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Constitutive overexpression of BAFF in autoimmune-resistant hosts drives only some aspects of systemic lupus erythematosus-like autoimmunity

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Abstract

Objective—To determine whether overexpression of BAFF can promote SLE-like autoimmunity in hosts that otherwise are autoimmune-resistant.

Methods—We utilized MHC II-deficient C57BL/6 (B6) mice as a model of resistance to SLE and *Sles1*-bearing B6 mice as a model of resistance to the autoantibody-promoting capacity of the *Sle1* region. We generated BAFF transgenic (Tg) counterparts to these respective mice and evaluated lymphocyte phenotype, serologic autoimmunity, renal immunopathology, and clinical disease in the BAFF-Tg and non-Tg mouse sets.

Results—Although constitutive BAFF overexpression did not lead to B cell expansion in MHC II-deficient B6 mice, it did lead to increased serum IgG autoantibody levels. Nevertheless, renal immunopathology was limited, and clinical disease did not develop. In B6 and B6.*Sle1* mice, constitutive BAFF overexpression led to increased B cells, CD4⁺ memory cells, and serum IgG and IgA autoantibody levels. Renal immunopathology was modestly greater in BAFF-Tg mice than in their non-Tg counterparts, but, again, clinical disease did not develop. Introduction of the *Sles1* region into B6.*Sle1*.Baff mice abrogated the BAFF-driven increase in CD4⁺ memory cells and the *Sle1*-driven, but not the BAFF-driven, increase in serum IgG anti-chromatin levels. Renal immunopathology was substantially ameliorated.

Conclusions—Although constitutive BAFF overexpression in otherwise autoimmune-resistant mice led to humoral autoimmunity, meaningful renal immunopathology and clinical disease did not develop. This raises the possibility that BAFF overexpression, even when present, may not necessarily drive disease in some SLE patients. This may help explain the heterogeneity in clinical response to BAFF antagonists in human SLE.

Introduction

B cell activating factor belonging to the TNF family (BAFF, also known as B lymphocyte stimulator [BLyS]) is a 285-amino acid type-II transmembrane protein member of the TNF ligand superfamily (1,2). Constitutive overexpression of BAFF in BAFF-transgenic (Tg) mice that otherwise are not autoimmune-prone leads to SLE-like features (3–5).

In humans, circulating BAFF levels are elevated in many systemic rheumatic disorders, including SLE (6,7). Importantly, overexpression of BAFF is not immutably associated with autoimmune disease. For example, patients with various hematologic malignancies or immunodeficiency disorders harbor elevated circulating BAFF levels (8–11). Thus, BAFF may be incapable of driving clinically important autoimmunity under certain *in vivo* conditions. To test this possibility, we turned to two murine models in which resistance to autoimmunity has been conferred through discrete genetic manipulations.

The first model utilizes C57BL/6 (B6) congenic mice that are genetically deficient in the I-A β chain (B6.A $\beta^{-/-}$ mice) and, therefore, do not express class II MHC (MHC II). Circulating autoantibody levels in SLE-prone mice are markedly reduced in the absence of MHC II (12,13), highlighting the vital role for MHC II in the development of humoral autoimmunity.

The second model utilizes B6 congenic mice homozygous for the *Sle1* region (B6.Sle1 mice) (14). These mice develop elevated circulating titers of IgG anti-chromatin autoantibodies but rarely develop renal disease (15,16). Full-blown disease develops when other genetic “insults” are superimposed, such as the *Sle2* or *Sle3* regions (17,18). Importantly, there are genetic modifiers, especially the *Sles1* region, which abrogate the ability of B6.Sle1 mice to generate SLE-associated autoantibodies (19). Circulating autoantibody levels in B6.Sle1.Sles1 mice are identical to those in B6 wild-type mice, and B6.Sle1.Sles1 mice do not develop end-organ (kidney) pathology even when the *Sle2* or *Sle3* region is introgressed.

In this report, we demonstrate that constitutive overexpression of BAFF in BAFF-Tg B6.A $\beta^{-/-}$ mice overcomes the resistance to autoimmunity associated with MHC II deficiency and promotes humoral autoimmunity. Nevertheless, renal immunopathology is very modest, and clinical disease does not develop. BAFF-Tg B6.Sle1.Sles1 mice also develop humoral autoimmunity, but the constitutive BAFF overexpression is incapable of reversing *Sles1*-mediated inhibition of *Sle1*-dependent autoimmunity, and renal immunopathology is very limited. Thus, the consequences of BAFF overexpression on *in vivo* autoimmunity may be highly dependent upon the genetic background of the host, raising the possibility that BAFF overexpression, even when present, may not necessarily be driving disease in some SLE patients. Accordingly, the clinical effects of therapeutic BAFF neutralization may be widely disparate across the heterogeneous human SLE patient population.

Materials and methods

General

All mice were maintained at USC, and the experiments were approved by the Institutional Animal Care and Use Committee.

Mice

B6.Sle1, B6.Sles1, B6.Sle1.Sles1, B6.A $\beta^{-/-}$, and B6 mice hemizygous for a murine BAFF Tg (B6.Baff mice) have been previously described, and B6.Sle1.Baff, B6.Sle1.Sles1.Baff and B6.A $\beta^{-/-}$.Baff mice were generated by crossing the relevant parents and screening by PCR and/or flow cytometry (13,14,19–21). Mice of either sex were assessed.

Cell surface staining

Murine spleen mononuclear cells were stained with fluorochrome-conjugated mAb specific for murine CD3, CD4, CD8, CD44, CD62L, CD45R (B220), CD19, CD21, or CD23 (BD

PharMingen, San Diego, CA, or eBioscience, San Diego, CA) and analyzed by flow cytometry (22).

Serum Ig and spleen Ig-secreting cells (IgSC)

Sera were assayed for levels of total IgM and total IgG by ELISA (21). Spleen IgSC were determined by the reverse hemolytic plaque assay (23).

Serum autoantibodies

Serum levels of IgM, IgA, IgG, and IgG subclass autoantibodies to chromatin were determined by ELISA (15). Pooled sera from B6.Sle1.Sle2.Sle3 mice were used to generate a standard curve. Values are presented in arbitrary units, with 100 U/ml defined as the activity of a 1:100 dilution of the pooled B6.Sle1.Sle2.Sle3 mouse sera. Since the Ig representing a unit differs across the individual Ig classes/subclasses, U/ml are comparable only within a given Ig class/subclass.

Kidney immunofluorescence (IF)

Sections of snap-frozen kidneys were stained for IgG, IgA, or C3 using FITC-conjugated goat antibodies against the respective targets (SouthernBiotech, Birmingham, AL, and MP Biomedicals, Solon, OH). From each mouse cohort, 4 kidneys were randomly chosen, and 14–25 glomeruli from each kidney were qualitatively scored (0, negative; 1, weak; 2, moderate; 3, strong).

Kidney histology

Sections of formalin-fixed kidneys were stained with H&E and were assessed for glomerular hypercellularity, mesangial matrix expansion, interstitial cellular infiltration, and tubular atrophy or dilation.

Statistical analysis

All analyses were performed using SigmaPlot software (SPSS, Chicago, IL). When needed, raw results were log-transformed to achieve normality. Parametric testing between two groups was performed by the t test, and parametric testing among three groups was performed by one-way ANOVA. When log-transformation failed to generate normally distributed data 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 groups. Since the IF scores are ordinal ones, non-parametric testing was performed.

Results

Divergent effects of constitutive BAFF overexpression on spleen B cells in B6.A $\beta^{+/-}$ and B6.A $\beta^{-/-}$ mice

Spleen lymphocyte phenotype was assessed in 4-month-old B6.A $\beta^{+/-}$, B6.A $\beta^{+/-}$.Baff, B6.A $\beta^{-/-}$, and B6.A $\beta^{-/-}$.Baff littermate mice (Figure 1). As expected, the phenotype of MHC II-deficient B6.A $\beta^{-/-}$ mice differed from that of their MHC II-sufficient B6.A $\beta^{+/-}$ littermates. Total spleen mononuclear cells were modestly increased ($p = 0.030$); CD3⁺ cells were insignificantly decreased ($p = 0.234$); CD4⁺ cells were markedly decreased ($p < 0.001$); CD8⁺ cells were insignificantly increased ($p = 0.224$); and total B220⁺ cells and marginal zone (MZ) B (B220⁺CD21⁺CD23^{lo}) cells were greatly increased ($p = 0.027$ and $p < 0.001$, respectively).

Constitutive BAFF overexpression had disparate effects on lymphocyte phenotypes in B6.A $\beta^{+/-}$ and B6.A $\beta^{-/-}$ littermates. In comparison to non-Tg B6.A $\beta^{+/-}$ mice, total spleen mononuclear cells in B6.A $\beta^{+/-}$. Baff mice were greatly increased ($p = 0.002$); CD3 $^{+}$ cells were modestly increased ($p = 0.058$); CD4 $^{+}$ cells and CD8 $^{+}$ cells were each insignificantly increased ($p = 0.109$ and $p = 0.587$, respectively); and total B220 $^{+}$ cells and MZ B cells were markedly increased ($p < 0.001$ for each comparison).

The phenotypic consequences of constitutive BAFF overexpression in B6.A $\beta^{-/-}$ mice were strikingly different. In comparison to non-Tg B6.A $\beta^{-/-}$ mice, no significant increases (or decreases) in total spleen mononuclear cells, CD3 $^{+}$ cells, CD4 $^{+}$ cells, CD8 $^{+}$ cells, total B220 $^{+}$ cells, or MZ B cells were appreciated in B6.A $\beta^{-/-}$. Baff mice ($p \geq 0.290$ for each comparison). Thus, although constitutive BAFF overexpression leads to marked expansion of spleen B cells in a MHC II-sufficient host, it fails to do so in a MHC II-deficient host.

BAFF-driven serologic autoimmunity in the absence of MHC II

BAFF-driven B cell expansion in B6.A $\beta^{+/-}$. Baff mice was paralleled by large increases in spleen IgSC and serum total IgG levels ($p < 0.001$ and $p = 0.008$, respectively; Figure 2A–B). Similar increases in spleen IgSC ($p = 0.009$) and serum total IgG levels ($p = 0.001$) were also observed in B6.A $\beta^{-/-}$. Baff mice despite the absence of BAFF-driven B cell expansion. Of note, spleen IgSC were greater in B6.A $\beta^{-/-}$ mice than in B6.A $\beta^{+/-}$ mice ($p < 0.001$) despite serum total IgG levels being considerably greater in the latter than in the former ($p < 0.001$). This likely reflects impaired Ig class switching in B6.A $\beta^{-/-}$ mice consequent to the marked reduction in CD4 $^{+}$ cells in B6.A $\beta^{-/-}$ mice. Consistent with this notion, serum total IgM levels were greater in B6.A $\beta^{-/-}$ mice than in B6.A $\beta^{+/-}$ mice ($p = 0.031$; Figure 2C). As expected, serum total IgM levels in B6.A $\beta^{+/-}$. Baff and B6.A $\beta^{-/-}$. Baff mice were each substantially greater ($p < 0.001$ and $p = 0.003$, respectively) than in their corresponding non-Tg littermates.

Serum IgG anti-chromatin antibody levels were also increased in both B6.A $\beta^{+/-}$. Baff and B6.A $\beta^{-/-}$. Baff mice relative to their non-Tg counterparts ($p = 0.003$ and $p = 0.002$, respectively; Figure 2D). This largely held true across all the tested IgG isotypes (IgG1: $p = 0.068$ and $p = 0.089$, respectively; IgG2b: $p = 0.009$ and $p = 0.028$, respectively; IgG3: $p = 0.005$ and $p = 0.212$, respectively; Figures 2G–I). In contrast, neither serum IgM anti-chromatin antibody levels (Figure 2E) nor serum IgA anti-chromatin levels (Figure 2F) were significantly greater in B6.A $\beta^{+/-}$. Baff and B6.A $\beta^{-/-}$. Baff mice relative to their non-Tg counterparts ($p = 0.224$ and $p = 0.577$, respectively, for IgM anti-chromatin; $p = 0.246$ and $p = 0.293$, respectively, for IgA anti-chromatin).

Limited BAFF-driven renal immunopathology in the absence of MHC II

To increase the likelihood of detecting renal immunopathology, we studied 12-month-old mice (Figure 3). Glomerular deposition of IgG, C3, and IgA in B6. Baff mice was not as strong as that previously observed by others (24), perhaps due to subtle genetic differences among the various B6. Baff mouse lines and/or differences in environmental factors. Regardless, no significant differences in IF scores for glomerular IgG deposition were appreciated between B6 and B6. Baff mice ($p = 0.185$) or between B6.A $\beta^{-/-}$ and B6.A $\beta^{-/-}$. Baff mice ($p = 0.129$). The IF score for glomerular C3 deposition was actually lower in B6. Baff mice than in B6 mice ($p = 0.005$), but it was greater in B6.A $\beta^{-/-}$. Baff mice than in B6.A $\beta^{-/-}$ mice ($p < 0.001$). The IF scores for glomerular IgA deposition were greater in B6. Baff and B6.A $\beta^{-/-}$. Baff mice than in B6 and B6.A $\beta^{-/-}$ mice ($p = 0.005$ and $p = 0.006$, respectively). Importantly, mesangial hypercellularity was noted only in B6. Baff mice but not in B6.A $\beta^{-/-}$. Baff (or B6 or B6.A $\beta^{-/-}$) mice. Consistent with the limited renal

immunopathology, BAFF overexpression did not promote overt clinical kidney disease (severe proteinuria and/or increased mortality) in any of these mice.

Divergent effects of constitutive BAFF overexpression on spleen T cells in B6.Sle1 and B6.Sle1.Sles1 mice

Since deficiency of MHC II leads to profound disturbances in T cell physiology, it remained uncertain whether constitutive BAFF overexpression could overcome an “autoimmune-resistant” state and promote autoimmunity under less severe *in vivo* conditions. Accordingly, we turned to B6.Sle1 and B6.Sle1.Sles1 mice in which expression of MHC II is intact.

The lymphocyte phenotype of B6.Sle1 mice (6 months of age) differed from that of age-matched B6 mice. Total spleen mononuclear cells, CD3⁺ cells, and CD4⁺ cells were each modestly increased ($p = 0.030$, $p = 0.021$, and $p = 0.038$, respectively; Figure 4A–C) whereas CD8⁺ cells were not ($p = 0.477$; data not shown). The increases in CD4⁺ cells included both memory (CD44⁺CD62L[−]) and naive (CD44[−]CD62L⁺) cells ($p = 0.061$ and $p = 0.013$, respectively; Figure 4D and data not shown). Total B220⁺ cells and MZ B cells were increased to an even greater extent ($p = 0.005$ and $p = 0.002$; Figures 4E–F). *In toto*, these results agree well with previous reports (17, 25, 26).

The effects of introducing a BAFF Tg were similar in B6 and B6.Sle1 mice (Figure 4A–F). In each case, total spleen mononuclear cells, total B cells, and MZ B cells were greatly increased ($p < 0.001$ for each comparison). Although expansions in CD3⁺ cells ($p = 0.601$ and $p = 0.102$, respectively), CD8⁺ cells ($p = 0.059$ and $p = 0.213$, respectively), and CD4⁺ naive cells ($p = 0.092$ and $p = 0.963$, respectively) were limited at most, CD4⁺ memory cells were substantially expanded ($p = 0.002$ and $p = 0.009$, respectively).

A second set of mice (12 months of age) was studied to assess the effect of the *Sles1* region on the lymphocyte phenotype. Although spleen total mononuclear cells, CD3⁺ cells, CD4⁺ cells, CD4⁺ memory cells, B (CD19⁺) cells, and MZ B cells were similar in B6.Sle1 and B6.Sle1.Sles1 mice ($p \geq 0.094$ for each comparison), the phenotypes of B6.Sle1. Baff and B6.Sle1.Sles1. Baff mice were strikingly different (Figure 4G–L). Whereas B6.Sle1. Baff mice harbored greater total spleen mononuclear cells ($p < 0.001$), CD4⁺ cells ($p = 0.034$), and CD4⁺ memory cells ($p = 0.003$) than did B6.Sle1 mice, none of these cell populations were significantly greater in B6.Sle1.Sles1. Baff than in B6.Sle1.Sles1 mice ($p \geq 0.113$ for each comparison). Nonetheless, B6.Sle1.Sles1. Baff mice did harbor greater CD19⁺ cells ($p < 0.001$) and MZ B cells ($p = 0.019$) than did B6.Sle1.Sles1 mice, similar to the expansions in these populations in B6.Sle1. Baff mice relative to B6.Sle1 mice ($p < 0.001$ for each comparison).

The disparate effects of constitutive BAFF overexpression in B6.Sle1. Baff mice from those in B6.Sle1.Sles1. Baff mice resulted in the latter having a T cell phenotype more similar to that of B6.Sle1 mice than to that of B6.Sle1. Baff mice but a B cell phenotype more similar to that of B6.Sle1. Baff mice than to that of B6.Sle1 mice. Total spleen mononuclear cells in B6.Sle1.Sles1. Baff mice were modestly lower than in B6.Sle1. Baff mice ($p = 0.021$) but were modestly greater than in B6.Sle1 mice ($p = 0.013$). These changes can be completely explained by selective effects on CD3⁺, total CD4⁺, and CD4⁺ memory cells. CD3⁺ cells in B6.Sle1.Sles1. Baff mice were substantially reduced in comparison to B6.Sle1. Baff mice ($p = 0.006$) and in comparison to B6.Sle1 mice ($p = 0.012$). Total CD4⁺ cells and CD4⁺ memory cells in B6.Sle1.Sles1. Baff mice were also greatly reduced in comparison to B6.Sle1. Baff mice ($p = 0.002$ and $p < 0.001$, respectively) and were modestly, albeit not significantly, reduced in comparison to B6.Sle1 mice ($p = 0.111$ and $p = 0.060$, respectively). In sharp contrast, total B (CD19⁺) and MZ B cells in B6.Sle1.Sles1. Baff mice

were identical to those in B6.Sle1. Baff mice ($p = 0.799$ and $p = 0.280$, respectively) and were markedly greater than those in B6.Sle1 mice ($p < 0.001$ for each comparison).

BAFF-driven serologic autoimmunity in B6.Sle1 and B6.Sle1.Sles1 mice

In agreement with previous reports (15,16,19), B6.Sle1 mice developed considerable serum IgG anti-chromatin levels, whereas levels in B6, B6.Sles1, or B6.Sle1.Sles1 mice were, with the exception of a single mouse, uniformly very low ($p < 0.001$ for comparison between the B6.Sle1 mice and the other non-Tg mice; Figure 5A). Constitutive BAFF overexpression led to development of serum IgG anti-chromatin antibodies in B6. Baff mice ($p < 0.001$ in comparison to B6 mice) which was qualitatively reflected across all tested IgG subclasses ($p = 0.017$ for IgG1, $p = 0.002$ for IgG2b, $p < 0.001$ for IgG3; Figure 5B–D). Of note, constitutive BAFF overexpression had no incremental effect in B6.Sle1. Baff mice on the already-elevated serum IgG anti-chromatin antibody levels in B6.Sle1 mice ($p = 0.443$), suggesting that the autoimmune process leading to such autoantibodies was already maximal in *Sle1*-bearing hosts. Importantly, serum IgG anti-chromatin levels in B6.Sle1.Sles1. Baff mice were neither reduced to the levels of those in B6 mice ($p < 0.001$) nor elevated to the levels of those in B6.Sle1. Baff mice ($p = 0.003$). Rather, serum IgG anti-chromatin levels in B6.Sle1.Sles1. Baff mice were identical with those in B6. Baff mice ($p = 0.896$).

Although, as expected, serum IgG anti-chromatin antibody levels in B6.Sle1 mice were considerably greater than those in B6 mice, serum IgA anti-chromatin levels were not ($p = 0.943$; Figure 5E). Nonetheless, serum IgA anti-chromatin levels were significantly increased in B6. Baff, B6.Sle1. Baff, and B6.Sle1.Sles1. Baff mice in comparison to the corresponding non-Tg mice ($p = 0.006$, $p < 0.001$, and $p = 0.003$, respectively). This is consistent with the increased serum IgA levels previously reported in BAFF-Tg mice (4, 27).

Limited BAFF-driven renal immunopathology in *Sles1*-bearing hosts

Glomerular deposition of IgG, C3, and IgA was assessed in 12-month-old B6.Sle1, B6.Sle1. Baff, B6.Sle1.Sles1, and B6.Sle1.Sles1. Baff mice (Figure 6A–L). As assessed by IF scores, glomerular IgG deposition and glomerular IgA deposition were each greater in B6.Sle1. Baff mice than in B6.Sle1 mice ($p < 0.001$ for each comparison), whereas glomerular C3 deposition was not ($p = 0.123$). Of note, IgG deposition and IgA deposition in B6.Sle1.Sles1 mice were each similar to those in B6.Sle1 mice ($p = 0.424$ and $p = 0.062$, respectively), whereas C3 deposition was markedly reduced ($p < 0.001$). Importantly, glomerular deposition of IgG, C3, and IgA was greater in B6.Sle1.Sles1. Baff mice than in B6.Sle1.Sles1 mice ($p < 0.001$, $p = 0.039$, and $p < 0.001$, respectively).

We previously reported that B6.Sle1. Baff mice developed glomerular pathology by 3 months of age (28). Similar renal pathology (predominantly mesangial hypercellularity) was noted in 12-month-old B6.Sle1. Baff mice but was not appreciated in B6.Sle1, B6.Sle1.Sles1, or B6.Sle1.Sles1. Baff mice (Figure 6M–P). None of these mice developed overt clinical disease by 12 months (data not shown).

Discussion

Constitutive overexpression of BAFF in non-autoimmune-prone hosts is known to lead to B cell expansion, high titers of circulating IgG autoantibodies, and, in some cases, Ig deposition in the kidneys (3–5). We presently report that constitutive BAFF overexpression can overcome some, but not all, of a host's genetic resistance to SLE-like autoimmunity.

The first model we studied took advantage of the strong dependence on MHC II expression that development of circulating autoantibodies and disease has in SLE-prone mice (12,13),

providing *prima facie* evidence that a MHC II-deficient state creates an autoimmune-resistant environment. In comparison to their MHC II-sufficient B6.A $\beta^{+/-}$ littermates, MHC II-deficient B6.A $\beta^{-/-}$ mice harbored increased spleen B cells and IgSC along with the expected marked decrease in CD4⁺ cells (Figures 1–2). Consistent with this decrease in CD4⁺ cells, total serum IgG levels were substantially lower in B6.A $\beta^{-/-}$ mice than in their B6.A $\beta^{+/-}$ littermates. Not surprisingly, serum levels of SLE-associated IgG autoantibodies were low in these non-autoimmune-prone B6.A $\beta^{-/-}$ and B6.A $\beta^{+/-}$ mice.

Introduction of a BAFF Tg into B6.A $\beta^{+/-}$ mice led, as expected, to increased spleen B cells and IgSC and to increased serum total IgG and IgG anti-chromatin antibody levels, although the increase in serum levels of neither IgM nor IgA anti-chromatin antibodies achieved statistical significance. Furthermore, although spleen B cells did not increase in B6.A $\beta^{-/-}$. Baff mice, spleen IgSC and serum levels of total IgG and IgG autoantibodies did despite the paucity of CD4⁺ cells and the complete absence of MHC II. Whether the inability of MHC II-deficient B cells to expand in response to BAFF overexpression reflects an inherent defect of such B cells or is consequent to the marked reduction in CD4⁺ cells remains to be determined. Definitive studies will require the generation of mice in which MHC II deficiency is limited solely to B cells.

Regardless, the increased levels of IgG autoantibodies in B6.A $\beta^{-/-}$. Baff mice proves that the effects of constitutive BAFF overexpression on humoral autoimmunity do not require expression of H-2^b, a haplotype that permits development of SLE-associated IgG autoantibodies in BALB/c and C3H mice (29). Moreover, the divergent effects of BAFF overexpression on B cells and IgG autoantibody levels in B6.A $\beta^{-/-}$ mice highlight the *in vivo* separability of B cell number from B cell-derived products. Along this vein, we have previously documented serologic autoimmunity in BAFF-deficient NZM 2328 mice despite their life-long profound reductions in B cells (30).

The absence of BAFF-driven B cell expansion in B6.A $\beta^{-/-}$ mice sharply contrasts with the BAFF-driven B cell expansion observed in wild-type B6 mice or B6 mice completely devoid of T cells (24). It is possible, but unlikely, that MHC II-deficient B cells inherently have impaired proliferative capacity to BAFF overexpression, since BAFF overexpression leads to increased IgSC and increased production of IgG autoantibodies by such MHC II-deficient cells. An alternative, non-mutually exclusive explanation invokes CD4⁺ T regulatory cells (T regs) which are increased in BAFF-Tg mice (24,31). CD4⁺FoxP3⁺ T regs in MHC II-deficient hosts differ phenotypically from those in MHC II-intact hosts (32), so their respective inhibitory effects on BAFF-driven B cell expansion may also differ. A third, non mutually-exclusive explanation invokes CD8⁺ cells. In contrast to MHC II-intact hosts in which most FoxP3⁺ cells are CD4⁺, FoxP3⁺ cells in MHC II-deficient hosts are evenly distributed between CD4⁺ and CD8⁺ subsets (32). Thus, the regulatory role for CD8⁺ T regs in MHC II-deficient hosts may be considerable, and such cells may modulate what otherwise would be unfettered BAFF-driven expansion of B cells. Additional experimentation, including the genetic depletion of CD8⁺ cells, will be required to definitively determine the mechanisms underlying the absence of BAFF-driven B cell expansion in B6.A $\beta^{-/-}$ mice.

The increase in circulating IgG autoantibodies in B6.A $\beta^{-/-}$.Baff mice was associated with no incremental glomerular deposition of IgG and only very modest incremental glomerular deposition of C3. Importantly, no renal histopathology was noted in B6.A $\beta^{-/-}$. Baff mice (Figure 3). These findings must be reconciled with a previous report of substantial renal immunopathology in BAFF-Tg, T cell-deficient B6 mice (24). It may be that CD4⁺ and/or CD8⁺ T regs present in MHC II-deficient hosts, but absent from T cell-deficient hosts, play a protective immunomodulatory role. Alternatively, subtle differences in genetic

backgrounds and/or local environments may have affected the immunopathological outcomes. Further investigation in this area is warranted.

The second model we studied took advantage of the ability of the *Sle1* region to confer development of IgG anti-chromatin autoantibodies upon B6 mice and the ability of the *Sles1* region to abrogate such autoimmunity (15,16,19). Accordingly, a *Sles1*-bearing host is resistant to *Sle1*-dependent autoimmunity (although not necessarily resistant to *Sle1*-independent autoimmunity).

In *Sle1*-expressing mice, functional changes are independently expressed in both B cells and T cells (26,33). The *Sle1* locus is a complex of multiple susceptibility alleles (25), including *Cr2* (34), 4 Slam family genes (*Slam*, *Ly108*, *Cd84*, and *Cd48*) (35), and *Fcgr2b* (36). Both *Sle1* (NZW) alleles of *Cr2* and *Fcgr2b* alter germinal center B cell function (34,36), and the *Sle1* (NZW) allele of *Ly108* impedes negative selection of transitional B cells (37). Importantly, both B cells and T cells (including CD4⁺ memory cells) were increased in B6.Sle1 mice (Figure 4), and introduction of a BAFF Tg led to further expansions in these cells. Determination of how BAFF overexpression interacts with the individual susceptibility alleles within the *Sle1* locus will require breeding of the BAFF Tg into the respective individual sub-congenic strains. Regardless, expansion of CD4⁺ memory cells may be especially important, since expansion of this population in SLE-prone NZM 2328 mice closely reflects onset and severity of clinical disease (22,38).

Remarkably, BAFF-driven expansion of T cells was abrogated in B6.Sle1.Sles1. BAFF mice, whereas BAFF-driven expansion of B cells was unaffected. Despite this lack of effect on BAFF-driven B cell expansion, the IgG autoantibody profile of B6.Sle1.Sles1. BAFF mice resembled that of B6. Baff mice rather than that of B6.Sle1. Baff mice (Figure 5), raising the possibility that the *Sle1*-associated component of the IgG autoimmune response is largely driven by T cells. Indeed, the *Sle1a* and *Sle1c* subregions can promote development of chromatin-specific autoreactive CD4⁺ cells (39). Since the *Sles1* locus maps to a small region that contains the MHC II genes and abrogates *Sle1*-driven T cell activation while leaving *Sle1*-driven ERK phosphorylation in B cells intact (40), it may inhibit autoreactive CD4⁺ cells in B6.Sle1.Sles1. BAFF mice, and such inhibition may be resistant to BAFF overexpression. Nonetheless, the *Sles1* region may also importantly affect *Sle1*-driven B cell activation (as opposed to B cell expansion) and/or autoantibody affinity maturation and, thereby, contribute to inhibition of *Sle1*-associated autoimmunity through T cell-independent means. Formal testing of these possibilities is needed, and B6.Sles1. BAFF mice could be especially informative in establishing the effects of the *Sles1* region on the biologic consequences of constitutive BAFF overexpression.

In any case, BAFF overexpression appears to be completely capable of promoting *Sle1*-independent IgG (and IgA) autoantibody production. The inability of a *Sles1*-bearing host to overcome the autoantibody-promoting effects of BAFF overexpression is reminiscent of the inability of a *Sles1*-bearing host to overcome the autoantibody-promoting effects of the *Sle2* or *Sle3* regions (19). Regardless, and in line with their elevated circulating IgG autoantibody levels, glomerular deposition of IgG and C3 in B6.Sle1.Sles1. Baff mice was greater than that in B6.Sle1.Sles1 mice. In contrast, no renal histopathology was noted in B6.Sle1.Sles1. Baff mice (Figure 6), and clinical disease did not develop.

Collectively, our findings in two disparate models of autoimmune resistance demonstrated a discordance among glomerular IgG deposition, glomerular C3 deposition, glomerular IgA deposition, and renal histopathology. Glomerular IgG deposition and glomerular IgA deposition in B6.Sle1. Baff mice were greater than in B6.Sle1 mice, but glomerular C3 deposition was similar in each. Conversely, glomerular C3 deposition and glomerular IgA

deposition in B6.Aβ^{-/-}. Baff mice were greater than in B6.Aβ^{-/-} mice, but glomerular IgG deposition in each was similar. Furthermore, no renal histopathology developed in B6.Sle1.Sles1. Baff mice despite considerable glomerular deposition of IgG, C3, and IgA.

Indeed, the limited renal immunopathology and absence of clinical disease in 12-month-old B6. Baff mice indicate that longstanding overexpression of BAFF requires additional factors to promote *bona fide* disease. The similar renal immunopathology in the 12-month-old B6.Sle1. Baff mice of the present study to the renal immunopathology previously identified in 3-month-old B6.Sle1. Baff mice (28) suggests that the immunopathology promoted by BAFF overexpression reaches a plateau. Only in the presence of other genetic and/or environmental factors might this finite BAFF-driven immunopathology progress to clinical disease.

The differential ability of BAFF overexpression to promote or permit different facets of autoimmunity may have important ramifications for human disease. Although BAFF antagonists have been highly effective in treating murine SLE (5,41–43), results in human SLE have been less impressive (44–46). It may be that in some patients, only certain autoimmune elements of SLE are promoted or permitted by BAFF. If clinical disease in any given patient is largely BAFF-independent, then that patient would not be expected to respond to a BAFF antagonist. Future studies will be needed to distinguish those patients whose disease is largely BAFF-driven from those whose disease is not.

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Figure 1. Spleen T and B cells in B6.Aβ^{+/−}, B6.Aβ^{+/−}·Baff, B6.Aβ^{−/−}, and B6.Aβ^{−/−}·Baff mice
 Spleen total mononuclear cells (panel A), CD3⁺ cells (panel B), CD4⁺ cells (panel C), CD8⁺ cells (panel D), total B (B220⁺) cells (panel E), and MZ B (B220⁺CD21⁺CD23^{lo}) cells (panel F) from the indicated littermate mice (4 months old) are plotted. Each symbol 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.

Figure 2. Spleen IgSC and serum total IgG and IgG, IgM, and IgA anti-chromatin antibody levels in B6.Aβ^{+/+}, B6.Aβ^{+/+}·Baff, B6.Aβ^{-/-}, and B6.Aβ^{-/-}·Baff mice

Spleen IgSC (panel A) and serum levels of total IgG (panel B), total IgM (panel C), IgG anti-chromatin (panel D), IgM anti-chromatin (panel E), IgA anti-chromatin (panel F), IgG1 anti-chromatin (panel G), IgG2b anti-chromatin (panel H), and IgG3 anti-chromatin (panel I) from the indicated littermate mice are plotted as in Figure 1.

Figure 3. Renal immunofluorescence and histology in B6, B6.Baff, B6.A β ^{-/-}, and B6.A β ^{-/-}.Baff mice

Kidney sections from B6 (panels A, E, I, M), B6.Baff (panels B, F, J, N), B6.A β ^{-/-} (panels C, G, K, O), and B6.A β ^{-/-}.Baff (panels D, H, L, P) mice were stained for IgG (panels A-D), C3 (panels E-H), and IgA (panels I-L) immunofluorescence or were stained with H&E (panels M-P) for standard histological evaluation. Representative sections are illustrated. The yellow arrows in panels E and H point to areas of true glomerular C3 deposition. The blue arrows in panels F and G point to areas of non-specific staining. The black arrow in panel J points to mesangial hypercellularity.

Figure 4. Spleen T and B cells in B6, B6.Baff, B6.Sle1, B6.Sle1.Baff, B6.Sle1.Sles1, and B6.Sle1.Sles1.Baff mice

Spleens from two separate sets (panels A–F and G–L, respectively) of the indicated mice at 6 months (panels A–F) or 12 months (panels G–L) of age were analyzed for total mononuclear cells (panels A, G), CD3⁺ cells (panels B, H), CD4⁺ cells (panels C, I), CD4⁺ memory (CD4⁺CD44⁺CD62L[−]) cells (panels D, J), total B (B220⁺ or CD19⁺) cells (panels E, K), and MZ B (B220⁺CD21⁺CD23^{lo}) cells (panels F, L). Results plotted as in Figure 1.

Figure 5. Serum IgG and IgA anti-chromatin antibody levels in B6, B6.Baff, B6.Sle1, B6.Sle1.Baff, B6.Sles1, B6.Sle1.Sles1, and B6.Sle1.Sles1.Baff mice
 Serum levels of IgG (panel A), IgG1 (panel B), IgG2b (panel C), IgG3 (panel D), and IgA (panel E) anti-chromatin from the indicated mice are plotted as in Figure 1.

Figure 6. Renal immunofluorescence and histology in B6.Sle1, B6.Sle1.Baff, B6.Sle1.Sles1, and B6.Sle1.Sles1.Baff mice

Kidney sections from B6.Sle1 (panels A, E, I, M), B6.Sle1.Baff (panels B, F, J, N), B6.Sle1.Sles1 (panels C, G, K, O), and B6.Sle1.Sles1.Baff (panels D, H, L, P) mice were stained for IgG (panels A–D), C3 (panels E–H), and IgA (panels I–L) immunofluorescence or were stained with H&E (panels M–P) for standard histological evaluation. Representative sections are illustrated. The yellow arrows in panels E and F point to areas of true glomerular C3 deposition. The blue arrow in panel G points to an area of non-specific staining. The black arrow in panel N points to mesangial hypercellularity.