# Targeting B-cells Mitigates Autoimmune Diabetes in NOD Mice: What Is Plan B?

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In this issue of *Diabetes*, Grey and colleagues (1) demonstrate that therapeutic B-cell depletion delays diabetes onset and reduces diabetes incidence in NOD mice. B-cell depletion in pre-diabetic NOD mice was accomplished using an extended regime of recombinant B-cell maturation antigen (BCMA)-Fc chimerized protein that targets B-lymphocyte stimulator (BLyS)/B-cell–activating factor of the tumor necrosis factor family (BAFF)—a cytokine critical for maintenance of the peripheral B-cell pool. This follows recent studies demonstrating significant effects of B-cell depletion on diabetes onset and severity (2–5); however, no other studies have reported profound and complete protection from hyperglycemia as observed here. Following B-cell depletion therapy during 9–15 weeks of age, NOD mice remained diabetes free for ≥50 weeks of age, even after B-cell reconstitution. An increase in $CD25^+Foxp3^+CD4^+$ regulatory T-cells (Tregs) following B-cell depletion may mediate prolonged tolerance given that the CD25 monoclonal antibody (mAb) treatment neutralized the long-term therapeutic benefits of B-cell depletion (Fig. 1). This mechanism may explain why B-cell–deficient NOD.μMT mice do not develop hyperglycemia given that autoimmune diabetes was also precipitated in these mice by Treg depletion.

Most diabetes-modifying immunotherapies target the T-cell compartment directly because β-cell destruction is primarily mediated by $CD4^+$ and $CD8^+$ T-cells. Examples include T-cell depletion, blockade of T-cell costimulation, and Treg induction. Therapies that reduce antigen-specific T-cell clonal expansion have profound effects during the pre-diabetic stages of disease, whereas few therapies reverse disease once the clinical manifestations of diabetes are evident. This limitation appears to hold for B-cell–directed therapies in NOD mice as well. Genetically, B-cell–deficient NOD mice generally lack islet infiltration or insulitis and are free of overt diabetes (6–9). Likewise, B-cell depletion by anti-μ antibody given from birth abrogates insulitis development in NOD mice (10). Recent B-cell depletion studies in NOD mice have included an anti-BLyS/BAFF mAb (3), anti-mouse CD20 mAb (2), anti-human CD20 mAb in human CD20 transgenic NOD mice (4), and anti-CD22 immunotoxin (5), which all target mature B-cells. While each study highlights particular findings or interpretations, the main observation is that the absence of B-cells alters an early trigger for diabetes onset, with the consistent conclusion that disease does not progress in the absence of B-cells. Whether the remarkable long-term tolerance indicated in the current study results from features unique to the BCMA-Fc chimerized protein is difficult to conclude because all of the reported B-cell depletion strategies have varied in approach, timing, and analysis among individual NOD mouse colonies. Nonetheless, now that B-cell depletion strategies with potential clinical efficacy have been found, standardized guidelines for side-by-side comparisons in NOD mice should be developed to identify important differences between therapeutic approaches and results.

B-cells are among the earliest cells to infiltrate the pancreatic islets of NOD mice, and autoantibodies against islet antigens indicate disease onset in humans and mice (11). Despite this, autoantibody production is not sufficient to initiate disease and is disconnected from the occurrence of diabetes and insulitis (11). Rather, B-cells are multifunctional and are crucial antigen-presenting cells (APCs) for priming proinflammatory T-cell responses to β-cell antigens (12–16). Thereby, obligatory B-cell APC function may set the stage for systemic autoreactivity in NOD mice because B-cell selection (17) and innate cell APC function are impaired (18) in NOD mice (Fig. 1). Consistent with this, diabetes resistance in congenitally B-cell–deficient NOD mice is lost following B-cell reconstitution (9,12). It is encouraging that the return of B-cells following prolonged depletion in the current study did not alter diabetes resistance given that the effect of mature B-cell depletion on the peripheral B-cell repertoire remains an open question. Presumably, unselected and potentially autoreactive pre–B-cells and immature B-cells that were not depleted during therapy repopulate the periphery. For this, pre–B-cell and B-cell depletion using CD19-directed therapies may have advantages (19). Because regulatory B-cells (B10-cells) also significantly affect autoimmunity (20), it will be important to determine whether they represent a significant component of the reconstituted B-cell pool. Nonetheless, B-cell depletion before disease onset may induce long-term tolerance through Tregs—a possibility that opens new avenues for investigation.

Transient B-cell depletion after the first signs of disease onset using anti-BLyS mAb also arrests diabetes progression and maintains NOD mice in a "honeymoon" state for extended periods (3). Because Treg numbers did not change 10 weeks posttreatment, the honeymoon was attributed to enhanced B-cell competition and selection as a result of limited BLyS/BAFF availability. However, that other B-cell depletion therapies similarly reduce diabetes incidence (2,4,5) argues that immediate β-cell protection results from changes in available APCs for autoreactive

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Received 3 April 2009 and accepted 8 April 2009.
DOI: 10.2337/db09-0497


**See accompanying article, p. 1568.**

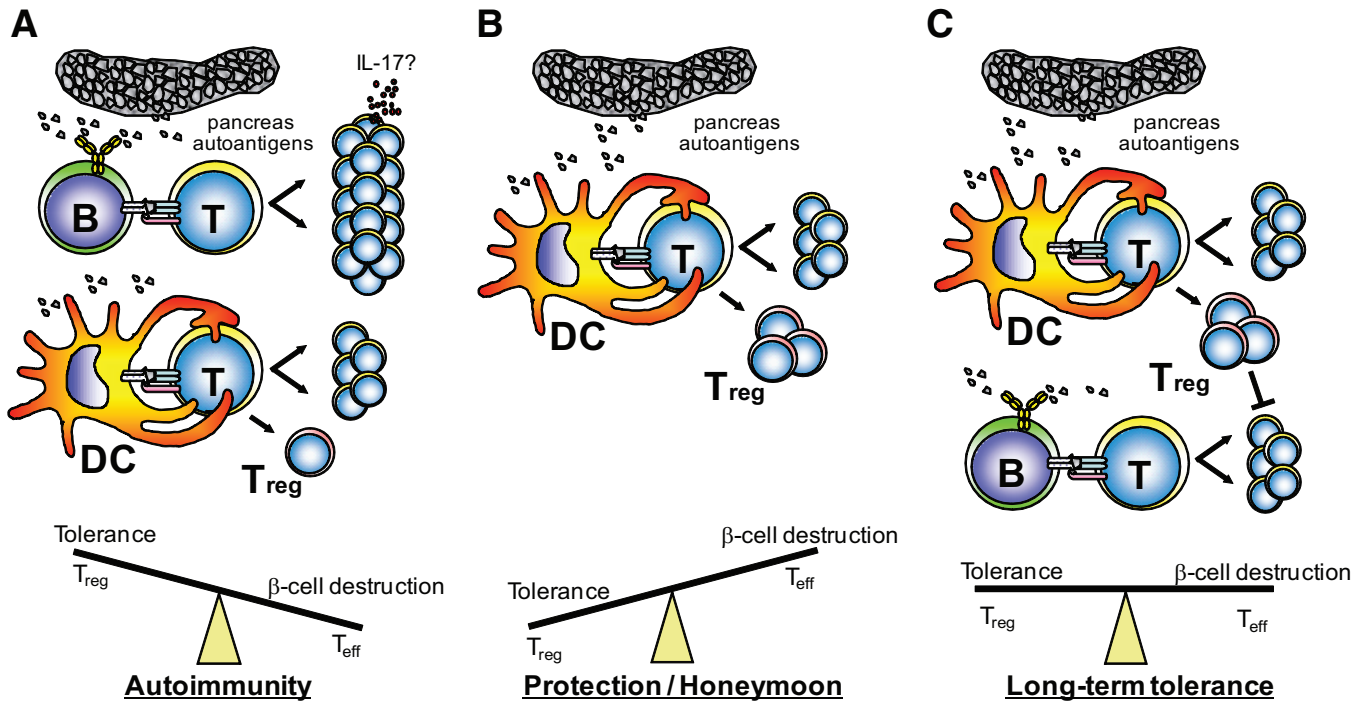


**FIG. 1. Model for autoantigen presentation in B-cell–depleted NOD mice. Autoantigen presentation is normally balanced between B-cells and dendritic cells (DCs) in mice (16). *A*: NOD autoantigen presentation. However, B-cell cognate presentation of pancreatic autoantigens may dominate in NOD mice because B-cell selection (17) and innate cell APC function are impaired (18). As a result, B-cell–driven pathogenic $CD4^+$ T-cell expansion and effector function leads to β-cell destruction and insulin deficiency. *B*: Presentation without B-cells. Mature B-cell depletion in NOD mice eliminates the initiating role of B-cells in disease pathogenesis but shifts antigen presentation to other APCs that may reduce $CD4^+$ T-cell activation in favor of Treg induction or expansion and lead to a tolerogenic state or honeymoon period without disease. *C*: Following B-cell reconstitution, previously induced Tregs may limit autoreactive T-cell activation and expansion, thereby enforcing long-term tolerance and protection from diabetes onset. Teff, effector T-cell.**

T-cell activation, where APC–T-cell interactions may also induce Treg expansion beyond that of pathogenic effector T-cells (Fig. 1). Future studies are needed to determine whether B-cell depletion uniformly induces Tregs and how. The effect of BCMA-Fc treatment during later stages of insulitis will also require further examination. If Tregs do accumulate as suggested by Grey and colleagues, B-cell depletion may be curative when administered before extensive loss of pancreatic β-cell function because immune regulatory mechanisms will not be beneficial once β-cell destruction is complete, except for subsequent islet transplants.

In summary, "Plan A" has shown that B-cell depletion is a powerful therapeutic tool for reducing diabetes severity and arresting islet destruction in NOD mice. This advance in understanding diabetes onset and progression provides the basis for "Plan B": identifying the optimal strategy for targeting B-cells for therapeutic benefit. Whether this involves the modulation of APC–T-cell interactions, the induction of regulatory B- and T-cell subsets, or both remains unanswered. Given the demonstrated importance of both T- and B-cells in diabetes initiation and pathogenesis, it is likely that modulating both arms of the adaptive immune response will be key for the development of protective immunotherapies.

## ACKNOWLEDGMENTS

This work was supported by National Institutes of Health grants AI56363, CA105001, and CA96547.

T.F.T. is a paid consultant and shareholder of Angelica Therapeutics and a consultant for MedImmune. No other potential conflicts of interest relevant to this article were reported.

We thank Dr. Damian Maseda and David Dilillo for their helpful comments.

## REFERENCES

1. Mariño E, Villanueva J, Walters S, Liuwantara D, Mackay F, Grey ST. $CD4^+CD25^+$ T-cells control autoimmunity in the absence of B-cells. Diabetes 2009;58:1568–1577
2. Xiu Y, Wong CP, Hamaguchi Y, Wang Y, Pop S, Tisch RM, Tedder TF. B lymphocytes depletion by CD20 monoclonal antibody prevents diabetes in NOD mice despite isotype-specific differences in FcγR effector functions. J Immunol 2008;180:2863–2875
3. Zekavat G, Rostami SY, Badkerhanian A, Parsons RF, Koeberlein B, Yu M, Ward CD, Migone TS, Yu L, Eisenbarth GS, Cancro MP, Naji A, Noorchashm H. In vivo BLyS/BAFF neutralization ameliorates islet-directed autoimmunity in nonobese diabetic mice. J Immunol 2008;181:8133–8144
4. Hu CY, Rodriguez-Pinto D, Du W, Ahuja A, Henegariu O, Wong FS, Shlomchik MJ, Wen L. Treatment with CD20-specific antibody prevents and reverses autoimmune diabetes in mice. J Clin Invest 2007;117:3857–3867
5. Fiorina P, Vergani A, Dada S, Jurewicz M, Wong M, Law K, Wu E, Tian Z, Abdi R, Guleria I, Rodig S, Dunussi-Joannopoulos K, Bluestone J, Sayegh MH. Targeting CD22 reprograms B-cells and reverses autoimmune diabetes. Diabetes 2008;57:3013–3024
6. Serreze DV, Chapman HD, Varnum DS, Hanson MS, Reifsnyder PC, Richard SD, Fleming SA, Leiter EH, Shultz LD. B lymphocytes are essential for the initiation of T cell-mediated autoimmune diabetes: analysis of a new "speed congenic" stock of NOD.$Ig\mu^{null}$ mice. J Exp Med 1996;184:2049–2053
7. Akashi T, Nagafuchi S, Anzai K, Kondo S, Kitamura D, Wakana S, Ono J, Kikuchi M, Niho Y, Watanabe T. Direct evidence for the contribution of B cells to the progression of insulitis and the development of diabetes in non-obese diabetic mice. Int Immunol 1997;9:1159–1164

8. Yang M, Charlton B, Gautam AM. Development of insulitis and diabetes in B cell-deficient NOD mice. J Autoimmun 1997;10:257–260
9. Chiu PP, Serreze DV, Danska JS. Development and function of diabetogenic T-cells in B-cell–deficient nonobese diabetic mice. Diabetes 2001;50:763–770
10. Noorchashm H, Noorchashm N, Kern J, Rostami SY, Barker CF, Naji A. B-cells are required for the initiation of insulitis and sialitis in nonobese diabetic mice. Diabetes 1997;46:941–946
11. Lehuen A, Bendelac A, Bach JF, Carnaud C. The nonobese diabetic mouse model. Independent expression of humoral and cell-mediated autoimmune features. J Immunol 1990;144:2147–2151
12. Serreze DV, Fleming SA, Chapman HD, Richard SD, Leiter EH, Tisch RM. B lymphocytes are critical antigen-presenting cells for the initiation of T cell-mediated autoimmune diabetes in nonobese diabetic mice. J Immunol 1998;161:3912–3918
13. Falcone M, Lee J, Patstone G, Yeung B, Sarvetnick N. B lymphocytes are crucial antigen-presenting cells in the pathogenic autoimmune response to GAD65 antigen in nonobese diabetic mice. J Immunol 1998;161:1163–1168
14. Noorchashm H, Lieu YK, Noorchashm N, Rostami SY, Greeley SA, Schlachterman A, Song HK, Noto LE, Jevnikar AM, Barker CF, Naji A. I-$A^{g7}$-mediated antigen presentation by B lymphocytes is critical in overcoming a checkpoint in T cell tolerance to islet β cells of nonobese diabetic mice. J Immunol 1999;163:743–750
15. Wong FS, Wen L, Tang M, Ramanathan M, Visintin I, Daugherty J, Hannum LG, Janeway CA Jr, Shlomchik MJ. Investigation of the role of B-cells in type 1 diabetes in the NOD mouse. Diabetes 2004;53:2581–2587
16. Bouaziz JD, Yanaba K, Venturi GM, Wang Y, Tisch RM, Poe JC, Tedder TF. Therapeutic B cell depletion impairs adaptive and autoreactive $CD4^+$ T cell activation in mice. Proc Natl Acad Sci U S A 2007;104:20882–20887
17. Quinn WJ 3rd, Noorchashm N, Crowley JE, Reed AJ, Noorchashm H, Naji A, Cancro MP. Cutting edge: impaired transitional B cell production and selection in the nonobese diabetic mouse. J Immunol 2006;176:7159–7164
18. Noorchashm H, Moore DJ, Noto LE, Noorchashm N, Reed AJ, Reed AL, Song HK, Mozaffari R, Jevnikar AM, Barker CF, Naji A. Impaired CD4 T cell activation due to reliance upon B cell-mediated costimulation in nonobese diabetic (NOD) mice. J Immunol 2000;165:4685–4696
19. Yazawa N, Hamaguchi Y, Poe JC, Tedder TF. Immunotherapy using unconjugated CD19 monoclonal antibodies in animal models for B lymphocyte malignancies and autoimmune disease. Proc Natl Acad Sci U S A 2005;102:15178–15183
20. Bouaziz J-D, Yanaba K, Tedder TF. Regulatory B cells as inhibitors of immune responses and inflammation. Immunol Rev 2008;224:201–214