–14), and BAFF expression correlates with disease activity (15, 16).

Importantly, elimination/neutralization of BAFF leads to prevention/amelioration of SLE. Genetic deficiency of BAFF protects SLE-prone NZM 2328 (NZM) mice from clinical disease (17), and both (NZBxNZW)F1 and MRL.*lpr* mice manifest enhanced survival in response to BAFF antagonists (11, 18, 19). Two phase-III clinical trials in human SLE of the anti-BAFF monoclonal antibody (mAb) belimumab documented its efficacy and safety (20, 21), prompting the FDA to approve belimumab for the treatment of SLE (reviewed in ref 22). In the phase-III trials, clinical response to belimumab was greatest among patients who were anti-dsDNA-positive and harbored low complement levels at baseline (23), suggesting that therapeutic benefit arising from BAFF neutralization is substantially mediated by inhibiting pathogenic autoreactive B cells and production of pathogenic autoantibodies.

BAFF has three receptors, BCMA, TACI, and BR3 (BAFFR), but it is not known which BAFF receptor(s) is required for the SLE-promoting effects of BAFF. Of note, single deficiency of the individual BAFF receptors in non-autoimmune mice yields markedly divergent phenotypes.

BCMA-deficient mice display a near-normal phenotype. They harbor normal numbers of lymphocytes and lymphocyte subsets; the *in vitro* functions of these cells are normal; and the mice manifest no obvious immunodeficiency (9, 24). However, immunized BCMA-deficient mice do not maintain as many antigen-specific long-lived Ig-secreting plasma cells (PC) in their bone marrow (BM) as do corresponding wild-type (WT) mice (25). In principle, BCMA deficiency in the context of SLE might reduce the numbers of pathogenic autoreactive long-lived PC and, thereby, attenuate SLE.

Mice deficient in BR3 display a phenotype close to that of BAFF-deficient mice. Spleen B cells, mature recirculating B cells in the BM, and baseline and antigen-induced serum Ig levels are markedly reduced (26, 27). In BM-chimeric mice harboring both WT B cells and B cells that bear a mutant BR3, the B cells bearing the mutant BR3 manifest decreased survival (28). Collectively, these observations point to BAFF/BR3 interactions as essential for the pro-survival effects of BAFF on peripheral B cells. Given the central role for B cells in SLE, one could anticipate that BR3 deficiency would markedly attenuate SLE.

The phenotype of TACI-deficient mice dramatically differs from those of BR3- or BCMA-deficient mice. B cells are increased in TACI-deficient mice (29, 30) and, as they age, develop elevated circulating titers of autoantibodies, immune complex-mediated glomerulonephritis, and premature death (31). Nevertheless, TACI deficiency could have a net down-modulatory effect in a SLE-prone host. TACI-deficient mice generate impaired Ig

responses to T-independent antigens (29, 30), and expression of TACI may control the ability of B cells to produce Ig in response to BAFF (32). Although TACI deficiency leads to expansion of T follicular helper cells, it concurrently impairs PC survival and leads to decreased long-lived PC in the BM (33, 34). Thus, TACI deficiency in a SLE-prone host may attenuate, rather than aggravate, SLE.

To directly assess the need for BMCA, TACI, or BR3 in the development SLE, we generated NZM.*Bcma*$^{-/-}$, NZM.*Taci*$^{-/-}$, and NZM.*Br3*$^{-/-}$ mice. Despite considerable divergence among these mice in their lymphocyte and serological phenotypes, all developed renal immunopathology and clinical disease to at least the same extent as did NZM WT mice. Thus, any single BAFF receptor, including BR3, is dispensable for full-blown clinical SLE in NZM mice, and lymphocyte and serological profiles can be dissociated from development of severe pathological and clinical disease. These observations may have important ramifications for B cell-directed therapies, and NZM.*Br3*$^{-/-}$ mice offer a model system in which full-blown clinical SLE develops in the face of chronic profound B cell depletion.

Materials and methods

General

All mice were maintained in specific pathogen-free quarters at USC, and the experiments were approved by the USC IACUC.

Mice

Female mice from five congenic NZM strains were studied: NZM WT, NZM.*Baff*$^{-/-}$, NZM.*Bcma*$^{-/-}$, NZM.*Br3*$^{-/-}$, and NZM.*Taci*$^{-/-}$. NZM.*Baff*$^{-/-}$ mice have previously been described (17). To generate NZM.*Bcma*$^{-/-}$, NZM.*Br3*$^{-/-}$, and NZM.*Taci*$^{-/-}$ mice, the *Bcma*$^{-/-}$, *Br3*$^{-/-}$, and *Taci*$^{-/-}$ genotypes from *Bcma*$^{-/-}$, *Br3*$^{-/-}$, and *Taci*$^{-/-}$ mice, respectively (9, 27, 29), were individually introgressed into NZM mice through a marker-assisted selection protocol, using microsatellite markers spanning the entire genome and including markers to ensure that all known SLE-susceptibility loci remained intact (17, 35). Results are presented for mice from the N7 backcross generation or later, at which time all mice bore the NZM genotype at all tested markers.

To detect the disrupted *Bcma*, *Br3*, or *Taci* gene fragments, genomic DNA extracted from mouse tail clippings was PCR-amplified for 30 cycles at 94°C for 60 sec, 55°C (62°C for *Taci*) for 60 sec, and 72°C for 60 sec. The primer sequences were:

Bcma (1): 5′-TCA_CTG_TGG_AAA_CAC_TGT_TGC_GCC_ATG-3′

Bcma (2): 5′-GAT_ATC_CTG_ATC_ATC_GGT_CTT_CAG_ATG_C-3′

Bcma (3): 5′-CTG_TCC_ACA_TTG_CAA_CTG_TTA_CCT_GGG-3′.

Br3 (1): 5′-CGC_GGT_TTC_ATT_CTA_GAC_TAC_AGG_G-3′

Br3 (2): 5′-ACA_CGC_AGT_TTC_TCA_CCA_GAG_GGT_CGA_AGC-3′

Br3 (3): 5′-ATC_CTG_ATC_ATC_GGT_CTT_CAG_ATG_C-3′

Taci (1): 5′-CCT_CAG_GCC_AGG_AGC_TTT_TAG_GGA_GAA-3′

Taci (2): 5′-CCA_GCA_TCC_CCT_CTG_CTC_TGG_TTT_TAT-3′

Taci (3): 5′-CCT_GGG_TGG_AGA_GGC_TTT_TTG_CTT_CCT-3′

All three primers for each gene fragment were added to a single reaction mix. Band size for the amplified PCR products are: intact *Bcma,* 325 bp; disrupted *Bcma*, 425 bp; intact *Br3*, 180 bp; disrupted *Br3*, 379 bp; intact *Taci*, 440 bp; disrupted *Taci,* 300 bp.

Cell surface staining

To determine T cell and B cell subsets, spleen mononuclear cells were stained with combinations of fluorochrome-conjugated mAb specific for CD3, CD4, CD8, CD44, CD62L, B220, CD19, CD21, CD23, or CD69 (BD PharMingen or eBioscience) and analyzed by flow cytometry (36). For PC determination, spleen or BM cells were stained with a combination of fluorochrome-conjugated mAb specific for CD4, CD8, Gr1, F4/80, B220, IgD, and CD138 (BD PharMingen or eBioscience). PC were taken as the $CD4^{-}CD8^{-}Gr1^{-}F4/80^{-}IgDB220^{-}$ $CD138^{+}$ cells. For BAFF receptor expression, B cells or PC were additionally stained with fluorochrome-conjugated mAb specific for BCMA, TACI (R&D Systems), or BR3 (eBioscience).

In vitro responsiveness of B cells to BAFF

Spleen mononuclear cells were stained by a combination of biotinylated mAb specific for CD4, CD8, F4/80, GR-1, and TER-119 (BD PharMingen or eBioscience), and untouched B cells were isolated by magnetic sorting with a MiniMACS column and streptavidin microbeads (Miltenyi Biotec). The B cells were then stained with a combination of fluorochrome-conjugated mAb specific for CD19, B220, CD93, CD23, and CD21/35 (BD PharMingen, eBioscience, or BioLegend) and were sorted. Transitional (TR) B cells were taken as $CD19^{+}B220^{+}CD93^{+}CD21/35^{-}$ cells, and follicular (FO) B cells were taken as $CD19^{+}B220^{+}CD93^{-}CD23^{+}CD21/35^{-}$ cells. Sorted B cells (1×10^{5}/0.2 ml) were cultured in triplicate in 96-well flat-bottomed plates in RPMI 1640 medium + 10% FCS with or without exogenous recombinant human BAFF (rhBAFF, 100 ng/ml; Human Genome Sciences). Viability of the cultured cells was determined at 48 hr by staining with DAPI (Invitrogen).

Serum total IgG and IgG autoantibody determinations

Serum levels of total IgG and anti-dsDNA antibodies were determined by ELISA (37). 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.

Serum BAFF determination

Serum BAFF was measured by ELISA as previously described (38).

Kidney histology

Sections of formalin-fixed kidneys were stained with hematoxylin and eosin (H&E), Masson's trichrome, and Jones' silver methenamine and were assessed by light microscopy for glomerular activity (hypercellularity, necrotizing lesions, karyorrhexis, cellular crescents, hyaline deposits), tubulointerstitial activity (interstitial cellular infiltration, tubular cell necrosis), chronic glomerular pathology (glomerulosclerosis, fibrous crescents), and chronic tubulointerstitial pathology (tubular atrophy, interstitial fibrosis). Each category was subjectively scored on a 0–3 scale, for a maximum composite score of 12 (39).

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) (37).

Assessment of 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.

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.

Results

Specific absence of the deleted BAFF receptor in NZM mice deficient in one BAFF receptor

To confirm at the protein level that the specific BAFF receptor was not expressed, spleen B ($CD19^+$) cells from NZM WT, NZM.$Bcma^{-/-}$, NZM.$Taci^{-/-}$, and NZM.$Br3^{-/-}$ mice were evaluated for surface expression of the individual BAFF receptors (Figure 1A). TACI was surface-expressed in all mice except for NZM.$Taci^{-/-}$ mice, and BR3 was surface-expressed in all mice except for NZM.$Br3^{-/-}$ mice. Surface BCMA was not detected in any of the mice, consistent with it being undetectable on non-terminally differentiated mature B cells in non-autoimmune mice (40, 41). We, therefore, analyzed spleen and BM PC and documented that BCMA was surface-expressed in NZM WT mice but was specifically not expressed in NZM.$Bcma^{-/-}$ mice (Figure 1B).

Serum BAFF levels and B cell-surface BAFF receptor expression among NZM mice deficient in one BAFF receptor

Loss of the individual BAFF receptors led to highly divergent serum BAFF levels. Whereas serum BAFF levels were much greater in NZM.$Taci^{-/-}$ and NZM.$Br3^{-/-}$ mice than in NZM WT mice, they were decreased in NZM.$Bcma^{-/-}$ mice (Figure 1C). As expected, BAFF was not detected in sera from NZM.$Baff^{-/-}$ mice.

The differences in serum BAFF levels among NZM.$Bcma^{-/-}$, NZM.$Taci^{-/-}$, and NZM.$Br3^{-/-}$ mice were associated with only limited effects on B cell surface-expression of the reciprocal two BAFF receptors. BR3, absent in NZM.$Br3^{-/-}$ mice, was highly and equally surface-expressed by B cells in NZM.$Bcma^{-/-}$, NZM.$Taci^{-/-}$, and NZM WT mice, and TACI, absent in NZM.$Taci^{-/-}$ mice, was highly expressed in the other mice, albeit somewhat lower in NZM.$Br3^{-/-}$ mice than in NZM.$Bcma^{-/-}$ or NZM WT mice (Figure 1A). Surface-expression of any BAFF receptor on T cells was minimal in all the mice and was not detectably up-regulated or down-regulated (data not shown).

Clinical disease and renal immunopathology among NZM mice deficient in one BAFF receptor

The clinical courses in NZM.$Bcma^{-/-}$, NZM.$Taci^{-/-}$, and, most surprisingly and unexpectedly, NZM.$Br3^{-/-}$ mice were indistinguishable from that in NZM WT mice. No differences among the mouse cohorts were detected in development of either severe proteinuria or mortality. Severe proteinuria began to develop in each of the cohorts at 4–5 months of age, and ~90% of the mice in each cohort were affected by 12 months of age. Mortality in each of the cohorts was noted as early as 6–7 months of age, with ~90% of the mice in each cohort being dead by 12 months of age (Figure 2A).

Development of renal immunopathology in NZM mice deficient in one BAFF receptor, including NZM.*Br3*$^{-/-}$ mice, was consonant with their clinical courses. Glomerular deposition of IgG and C3 was as robust in NZM.*Br3*$^{-/-}$ mice as in NZM WT mice, with substantial IgG and C3 deposition in the glomeruli of NZM.*Bcma*$^{-/-}$ and NZM.*Taci*$^{-/-}$ mice as well (Figure 2B). Moreover, renal pathology, including glomerular hypercellularity, glomerular crescents, mesangial matrix deposition, interstitial inflammation and fibrosis, tubular atrophy, and perivascular leukocyte infiltration, was at least as severe in all the BAFF receptor-deficient NZM mice (including NZM.*Br3*$^{-/-}$ mice) as in NZM WT mice (Figure 2C). Indeed, renal histology scores were actually greater in NZM.*Taci*$^{-/-}$ and NZM.*Br3*$^{-/-}$ mice than in NZM WT mice at 5 months of age and were still greater in NZM.*Taci*$^{-/-}$ mice than in NZM WT mice at 8 months of age (Figure 2D).

B cell phenotypes among NZM mice deficient in one BAFF receptor

Their similar clinical courses and renal immunopathologic findings notwithstanding, the B cell phenotypes of NZM.*Br3*$^{-/-}$, NZM.*Taci*$^{-/-}$, and NZM.*Bcma*$^{-/-}$ mice were very different from each other and from that of NZM WT mice. In comparison to NZM WT mice, total, FO, and marginal zone (MZ) B cells were greatly reduced in NZM.*Br3*$^{-/-}$ mice (Figure 3Aa–c). Of note, activated (CD69^{+}) B cells and B cells expressing the costimulatory CD80 molecule were similar in number in NZM.*Br3*$^{-/-}$ and NZM WT mice (Figure 3Ad–e), whereas the difference between NZM.*Br3*$^{-/-}$ and NZM WT mice in B cells expressing the costimulatory CD86 molecule was significant (Figure 3Af). Although indisputably abnormal, the B cell phenotype of NZM.*Br3*$^{-/-}$ mice was not as severe as that of NZM.*Baff*$^{-/-}$ mice, with numbers of total B cells and every B cell subset tested (other than MZ B cells) being significantly greater in NZM.*Br3*$^{-/-}$ mice than in NZM.*Baff*$^{-/-}$ mice (Figure 4a–f).

In contrast to the reductions in total B cells and in several B cell subsets in NZM.*Br3*$^{-/-}$ mice, total B cells and individual B cell subsets were expanded in NZM.*Taci*$^{-/-}$ and NZM.*Bcma*$^{-/-}$ mice (Figure 3A). Total, FO, and MZ B cells were all greatly increased in NZM.*Taci*$^{-/-}$ mice as were CD69^{+}, CD80^{+}, and CD86^{+} B cells. Total B cells were also expanded in NZM.*Bcma*$^{-/-}$ mice, albeit not to the extent as in NZM.*Taci*$^{-/-}$ mice. Whereas the number of MZ B cells in NZM.*Bcma*$^{-/-}$ mice was not different from that in NZM WT mice, the expansions of CD69^{+}, CD80^{+}, and CD86^{+} B cells in NZM.*Bcma*$^{-/-}$ mice were very similar to those in NZM.*Taci*$^{-/-}$ mice.

T cell phenotypes among NZM mice deficient in one BAFF receptor

The T cell profiles among NZM.*Br3*$^{-/-}$, NZM.*Taci*$^{-/-}$, and NZM.*Bcma*$^{-/-}$ mice were less divergent than the B cell profiles. Total, CD4^{+}, CD8^{+}, and CD4^{+} naive cells were each increased in NZM mice deficient in any one BAFF receptor relative to those in NZM WT mice (Figure 3Ba–d). Moreover, CD4^{+} memory cells were increased in NZM.*Bcma*$^{-/-}$ mice but not in NZM.*Taci*$^{-/-}$ or NZM.*Br3*$^{-/-}$ mice (Figure 3Be). The expansion of CD4^{+} memory cells in NZM.*Bcma*$^{-/-}$ mice was proportionate to the expansion of CD4^{+} naive cells, resulting in CD4^{+} naive/memory cell ratios in these mice in line with those in NZM WT mice, whereas the expansion of CD4^{+} naive cells in NZM.*Br3*$^{-/-}$ mice, and to a lesser extent in NZM.*Taci*$^{-/-}$ mice, was out of proportion to the expansion of CD4^{+} memory cells, leading to increased CD4^{+} naive/memory cell ratios (Figure 3Bf). In striking contrast to the T cell profile in NZM.*Br3*$^{-/-}$ mice, total T cells and T cell subsets were markedly reduced in NZM.*Baff*$^{-/-}$ mice (Figure 4g–k). This does not appear to reflect a difference in T cell activation between these mice, since no difference in their CD4^{+} naive/memory ratios was observed (Figure 4l).

Differential *in vitro* responsiveness to BAFF of B cells from NZM mice deficient in one BAFF receptor

The increased circulating BAFF levels in NZM.*Br3*$^{-/-}$ mice coupled to their marked reduction in B cells strongly suggested that such B cells were highly unresponsive to BAFF. To confirm this impression, TR B cells and FO B cells from NZM.*Bcma*$^{-/-}$, NZM.*Taci*$^{-/-}$, NZM.*Br3*$^{-/-}$, and NZM WT mice were cultured with or without exogenous rhBAFF and assessed for viability after 48 hr. Whereas rhBAFF enhanced the *in vitro* survival of TR and FO B cells from NZM.*Bcma*$^{-/-}$, NZM.*Taci*$^{-/-}$, or NZM WT mice, rhBAFF had no discernible effect on these cells from NZM.*Br3*$^{-/-}$ mice (Figure 5). Of interest, *in vitro* survival of B cells (especially follicular B cells) from NZM.*Bcma*$^{-/-}$ or NZM.*Taci*$^{-/-}$ mice in either the absence or presence of rhBAFF was greater than that of B cells from NZM WT mice, consistent with the greater numbers of spleen B cells harbored by NZM.*Bcma*$^{-/-}$ or NZM.*Taci*$^{-/-}$ mice than by NZM WT mice (Figure 3A).

PC and serologic profiles among NZM mice deficient in one BAFF receptor

In NZM.*Baff*$^{-/-}$ mice, the reduction in B cells is paralleled by reductions in spleen and BM PC (17, 39). Strikingly, a diminution in neither spleen nor BM PC was observed in NZM.*Br3*$^{-/-}$ mice despite their low numbers of B cells (Figure 6A–B). In NZM.*Bcma*$^{-/-}$ and NZM.*Taci*$^{-/-}$ mice, spleen PC were greater than in NZM WT mice, although BM PC were similar across all the mouse cohorts.

Neither serum total IgG levels nor serum IgG anti-dsDNA levels immutably paralleled PC numbers. Despite their differences in spleen PC, serum total IgG and IgG anti-dsDNA levels differed in neither NZM.*Bcma*$^{-/-}$ nor NZM.*Taci*$^{-/-}$ mice from those in NZM WT mice at any age tested (Figure 6C–D). In contrast, serum total IgG levels and IgG anti-dsDNA levels were each significantly reduced in NZM.*Br3*$^{-/-}$ mice at 4–6 months of age despite the WT-like numbers of PC harbored by these mice. Of note, serum IgG anti-dsDNA levels in NZM.*Br3*$^{-/-}$ mice by 7–9 months of age were no longer lower than those in age-matched NZM WT mice, yet serum total IgG levels in NZM.*Br3*$^{-/-}$ mice remained substantially reduced.

Discussion

Whereas the contribution of BAFF to SLE has been firmly established (11, 17–21), the contribution of any individual BAFF receptor to SLE had not previously been investigated. To that end, we generated and investigated SLE-prone NZM mice deficient in BCMA, TACI, or BR3, and several remarkable conclusions can be drawn from our findings.

First, the B cell phenotypes are highly divergent. Whereas B cells are expanded in NZM.*Bcma*$^{-/-}$ and NZM.*Taci*$^{-/-}$ mice, they are dramatically reduced in NZM.*Br3*$^{-/-}$ mice. These observations are congruent with the B cell expansion in TACI-deficient non-autoimmune mice (29, 30), the B cell expansion in BCMA-deficient B6.*lpr* and B6.Nba2 mice (42), and the great reduction in B cells in BR3-deficient non-autoimmune mice (26, 27). Since BCMA and TACI each bind APRIL (43) and, therefore, could normally serve as a “sink” for APRIL, increased circulating APRIL levels in NZM.*Bcma*$^{-/-}$ and NZM.*Taci*$^{-/-}$ mice may have contributed to the B cell expansion in these mice. Unfortunately, repeated attempts at developing a quantitative ELISA for murine APRIL were unsuccessful, so we were not able to measure serum APRIL levels.

Of note, the reduction of B cells in NZM.*Br3*$^{-/-}$ mice is not as great as that in NZM.*Baff*$^{-/-}$ mice. Although engagement of BR3 by BAFF indisputably plays a vital role in B cell survival (26–28), our observations suggest a modest, yet discernible, contribution to B cell survival from engagement of TACI and/or BCMA as well. Since B cells are expanded,

rather than contracted, in NZM. *April*$^{-/-}$ mice (39), it is likely that BAFF/TACI and/or BAFF/BCMA interactions, rather than APRIL/TACI and/or APRIL/BCMA interactions, are the relevant ones.

Second, there is a divergence in spleen and BM PC and in serum levels of total IgG and IgG anti-dsDNA among the BAFF receptor-deficient NZM mice. Although spleen PC are modestly increased in NZM.*Bcma*$^{-/-}$ and NZM.*Taci*$^{-/-}$ mice, BM PC are not. This dichotomy between spleen PC and BM PC suggests that regulation of spleen PC is more dependent upon BCMA and TACI than is regulation of BM PC. Additional experimentation is needed to evaluate this possibility.

The WT-like numbers of BM PC in NZM.*Bcma*$^{-/-}$ and NZM.*Taci*$^{-/-}$ mice were unexpected, since BCMA-deficient and TACI-deficient non-autoimmune mice do not harbor as many long-lived antigen-specific BM PC as do their BCMA- or TACI-sufficient counterparts (25, 33, 34). The nature of the antigen inducing the long-lived PC response may be important. BCMA and TACI may play vital roles in long-lived PC responses to foreign proteins (predominantly T cell-dependent responses), whereas they may play little role in long-lived PC responses to self polynucleotides (predominantly T cell-independent responses). Studies are currently under way to delineate what, if any, differential role BCMA and TACI have in T cell-dependent vs T cell-independent PC responses.

In any case, serum levels of total IgG and IgG anti-dsDNA in NZM.*Bcma*$^{-/-}$ and NZM.*Taci*$^{-/-}$ mice are no different from those in NZM WT mice. Our findings contrast with the increased circulating levels of autoantibodies in BCMA-deficient B6.*lpr* or B6.Nba2 mice (42) and with the profound serological autoimmunity that develops in TACI-deficient non-autoimmune mice (31). Our results highlight the importance of *in vivo* models of *bona fide* SLE when investigating a given molecule or biological pathway. Results obtained in non-autoimmune models or “incomplete SLE” models may not accurately predict outcomes in *bona fide* SLE.

Most surprisingly, NZM.*Br3*$^{-/-}$ mice harbor WT-like numbers of spleen and BM PC. Whereas PC in NZM.*Baff*$^{-/-}$ mice are substantially reduced in parallel with the marked reduction in B cells (17, 39), the reduction in B cells in NZM.*Br3*$^{-/-}$ mice is not accompanied by reductions in spleen or BM PC. Since expression of BR3 declines, and expression of BCMA increases, as B cells differentiate into plasmablasts/PC in BAFF receptor-intact hosts (25, 44), the engagement of BCMA (and/or TACI) by the increased levels of BAFF in NZM.*Br3*$^{-/-}$ mice appears capable of supporting WT-like PC responses. Although TR B cells and FO B cells in NZM.*Br3*$^{-/-}$ mice are much less responsive to BAFF in terms of survival than are the corresponding cells in NZM.*Bcma*$^{-/-}$, NZM.*Taci*$^{-/-}$, or NZM WT mice, BAFF continues to promote the differentiation of the small fraction of B cells that does survive in the absence of BR3.

Of interest, serum levels of total IgG in NZM.*Br3*$^{-/-}$ mice at 4–6 months of age are lower than those in age-matched NZM WT mice, and this difference is even more apparent at 7–9 months of age. Importantly, whereas serum IgG anti-dsDNA levels are initially lower in NZM.*Br3*$^{-/-}$ mice than in NZM WT mice, they “catch up” by 7–9 months of age. One possibility is that autoreactive B cells are more dependent upon BAFF than are non-autoreactive B cells (45, 46). The elevated circulating BAFF levels in NZM.*Br3*$^{-/-}$ mice may preferentially support the autoreactive B cells within the contracted B cell pool, so autoantibody titers increase out of proportion to increases in total IgG. Alternatively, there may be a BAFF-independent mechanism that drives autoantibody production similar to that in NZM.*Baff*$^{-/-}$ mice, which develop robust serological autoimmunity over time (17).

APRIL may be one such contributor, given that serum IgG autoantibody levels are lower in NZM.*Baff*$^{-/-}$.*April*$^{-/-}$ mice than in NZM.*Baff*$^{-/-}$ mice (39).

Third, T cell expansion is a feature of NZM mice deficient in any single BAFF receptor. This contrasts with the normal numbers of T cells in the corresponding non-autoimmune mice (9, 24, 26, 27, 29, 30) and the decreased number of T cells in NZM.*Baff*$^{-/-}$ mice (17, 39). In light of the substantial reduction in total B cells in both NZM.*Baff*$^{-/-}$ mice and NZM.*Br3*$^{-/-}$ mice, the divergent T cell profiles are especially noteworthy. It may be that the preservation of activated B cells in NZM.*Br3*$^{-/-}$ mice is crucial to the T cell expansion. Regardless, the T cell response in NZM.*Bcma*$^{-/-}$, NZM.*Taci*$^{-/-}$, and NZM.*Br3*$^{-/-}$ mice is not monolithic. CD4^{+} naive cells expanded similarly in all these mice, whereas CD4^{+} memory cells significantly expanded only in NZM.*Bcma*$^{-/-}$ mice. Expansions of both CD4^{+} naive and memory cells were also observed in BCMA-deficient B6.*lpr* mice (42).

Fourth, serum BAFF levels among the BAFF receptor-deficient NZM mice are highly divergent. In NZM.*Taci*$^{-/-}$ and NZM.*Br3*$^{-/-}$ mice, serum BAFF levels are increased. This is expected, since loss of BAFF receptor “sinks” for circulating BAFF should lead to decreased removal of BAFF from circulation. The ensuing increased BAFF levels likely contribute to the B cell and T cell expansions in NZM.*Taci*$^{-/-}$ mice and to the T cell expansion in NZM.*Br3*$^{-/-}$ mice, since BAFF not only is a potent B cell survival factor but has agonist effects on T cells as well (47, 48).

In contrast, serum BAFF levels in NZM.*Bcma*$^{-/-}$ mice are lower than those in NZM WT mice. BCMA expression on mature B cells is very low (40, 41), so BCMA is much less of a “sink” for BAFF than is either TACI or BR3. As a result, BAFF levels in NZM.*Bcma*$^{-/-}$ mice might be expected to be equal to those in NZM WT mice. Why BAFF levels are actually lower in NZM.*Bcma*$^{-/-}$ mice remains unknown, and further investigation is warranted.

Regardless, the reduced serum BAFF levels in NZM.*Bcma*$^{-/-}$ mice sharply contrast with the increased serum BAFF levels in BCMA-deficient B6.*lpr* mice (42). BCMA deficiency in combination with the dysregulated Fas pathway in *lpr* mice may promote BAFF production, whereas BCMA deficiency in the context of an intact Fas pathway may not. Since B cells and T cells are expanded in both NZM.*Bcma*$^{-/-}$ and B6.*lpr* mice despite the marked disparity in serum BAFF levels, B cell and/or T cell expansion in a BCMA-deficient environment may be relatively BAFF-independent. Again, further investigation is warranted.

Fifth, the cellular and serological differences among the BAFF receptor-deficient NZM mice notwithstanding, development of renal immunopathology in them was at least as severe as in NZM WT mice and, most strikingly, the time courses of clinical disease among all the mice were indistinguishable. That is, even NZM.*Br3*$^{-/-}$ mice, which bear a disrupted *Br3* gene, do not express BR3 on their B cells, harbor vastly reduced numbers of mature B cells, and whose TR and FO B cells are markedly less responsive to BAFF in terms of survival than are the corresponding cells from NZM.*Bcma*$^{-/-}$, NZM.*Taci*$^{-/-}$, or NZM WT mice, develop clinical and pathological SLE to at least the same extent as do NZM WT mice. This unequivocally demonstrates that any single BAFF receptor, including BR3, is dispensable to the development of clinical SLE. That is, the presence of any two BAFF receptors is sufficient for full development of clinical and pathological disease. NZM mice expressing only BCMA, TACI, or BR3 (i.e., deficient in two BAFF receptors) are currently being generated to determine whether any single BAFF receptor is sufficient for development of SLE.

Our findings in NZM.*Br3*$^{-/-}$ mice may explain an apparent clinical paradox and, thereby, have important therapeutic ramifications for human SLE. Belimumab, an agent which results in only modest depletion of B cells, was successful in two independent phase-III SLE trials (20, 21), whereas rituximab, an agent which results in considerable B cell depletion, failed to achieve its endpoints in two independent phase-II/III SLE trials (49, 50). Since circulating BAFF levels in SLE patients rise following rituximab treatment (51), these increased BAFF levels may preferentially drive the remaining autoreactive B cells and promote ongoing disease activity. Therapeutic approaches that inactivate B cells rather than depleting them may circumvent this problem by not triggering a rise in BAFF levels. Such agents are in development (52), and clinical trials with such agents should be highly informative.

Acknowledgments

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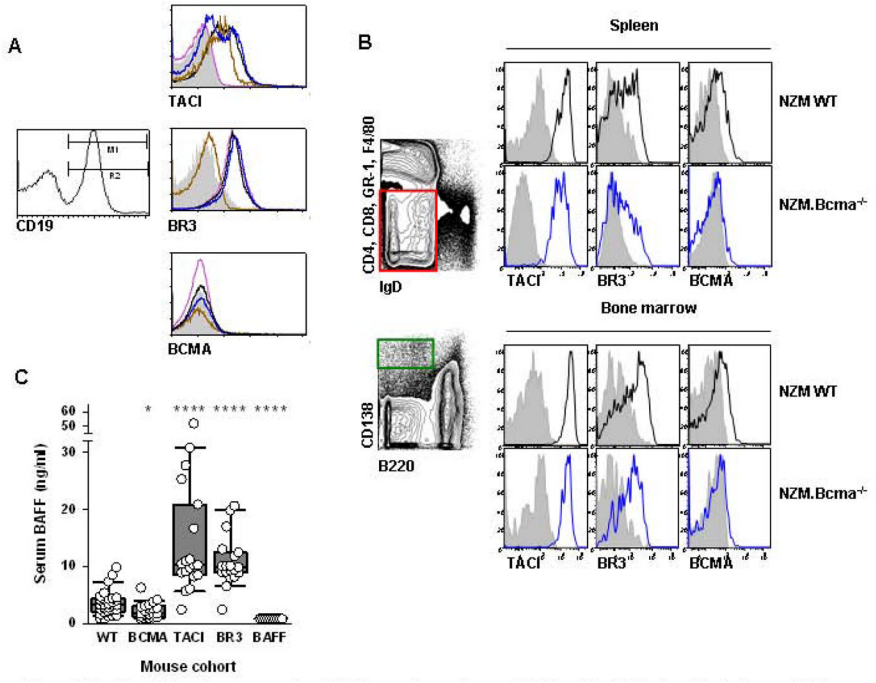

Figure 1. B cell and PC surface-expression of BAFF receptors and serum BAFF levels in NZM mice deficient in one BAFF receptor

Panel A: Spleen cells from NZM WT (black line), NZM.*Bcma*$^{-/-}$ (blue line), NZM.*Taci*$^{-/-}$ (pink line), and NZM.*Br3*$^{-/-}$ (brown line) mice were double-stained for CD19 and the indicated BAFF receptor. Cells were gated on the CD19^{+} population (left), and the histograms for CD19^{+} cells were plotted (right). The shaded areas indicate fluorescence intensity with isotype control mAb. Panel B: The gating strategy for PC is shown at the left. CD4^{-}CD8^{-}Gr1^{-}F4/80^{-}IgD^{-} cells (red box) were then gated for B220^{-}CD138^{+} cells (green box), and these cells isolated from spleen or BM were analyzed for BAFF receptor expression. Panel C: Serum BAFF levels from NZM WT (WT; n = 30), NZM.*Bcma*$^{-/-}$ (BCMA; n = 21), NZM.*Taci*$^{-/-}$ (TACI; n = 19), NZM.*Br3*$^{-/-}$ (BR3; n = 19), and NZM.*Baff*$^{-/-}$ (BAFF; n = 10) mice (3–5 months old) are plotted. Each circle represents an individual mouse. The composite results are plotted as box plots. The lines inside the boxes indicate the medians; the outer borders of the boxes indicate the 25th and 75th percentiles; and the bars extending from the boxes indicate the 10th and 90th percentiles. “*” indicates $p \leq 0.05$; “**” indicates $p \leq 0.01$; “***” indicates $p \leq 0.005$; “****” indicates $p \leq 0.001$ in comparison to NZM WT mice.

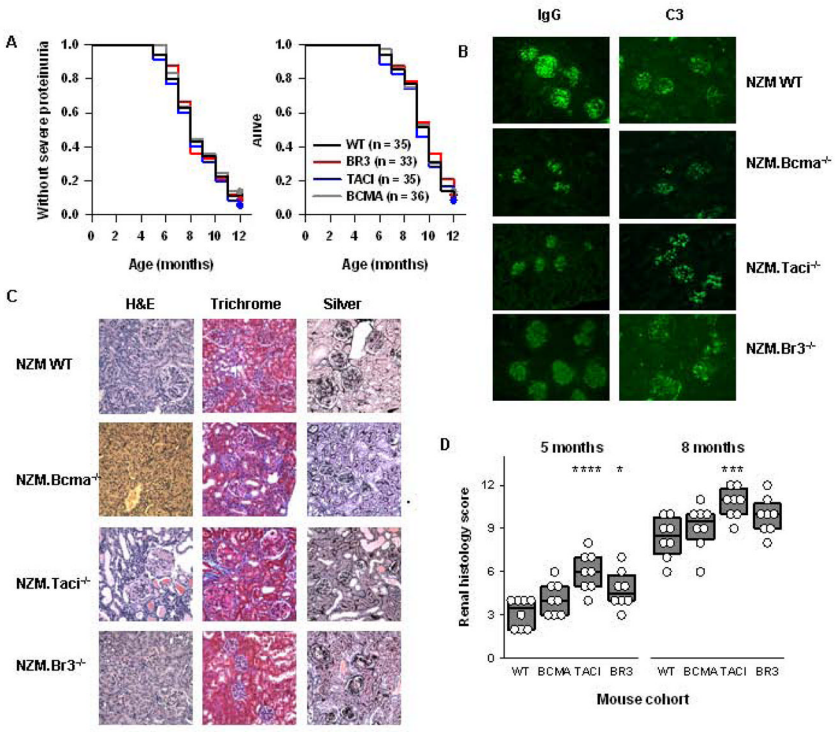

Figure 2. Clinical disease and renal immunopathology among NZM mice deficient in one BAFF receptor

Panel A: WT (n = 35, black lines), BCMA (n = 36, gray lines), TACI (n = 35, blue lines), and BR3 (n = 33, red lines) mice were monitored for 12 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). Panel B: Kidney sections from 8- month-old NZM WT, NZM.*Bcma*$^{-/-}$, NZM.*Taci*$^{-/-}$, and NZM.*Br3*$^{-/-}$ mice were stained for IgG (left) or C3 (right) immunofluorescence. Representative sections are illustrated. Original magnification 400X. Panel C: Kidney sections from 8-month-old NZM WT, NZM.*Bcma*$^{-/-}$, NZM.*Taci*$^{-/-}$, and NZM.*Br3*$^{-/-}$ mice were stained with H&E (left), Masson's trichrome (Trichrome; center), or Jones' silver methenamine (Silver; right) for histological evaluation. Representative sections are illustrated. Panel D: The renal histology scores for the 8 mice in each cohort are plotted.

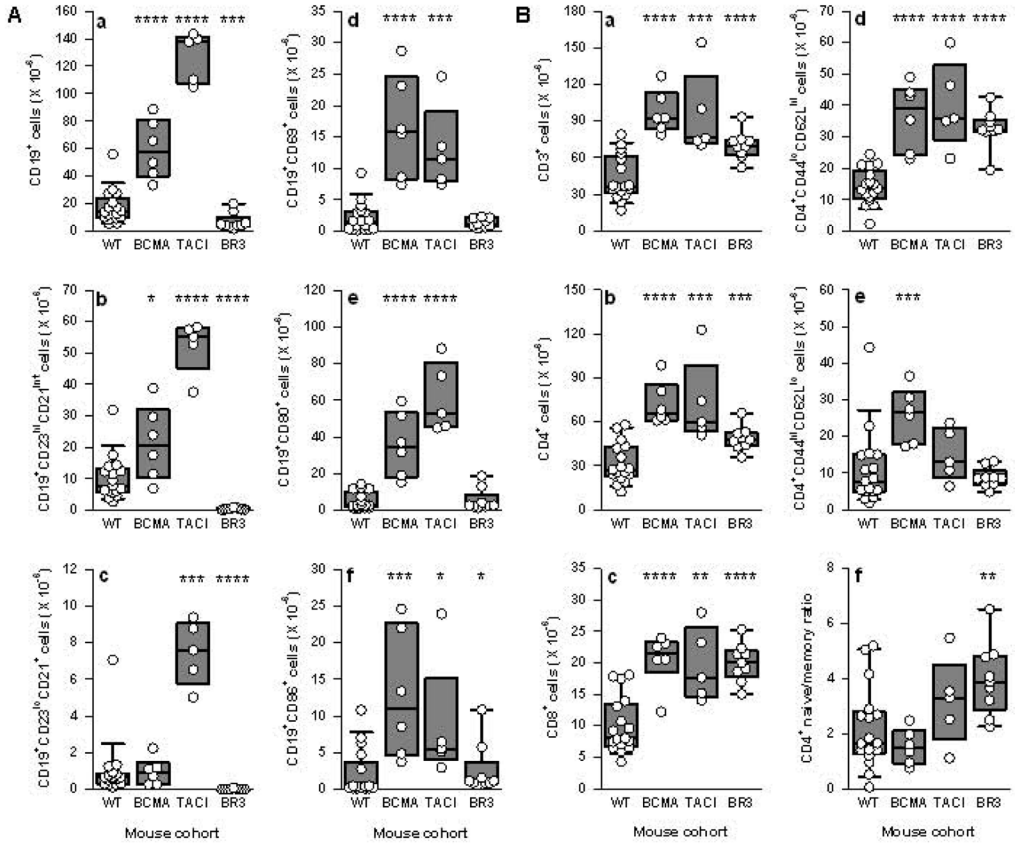

Figure 3. B and T cell phenotypes in NZM mice deficient in one BAFF receptor

Panel A: Spleen total B ($CD19^+$) cells (a), FO ($CD23^{hi}CD21^{int}$) B cells (b), MZ ($CD23^{lo}CD21^+$) B cells (c), activated ($CD69^+$) B cells (d), $CD80^+$ B cells (e), and $CD86^+$ B cells (f) from WT (n = 17), BCMA (n = 6), TACI (n = 5), and BR3 (n = 9) mice (4–5 months old) are plotted. Panel C: Spleen total T cells (a), $CD4^+$ T cells (b), $CD8^+$ T cells (c), $CD4^+$ naive cells (d), and $CD4^+$ memory cells (e) and $CD4^+$ naive/memory cell ratios (f) from the mice in panel A are plotted. "*" indicates $p \leq 0.05$; "**" indicates $p \leq 0.01$; "***" indicates $p \leq 0.005$; "****" indicates $p \leq 0.001$ in comparison to NZM WT mice.

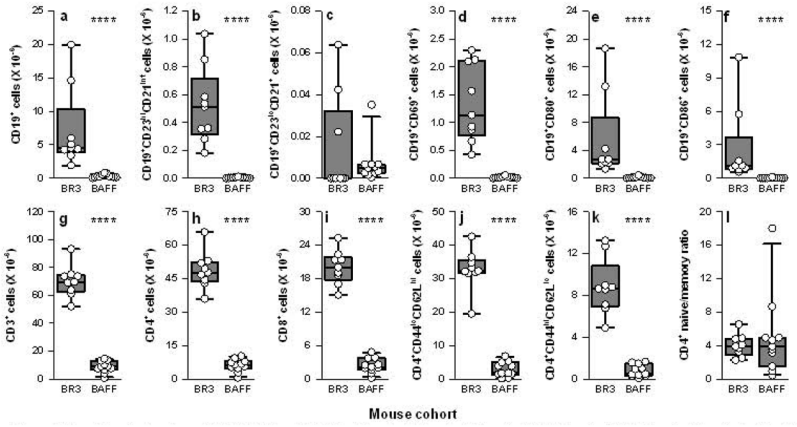

Figure 4. B and T cell phenotypes in NZM.*Br3*$^{-/-}$ and NZM.*Baff*$^{-/-}$ mice

Spleen total B cells (a), FO B cells (b), MZ B cells (c), activated B cells (d), CD80^{+} B cells (e), CD86^{+} B cells (f), total T (CD3^{+}) cells (g), CD4^{+} T cells (h), CD8^{+} T cells (i), CD4^{+} naive (CD44^{lo}CD62L^{hi}) cells (j), and CD4^{+} memory (CD44^{hi}CD62L^{lo}) cells (k), and CD4^{+} naive/memory cell ratios (l) from the BR3 mice shown in Figure 3 and NZM.*Baff*$^{-/-}$ (BAFF; n = 11) mice (4–5 months old) are plotted. "****" indicates $p \leq 0.001$ in comparison to NZM.*Br3*$^{-/-}$ mice.

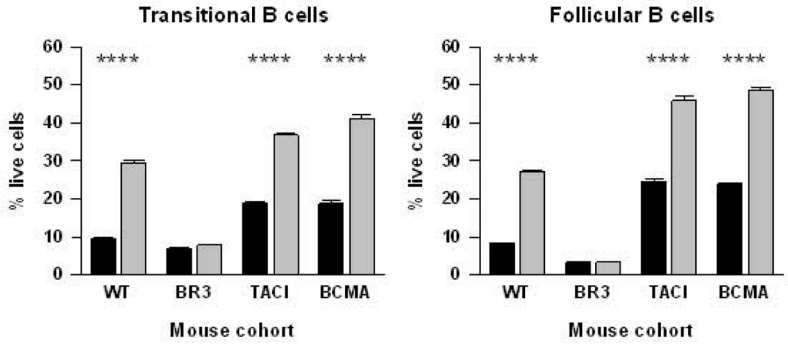

Figure 5. Differential *in vitro* responsiveness to BAFF of B cells from NZM mice deficient in one BAFF receptor

Transitional (left) and follicular (right) B cells from the indicated mice were cultured in triplicate in the absence (black bars) or presence (gray bars) of exogenous rhBAFF (100 ng/ml) and were assessed for viability after 48 hr. Results are presented as mean ± SEM. Significance comparisons are between the rhBAFF-containing and the corresponding rhBAFFnon- containing cultures.

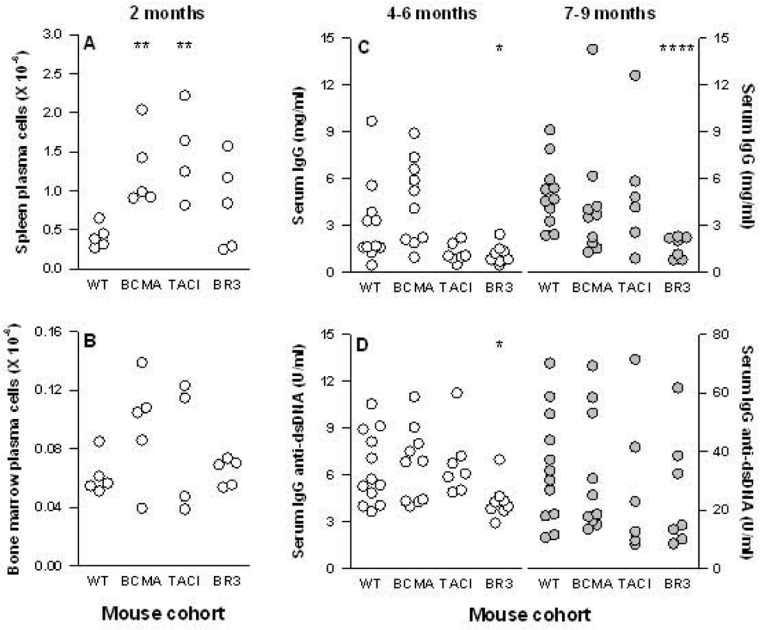

Figure 6. PC and serologic profiles among NZM mice deficient in one BAFF receptor

Spleen (panel A) and BM (panel B) PC (n = 4–5 per group) and serum levels of total IgG (panel C) and IgG anti-dsDNA (panel D) (n = 6–12 per group) from WT, BCMA, TACI, and BR3 mice of the indicated ages are plotted. Note the change in scale for serum IgG anti-dsDNA levels in 7–9- month-old mice.