CD4+CD25+ T-Cells Control Autoimmunity in the Absence of B-Cells

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OBJECTIVE—Tumor necrosis factor ligand family members B-cell—activating factor (BAFF) and a proliferation-inducing ligand (APRIL) can exert powerful effects on B-cell activation and development, type 1 T-helper cell (Th1) immune responses, and autoimmunity. We examined the effect of blocking BAFF and APRIL on the development of autoimmune diabetes.

RESEARCH DESIGN AND METHODS—Female NOD mice were administered B-cell maturation antigen (BCMA)-Fc from 9 to 15 weeks of age. Diabetes incidence, islet pathology, and T- and B-cell populations were examined.

RESULTS—BCMA-Fc treatment reduced the severity of insulitis and prevented diabetes development in NOD mice. BCMA-Fc–treated mice showed reduced follicular, marginal-zone, and T2MZ B-cells. B-cell reduction was accompanied by decreased frequencies of pathogenic CD4+CD40− T-cells and reduced Th1 cytokines IL-7, IL-15, and IL-17. Thus, T-cell activation was blunted with reduced B-cells. However, BCMA-Fc–treated mice still harbored detectable diabetogenic T-cells, suggesting that regulatory mechanisms contributed to diabetes prevention. Indeed, BCMA-Fc–treated mice accumulated increased CD4+CD25+ regulatory T-cells (Tregs) with age. CD4+CD25+ cells were essential for maintaining euglycemia because their depletion abrogated BCMA-Fc–mediated protection. BCMA-Fc did not directly affect Treg homeostasis given that CD4+CD25+Foxp3+ T-cells did not express TACI or BR3 receptors and that CD4+CD25+Foxp3+ T-cell frequencies were equivalent in wild-type, BAFF−/−, TACI−/−, BCMA−/−, and BR3−/− mice. Rather, B-cell depletion resulted in CD4+CD25+ T-cell–mediated protection from diabetes because anti-CD25 monoclonal antibody treatment precipitated diabetes in both diabetes-resistant NOD,µMT−/− and BCMA-Fc–treated mice.

CONCLUSIONS—BAFF/APRIL blockade prevents diabetes. BCMA-Fc reduces B-cells, subsequently blunting autoimmune activity and allowing endogenous regulatory mechanisms to preserve a prehyperglycemic state. Diabetes 58:1568–1577, 2009

The members of the tumor necrosis factor (TNF) ligand family of molecules B-cell—activating factor (BAFF) (also known as BlyS, TNFSF13b) and a proliferation-inducing ligand (APRIL) can exert powerful effects on B-cell development, survival, and function; T-cell activation; and type 1 T-helper cell (Th1) immune responses and autoimmunity (1). BAFF exists as both a soluble and a membrane bound molecule and is expressed by a wide range of inflammatory-activated cells, including monocytes, macrophages, dendritic cells, and T-cells (2). In contrast, APRIL is processed intracellularly and exerts its function as a soluble protein. BAFF and APRIL can bind to one of two receptors: B-cell maturation antigen (BCMA) (3) or transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI) (3,4), whereas BAFF can also bind to BR3 (otherwise known as BAFF-R) (5). These receptors are found on a wide range of B-cell subsets including immature, transitional, mature, memory, and germinal center B-cells, as well as on plasma cells (2). Further, activated T-cells can express the receptors BR3 and TACI (4,6).

BAFF has emerged as an important player in the development of autoimmunity. Elevated BAFF and APRIL levels have been detected in sera from human patients with rheumatoid arthritis, lupus, and Sjogren’s syndrome (7–9). Moreover, BAFF-transgenic mice harbor increased titers of self-reactive antibodies and develop autoimmune symptoms very similar to those of lupus and Sjogren’s syndrome (10,11). Forced expression of BAFF also results in symptoms very similar to those of lupus and Sjogren’s syndrome (10,11). Forced expression of BAFF also results in a marked expansion of marginal-zone B-cells (MZBs) (12)—a B-cell subset associated with autoimmune conditions including lupus (13), Sjogren’s syndrome (11), and, more recently, type 1 diabetes (14,15). Thus, the BAFF/APRIL system can be considered a proinflammatory pathway associated with the development of autoimmunity (7,8). Indeed, studies designed to explore the therapeutic potential of BAFF pathway blockers for the treatment of autoimmune conditions are underway (16,17). This background makes targeting the BAFF/APRIL system a potential therapeutic candidate for the treatment of type 1 diabetes. This study was undertaken to test the hypothesis that targeting the BAFF/APRIL system would have multiple inhibitory effects on the spontaneous development of autoimmune diabetes in the NOD model.

RESEARCH DESIGN AND METHODS

C57BL/6, NOD,SCID, and NOD/Lt (NOD) mice were obtained from The Walter and Eliza Hall Institute of Medical Research (WEHI). NOD,µMT−/− mice were provided by Dr. Serreze (18). BAFF−/−, BCMA−/−, and TACI−/− mice were provided by Dr. Susan Kalled (Biogen Idec). BR3−/− mice were a gift from Dr. Rajewsky (19). All animal experiments...
were approved by the St. Vincent's Campus Animal Experimentation and Ethics Committee.

**Diabetes incidence studies.** NOD mice were administered BCMA-Fc (150 μg per treatment) based on previous studies (20). BCMA-Fc is a fusion protein—the extracellular portion of BCMA fused to the Fc domain of human IgG. BCMA-Fc was provided by Dr. Susan Kalled (Biogen Idec). Controls were treated with PBS or intravenous globulin (HuIvlg) (150 μg). For adoptive transfer studies, splenocytes (1 × 10⁷) from pre-diabetic 16-week-old female NOD donors or BCMA-Fc–treated mice were transferred intravenously into NOD.SCID recipients. Glucose levels were monitored twice weekly from 10 weeks of age onward for BCMA-Fc–treated mice or starting with transfer of splenocytes; a blood glucose level >12.0 mmol/l on two consecutive readings was scored as indicative of diabetes.

**Phenotypic analysis of mononuclear cells.** Lymphocytes were isolated and analyzed by flow cytometry exactly as previously described (15). T-cell subpopulations were identified as follows: CD8α (Ly2)(53-6-7) and memory-effector cells CD44hiCD62Llow and regulatory T-cells (Tregs) CD4+ (L-243) (GK1.5), CD25+ (7D4) and (PC61), and Foxp3+ (Foxp3-staining kit; eBioscience, San Diego, CA). Diabetogenic T-cell clones were identified based on expression of CD4 (H122.19) and CD40 (3/23) as previously described (21). B-cell subpopulations were identified exactly as previously described (15): follicular B-cells (FoB) (CD23hiCD52low, IgM+, IgG1+, and IgG2b+), MZls (CD21hi, IgMhi, and IgG1+, transitional type 1 (T1) cells (CD23+, IgMhi, and IgG1+), and transitional type 2 (T2MZ) cells (CD23+, IgM+, and IgG1+, IgG2b+), Isotype controls included IgG1, IgG2a, IgG2b, and IgG3. Flow cytometric analysis was conducted on a FACScalibur flow cytometer (BD Biosciences, San Jose, CA).

**Cytokine analysis.** Cytokine profile of sera samples was performed with a LINCOplex mouse 9-plex cytokine kit from Lincro Research (St. Charles, MO), following the manufacturer’s instructions. The assays were carried out at The University of New South Wales Inflammation Disease Unit in conjunction with Taline Hampartzoumian.

**Histopathology.** Formaldehyde-fixed, paraffin-embedded pancreata sections (5 μm) were hematoxylin and eosin stained. Insulitis was scored (100× magnification) as follows: grade 0, no insulitis; grade 1, peri-insulitis; grade 2, insulitis involving <25% islet; grade 3, insulitis involving >25% islet; and grade 4, insulitis involving >75% and/or complete islet infiltration. Photos were taken using a Leica DC800 camera on a Leica DMIRE microscope (BD Biosciences, San Jose, CA). Diabetes incidence studies were graphed as Kaplan-Meier survival plots and analyzed using the Mantel-Cox log-rank method with 2 degrees of freedom (GraphPad Prisms; GraphPad Software). *P* values represent comparison between different treatments as indicated in the figure legends.

**RESULTS**

**Disrupting the BAFF/APRIL pathway in the preclinical phase prevents diabetes onset.** To test the effect of disrupting the BAFF/APRIL pathway before the onset of hyperglycemia, we injected NOD mice with 150 μg BCMA-Fc intraperitoneally (i.p.) twice weekly from 9 to 15 weeks of age (12 injections over a 6-week period); control groups were administered PBS or HuIvlg (150 μg i.p.) over the same period (Fig. 1). We found that all NOD mice treated with PBS or HuIvlg from 9 to 15 weeks of age developed diabetes with the expected high frequencies. There were no significant differences in diabetes incidence between PBS- and HuIvlg-treated groups (*P* = 0.1309; *n* ≥ 10). In contrast, we found that NOD mice treated with BCMA-Fc from 9 to 15 weeks of age were completely protected from diabetes (diabetes incidence 0 of 10 at 50 weeks of age; *P* = 0.0041, *n* ≥ 10, log-rank vs. HuIvlg).

**Effect of BCMA-Fc treatment on peripheral B-cell populations.** Examination of peripheral lymphoid populations before and at the cessation of the 9- to 15-week BCMA-Fc treatment was carried out by flow cytometry. Given the well-described requirement of BAFF in the regulation of steady-state B-cell homeostasis (1), we first examined B-cell populations. As shown in Fig. 2A, all three known BAFF and APRIL receptors were expressed by NOD IgM+ B220+ B-cells, suggesting that NOD B-cells would be sensitive to BAFF/APRIL blockade. Tracking IgM+ B220+ cells in the blood during the course of BCMA-Fc and HuIvlg treatment revealed a steady reduction in the peripheral B-cell frequency during the BCMA-Fc treatment period, reaching a nadir at ~4 weeks (Fig. 2B).

Further analysis conducted at the end of the 9- to 15-week treatment period demonstrated that BCMA-Fc treatment reduced the frequencies of mature follicular and MZB subsets, as well as the immature T2MZ cells in the spleen and pancreatic lymph node (PLN) (Fig. 2D). Similarly, the absolute numbers of the follicular, marginal-zone, and T2MZ subsets were reduced by ~80–90% (Fig. 2E).

In contrast, the frequency and absolute numbers of T1 precursor cells in the spleen were less affected by BCMA-Fc treatment (Fig. 2D and E), a result consistent with the role of BAFF in promoting B-cell development after the T1 checkpoint (22).

**Effect of BCMA-Fc treatment on peripheral T-cell populations and Th1 cytokines.** In contrast to its effect on B-cell populations, administration of BCMA-Fc from 9 to 15 weeks of age did not impact the absolute number of peripheral T-cells or CD4+ and CD8+ T-cell subsets (Fig. 3A). However, the frequency of splenic CD4+ and CD8+ T-cells was proportionally increased, presumably as a result of the decreased number of B-cells (data not depicted). To determine how BCMA-Fc treatment affected the activation of effector T-cell clones, we analyzed the expression of CD44 and CD40 on peripheral CD4 and CD8 T-cell populations. CD44 is expressed by activated T-cells, whereas CD40 has been identified as a marker for highly diabetogenic T-cell clones (21). BCMA-Fc treatment did not alter the frequency of CD44highCD4+ or CD44highCD8+ T-cells (data not depicted); however, the frequency of CD4+ and CD8+CD40+ T-cells was reduced in both the spleen and PLN of BCMA-Fc–treated mice (Fig. 3B). The reduction in frequency of pathogenic CD40+ T-cells in BCMA-Fc–treated mice was associated with a decrease in...
the circulating levels of interleukin (IL)-7, IL-15, and IL-17 (Fig. 3C), cytokines critical for the expansion and activation of effector T-cells.

**Effect of BCMA-Fc treatment on B-cell repopulation.** To examine how BCMA-Fc treatment effected B-cell repopulation, further analysis was carried out in long-term...
16-week-old NOD mice treated with PBS ( ), HuIvIg ( ), or BCMA-Fc ( ) from 9 to 15 weeks of age. Values from individual mice are shown (n ≥ 8 per group). B: Representative fluorescence-activated cell sorting plots illustrating frequency of CD4⁺/CD40⁻ (upper panel) and CD8⁺/CD40⁺ (lower panel) T-cells from treated mice at 16 weeks of age. Black line, CD40; gray line, isotype control (n = 7 per group). C: Serum cytokine levels in HuIvIg- ( ) and BCMA-Fc- ( ) treated NOD mice at 16 weeks of age. Values from individual mice are shown (n ≥ 3 per group). Significant differences between sample means are indicated. Bar represents median value. *P < 0.05 and ***P < 0.001. SPLN, spleen.

Effect of BCMA-Fc treatment on the pancreatic infiltrate. We conducted histological analysis of the pancreata from the BCMA-Fc-protected NOD mice at 16 and ≥50 weeks of age and a comparison with treated control mice. Representative histology for each group is shown in Fig. 5A, and insulitis scores for these mice are shown in Fig. 5C. Although BCMA-Fc–treated NOD mice did exhibit insulitis at the 16-week time point, the severity was reduced compared with that in HuIvIg-treated and diabetic control NOD mice at 16 weeks. Indeed, the frequency of islets exhibiting heavy insulitis (grades 3 and 4) at 16 weeks was only ~20 vs. ~45–50% in control groups. Flow cytometric analysis of the pancreatic infiltrate revealed that the frequency of FoB was markedly reduced (P =
0.014; n ≥ 5) in the BCMA-Fc–treated mice at 16 weeks (Fig. 5D). No changes were observed in the ratios of CD4+ to CD8+ T-cells, though their frequencies were increased (Fig. 5D), again, most probably as a result of the decrease in FoB.

The proportion of severely infiltrated islets in the BCMA-Fc–treated mice did not increase over time, as evidenced by analysis of the long-term–protected mice (e.g., ≥50-week-old mice) (Fig. 5A and C). Thus, although BCMA-Fc–treated mice did exhibit evident insulinis, the severity of insulinis was maintained at a level equivalent to that exhibited by prehyperglycemic 8- to 15-week-old NOD mice.

Given that NOD mice exhibit a progressive insulinis from the preclinical to hyperglycemic phase, these data suggest that BCMA-Fc treatment has not reversed the autoimmune process as determined by the persistence of a mononuclear cell pancreatic infiltrate but, rather, halted the progression to fulminant diabetes. Thus, we questioned whether this related to a change in the nature of the insulitic lesion or active regulation. Analysis of the B- and T-cell subsets infiltrating the pancreas in the protected BCMA-Fc–treated mice showed that the frequencies of infiltrating B- and T-cell subsets were similar to those of treated control mice (Fig. 5D). Thus, in long-term–protected mice, B-cells are not prohibited from forming a part of the insulitic lesion. To test whether the BCMA-Fc–treated NOD mice still harbored T-cells with self-reactive potential, splenocytes from normoglycemic BCMA-Fc–treated mice still harbored T-cells with self-reactive potential, but these T-cells are unable to precipitate diabetes in their BCMA-Fc–treated hosts.

**Time-dependent accumulation of CD4+CD25+ T-cells in BCMA-Fc–treated NOD mice.** The long-term protection afforded by BCMA-Fc treatment despite the persistence of self-reactive T-cells prompted us to investigate possible regulatory mechanisms. Tregs that express the markers CD4+ and CD25+ can control the progression to overt diabetes in the NOD model (23). We analyzed the frequency and number of CD4+CD25+ T-cells in the spleen, PLN, and pancreas of BCMA-Fc–treated NOD mice at 16 weeks of age. As shown in Fig. 6A and B, compared with treated control NOD mice, 9- to 15-week BCMA-Fc–treated mice harbored an increased frequency of splenic CD4+CD25+ T-cells at 16 weeks of age. The majority (>90%) of these CD4+CD25+ T-cells were also Foxp3+ (Fig. 6C and D), demonstrating that they belonged to the set of Tregs. Analysis of the long-term (≥50 weeks of age) BCMA-Fc–protected mice revealed increased (twofold or more) frequencies of CD4+CD25+ T-cells in both the spleen and PLN (Fig. 6E). Further, in contrast with the 16-week time point, the increased frequency of Tregs was also reflected as an increase in the absolute numbers of CD4+CD25+ T-cells in both spleen and PLN (Fig. 6E). Thus, BCMA-Fc–treated mice showed an accumulation of Treg numbers over time. The increased number of Tregs was associated with a halting of the autoimmune attack and permanent euglycemia.
BCMA-Fc–mediated protection from diabetes requires the presence of CD4⁺CD25⁺ T-cells. We next determined whether CD4⁺CD25⁺ T-cells were required for BCMA-Fc–mediated protection from diabetes. To achieve this, mice were administered the anti-CD25 mAb PC61. Control mice received PBS or rat IgG1. In preliminary experiments, we could show that administration of a single dose of PC61 (200 μg i.p.) induced an
~90% reduction in the frequency of peripheral CD4+CD25+Foxp3+ T-cells for ~14 days (Fig. 7A). For the experiment, NOD mice were first treated with BCMA-Fc from 9 to 15 weeks of age; at the cessation of BCMA-Fc treatment, mice were administered the anti-CD25 mAb PC61 (200 µg i.p.) every 14 days (a total of four injections), and blood glucose levels were monitored. Kaplan-Meier survival analysis showed that BCMA-Fc–treated NOD mice remained diabetes free, whereas control PC61-treated NOD mice developed hyperglycemia with the expected frequency (Fig. 7B). In contrast, subsequent administration of PC61 after BCMA-Fc treatment precipitated diabetes in 100% of mice (P = 0.001 BCMA-Fc vs. BCMA-Fc plus PC61; n ≥ 5).

**Blocking the BAFF/APRIL pathway indirectly modulates CD4+CD25+Foxp3+ T-cells.** We next focused on determining the mechanism by which BCMA-Fc treatment would effect protection in a CD4+CD25+ T-cell–depen-
TACI and BR3 expression in CD4+ T-cells. To determine whether BAFF or APRIL could engage Tregs directly, we administered BCMA-Fc from 9 to 15 weeks of age (solid black line) or PC61 (solid gray line) (Fig. 8A). Also shown in Fig. 8A, the frequency of peripheral CD4+CD25+Foxp3+ T-cells in BAFF−/−, BR3−/−, BCMA−/−, and TACI−/− mice was comparable with that in wild-type mice. Further, to determine whether BAFF or APRIL could engage Tregs directly, we examined TACI and BR3 expression in CD4+CD25+ T-cells. CD4+CD25+ T-cells did not express significant levels of the receptors BR3 or TACI (Fig. 8B) in contrast to splenic B-cells. These data indicate that it is unlikely that targeting the BAFF/APRIL system resulted in increased CD4+CD25+Foxp3+ T-cells via a direct mechanism.

**CD4+CD25+ T-cells reign in destructive T-cells in B-cell-deficient NOD mice.** B-cell subpopulations were severely reduced by BCMA-Fc treatment. To explore the relationship between a reduction in B-cells and CD4+CD25+ T-cells, we used B-cell-deficient NOD,μMT−/− mice (18). NOD,μMT−/− mice exhibit a number of features, with regard to diabetes development, reminiscent of those in BCMA-Fc-treated mice. These include a reduced degree of insulitis (Fig. 5B and C) and resistance to diabetes development (Fig. 8C). To assess whether CD4+CD25+ T-cells were important in maintaining euglycemia in NOD,μMT−/− mice, ~16-week-old mice were treated with PC61 (200 μg.i.p. each) every 14 days for a total of four injections and blood glucose levels were followed. This treatment reduced the frequency of peripheral
CD4⁺CD25⁺Foxp3⁺ T-cells as observed for B-cell–sufficient NOD mice (Fig. 7A). Treatment with PC61 precipitated diabetes in ~80% (P = 0.0184; n = 10) of NOD.µMT⁻/⁻ mice (Fig. 8C)—a dramatic result when compared with data in control NOD.µMT⁻/⁻ mice. Therefore, targeting CD25⁺ regulatory populations triggered diabetes onset in B-cell–deficient NOD.µMT⁻/⁻ mice that are otherwise resistant.

**DISCUSSION**

Emerging evidence demonstrates that B-cells can impact multiple stages in the pathogenesis of autoimmune diabetes (24). BAFF and APRIL play critical roles in supporting B-cell survival (1), such that BCMA-Fc–treated mice harbored a much reduced B-cell pool, providing one potential mechanism by which BCMA-Fc prevented diabetes in NOD mice. BAFF-activated B-cells show an enhanced antigen-presenting cell (APC) capacity (25), and in vivo BAFF-activated B-cells facilitate heightened Th1 T-cell responses (26). In the NOD model, B-cells undergo a marked expansion from ~9 to 15 weeks of age (15), show an increased capacity to act as APCs during this time (15,27), and can present captured autoantigen to self-reactive T-cells (15,28,29). The APC function of B-cells is essential for the activation (30) and expansion (31) of the CD4⁺ T-cell repertoire from ~9 to 15 weeks of age, i.e., before the onset of hyperglycemia. Thus, by reducing the availability of B-cells to act as APCs, BCMA-Fc treatment curtailed B- and T-cell interactions during the critical 9- to 15-week time period, halting the progression from clinically silent insulitis to overt hyperglycemia. This concept is consistent with our observation that BCMA-Fc–treated mice exhibited a reduced proportion of B-cells in association with a diminished frequency of CD4⁺CD40⁺ diabetogenic T-cells and a reduced level of Th1 T-cell–derived cytokines at 16 weeks of age. Additional evidence to support this hypothesis comes from studies in which NOD mice expressing human CD20 have been treated with an anti-CD20 mAb (32); in this case, reducing B-cells from 9 weeks of age delays diabetes onset. Depletion of B-cells with anti-CD20 impairs activation of adaptive and autoreactive CD4⁺ T-cell responses (32,33), further demonstrating the required role for B-cells in the activation of self-reactive CD4⁺ T-cells (30,34).

Our studies uncover an additional and perhaps unexpected mechanism by which targeting the BAFF and APRIL system can prevent diabetes, namely through increasing CD4⁺CD25⁺ T-cells. Tregs expressing CD25⁺ and Foxp3 can control the development of autoimmune diabetes (35), and we found that CD4⁺CD25⁺ T-cells were required for the continued maintenance of a hyperglycemia-free state. Further, these CD4⁺CD25⁺ T-cells expressed the Treg lineage marker Foxp3, suggesting that they belonged to the set of natural Tregs (36). To address the question of how BCMA-Fc might affect CD4⁺CD25⁺ T-cell homeostasis, we examined Foxp3⁺ Treg frequencies in mice in which BAFF and APRIL signaling were disrupted. These data demonstrated that loss of BAFF or APRIL did not increase the frequency of Foxp3⁺ Tregs per se, suggesting that BCMA-Fc altered Treg homeostasis through an indirect mechanism. Of interest, an increased frequency of Tregs was demonstrated in two recent studies in which NOD mice were treated with the B-cell depleting agents: anti-CD20 or anti-CD22 mAb, respectively (32,37). Both studies also reported that NOD mice treated between 9 and 15 weeks exhibited a delayed and reduced incidence of diabetes. Together with our study results, these data indicate that reducing B-cells in autoimmune NOD mice is associated with an increased frequency of cells with a Treg phenotype. Significantly, our present data demonstrate that in the absence of B-cells, endogenous Tregs control the progression of autoimmunity in NOD mice. This conclusion is further supported by our analysis of NOD.µMT⁻/⁻ mice that lack B-cells and are resistant to diabetes (18). Treatment of NOD.µMT⁻/⁻ mice with the anti-CD25 mAb PC61 precipitated the onset of diabetes, demonstrating that the hyperglycemia-free state in NOD.µMT⁻/⁻ mice is also dependent on CD25⁺ regulatory cells.

These are, to our knowledge, the first results to show that the resistance of B-cell–deficient NOD mice to diabetes can be due to the activity of Tregs. However, they are consistent with emerging results from other models of autoimmune disease. Indeed, NOD.H-2h4 mice develop spontaneous autoimmune thyroiditis, whereas B-cell–deficient NOD.H-2h4 mice are protected from autoimmunity (38). Treatment of B-cell–deficient NOD.H-2h4 with PC61 induced the rapid onset of autoimmunity (39), similar to the results obtained in NOD.µMT⁻/⁻ mice, suggesting a common mechanism whereby the absence of B-cells allows a regulatory pathway to prevent autoimmunity. A number of possibilities exist to explain this intriguing relationship between B-cells and Tregs. As an example, Olson et al. (40) using a model of Crohn’s disease demonstrated that B-cells could block Treg activity in a glucocorticoid-induced tumor necrosis factor receptor (GITR)-dependent manner. Thus, activated NOD B-cells may directly suppress the activity of Tregs through mechanisms including expression of GITR ligand (40). Alternatively, because the number of B-cells increases as disease progresses (15), the opportunity for T-cells to engage activating APCs (e.g., B-cells) (30,31) versus a tolerizing APC (e.g., macrophages or dendritic cells) (41) may decrease. In this scenario, BCMA-Fc treatment could be protective via two potential mechanisms. First, BCMA-Fc, by reducing B-cells, may alter the balance in the production of effector cells versus Tregs, leading to an accumulation of CD4⁺CD25⁺ Tregs. Second, without B-cells to expand up self-reactive T-cell clones, endogenous regulatory cells may simply be able to reign in the present autoreactive T-cell pool, preventing the progression to hyperglycemia. These hypotheses should be explored in further studies.

In this study, we demonstrate that targeting the BAFF and APRIL pathway with BCMA-Fc before the onset of hyperglycemia prevented diabetes in spontaneously diabetic NOD mice. We hypothesize that the major action achieved by blocking BAFF and APRIL was a reduction in peripheral B-cells, thereby limiting their involvement in diabetes pathogenesis. This prevented diabetes progression through two mechanisms: a dampening of T-cell autoimmune activity and subsequent elaboration of cytokines, most likely achieved by decreasing the availability of B-cells to act as APCs; a second mechanism also related to decreased B-cells, which induced or allowed Tregs to reign in the autoreactive potential of diabetogenic T-cells. This second mechanism highlights a novel pathway by which targeting B-cells may provide resistance to autoimmunity.

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