Paucity of Clinical Disease despite Serological Autoimmunity and Kidney Pathology in Lupus-Prone New Zealand Mixed 2328 Mice Deficient in BAFF

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BaFF has therapeutic ramifications as illustrated by the clinical observations made in human systemic rheumatoid arthritis (SLE) and related diseases. In SLE patients, serum total Ig and autoantibody levels are reduced at 4–6 mo but approached wild-type levels with increasing age, indicating that autoreactive B cells can survive and secrete autoantibodies despite the complete absence of BAFF. At least some of these autoantibodies are nephrophilic in that glomerular deposition of total Ig and IgG1 (but not of IgG2a, IgG2b, or C3) was substantial in NZM. BAFF-deficient mice by 6–7 mo of age, severe proteinuria and mortality were greatly attenuated. These results demonstrate that the lifelong absence of BAFF does not protect NZM 2328 mice from serological autoimmunity and renal pathology. Nevertheless, the character of the renal pathology is altered, and the mice are largely spared from clinically overt disease (severe proteinuria and premature death). These observations may have profound ramifications for the use of BAFF antagonists in human SLE and related diseases. The Journal of Immunology, 2006, 177: 2671–2680.

The most widely appreciated biologic property of BAFF is its ability to inhibit B cell apoptosis (11–17). Not surprisingly, administration of exogenous BAFF to mice at the time of immunization enhances Ag-specific Ab production (12). Moreover, repeated administration of BAFF to mice, even without intentional antigen immunization, results in B cell expansion and polyclonal hypergammaglobulinemia (2). Some of the increased Ig production likely is directed to common environmental (foreign) Ags, but some of the increased Ig production likely is directed to self Ags. Indeed, constitutive overexpression of BAFF in BAFF-transgenic (Tg) non-autoimmune-prone mice often leads to systemic lupus erythematosus (SLE)-like features (elevated circulating titers of multiple autoantibodies and renal Ig deposits) (6, 18, 19) and accelerates development of target-organ (kidney) pathology in autoimmune-prone mice (20). Even without “artificial” Tg-driven BAFF overexpression, BAFF overexpression is a feature of murine and human SLE. Circulating BAFF levels are elevated in (New Zealand Black (NZB) × New Zealand White (NZW)) F1 and MRL-lpr/lpr mice at the time of disease onset (6), and circulating BAFF levels are increased in as many as 50% of SLE patients (21–23), with BAFF expression correlating with clinical disease activity (24, 25). The pathogenicity of excessive BAFF production notwithstanding, “normal” expression of BAFF is vital to normal B cell development. Nonautoimmune-prone mice genetically rendered BAFF-deficient display considerable global reductions in mature B2 B cells (with nearly intact numbers of peritoneal B1 B cells and immature bone marrow B cells) and in baseline serum Ig levels and Ig responses to T cell-dependent and T cell-independent Ags (26, 27). The dependence of B cell survival and differentiation on BAFF has therapeutic ramifications as illustrated by the clinical
response (decreased disease progression and improved survival) of (NZB × NZW)F1, and MRL-lpr/lpr mice to treatment with BAFF antagonists (6, 28–30).

Although neutralization of BAFF can ameliorate the severity of established SLE disease, it is not known whether the absence of BAFF can actually prevent de novo onset of disease. Because the survival of autoreactive B cells may be much more dependent upon BAFF than is the survival of nonautoreactive B cells (31, 32), we postulated that the de novo development of autoimmunity in BAFF-deficient hosts would be profoundly attenuated, if not completely eliminated. To address this issue, we used the lupus-prone (NZB × NZWF1)-derived inbred New Zealand Mixed (NZM) 2328 mouse strain whose phenotype closely resembles that of the original F1 mice (33). To our surprise, development of serological autoimmunity in BAFF-deficient NZM 2328 mice was considerable. Substantial end-organ (kidney) pathology also developed in these mice, but it qualitatively differed from that in their BAFF-intact counterparts. Despite the serological autoimmunity and kidney pathology, clinically overt disease (severe proteinuria, premature mortality) in BAFF-deficient hosts was very limited.

Materials and Methods

**Mice**

All mice were maintained at the University of Southern California (Los Angeles, CA), and the experiments were approved by the Institutional Animal Care and Use Committee. BAFF-deficient (Baff+/−) mice on a B6/129 mixed background (27) were backcrossed into SLE-prone NZM 2328 Baff−/− mice (34). The N8 backcross generation (NZM.Baff−/−) was fully congenic. NZM.Baff−/− males and females were crossed, yielding NZM mice that were Baff+/+, Baff−/−, and Baff+/− at the expected 1:2:1 ratio. The Baff gene is located on mouse chromosome 8, ~10.52 megabases from the top, in a region not considered to be associated with susceptibility to, or resistance from, SLE. Thus, it is unlikely that inadvertent introduction and/or elimination of susceptibility and/or resistance genes had occurred consequent to the introgression. Only female mice were studied.

**Detection of Baff genotype**

Genomic DNA extracted from mouse tail clippings was PCR-amplified for 25 cycles each at 94°C for 1 min, 65°C for 1.5 min, and 72°C for 1 min. The primer sequences used were: 5′-GCAGATGGACAAATCTGGAAGGCCA-3′, 5′-TGGCAGGCTTTGACGACTCATCCAT-3′, 5′-CAAGGTGTCCTGCAACCAAAGGCAC-3′.

The PCR products were subjected to electrophoresis in agarose gels containing ethidium bromide, and bands were visualized using UV light. Band size for the intact Baff gene fragment is 234 bp and for the disrupted Baff gene is 336 bp.

**Cell surface staining**

Mouse spleen mononuclear cells were stained with combinations of FITC-PE, PerCP, aliphocyanin, and/or CyChrome-conjugated mAb specific for murine CD3, CD4, CD5, CD8, CD11b, CD19, CD21, CD23, CD44, CD45R (B220), CD62L, IgD, or IgM (BD Pharmingen) and analyzed by flow cytometry (37).

**Serum Ig and spleen Ig-secreting cells (IgSC) determinations**

Sera were assayed for levels of total IgG and total IgM by ELISA (37). Spleen cells were assayed for numbers of total IgSC by the reverse hemolytic plaque assay (38, 39). Each plaque-forming cell was taken as an IgSC.

**Serum autoantibody determinations**

Sera were assayed for levels of IgG and IgM anti-chromatin, anti-histone, and anti-dsDNA autoantibodies by ELISA (40, 41). Five sera from 36-wk-old (NZB × NZWF1) mice at a 1:200 dilution were assayed on each plate, and the average OD of these sera for each autoantigen was arbitrarily set at a value of 100. Values for the test sera were calculated as (ODserum/ODcontrol) × 100.

**Serum BAFF determination**

Serum BAFF levels were determined by a sandwich ELISA. Quantitative values were calculated from a standard curve of known concentrations of recombinant soluble murine BAFF (Biogen Idec). Because there is some batch-to-batch variation in the recombinant soluble murine BAFF used as a standard, the values should be viewed in relative terms rather than in absolute terms. The lower level of detection is 10 ng/ml.

**Spleen immunofluorescence**

OCT-embedded frozen spleen sections were stained with PE-conjugated anti-CD45R/B220 mAb (BD Biosciences), FITC-conjugated anti-MOMA-1 mAb (Serotec), or Alexa 546-conjugated peanut agglutinin (PNA) (Invitrogen Life Technologies) for 45 min at room temperature and mounted with Fluoromount G (Electron Microscopy Sciences). Stained sections were examined by fluorescence microscopy (Nikon E600).

**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.

**Kidney immunofluorescence**

OCT-embedded frozen kidney sections were incubated with FITC-conjugated Fab(ab)2 of goat anti-mouse IgG γ-chain, FITC-conjugated goat anti-mouse IgG1, IgG2a, or IgG2b isotypes (Jackson ImmunoResearch Laboratories), or goat anti-mouse C3 (MP Biomedicals) followed by FITC-conjugated anti-goat IgG (Jackson Immunoresearch Laboratories). Stained sections were examined by fluorescence microscopy (Nikon).

**Kidney histology**

Individual sections of formalin-fixed kidneys were stained with H&E and were examined by light microscopy by one of us (M. N. Koss) who was blinded to the genotype of the mouse. Each case was assessed for glomerulonephritis (GN) using a modification of the World Health Organization classification for lupus nephritis (20). Class I was assigned for normal histology by light microscopy; class II was assigned for increases in mesangial matrix and/or cells; class III was assigned for focal proliferative GN (>50% of glomeruli showing endocapillary proliferative changes with or without crescents); and class IV was assigned for diffuse proliferative GN (>50% of glomeruli showing endocapillary proliferative changes with or without crescents).

**Statistical analysis**

All analyses were performed using SigmaStat software (SPSS). When necessary, raw results were log transformed to achieve normality and/or to satisfy the equal variance test. 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, nonparametric 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. Correlations were determined by Pearson product moment correlation for interval data and by Spearman rank order correlation for ordinal data or for interval data which did not follow a normal distribution. Survival data were analyzed by the log rank test with multiple comparisons by the Holm-Sidak method.

**Results**

**Serum BAFF levels in NZM 2328 mice**

To confirm that NZM.Baff−/− mice were not “leaky”, serum BAFF levels were measured in a randomly selected group of NZM.Baff−/−, NZM.Baff+/+, and NZM.Baff−/− mice at 2 mo of age. BAFF was not detectable in the serum of any of the NZM.Baff−/− mice, whereas serum BAFF concentrations were readily measurable in the NZM.Baff−/+ and NZM.Baff+/− mice (Fig. 1). BAFF was also not detectable in the sera of a second group of NZM.Baff−/− mice bled at 6–7 mo of age.

**Effect of BAFF deficiency on splenic B cells, IgSC, and T cells in NZM 2328 mice**

In nonautoimmune-prone mice (B6/129 mixed background), BAFF deficiency leads to marked reduction in splenic B cells (27),
In SLE-prone NZM 2328 mice, BAFF deficiency had a similar effect. The geometric mean spleen B cell number in NZM.Baff/−/− mice was reduced by 96 and 92% in comparison with those in NZM.Baff+/+ and NZM.Baff+/− mice, respectively (p < 0.001, Fig. 2A). Similarly, the geometric mean spleen IgSC number in NZM.Baff/−/− mice was reduced by 91 and 81% in comparison with those in NZM.Baff+/+ and NZM.Baff+/− mice, respectively (p < 0.001, Fig. 2B). As expected, the correlation between spleen B cells and spleen IgSC across all the congenic NZM 2328 mice was very strong (Fig. 2E).

In agreement with previous observations in BAFF-deficient nonautoimmune-prone mice (42), CD23 expression was profoundly reduced on B220+ B cells from NZM.Baff/−/− mice (Fig. 3). Moreover, staining for surface IgM and IgD established that the mature (IgMlowIgDhigh) B cells were preferentially lost with relative preservation of the immature (IgMhighIgD) B cells. Immunohistochemical analysis of spleen sections (Fig. 4) confirmed the reduction in B220+ cells and documented disruption of the underlying splenic architecture, as illustrated by the altered in situ staining pattern of MOMA-1+ cells. In addition, germinal centers illustrated by clusters of PNA+ cells were smaller and fewer in number in NZM.Baff/−/− mice, consistent with the effects of BAFF deficiency in nonautoimmune-prone mice (43, 44).

Peritoneal CD5+ (B1a) B cells remain nearly intact in BAFF-deficient mice bearing a nonautoimmune-prone background (26, 27). Although CD5+ B cells variably comprise ~5% of splenic B cells in most mouse strains (45), NZB mice harbor an expanded spleen CD5+ B cell population (46). Because the genetic constitution of NZM 2328 mice is partly derived from NZB mice, we assessed additional NZM.Baff+/+ and NZM.Baff/−/− mice for spleen CD5+ B cells. CD5+ B cells were markedly expanded in 4-mo-old NZM.Baff+/+ mice (Table I). Most of these cells also expressed high levels of surface IgM, whereas the great majority of the spleen CD5+ B cells were CD11b−. The expansion of CD5+ B cells in the spleens of NZM.Baff/−/− mice is even greater than that previously observed in NZB mice (46), and the percentages of IgM+ cells or CD11b+ cells among spleen CD5+ B cells are consistent with previous observations in nonautoimmune BALB/c mice (47). Very small percentages of B1b-like cells (CD19+CD5+CD11b+IgM+) were also present in the spleens of NZM.Baff+/+ mice. In both age-matched and much older (14–18 mo of age) NZM.Baff/−/− mice, the percentages of spleen CD5+ B cells were modestly greater than those in NZM.Baff+/+ mice, but the absolute numbers of all the individual B cell populations in NZM.Baff/−/− mice were markedly lower than those in NZM.Baff+/+ mice.

**FIGURE 1.** Serum levels of BAFF in NZM 2328 mice. Sera from 2-mo-old Baff+/+ (int; n = 2), Baff+/− (het; n = 3), and Baff−/− (def; n = 8) NZM 2328 mice were analyzed for BAFF levels. Sera from a second group of NZM.Baff+/− mice (def II; n = 9) bled at 6–7 mo of age were also analyzed for BAFF levels.

**FIGURE 2.** Spleen B cells, IgSC, and T cells in NZM.Baff+/+, NZM.Baff+/−, and NZM.Baff−/− mice. A–D, Spleens from Baff+/+ (int; n = 8; age 4–8 mo), Baff+/− (het; n = 8; age 11–13 mo), and Baff−/− (def; n = 9; age 12–13 mo) NZM 2328 mice were analyzed for B (B220+) cells (A), IgSC (B), CD4+ cells (C), and CD8+ cells (D). 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. E–G, Correlations among all NZM 2328 mice (Baff+/+; ○; Baff+/−; □; Baff−/−; ◻) are illustrated for spleen B (B220+) cells vs spleen IgSC (E), spleen B (B220+) vs spleen CD4+ cells (F), and spleen B (B220+) vs spleen CD8+ cells (G).
NZM. Baff−/− mice at 10–13 mo of age (Fig. 5C) (most NZM. Baff−/− mice were dead by this age, so serum samples from this age group were not available for testing). A similar reduction in serum total IgM levels in NZM. Baff−/− mice at 4–6 mo of age and an equalization of total IgM levels among NZM. Baff+/+., NZM. Baff−/−, and NZM. Baff−/− mice with increasing age were also observed (data not shown).

Comparable patterns were observed for the tested serum IgG autoantibodies. At 4–6 mo of age, IgG anti-chromatin Ab levels were significantly lower (p < 0.001) in either NZM. Baff+/+ or NZM. Baff−/− mice than in NZM. Baff+/+ mice (Fig. 5D). By 7–9 mo of age, IgG anti-chromatin Ab levels in NZM. Baff−/− mice were no longer significantly lower than those in NZM. Baff+/+ mice (Fig. 5E), and by 10–13 mo of age, IgG anti-chromatin Ab levels in NZM. Baff−/− mice were no longer significantly lower than those in NZM. Baff+/+ mice (Fig. 5F). For IgG anti-histone Abs, the modest significant decrease (p = 0.002) in serum levels in NZM. Baff+/+ or NZM. Baff−/− mice at 4–6 mo relative to those in corresponding NZM. Baff+/+ mice completely disappeared by 7–9 mo of age (Figs. 5, G–I). For IgG anti-dsDNA Abs, serum levels in NZM. Baff+/+ mice were not significantly different from those in NZM. Baff−/− mice at any time point (Fig. 5, J–L). Although levels in NZM. Baff+/+ mice were significantly greater than those in NZM. Baff−/− mice at both 4–6 mo and 7–9 mo of age (p < 0.001 and p = 0.002, respectively), they were surpassed in NZM. Baff−/− mice by 10–13 mo of age. The serum IgG autoantibody levels harbored by 10–13-mo-old NZM. Baff+/+ mice were similar to those harbored by 11–22-mo-old BAFF-sufficient nonautoimmune-prone C57BL/6 female mice (n = 7; IgG anti-chromatin 21–98 U/ml; IgG anti-histone 6–30 U/ml; IgG anti-dsDNA 21–148 U/ml). Serum levels of the corresponding IgM autoantibodies in NZM. Baff−/− and NZM. Baff−/− mice also were similar to those in NZM. Baff+/+ or NZM. Baff−/− mice by 7–9 mo of age (data not shown).

**Effect of BAFF deficiency on kidney pathology in NZM 2328 mice**

The development, albeit delayed, of serologic autoimmunity in BAFF-deficient NZM 2328 mice raised the possibility that target-organ (kidney) pathology might be substantial in these mice. Immunofluorescence analysis of kidneys from NZM. Baff+/+ mice (6–7 mo of age) demonstrated widespread glomerular deposition of total IgG and C3 (Fig. 6, A and D). In contrast, glomerular total IgG deposition in kidneys from age-matched NZM. Baff−/− mice was greatly reduced, and glomerular C3 deposition was barely discernible (Fig. 6, B and E). In much older NZM. Baff−/− mice (12–13 mo of age), deposition of total IgG had become substantial and was qualitatively similar to that in 6- to 7-mo-old NZM. Baff+/+ mice (Fig. 6C). Nevertheless, deposition of C3 was still barely discernible (Fig. 6F). This dichotomy in old NZM. Baff−/− mice between glomerular total IgG deposition and glomerular C3 deposition may have been due to preferential glomerular deposition of IgG1 (poor at complement fixation) rather than IgG2a or IgG2b (good at complement fixation). Indeed, glomerular IgG1 deposition in old NZM. Baff−/− mice was considerable, whereas glomerular deposition of IgG2a and IgG2b in these mice was very limited (Fig. 6, I, L, and O).

The striking reductions in glomerular IgG2a, IgG2b, and C3 deposition among NZM. Baff−/− mice were associated with less severe kidney pathology. Kidneys from eight NZM. Baff−/− mice 6–7 mo of age and from nine NZM. Baff−/− mice 11–13 mo of age were evaluated histologically. Among the former, one had class IIB GN, three had class III GN, and four had class IV GN (NZM. Baff+/+ mice routinely have class IV GN by 6–7 mo of age).
B220  MOMA-1  PNA

NZM.Baff+/+

NZM.Baff−/−

FIGURE 4. Spleen immunofluorescence in NZM.Baff+/+ (A–C) and NZM.Baff−/− (D–F) mice. Spleen sections from the indicated mice were stained with anti-B220 mAb, MOMA-1, or PNA. Original magnification is ×10.

age). No obvious progression of the renal pathology to the more severe forms (i.e., class IV) was appreciated among the 11- to 13-mo-old NZM.Baff−/− mice, with three having class IIB GN, three having class III GN, and three having class IV GN. Qualitatively, the pathology even among the NZM.Baff−/− mice was more characterized by occasional foci of interstitial nephritis (green arrow), perivascular inflammation (red arrow), and widespread hyaline thrombi (blue arrows), the last of which being virtually undetected in NZM.Baff−/− mice.

Effect of BAFF deficiency on clinical disease in NZM 2328 mice

Consistent with previous observations (48), NZM.Baff+/+ mice started developing ≥3+ proteinuria at 5–6 mo of age, with >90% being affected by 10 mo of age. Death followed shortly after development of this severe proteinuria (Fig. 8). Although renal pathology was substantial in NZM.Baff−/− mice, development of severe proteinuria and mortality was markedly attenuated in these mice (p < 0.001 for each in comparison to NZM.Baff+/+ and NZM.Baff−/− mice). Development of severe proteinuria and mortality in NZM.Baff−/− mice was not significantly different from those in NZM.Baff+/+ mice (p = 0.165 and p = 0.128, respectively).

Discussion

In nonautoimmune-prone mice (B6/129 mixed background), lifelong absence of BAFF results in profound reductions in total B cells (preferentially affecting mature B cells) and in circulating Ig (27). NZM 2328 mice, despite their strong underlying autoimmune diathesis, also displayed profound lifelong reductions in total B cells (preferentially affecting mature IgD+ B cells) and spleen IgSC in response to lifelong BAFF deficiency (Figs. 2, A and B, and 3). Splenic architecture was altered and expression of B cell surface CD23 was markedly diminished in NZM.Baff−/− mice in comparison to NZM.Baff+/+ mice as were the numbers and size of spleen germinal centers (Figs. 3 and 4), similar to observations made in nonautoimmune-prone BAFF-deficient mice (42–44).

Whereas BAFF-deficient NZM 2328 mice recapitulated many B cell phenotypic features of BAFF-deficient nonautoimmune-prone mice, there was one apparent exception. CD5+ B cells were markedly expanded in NZM 2328 mice, with >50% of spleen B cells expressing both CD19 and CD5 (Table I). However, whereas the absolute numbers of CD5+ (B1a) B cells remained nearly intact in nonautoimmune-prone BAFF-deficient mice (26, 27), the absolute numbers of such cells (along with CD5− B1b cells) were markedly

Table I. B1 cells in NZM.Baff+/+ and NZM.Baff−/− mice

<table>
<thead>
<tr>
<th>Mice</th>
<th>% CD19+</th>
<th>% CD5+</th>
<th>% CD5+11b−</th>
<th>% CD5+IgM−</th>
<th>% CD5+11b−IgM−</th>
<th>CD19+</th>
<th>CD5+11b+</th>
<th>CD5+11b−IgM−</th>
<th>CD5+11b−IgM−</th>
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<tr>
<td>Baff+/+</td>
<td>17.4</td>
<td>56.1</td>
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<td>52.5</td>
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<td>18.4</td>
<td>10.2</td>
<td>0.86</td>
<td>9.5</td>
</tr>
<tr>
<td>Baff−/− (young)</td>
<td>1.6</td>
<td>71.4</td>
<td>17.8</td>
<td>44.4</td>
<td>3.1</td>
<td>0.60</td>
<td>0.44</td>
<td>0.095</td>
<td>0.27</td>
</tr>
<tr>
<td>Baff−/− (old)</td>
<td>3.3</td>
<td>68.2</td>
<td>12.2</td>
<td>42.2</td>
<td>10.7</td>
<td>0.65</td>
<td>0.44</td>
<td>0.076</td>
<td>0.27</td>
</tr>
</tbody>
</table>

a Spleen cells from NZM.Baff+/+ mice (age 4 mo, n = 4) and young (age 4–6 mo, n = 5) and old (age 14–18 mo, n = 5) NZM.Baff−/− mice were stained for the indicated populations.

b Median percentages of spleen mononuclear cells.

c Median percentages of spleen CD19+ cells.

d Geometric mean cell numbers × 10^6.

p < 0.001 compared to Baff+/+.

p = 0.006 compared to Baff+/+.

p = 0.004 compared to Baff+/+.

Median value; p = 0.032 compared to Baff+/+.

p = 0.008 compared to Baff+/+. 
reduced in NZM.Baff−/− mice compared with those in NZM.Baff+/+ mice. It must be stressed that very few CD5+B cells are present in the spleens of most mouse strains, so reductions in spleen CD5+B cells would be difficult to perceive. That is, previous reports claiming BAFF independence by B1 cells may have been overstated, and absence of BAFF even in nonautoimmune-prone mice may severely affect total CD5+B cell number. Nevertheless, the possibility remains that the contribution of BAFF to the maintenance of discrete B cell populations may differ in an autoimmune-prone host from that in a nonautoimmune-prone host. The precise specificities and contributions to autoimmunity of the expanded individual CD5+B cell subsets as well as those of CD5−B1b cells in NZM.Baff+/+ and NZM.Baff−/− mice will require further experimental elucidation.
BAFF-deficient NZM 2328 mice also displayed reductions in CD4<sup>+</sup> and CD8<sup>+</sup> cells (Fig. 2, C and D), albeit not as dramatic as the reductions in B cells and IgSC. Neither the T cell activation state (as determined by surface expression of CD44 and CD62L) nor the percentages of CD4<sup>+</sup>CD25<sup>high</sup> cells differed among NZM<sup>Baff</sup> mice as a function of Baff genotype (data not shown). The strong correlations between spleen B cell and T cell numbers (Fig. 2, F and G) suggests their possible coordinate regulation. Although the three BAFF receptors (B cell maturation Ag (BCMA), transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI), BAFFR/BR3) are predominantly expressed by B cells (49–52), some T cells express TACI and/or BAFFR (50, 53, 54) and can be costimulated by BAFF (10, 54, 55). Thus, the absence of BAFF-mediated trophic effects on B cells and T cells in NZM.<sup>Baff</sup> mice could have led to parallel reductions in both. Alternatively, decreased numbers of B cells, with the attendant reduction in net B cell APC function, may have resulted in a secondary decrease in T cells.

In accord with the profound diminution in B cells, serum total Ig and autoantibody levels were significantly reduced in NZM.<sup>Baff</sup> mice relative to those in age-matched NZM.<sup>Baff</sup> mice through ~6 mo of age. With increasing age, however, serum total Ig and autoantibody levels in NZM.<sup>Baff</sup> mice continued to rise to the point where the overlap in values among NZM.<sup>Baff</sup><sup>+/+</sup>, NZM.<sup>Baff</sup><sup>++/+</sup>, and NZM.<sup>Baff</sup> mice was considerable (Fig. 5, and data not shown). This increase occurred despite the profound persistent decrease in spleen IgSC in NZM.<sup>Baff</sup> mice (Fig. 2B), indicating that nonspleen IgSC (e.g., bone marrow IgSC) had greatly increased in number and/or that Ig secreting by each IgSC had increased in a BAFF-independent manner. The serum levels of IgG autoantibodies in 10- to 13-mo-old NZM.<sup>Baff</sup> mice were similar to those in 11- to 22-mo-old BAFF-sufficient C57BL/6 mice, raising the possibility that development of serological autoimmunity with age in BAFF-sufficient hosts may have a BAFF-independent component. In any case, the identity of the BAFF-independent factor(s) that promoted B cell survival and differentiation in NZM.<sup>Baff</sup> mice remains speculative but may have involved overexpression of Bcl-2 and/or Bcl-xL. Plasma blasts that overexpress Bcl-2 are resistant to the proapoptotic effects of BAFF neutralization (56), and overexpression of Bcl-2 or Bcl-xL can reverse the B cell-depleting effects of BAFF or BAFFR deficiency (57–60).

Alternatively, a proliferation-inducing ligand (APRIL) may have been playing a critical role in promoting autoimmunity in NZM.<sup>Baff</sup> mice. Although APRIL does not bind to BAFFR (51, 52), APRIL does bind to the other two BAFF receptors, BCMA and TACI (61–64). Engagement of either receptor by APRIL could facilitate development and/or maintenance of an autoimmune state. BCMA likely plays an important role in survival of long-lived plasma cells (65) and may play an important role in

**FIGURE 6.** Kidney immunofluorescence in NZM.<sup>Baff</sup><sup>+/+</sup> and NZM.<sup>Baff</sup><sup>−/−</sup> mice. Sections of fresh-frozen kidneys from a 6-mo-old NZM.<sup>Baff</sup> mouse (A, D, G, J, and M), a 7-mo-old NZM.<sup>Baff</sup> mouse (B, E, H, K, and N), and a 13-mo-old NZM.<sup>Baff</sup> mouse (C, F, I, L, and O) were stained for total IgG, C3, IgG1, IgG2a, and IgG2b. Original magnification is ×40.
augmenting B cell APC function (66). TACI, in addition to its role in promoting T cell-independent responses (67, 68), is likely involved in Ig class switching (69) as is APRIL (70). APRIL-deficient mice manifest impaired Ig class switching (71), so APRIL/TACI interactions may have been vital to development of IgG autoantibodies in BAFF-deficient NZM 2328 mice. Indeed, Ag-specific Ab responses are enhanced in APRIL-Tg mice (72), so APRIL may readily promote autoantigen-specific Ab responses in autoimmune-prone hosts.

Based on studies in hen egg lysozyme (HEL)/anti-HEL double-Tg mice, it has been suggested that survival of autoreactive B cells is more dependent upon BAFF than is survival of nonautoactive B cells (31, 32). The observed steady rise in serum IgG autoantibody levels with age in NZM.Baff−/− mice does not necessarily refute this view. Nevertheless, it does convincingly demonstrate that, despite the complete absence of BAFF, autoreactive B cells can survive in an autoimmune-prone (NZM 2328) environment and differentiate into IgG autoantibody-secreting cells. Although BAFF may preferentially promote survival of autoreactive B cells, BAFF is clearly not indispensable for the development of autoimmunity per se.

At least some of the autoantibodies that can emerge in a BAFF-deficient environment are nephrophilic, since glomerular IgG deposition developed in NZM.Baff−/− mice, albeit with delayed kinetics relative to development of glomerular IgG deposition in NZM.Baff+/+ mice (Fig. 6). Although glomerular IgG deposition was minimal in 6- to 7-mo-old NZM.Baff−/− mice, glomerular IgG deposition in these mice was considerable by 12- to 13-mo of age. Nevertheless, glomerular C3 deposition was barely detectable in these mice was considerable by 12–13 mo of age. Nevertheless, glomerular C3 deposition was barely detectable in these mice was considerable by 12–13 mo of age. Nevertheless, glomerular C3 deposition was barely detectable in these mice was considerable by 12–13 mo of age. Nevertheless, glomerular C3 deposition was barely detectable in these mice was considerable by 12–13 mo of age. Nevertheless, glomerular C3 deposition was barely detectable.

Kidney pathology frequently developed in NZM.Baff−/− mice by 6–7 mo of age, with class IV GN being present in >30% of these mice (Fig. 7). Nevertheless, there were clear-cut qualitative differences between the class IV GN in NZM.Baff−/− mice and the class IV GN in NZM.Baff+/+ mice. Most striking were the greater degree of endocapillary proliferation in the latter compared with that in the former and the widespread hyaline thrombi in the former which were very rare in the latter (both types of pathology are seen in human lupus nephritis, although the extensive nature of the hyaline thrombi seen in NZM.Baff−/− mice may outstrip that seen in human disease).

In addition to any role having been played by the delay in IgG deposition and the near absence of C3 deposition in the glomeruli, the alteration in renal pathology in NZM.Baff−/− mice may have been related to the substantial, albeit incomplete, depletion of B cells per se in a BAFF-deficient environment. MRL-lpr/lpr mice completely deficient in B cells do not display heightened T cell activation (76), and, in the absence of B cells, neither MRL-lpr/lpr nor NZM 2328 mice develop disease at all (Ref. 77 and C. O. Jacob, unpublished observations). However, MRL-lpr/lpr mice
that bare B cells but whose B cells are incapable of secreting Ig (including autoantibodies) do develop heightened T cell activation and do develop disease, but the disease is attenuated relative to that in wild-type MRL-lpr/lpr mice (78). How much of the altered renal pathology in NZM.Baff mice is due to the reduction in glomerular IgG deposition and/or absence of glomerular C3 deposition and how much is due to the reduction in B cell number and attendant autoantibody-independent B cell APC function remains to be determined through future investigation.

In any case, the alteration in kidney pathology is likely directly responsible for the considerable delay and attenuation of severe proteinuria and mortality (Fig. 8). The paradox of little clinical responsible for the considerable delay and attenuation of severe proteinuria and mortality (Fig. 8). The paradox of little clinical

References


AUTOIMMUNITY IN BAFF-DEFICIENT NZM 2328 MICE


