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Global T Cell Dysregulation in Non-Autoimmune-Prone Mice Promotes Rapid Development of BAFF-Independent, Systemic Lupus Erythematosus-Like Autoimmunity

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In otherwise non-autoimmune-prone C57BL/6 (B6) mice rendered genetically deficient in CD152 (CTLA-4), polyclonal hypergammaglobulinemia with increased levels of systemic lupus erythematosus (SLE)-associated IgG autoantibodies, glomerular IgG and C3 deposition, and interstitial nephritis all developed by 3–5 wk of age. Remarkably, superimposing genetic deficiency of BAFF (B cell-activating factor belonging to the TNF family) onto CD152 deficiency did not substantially attenuate humoral autoimmunity and immunopathology in these mice, despite the resulting marked reduction in B-lineage cells. Although superimposing a BAFF transgene (resulting in constitutive BAFF overexpression) onto CD152-deficient mice did lead to increases in B-lineage cells and serum levels of certain SLE-associated IgG autoantibodies, renal immunopathology remained largely unaffected. Taken together, these results demonstrate that global T cell dysregulation, even in an otherwise non-autoimmune-prone host, can promote systemic humoral autoimmunity and immunopathology in a BAFF-independent manner. Moreover, supraphysiologic expression of BAFF in the setting of ongoing autoimmunity does not necessarily lead to greater immunopathology. These findings may help explain the limited clinical efficacy appreciated to date of BAFF antagonists in human SLE. The Journal of Immunology, 2008, 181: 833–841.
aged (31). This clearly demonstrated that B-cell-based autoimmunity could emerge despite the lifelong absence of BAFF, but the underlying driving force(s) remained uncertain.

Since T cell dysregulation is a feature of SLE, one plausible explanation for development of autoimmunity in BAFF-deficient NZM 2328 mice is that dysregulated T cell activation promotes differentiation of, and Ig production by, the limited numbers of B cells extant in the BAFF-deficient, but otherwise SLE-prone, hosts. However, a myriad of T cell-intrinsic abnormalities, including those involving B cells and/or the innate immune system, in these mice could potentially contribute in a meaningful way to development of autoimmunity. That is, development of BAFF-independent autoimmunity in NZM 2328 mice may not necessarily be (solely) T cell driven. To unambiguously address development of T cell-driven BAFF-independent autoimmunity and immunopathology, we turned to a model system based on deficiency of CD152 (CTLA-4) in an otherwise non-autoimmune-prone host.

CD152 is a vital homeostatic regulator of T cell activation. The suppressor effects of CD4+CD25+ regulatory T cells are mediated, at least in part, by CD152 (32, 33), and engagement of CD152 is crucial to development and/or maintenance of tolerance (34, 35). C57BL/6 (B6) or BALB/c mice genetically deficient in CD152 (cd152−/− mice) spontaneously develop massive systemic T cell expansion and infiltration into vital organs, which is lethal by as early as 3 wk of age (36–38). Negative and positive selection in the thymus are normal in cd152−/− mice (39, 40), indicating that the physiologic defect is in control of peripheral T cell activation rather than in central T cell development. Because the accelerated T cell activation, T cell expansion, and mortality are markedly attenuated in TCR-Tg cd152−/− mice that express highly limited T cell repertoires (40–43), it is likely that the proliferating T cells in non-TCR Tg cd152−/− mice respond to highly prevalent environmental Ags and/or self-Ags. The diverse and unbiased TCR repertoire in these non-TCR Tg cd152−/− mice (44) indicates that no individual self (or environmental)-Ag is uniquely driving the pathologic response, but it does make it likely that autoreactive T cells are represented among the activated and proliferating T cells.

Development of humoral autoimmunity in cd152−/− mice has, to date, not been reported. In this report, we demonstrate that cd152−/− mice bearing a non-autoimmune-prone B6 genetic background (B6.cd152−/− mice) develop, by 3–5 wk of age, high circulating levels of SLE-associated IgG autoantibodies along with renal deposition of Ig and complement and interstitial nephritis. Remarkably, BAFF-deficient B6.cd152−/− (B6.cd152−/−, baff−/−) mice develop similarly robust autoimmunity and immunopathology, demonstrating that dysregulated T cells, even in the context of an otherwise non-autoimmune-prone environment, can promote humoral autoimmunity and immunopathology in a BAFF-independent manner. These findings may have profound ramifications for BAFF-targeted therapeutic approaches in human autoimmune diseases.

Materials and Methods

General

All reported studies were approved by the University of Southern California Institutional Animal Care and Use Committee. All assays and tissue evaluations described below were performed by individuals who were blinded to the genotypes of the mice.

Mice

All mice were housed at the University of Southern California in a single specific pathogen-free room. To generate BAFF-deficient (baff−/−) cd152−/− mice, baff−/− mice (30) that had been backcrossed to B6 mice for more than nine generations (B6.baff−/− mice) were first crossed with (baff−/−) B6.cd152−/− mice (45), and the pups were screened by PCR for the cd152−/− genotype (45) and the baff−/− genotype (31). The resulting B6.baff−/−cd152−/− mice were backcrossed to B6.baff−/− mice, and the pups were screened for the cd152−/− and baff−/− genotypes. The resulting male and female B6.baff−/−cd152−/− mice were intercrossed, giving rise to B6.baff−/−cd152−/−, B6.baff−/−cd152+/-, and B6.baff+/-cd152−/− mice.

To generate BAFF-Tg cd152−/− mice, B6.cd152−/− mice were first crossed with (cd152+/-) BAFF-Tg B6 (B6.BTg) mice (26, 46), and the pups were screened by PCR for the BAFF transgene (46) and for the cd152−/− genotype (45). The resulting B6.BTg.cd152−/− mice were then crossed with B6.cd152−/− mice, giving rise to B6.cd152−/−, B6.cd152+/-, B6.cd152+/-, B6.BTg.cd152−/−, and B6.BTg.cd152+/- mice.

Cell surface staining

Single-cell suspensions from spleen or BM were stained with combinations of the following conjugated mAb: FITC-conjugated anti-CD43 (BD Biosciences), anti-IgA (Southern Biotechnologies Associates), and anti-IgM (Southern Biotechnologies); allophycocyanin-conjugated anti-CD19 (BD Biosciences) and anti-CD93 (eBioscience); and aliphycocyanin Cy7-conjugated anti-B220 (eBioscience); PE-conjugated anti-IgA (Southern Biotechnologies Associates), anti-IgM (Southern Biotechnologies Associates), anti-CD23 (BD Biosciences), and anti-CD138 (BD Biosciences); PE Cy5-conjugated anti-GR1 (BD Biosciences), anti-CD4 (BD Biosciences), anti-CD8 (BD Biosciences), and anti-F4/80 (BD Biosciences); PE Cy5.5-conjugated anti-CD21/35 (Allman Laboratory, University of Pennsylvania, Philadelphia, PA); PE Cy7-conjugated anti-IgM (eBioscience); biotin-conjugated anti-CD138 (BD Biosciences), anti-CD23 (BD Biosciences), and anti-IgD (Southern Biotechnologies Associates); and streptavidin-conjugated PerCP Cy5.5 (BD Biosciences) and PacificBlue (Invitrogen). For intracellular Ag discrimination, samples were fixed and rendered permeable with Solutions A and B (Invitrogen) before addition of the Abs toward intracellular Ags. Dead cells were excluded from analyses with Aqua Fixable Live/Dead Kits (Invitrogen). Analyses were performed on a 17-color four-laser LSRII (BD Biosciences). Flow cytometry data were analyzed using FlowJo 8.6 software (Tree Star).

Spleen immunofluorescence

OCT-embedded frozen spleen sections were stained with PE-conjugated anti-CD45RB/B220 mAb (BD Biosciences) and FITC-conjugated anti-MOMA-1 mAb (Serotec) for 1 h at room temperature and mounted with Fluoromount G (Electron Microscopy Sciences). Stained sections were examined with a Nikon E600 fluorescence microscope.

Serum Ig and autoantibody determinations

For quantification of individual total Ig class or subclass concentrations, serial dilutions of mouse sera were added to ELISA plates that had been coated with capture Abs against the indicated specific Ig class or subclass. This was followed by alkaline phosphatase-conjugated detection Abs to the specific Ig class or subclass (Southern Biotechnologies Associates) (47). The Ig concentrations were calculated from standard curves concurrently generated with purified mouse myeloma proteins (Sigma-Aldrich).

For quantification of IgG autoantibody concentrations, mouse sera (1/250 dilution) were added to ELISA plates that had been coated with chromat (5 μg/ml), histone (20 μg/ml), ssDNA (100 μg/ml), or dsDNA (100 μg/ml) followed by alkaline phosphatase-conjugated goat anti-mouse IgG (Southern Biotechnologies Associates) (48). Each sample was normalized to the mean OD of serum from 5-mo-old MRL-1pr/lpr mice, the latter being arbitrarily assigned a value of 100 U/ml.

Kidney immunofluorescence

OCT-embedded frozen kidney sections were incubated with FITC-conjugated Fr(ab)2 fragments of goat anti-mouse IgM or IgG γ-chain, FITC-conjugated goat anti-mouse IgG1, IgG2b, or IgG3 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 5-μm sections of formalin-fixed kidneys were stained with HE, periodic acid-Schiff, and Masson’s trichrome stain and examined by light microscopy. Each case was assessed for GN using a modification of the World Health Organization classification for lupus nephritis (46) as previously described (31) and for interstitial nephritis.
B6.BTg, and B6. They undergo massive systemic T cell expansion and activation. A large cell dysregulation, inasmuch as such mice spontaneously un-

We chose CD152-deficient mice as a model of robust global T cell responses. The percentages of pro/pre (T1, T2, and T3) B cells. The numbers adjacent to the rectangles in a–f indicate the percentages of T1 (upper left), T2 (upper right), and T3 (lower right) B cells. The numbers adjacent to the ovals and quadrangles in g–l, respectively, indicate the percentages of FO and MZ B cells. C, Contour plots of surface CD138 and intracellular k/i (intracellular) staining of gated BM (a–f) or spleen (g–l) CD4 CD8– GR-1– B220low/– IgM– cells from the indicated mice are illustrated. The numbers inside the rectangles indicate the percentages of plasma cells. D, Sections of fresh-frozen spleens from the indicated mice (a–d) were stained with anti-B220-PE (red) plus anti-MOMA-1-FITC (green). Original magnification, ×10.

Statistical analysis

All analyses were performed using SigmaStat software (SPSS). If the raw (untransformed) data did not follow a normal distribution, they were log-

Results

Effects of CD152 deficiency on B cell phenotype in B6. B6.BTg, and B6. Baff mice led to modest increases in the percentages of BM pre/pro B cells and to substantial reductions in percentages of BM-immature B cells (Fig. 1A, a and d). These changes were mirrored in B6. baff mice but were blunted in B6.BTg mice (Fig. 1A, a, c, e, and f). (In all staining combinations, results for cd152 mice were identical with those for cd152 mice; therefore, results only for the latter are shown.) Importantly, CD152 deficiency in B6. baff mice did not lead to a reduction in the percentages of BM-mature recirculating B cells (Fig. 1A, g and j). Although the percentages of these cells in CD152-deficient B6.BTg and B6. baff mice were, respectively, greater than and less than that in CD152-deficient B6. baff mice, CD152 deficiency failed to lead to any relative reductions in the percentages of BM-mature recirculating B cells in either B6.BTg or B6. baff mice.
Among spleen transitional B cells, CD152-sufficient B6.BTg mice harbored greater percentages of late transitional (T3) B cells and lesser percentages of early transitional (T1) B cells than did CD152-sufficient B6.\textit{baff}^{+/+} mice, consistent with the ability of BAFF to serve as a B cell differentiation factor (21, 22). CD152 deficiency in both B6.\textit{baff}^{+/+} and B6.BTg mice led to increases in the percentages of T3 B cells and decreases in the percentages of T1 B cells (Fig. 1B, a, b, d, and e). Strikingly, transitional B cell progression beyond the T1 stage was largely arrested in CD152-sufficient B6.\textit{baff}^{+/+} mice, consistent with previous reports (29, 30). Nevertheless, there was an unmistakable increase in the percentages of the more mature T2 and T3 B cells in B6.\textit{baff}^{+/+} cd152^{−/−} mice (Fig. 1B, c and f), demonstrating that the consequences of BAFF deficiency on transitional B cell maturation could be at least partly overcome.

In addition to the substantial reduction in T2 and T3 B cells in BAFF-deficient (CD152-sufficient) mice, follicular (FO) and marginal zone (MZ) B cells are also substantially reduced in them (29, 30). In contrast, MZ B cells are expanded in BAFF Tg (CD152-sufficient) mice (16, 26). Analysis of the FO and MZ B cell subsets in CD152-sufficient B6.\textit{baff}^{+/+} and B6.BTg mice confirmed their respective reductions and expansions relative to their B6.\textit{baff}^{+/+} counterparts (Fig. 1B, g–i). Regardless of the \textit{baff} genotype, CD152-deficient mice harbored substantially lower percentages of both FO and MZ B cells than did the corresponding CD152-sufficient mice (Fig. 1B, j–l), although the effect on MZ B cells was difficult to appreciate in B6.\textit{baff}^{+/+}.cd152^{−/−} mice due to the very low percentages of MZ B cells harbored by B6.\textit{baff}^{+/+}.cd152^{+/+} and B6.\textit{baff}^{+/+}.cd152^{−/−} mice.

Preservation of plasma cells (PC) and development of hypergammaglobulinemia and SLE-associated autoantibodies in CD152-deficient B6.\textit{baff}^{+/+}, B6.BTg, and B6.\textit{baff}^{−/−} mice

In contrast to the reductions in percentages of T2, T3, FO, and MZ B cells in CD152-deficient mice relative to their CD152-sufficient counterparts, CD152 deficiency in B6.\textit{baff}^{+/+}, B6.BTg, and B6.\textit{baff}^{−/−} mice led to no change or modest increases in the percentages of BM and/or spleen PC (Fig. 1C, a–l), populations which include both CD138⁺ and CD138⁻ cells (49). Moreover, the collapsed spleen follicles seen in CD152-sufficient B6.\textit{baff}^{+/+} mice were substantially restored in their CD152-deficient counterparts (Fig. 1D, a and b). The paucity of MOMA-1⁺ rings surrounding the spleen follicles in B6.\textit{baff}^{+/+}.cd152^{−/−} mice is likely a feature of CD152 deficiency per se, inasmuch as a similar paucity of MOMA-1⁺ rings was observed in CD152-deficient, but not CD152-sufficient, B6.\textit{baff}^{+/+} mice (Fig. 1D, c and d).

Consistent with the relative increase in PC and, in the case of B6.\textit{baff}^{−/−} mice, the restoration of FO structure, serum levels of total IgM, IgG1, and IgG2b were significantly greater in CD152-deficient mice than in corresponding CD152-sufficient mice, regardless of the \textit{baff} genotype (\(p \leq 0.006\) for total IgM; \(p \leq 0.001\) for total IgG1; \(p \leq 0.005\) for total IgG2b; Fig. 2A, a–c). Although serum total IgM and IgG2b levels in B6.\textit{baff}^{+/+}.cd152^{−/−} mice were significantly lower than those in B6.\textit{baff}^{+/+}.cd152^{+/+} or B6.BTg.cd152^{−/−} mice (\(p < 0.001\) for each comparison), there were no significant differences in serum total IgG1 levels among these discrete mouse cohorts.

Not only did hypergammaglobulinemia develop in CD152-deficient mice regardless of their \textit{baff} genotype, but these mice also harbored considerable levels of serum SLE-associated IgG autoantibodies, including autoantibodies directed against chromatin, histone, ssDNA, and dsDNA (Fig. 2B, a–d). In contrast, serum levels of these autoantibodies were low in CD152-sufficient hosts, with no significant differences being appreciated between \textit{cd152}^{+/+} and \textit{cd152}^{−/−} mice. Of note, serum autoantibody levels were no greater in CD152-deficient B6.BTg mice than in CD152-sufficient B6.\textit{baff}^{−/−} mice, consistent with previous observations that elevated circulating IgG anti-chromatin and IgG anti-dsDNA autoantibodies were not detected in 3-mo-old B6.BTg (\textit{cd152}^{+/+}) mice (46). For any \textit{baff} genotype, autoantibody levels against each of the four tested specificities were significantly greater in CD152-deficient mice than in corresponding CD152-sufficient mice (\(p \leq 0.008\) for IgG anti-chromatin; \(p \leq 0.003\) for IgG anti-histone; \(p \leq 0.006\) for IgG anti-dsDNA; and \(p \leq 0.006\) for IgG anti-ssDNA).

The only IgG autoantibodies whose serum levels were statistically greater in CD152-deficient B6.BTg mice than in the other tested CD152-deficient mice were anti-ssDNA (\(p = 0.003\)) and anti-dsDNA (\(p = 0.032\)). That is, constitutive overexpression of BAFF in CD152-deficient B6 mice did not discernibly promote greater IgG anti-chromatin or anti-histone Ab responses. Strikingly, serum IgG anti-histone, anti-ssDNA, and anti-dsDNA levels were no different in CD152-deficient B6.\textit{baff}^{−/−} mice than in CD152-deficient B6.\textit{baff}^{+/+} mice. The only IgG autoantibody whose serum levels were statistically lower in the former than in the latter was anti-chromatin (\(p < 0.001\)). Although modest quantitative differences were detected among the individual CD152-deficient mouse cohorts in IgG subclass distribution of the SLE-associated IgG autoantibodies (data not shown), the serologic studies collectively indicate that the generation and maintenance of elevated levels of at least some of these autoantibodies in CD152-deficient B6 mice do not require BAFF. Moreover, when levels of such autoantibodies are already high, they frequently are not meaningfully augmented further by BAFF overexpression.

Development of renal immunopathology in CD152-deficient B6.\textit{baff}^{+/+}, B6.BTg, and B6.\textit{baff}^{−/−} mice

The development of serologic autoimmunity in CD152-deficient mice by 3–5 wk of age raised the possibility that development of target-organ (kidney) immunopathology might also be rapid in these mice. Immunofluorescence studies of kidney sections revealed discrete patterns of Ig deposition among the tested groups of mice. Renal IgM deposition was detected in CD152-sufficient B6.\textit{baff}^{+/+} and B6.BTg mice (Fig. 3A, a and c), with staining intensity being similar to that in 2-mo-old (before development of clinical autoimmunity) NZM 2328 mice (Fig. 3Ag). In contrast, no renal IgM deposition was appreciated in CD152-sufficient B6.\textit{baff}^{−/−} mice (Fig. 3Ar). However, renal IgM deposition was strong in all CD152-deficient mice, including B6.\textit{baff}^{+/+}, cd152^{+/−} mice, with staining intensity approaching that in 6-mo-old (clinically sick) NZM 2328 mice (Fig. 3A, b, d, f, and h).

Deposition of IgG in the kidneys of the tested mice was striking in its prevalence. Although renal IgG deposition was not appreciated in any of the CD152-sufficient mice (Fig. 3A, i, k, and m), all of the CD152-deficient mice, including B6.\textit{baff}^{−/−}.cd152^{−/−} mice, developed considerable renal IgG deposition (Fig. 3A, j, l, and n). This renal IgG deposition by 3–5 wk of age in the CD152-deficient mice is especially remarkable, inasmuch as no renal IgG deposition was yet appreciated in 2-mo-old (clinically healthy) NZM 2328 mice despite their development of considerable renal IgG deposition by 6 mo of age (when clinically sick) (Fig. 3A, o and p). In general, renal deposition of C3 paralleled deposition of IgG (Fig. 3A, q–x), although occasionally, some interstitial C3 deposition was also observed, irrespective of the \textit{baff} or cd152 genotypes of the mice (Fig. 3Au).

In BAFF-deficient NZM 2328 mice, deposition of IgG in the kidneys was substantial, albeit delayed in time relative to that in corresponding BAFF-sufficient mice, but the distribution of IgG subclasses deposited in the kidneys of BAFF-deficient NZM 2328
mice differed from that in BAFF-sufficient NZM 2328 mice. In the former, IgG1 deposition predominated with limited IgG2a or IgG2b deposition. In contrast, all three subclasses were amply represented in the latter (31).

Given the dramatic deposition of IgG in the kidneys of both B6.baff+/-.cd152−/− mice and B6.baff−/- cd152−/− mice, we assessed the distribution of IgG subclasses in the renal deposits. Consistent with the lack of detectable deposition of total IgG in CD152-sufficient mice of any baff genotype, no renal IgG1, IgG2b, or IgG3 deposition was detected in any CD152-sufficient mouse (Fig. 3B). In contrast, renal IgG1 deposition was abundant in all CD152-deficient mice of any baff genotype.
genotype (Fig. 3B, b, d, and f). Renal IgG2b deposition was also detected in all CD152-deficient mice, but among these mice, the staining appeared to be less intense in B6.\textit{baff}\textsuperscript{-/-}\textit{cd152}\textsuperscript{-/-} mice (Fig. 3B, j, l, and n). Of note, renal IgG3 deposition was not detected in any CD152-deficient mouse tested (Fig. 3B, r, t, and v), which contrasts with the substantial renal deposition of IgG1, IgG2b, and IgG3 in 6-mo-old, but not 2-mo-old, NZM 2328 mice (Fig. 3B, g, h, o, p, w, and x).

In contrast to the severe and widespread GN that develops with age in either BAFF-sufficient or BAFF-deficient NZM 2328 mice (31), GN in 3- to 5-wk-old CD152-deficient (or CD152-sufficient) mice was quite limited, with endocapillary proliferation and mesangial hypercellularity only occasionally seen. In contrast, a very different picture emerged with regard to interstitial nephritis. Whereas interstitial nephritis was largely absent in B6.\textit{baff}\textsuperscript{-/-}\textit{cd152}\textsuperscript{-/-} mice at 3–5 wk of age, it was prominent in B6.\textit{baff}\textsuperscript{-/-}\textit{cd152}\textsuperscript{-/-} mice of the same age (Fig. 4, a and c, and our unpublished data). Strikingly and unexpectedly, interstitial nephritis was also a feature of CD152-sufficient B6.\textit{baff}\textsuperscript{-/-} mice and was further intensified in CD152-deficient B6.\textit{baff}\textsuperscript{-/-} mice (Fig. 4, b and d). Taken together, not only were the immunopathologic consequences of CD152 deficiency for the kidney not inhibited by BAFF deficiency, but they may actually have been aggravated by BAFF deficiency.

**Discussion**

Two striking conclusions can be drawn from the present study. First, the complete absence of CD152 leads to development of hypergammaglobulinemia and considerable humoral autoimmunity, including the production of nephrophilic autoantibodies and/or autoantibodies commonly associated with SLE (IgG anti-chromatin, IgG anti-histone, IgG anti-dsDNA, and

![](image1.png)

**FIGURE 3.** Renal Ig and C3 deposition in CD152-sufficient and CD152-deficient B6.\textit{baff}\textsuperscript{-/-}, B6.BTg, and B6.\textit{baff}\textsuperscript{-/-} mice. A, Sections of fresh-frozen kidneys from the indicated mice were stained for total IgM (a–h), total IgG (i–p), and C3 (q–x). B, Kidneys sections were stained for IgG1 (a–h), IgG2b (i–p), and IgG3 (q–x). Original magnification for all photomicrographs is ×40.

![](image2.png)

**FIGURE 4.** Renal histology in CD152-sufficient and CD152-deficient B6.\textit{baff}\textsuperscript{-/-} and B6.\textit{baff}\textsuperscript{-/-} mice. Representative sections of formalin-fixed kidneys from B6.\textit{baff}\textsuperscript{-/-}\textit{cd152}\textsuperscript{-/-} (a), B6.\textit{baff}\textsuperscript{-/-}\textit{cd152}\textsuperscript{-/-} (b), B6.\textit{baff}\textsuperscript{-/-}\textit{cd152}\textsuperscript{-/-} (c), and B6.\textit{baff}\textsuperscript{-/-}\textit{cd152}\textsuperscript{-/-} (d) mice were stained with H&E. The arrows point to overt areas of interstitial nephritis. Original magnification, ×400.
IgG anti-ssDNA Abs). The development of such humoral autoimmunity in CD152-deficient B6 mice (which harbor ostensibly normal B cells, a normal innate immune system, and an otherwise non-autoimmune-prone environment) by 3–5 wk of age is far more rapid than that which occurs in bona fide SLE mice (e.g., NZM 2328 mice). Deposition of IgG and C3 in the glomeruli of CD152-deficient B6 mice is readily demonstrable and these mice develop interstitial nephritis to a much greater degree than do their CD152-sufficient counterparts. Of interest, dramatic glomerular histologic changes are not associated with the renal IgG and C3 deposition. The paucity and early stage of histologic GN may reflect the very young age of the mice at the time of their sacrifice. It is possible that widespread GN would develop were the mice able to live longer. This speculation, however, is inherently indeterminate due to the uniformly early mortality of CD152-deficient mice from failure of nonrenal vital organs (e.g., heart, liver).

The humoral autoimmunity and immunopathology that develop in CD152-deficient hosts are almost certainly due to global T cell dysregulation rather than due to any intrinsic B cell abnormality. Experiments with chimeric mice harboring CD152-sufficient T cells and CD152-deficient B cells have demonstrated that the frequencies of peripheral B cells, total circulating IgM and IgG levels, and primary and secondary Ag-specific IgG responses are no different from those in chimeric mice harboring CD152-sufficient B cells rather than CD152-deficient B cells (50).

Although complete absence of CD152 has never been associated with human autoimmune disease, polymorphisms within the Cd152 gene have been associated with several human disorders characterized by autoantibody production, including SLE (51–53). This raises the possibility that alterations in CD152 function may importantly affect development of autoantibodies, including potentially pathogenic ones, not just in mice but in humans as well. Moreover, autoantibodies against CD152 circulate in some patients with systemic immune-based rheumatic diseases (54) and, thereby, may further compromise the regulatory function of CD152 and aggravate the autoimmune process.

The second and, perhaps, more striking conclusion from the present study is that the entire spectrum of autoimmune features observed in CD152-deficient mice can develop in the complete absence of BAFF. Regardless of their genetic background (non-autoimmune prone or autoimmune prone), there is a ~90% reduction in spleen B cells (including transitional, FO, and MZ B cells) among baфф−/− mice relative to their baфф+/+ counterparts (30, 31). Although CD152 deficiency, regardless of the baфф genotype, considerably affected the distribution of B cells among phenotypically defined B cell subsets, CD152 deficiency did not result in appreciable expansion of B cells and, of great importance, did not restore B cell numbers in B6.baфф−/− mice to levels observed in B6.baфф+/+ mice. That is, CD152-deficient B6.baфф−/− mice displayed a marked B cell deficiency, similar in degree to that displayed by CD152-sufficient B6.baфф+/+ mice.

This B cell deficiency notwithstanding, hypergammaglobulinemia developed in CD152-deficient mice by 3–5 wk of age. Although serum levels of total IgM and total IgG2b were significantly lower in B6.baфф−/−.cd152−/− mice than in B6.baфф+/+.cd152+/+ mice, serum total IgG1 levels in these respective mice were similar. Because BAFF can preferentially promote Th1 responses (55), the preferential global production of IgG1 in BAFF-deficient hosts may be a reflection of a shift from Th1 responses to Th2 responses (which would favor production of IgG1 over IgG2b).

In any case, there was only a limited difference among B6. baфф−/−.cd152−/− mice, B6.BTg.cd152−/− mice, and B6. baфф+/+.cd152−/− mice when it came to production of SLE-associated autoantibodies. Although serum levels of IgG anti-chromatin Abs in B6.baфф−/−.cd152−/− mice were modestly (albeit significantly) lower than those in the other two cohorts, serum levels of IgG anti-histone, IgG anti-ssDNA, and IgG anti-dsDNA Abs were essentially identical in B6.baфф−/−.cd152−/− and B6.baфф+/+.cd152−/− mice. Importantly, despite serum levels of IgG anti-ssDNA and IgG anti-dsDNA being greater in B6.BTg.cd152−/− mice than in the other CD152-deficient cohorts, glomerular deposition of IgG1 and IgG2b in B6.baфф−/−.cd152−/− mice, B6.BTg.cd152−/− mice, and B6.baфф+/+.cd152−/− mice was comparable and was associated with concurrent deposition of C3. Thus, global T cell dysregulation arising from CD152 deficiency drove considerable serologic autoimmunity and renal IgG and C3 deposition in non-autoimmune-prone B6 mice by 3–5 wk of age. This was not substantially affected either by the complete absence of BAFF or by constitutive supraphysiologic overexpression of BAFF. The fact that some differences exist between B6.baфф−/−, cd152−/− and B6.baфф+/+.cd152−/− or B6.BTg.cd152−/− and bona fide SLE-prone NZM 2328 mice, such as the renal deposition of IgG3 or the intact MOMA-1+ rings surrounding spleen follicles in the latter but not in the former, likely demonstrates that the numerous genetic differences between NZM 2328 and CD152-deficient B6 mice do lead to phenotypic differences.

Although we have tacitly assumed that the B cells in B6.baфф−/− mice are normal, this may not necessarily be strictly correct. Because all of the B cells in BAFF-deficient hosts will have matured and differentiated along a BAFF-independent pathway, they may be more sensitive and/or responsive to alternate maturation/differentiation factors than are B cells that mature and differentiate in a BAFF-sufficient environment. Future studies that assess the effects of pharmacologic inhibition of BAFF on autoimmunity in CD152-deficient BAFF-deficient hosts should lend insight into this matter.

Given that widespread T cell expansion and T cell infiltration into vital organs are cardinal features of CD152-deficient mice (36–38), it is not surprising that we documented the development of substantial interstitial nephritis in our CD152-deficient mice. What was unexpected was the considerable “baseline” interstitial nephritis that developed in CD152-sufficient B6.baфф−/− mice by 3–5 wk of age. Previous reports of BAFF-deficient (non-autoimmune-prone) mice have not included descriptions of kidney histology (29, 30, 56, 57); therefore, the clinical importance of interstitial nephritis in these mice and the underlying mechanisms warrant further investigation.

The unexpected finding of interstitial nephritis in B6.baфф−/− mice notwithstanding, the development of autoimmunity in the absence of BAFF should not be taken to mean that BAFF plays no contributory role in disease pathogenesis. Indeed, BAFF overexpression can promote SLE-like autoimmunity even in the absence of T cells (28). That is, abnormal T cell help may be dispensable for SLE-like features. Disease that develops in hosts bearing certain intrinsic “abnormalities” of B cells and/or the innate immune system might be highly responsive to BAFF elimination-neutralization. Nevertheless, the rapid T cell-driven development of substantial serologic autoimmunity and end-organ (kidney) immunopathology in a host that harbors an otherwise non-autoimmune-prone environment despite the complete absence of BAFF does raise some doubt regarding the ultimate utility of BAFF antagonists in clinical practice. Although treatment of SLE-prone (NZB × NZW)F1 or MRL-1pr/lpr mice with the BAFF antagonist TACI-Ig did attenuate disease and enhance survival (6), at least some of the clinical efficacy may have come from the ability of TACI-Ig to neutralize not just BAFF but APRIL as well. Although BAFFR-Ig, a BAFF-specific antagonist without any APRIL-neutralizing activity, was able to attenuate end-organ pathologic
changes in murine SLE (58, 59), only short-term effects were assessed. It remains plausible that in the long term, disease would have emerged in these mice despite the neutralization of BAFF.

Indeed, the development of serologic and pathologic features of SLE in BAFF-deficient NZM 2328 mice (31) and the development of full-blown serologic, pathologic, and clinical disease in MRL-lpr/lpr mice bearing a mutant BAFF incapable of transducing BAFF-triggered signals (60) support this premise.

Human experience to date also raises concerns regarding the efficacy of BAFF antagonism. Although clinical trials in human SLE with a neutralizing anti-BAFF mAb have documented a highly favorable safety profile, they have demonstrated, at most, only modest clinical benefit (61, 62). Although these observations should not be interpreted as showing the absence of any benefit from solely targeting BAFF, they do strongly suggest that therapeutic targeting of BAFF alone is inadequate. Optimal therapeutic utility of BAFF antagonism may require combination with other agents. Further investigation in both murine models and in human subjects will be needed to delineate the precise therapeutic niche for BAFF antagonists.

Disclosures

The authors have no financial interest of conflict.

References


