TACI deletion protects against progressive murine lupus nephritis induced by BAFF overexpression

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B cells are known to promote the pathogenesis of systemic lupus erythematosus (SLE) via the production of pathogenic anti-nuclear antibodies. However, the signals required for autoreactive B cell activation and the immune mechanisms whereby B cells impact lupus nephritis pathology remain poorly understood. The B cell survival cytokine B cell activating factor of the TNF Family (BAFF) has been implicated in the pathogenesis of SLE and lupus nephritis in both animal models and human clinical studies. Although the BAFF receptor has been predicted to be the primary BAFF family receptor responsible for BAFF-driven humoral autoimmunity, in the current study we identify a critical role for signals downstream of Transmembrane Activator and CAML Interactor (TACI) in BAFF-dependent lupus nephritis. Whereas transgenic mice overexpressing BAFF develop progressive membranoproliferative glomerulonephritis, albuminuria and renal dysfunction, TACI deletion in BAFF-transgenic mice provided long-term (about 1 year) protection from renal disease. Surprisingly, disease protection in this context was not explained by complete loss of glomerular immune complex deposits. Rather, TACI deletion specifically reduced endocapillary, but not mesangial, immune deposits. Notably, although excess BAFF promoted widespread breaks in B cell tolerance, BAFF-transgenic antibodies were enriched for RNA- relative to DNA-associated autoantigen reactivity. These RNA-associated autoantibody specificities were specifically reduced by TACI or Toll-like receptor 7 deletion. Thus, our study provides important insights into the autoantibody specificities driving proliferative lupus nephritis, and suggests that TACI inhibition may be novel and effective treatment strategy in lupus nephritis.

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The B-cell survival cytokine B-cell activating factor of the tumor necrosis factor (TNF) family (BAFF, also known as BLyS) has been implicated in the pathogenesis of lupus nephritis.[1] For example, independently generated BAFF-Tg (transgenic) mouse strains exhibit humoral autoimmunity recapitulating several cardinal features of systemic lupus erythematosus (SLE) including polyclonal B-cell activation, antinuclear antibody production, and the development of immune complex (IC)–mediated glomerulonephritis.[2,3] In addition, a prominent subset of patients with lupus exhibit increased serum BAFF[4] and an insertion–deletion variant in human *TNFSF13B* (encoding BAFF) that result in higher serum BAFF levels was recently demonstrated to confer an increased risk of SLE.[5] Finally, the BAFF-inhibiting therapeutic antibody belimumab received US Food and Drug Administration approval for the treatment of SLE,[6,7] and a *post hoc* analysis of renal disease parameters using pooled data from the phase 3 SLE trials demonstrated reduced nephritis in belimumab-treated lupus patients.[8] Although the clinical efficacy of belimumab in these human lupus trials has been relatively modest, these combined animal and human data indicate an important role for BAFF in the pathogenesis of SLE.

BAFF and the related TNF family cytokine A proliferation-inducing ligand (APRIL) promote B-cell survival and activation by binding distinct receptors on peripheral B cells. BAFF binds both the BAFF receptor and transmembrane activator and CAML interactor (TACI), whereas cytokine A proliferation-inducing ligand preferentially activates cells expressing TACI or B-cell maturation antigen.[1] Since BAFF receptor deletion results in a loss of mature B cells,[9] this receptor was predicted to be the likely target for BAFF-driven B-cell activation in autoimmunity. In contrast to this idea, we and others recently reported the unexpected finding that BAFF-driven production of certain autoantibody specificities requires TACI.[10,11] Although these data did not preclude additional roles for BAFF receptor and B-cell maturation antigen signals in BAFF-dependent autoantibody production, these findings highlighted the critical importance of TACI

activation in facilitating BAFF-driven breaks in B-cell tolerance.

Importantly, whether loss of TACI signals protects against BAFF-driven glomerulonephritis has not been fully addressed. Figgett *et al.*[10] reported decreased glomerulonephritis following TACI deletion. However, renal disease was evaluated at only 12 weeks after reconstitution of lethally irradiated BAFF-Tg mice with *Taci*$^{-/-}$ bone marrow, and previous studies of BAFF-Tg animals described the development of glomerulonephritis in aged animals (~10–17 months old).[12,13] Therefore, disease quantification at early time points may not adequately address whether TACI deletion prevents kidney disease, especially when one accounts for the several weeks required to reconstitute the B-cell compartment following irradiation.

For this reason, we examined the long-term (up to 1 year) impact of TACI deletion on BAFF-driven kidney disease. We observed protection from progressive glomerulonephritis, albuminuria, and renal dysfunction in *Taci*$^{-/-}$.BAFF-Tg mice. Surprisingly, rather than uniformly blocking BAFF-driven autoantibody formation, TACI deletion specifically reduced titers of class-switched antinuclear antibody against predominantly RNA-associated autoantigens. Loss of these autoantibody specificities correlated with absent endocapillary, but not mesangial, IC deposits in *Taci*-deficient BAFF-Tg mice. In addition to identifying TACI as a potential therapeutic target in SLE, these data strongly support a model in which RNA-associated autoantibody specificities can promote endocapillary IC formation and the pathogenesis of proliferative lupus nephritis.

RESULTS

TACI deletion prevents immune activation and hypergammaglobulinemia in BAFF-Tg mice

To test the impact of TACI signals on BAFF-driven autoimmunity, we crossed BAFF-Tg and *Taci*$^{-/-}$ mice.[14] Of the several BAFF-Tg models available, we utilized a model overexpressing BAFF within the myeloid compartment under the control of the human CD68 promoter,[3] as this most closely mirrors the tissue expression pattern for endogenous BAFF. Importantly, TACI deletion did not reduce serum BAFF levels in BAFF-Tg mice, suggesting that the absence of TACI expression does not indirectly impact BAFF-driven autoimmunity (Figure 1a). To assess the impact of increased serum BAFF over time, cohorts were killed at both early (3-month) and late (10- to 12-month) time points. BAFF-Tg mice exhibited pronounced splenomegaly that increased with age, but was attenuated by TACI deletion (Figure 1b). Splenic enlargement was accounted for by marked B-cell hyperplasia as well as the TACI-dependent expansion of myeloid cells, including CD11b^{+}GR1^{lo} monocyte/macrophages and CD11b^{+}GR1^{+} neutrophils, in BAFF-Tg mice (Figure 1c–e). Although the total number of splenic T cells was equivalent (Figure 1f), an increased proportion of CD4^{+} T cells from BAFF-Tg, but not *Taci*$^{-/-}$.BAFF-Tg, mice exhibited an activated effector/memory (CD44^{hi}CD62L$^{lo/hi}$) phenotype (Figure 1g and h).

Despite similar B-cell numbers in 3-month-old BAFF-Tg and *Taci*$^{-/-}$.BAFF-Tg animals, TACI signals promoted B-cell activation in BAFF-Tg mice. Specifically, aged BAFF-Tg mice exhibited a prominent expansion of splenic B220^{lo}CD138^{+}

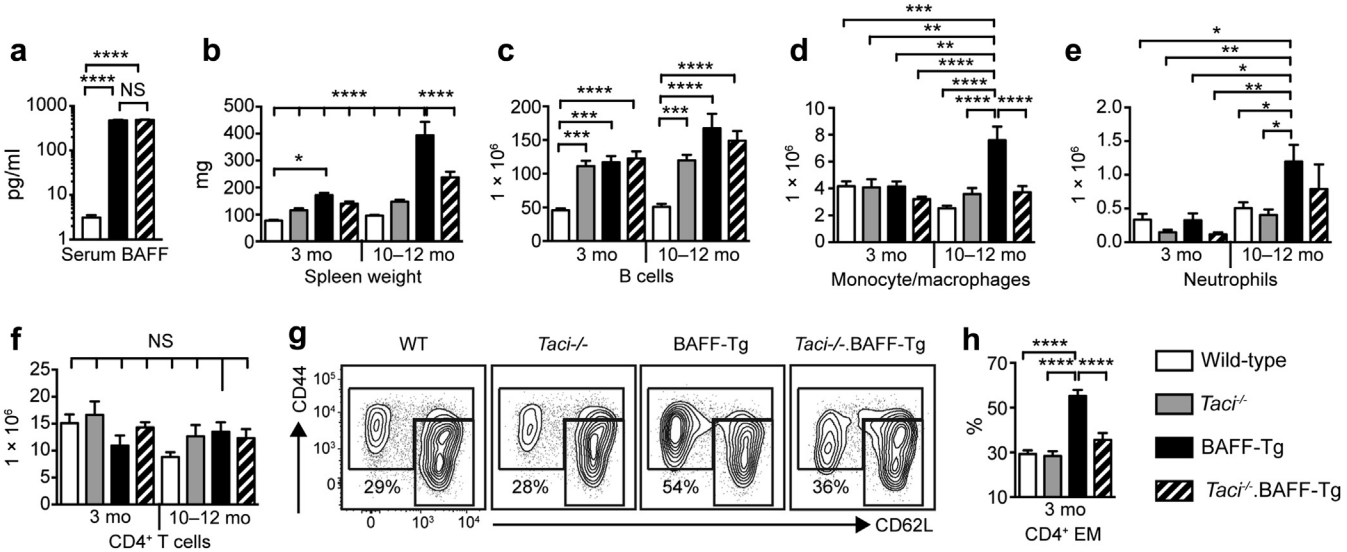

Figure 1 | Excess B-cell activating factor of the tumor necrosis factor family (BAFF) promotes progressive, transmembrane activator and CAML interactor (TACI)–dependent immune activation. (**a**) Serum BAFF levels in 6-month-old wild-type (WT) ($N = 5$), BAFF-transgenic (Tg) ($N = 8$), and *Taci*$^{-/-}$. BAFF-Tg ($N = 8$) mice. Spleen weight (**b**) and total number of splenic CD19^{+} B cells (**c**), CD11b^{+}GR1^{lo} monocyte/macrophages (**d**), CD11b^{+}GR1^{+} neutrophils (**e**), and CD4^{+} T cells (**f**) in WT, *Taci*$^{-/-}$, BAFF-Tg, and *Taci*$^{-/-}$. BAFF-Tg mice killed at 3 and 10 to 12 months old. Representative flow plots (**g**) (gated on CD4^{+} T cells) and percentage of CD4^{+} T cells exhibiting CD44^{hi}CD62L$^{lo/hi}$ effector/memory phenotype (**h**) from 3-month-old mice of indicated genotypes. Number equals the percentage in CD44^{hi}CD62L$^{lo/hi}$ EM gate. (**b**–**h**) $N = 11$ to 16 mice analyzed per group. (**a**–**h**) Error bars indicate SEM. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$. NS (not significant) by 1-way analysis of variance followed by Tukey's multiple comparison test. EM, electron microscopy; NS, not significant.