Published in final edited form as:

Curr Opin Endocrinol Diabetes Obes. 2009 August ; 16(4): 293–298. doi:10.1097/MED.0b013e32832e06a7.

Change you can B(cell)eive in: recent progress confirms a critical role for B cells in type 1 diabetes

Shannon K. O'Neill^a, Edwin Liu^b, and John C. Cambier^a

^a University of Colorado Denver and National Jewish Health, 1400 Jackson Street, Denver, Colorado

^b Barbara Davis Center for Childhood Diabetes, University of Colorado Denver, Aurora, Colorado, USA

Abstract

Purpose of review—Here we review extant recent findings regarding the multiple roles of B cells in type 1 diabetes (T1D) and discuss how autoreactive B cells may become activated by a breach in B cell tolerance, and thereby initiate disease. Finally, we discuss the use of B cell-targeted therapies for treatment of autoimmunity.

Recent findings—Anti-CD20-specific depletion of B cells prevents and reverses diabetes in humanCD20/non-obese diabetic (NOD) mice. Correspondingly, in nontransgenic NOD mice B cells are effectively depleted with high dose antimouse CD20 mAbs of varying isotypes, and this also prevents diabetes in more than 60% of the mice when administered early, and significantly delays disease in 15-week-old animals. A separate study revealed that targeting B cells with anti-CD22/cal monoclonal antibody therapy delays diabetes onset in prediabetic NOD mice and restores normoglycemia in new-onset hyperglycemic NOD mice. In humans, a clinical trial of rituximab in new onset type 1 diabetics has yielded promising preliminary findings.

Summary—B cells are major players in T1D in humans, and clearly essential for disease development in the NOD mouse model of T1D. In this review, we discuss the silencing of autoreactive B cells and how failure of this process may contribute to autoimmunity. Further, we describe the most recent advances in studies of therapeutic effects of B cell depletion in T1D, and provide recent data indicating the diverse functions by which B cells may mediate disease.

Keywords

autoimmunity; B cell depletion therapy; B lymphocytes; non-obese diabetic mice; type 1 diabetes

Introduction

The failure to find a cure for type 1 diabetes has led to the exploration of efficacious immunotherapies already approved for other diseases. A recent TrialNet study exploring the use of rituximab in new onset type 1 diabetics has been completed with positive outcome, stimulating interest in the role of B cells in autoimmunity. Studies in NOD mice initially identified the importance of B cells in this disease. In this context B cells likely function by mechanisms that include antigen presentation to CD4⁺ T cells and cytokine production. Furthermore, immune tolerance of autoreactive B cells is disrupted in NOD mice, leading to the secondary activation of insulin-specific T cells and onset of an autoimmune response. This

Correspondence to John C. Cambier, PhD, Ida and Cecil Green Professor and Chairman, Department of Immunology, University of Colorado Denver School of Medicine and National Jewish Health, 1400 Jackson Street, K803 Denver, CO 80206, USA, Tel: +1 303 398 1325; fax: +1 303 270 2325; cambierj@njhealth.org.

review will examine how autoreactive B cells may escape tolerance to initiate T1D, and describe genetic and cellular evidence that B cells are participants in disease development. Further, B cell depletion therapy in patients will be discussed in terms of the current clinical trial involving rituximab in new onset type 1 diabetics. Finally, we will discuss prospects for development of alternate and potentially complementary B cell targeting therapies.

How are self-reactive B cells prevented from entering into immune responses?

The construction of B cell antigen receptors through random use of heavy chain V-D-J and light chain V-J recombination leads to generation of a diverse repertoire of antigen specificities. Thus, the potential for B cells in diverse repertoires to react with self is vast, as is underscored by the fact that 55–75% of early immature B cells in human bone marrow are autoreactive [1]. There are two major developmental checkpoints at which autoreactive B cells are silenced: the immature B cell stage in the bone marrow, and the transition between new emigrant and mature B cells in the periphery [1]. The ‘system’ must also silence B cells that acquire autoreactivity by somatic mutation of variable regions in germinal centers.

Three primary mechanisms mediate silencing of autoreactive B cells. The first, known as receptor editing involves secondary Ig gene rearrangements mainly at the Igk locus (V-to-Jk) that generate new, hopefully innocuous, antigen receptor specificities. It has been demonstrated that receptor editing occurs very efficiently among autoreactive B cells within polyclonal B cell populations [2]. Receptor editing is initiated when immature or transitional B cells bind autoantigens with high avidity [3,4] and it is estimated that approximately 1/3 of newly generated autoreactive cells are successfully silenced by this mechanism [5]. A separate mechanism of silencing self-reactive B cells, termed clonal deletion, is also a consequence of high avidity antigen binding, but occurs only in B cells that have failed to eliminate autoreactivity by receptor editing.

B cell binding of low avidity autoantigens leads to silencing by a different mechanism(s). Studies in several murine models have demonstrated under such circumstances autoreactive B cells successfully reach the periphery yet are unresponsive to antigen, and are therefore referred to as ‘anergic’. The classical model of anergy utilized mice (MD4) that coexpress immunoglobulin heavy chain (μ and δ) and light chain transgenes that encode a BCR with a high affinity for hen egg lysozyme (HEL) [6]. When these mice are crossed to soluble HEL transgenic animals (ML5), the anti-HEL B cells are rendered anergic. These B cells express normal mIgD levels with reduced mIgM, exhibit a reduced lifespan and, strikingly, fail to mount an adaptive immune response following immunization [7]. Although all models of B cell anergy described to date display varying degrees of this anergic phenotype, anergy is also associated with aberrant B cell signaling subsequent to mIgM stimulation: such responses include reduced Ig α /Ig β and Syk phosphorylation, reduced Ca²⁺ mobilization, decreased proliferation and failure to express activation markers [7].

The recently described anergic B cell population (An1) found within natural polyclonal murine B cell repertoires is enriched in autoreactive cells and displays many features of anergy, including attenuated BCR mediated signaling and a shortened half-life [8]. Interestingly, based on the decreased lifespan of An1 cells, (approximately 5 days) compared with most peripheral B cells (about 40 days), and the frequency of An1 cells, it was determined that nearly 50% of newly formed B cells in wild-type mice are destined to become anergic [7]. These findings are consistent with the frequency of autoreactive cells among newly produced B cells, and establish that anergy is the dominant mechanism by which autoreactive B cells are silenced.

B cell anergy is initiated and maintained by continuous autoantigen encounter and stimulation of the BCR in the absence of T cell help or innate immune activation. The preservation of anergy is dependent upon signals transduced by continuously self-antigen-occupied BCRs [9]. B cell anergy can be disrupted by competitive dissociation of self-antigen from the BCR, and this is followed by rapid acquisition of a follicular phenotype and increased lifespan [9]. Crosslinking of antigen receptors on these cells leads to naïve cell-like signaling and upregulation of molecules that function in productive activation of CD4⁺ T cells. Anergic cells, therefore, represent a significant hazard for participation, even initiation, of autoimmunity. Their unresponsiveness is reversible, and they persist in the periphery where they are potentially exposed to spurious innate and adaptive immune signals that may compromise their anergic state.

Genetic evidence for the importance of B cells in T1D

At least twenty-five *Idd* genetic loci contribute to the onset of autoimmune diabetes in the NOD mouse [10,11]. Importantly, the primary NOD susceptibility locus (*Idd1*) maps to the major histocompatibility complex (MHC), as in human disease. B cell specific deletion of the MHC class II I-A^{g7} prevented spontaneous autoimmune diabetes in NOD mice, highlighting the essential function of MHC class II expression on B cells for the development of T1D [12]. In addition, there exist MHC-independent genetic loci in the mouse that promote the activation of autoreactive B cells. Genes on proximal chromosome 1 (*Idd5*) and distal chromosome 4 (*Idd9/11*) were both found to directly control diabetes development by regulating B cells in NOD mice, specifically by preventing the induction of B cell anergy following low-avidity BCR engagement by self-antigen [13]. Further, another locus that mapped to a region on chromosome 1 in the vicinity of the *Idd5.4* locus, controls TACI (transmembrane activator and calcium-modulating and cyclophilin ligand interactor) expression on B cells. Finally, a locus on chromosome 8 near *Idd22* temporally regulates the expression of TACI on NOD B cells [14]. Taken together, the NOD model has allowed identification of B cell intrinsic contributions to the development of T1D, and will likely continue to provide an invaluable tool for elucidation of more complex genetic contributions to development of diabetes.

In human studies of T1D, HLA class II genes on chromosome 6p21 within the MHC locus have the most compelling association and largest effect on disease. A recent genetic analysis indicates that specific alleles at the HLA-DRB1, DQA1 and DQB1 loci confer the highest risk or protection from T1D, based on the precise combinations of alleles that are expressed [15^{*}]. Recently, two human genome wide association studies identified 8 independent loci associated with type 1 diabetes, and follow-up studies provided confirmation of five novel genetic associations involving *ERBB3*, *C12orf30*, *KIAA3050*, *PTPN2* and *CD226* [16,17]. Although B cell-specific genetic defects aside from HLA class II have not been identified in T1D patients, it is likely based on genetic studies in NOD mice that alterations in genes that contribute to B cell anergy also participate in T1D development.

Overview of B cell functions in the non-obese diabetic model of T1D

B cells could promote autoimmunity by several mechanisms including: production of autoantibodies with consequent generation of immune complexes (IC), antigen presentation to generate primary autoreactive T cell responses, contribution to the maintenance of CD4⁺ T cell memory, or production of pro-inflammatory cytokines. Although autoantibodies alone may directly mediate certain autoimmune pathologies, some autoimmunities are B cell-dependent, yet independent of antibody production. In these cases B cells may contribute to disease via activation of autoreactive T cell responses or the maintenance of T cell memory [12,18–22,23^{**},24]. In addition, B cells produce a vast array of cytokines that can regulate the development, expansion or differentiation of Th1 and Th2 cells, as well as antibody production

[25*,26]. Thus, B cells probably contribute to autoimmune disease through diverse mechanisms.

B cells contribute to T1D development by mechanisms distinct from antibody production

The importance of B cells in the spontaneous development of autoimmune diabetes in NOD mice was clearly established using NOD.Igμ^{null} mice that lack B cells [27,28]. These mice are resistant to disease induction. In a separate study, in-vivo depletion of B cells by anti-IgM antibody treatment prevented the development of insulinitis and sialitis in NOD mice [29]. Furthermore, passive treatment of NOD Igμ^{null} mice with immunoglobulin from overtly diabetic NOD donors did not induce disease or insulinitis [24]. To confirm the apparent antibody independence of B cell function in T1D, NOD transgenic mice were produced in which B cells could express membrane but not secreted IgM [30]. As a consequence, they have the ability to internalize and present antigen, and mediate functions such as cytokine production. Female mice with this defect had a significantly increased incidence of diabetes compared with nontransgenic littermates that lacked B cells altogether, indicating that secreted antibodies are not required to induce disease. Parenthetically, T1D did not develop in experiments in which transmission of maternal autoantibodies to NOD pups was prevented, suggesting that autoantibodies may play some indirect licensing role in disease [31].

Antigen-specific B cells are crucial for T1D development

In T1D patients autoantibodies are consistently detected that react with a restricted, yet diverse set of pancreatic beta cell proteins, including insulin, glutamic acid decarboxylase (GAD), protein tyrosine phosphatase IA2 and the newly discovered target, ZnT8 [32]. Although autoantibodies may not be directly pathogenic, their existence is indicative of an ongoing antigen-specific autoimmune response. Hulbert *et al.* [33] addressed the question of whether specificity was important for B cell participation in T1D by generating NOD mice transgenic for a heavy chain (VH125) that produces a BCR repertoire with increased capacity to bind insulin. VH125 tg NOD mice developed diabetes at an accelerated rate compared with nontransgenic littermates, whereas control VH281 tg NODs, which have a B cell repertoire with a decreased potential to bind insulin, were significantly less susceptible to disease. In a separate study, NOD mice that express an irrelevant BCR (anti-HEL) by transgenesis were resistant to diabetes, suggesting that B cell specificity for autoantigen is required for disease development. Parenthetically, NOD.IgHEL mice that express some endogenous Ig genes in addition to anti-HEL BCRs did develop diabetes, albeit with delayed kinetics [34]. It is, therefore, predicted that the unique ability of B cells to capture and internalize antigens through cell surface Ig allows them to more efficiently activate diabetogenic T cells, compared with other APC populations in NOD mice [34]. These studies substantiate the importance of B cell autoantigen-specificity in the development of T1D in NOD mice.

On the basis of the requirement for B cell-specificity in T1D, it is easy to understand how T cell-specific responses may be altered in disease, whether B cells contribute to disease by initial activation of autoreactive T cells or by ensuring the quality of the CD4⁺ T cell memory response. Several studies indicate that B cells are the dominant APCs in T1D [24,30,34]. It would be interesting to explore whether autoreactive B cells in NOD mice dictate the persistence of memory T cells that are essential for the demise of islet beta cells.

Although it is clear that antibody-independent B cell functions mediate T1D in animal models, the situation is less clear in humans. Deciphering how B cells perpetuate disease will be important to inform the design of future therapies.

Defective B cell anergy may promote autoimmunity in non-obese diabetic mice

Participation of autoreactive B cells in T1D implies a loss of B cell tolerance, or alternatively, activation of autoreactive cells that are ignorant of their antigen due to low concentration or monovalency. One study showed that on the NOD background anti-HEL B cells undergo efficient clonal deletion upon encounter of a membrane bound self-antigen (membrane HEL). Similarly, receptor editing appears normal in NOD [35]. However, studies using anti-HEL $\times$ soluble HEL tg (MD4 $\times$ ML5) mice indicate that B cell anergy may be defeated by NOD genes [35]. Thus the B cell intrinsic mechanisms that normally function to induce and maintain B cell anergy to soluble self-antigen are likely defective in NOD mice. Accordingly, loss of B cell tolerance to insulin, GAD or other islet antigens may be an initiating event in diabetes, leading to activation of autoreactive CD4 T cells.

B cell targeted therapies prevent and reverse T1D in non-obese diabetic mice

Antigen-specific B cells clearly promote autoimmune diabetes in NOD mice, and recognition of this role has led to testing of B cell depletion therapies in T1D. Recently published studies of the effects of B cell depletion on T1D in NOD mice have shown significant promise in disease prevention and reversal, and have provided insight regarding how B cell depletion therapy may act mechanistically [36*,37*,38].

Anti-CD20 therapy ameliorates autoimmune diabetes in non-obese diabetic mice

The recent generation of transgenic NOD mice in which B cells express human CD20 (hCD20/NOD mice) has allowed analysis of the therapeutic effects of B cell-depletion using the murine anti-CD20 monoclonal antibody (2H7), which targets the same epitope as rituximab [38,39]. Anti-CD20 cell treatment of mice in the pre-clinical stages of diabetes development significantly delayed disease progression. Further, disease involving clinical hyperglycemia was effectively reversed in 36% of diabetic mice, suggesting a B cell role after initial T cell priming [38]. Interestingly a novel population of regulatory B cells and T cells emerged in antiCD20 treated mice that suppressed the rate at which diabetogenic splenocytes could transfer disease. In a separate study, antimouse CD20 mAb successfully depleted B cells and prevented diabetes in nontransgenic NOD female mice [36*]. Although NOD mice were more resistant than C57BL/6 mice to the depleting effects of varying isotypes of anti-CD20 mAbs due to a lack of Fc γ RI function and reduced monocyte numbers, high-dose anti-CD20 therapy prevented diabetes onset in more than 60% of 5 week old, but not 15 week old treated NOD mice [36*]. These studies highlight the ability of anti-CD20 therapy to prevent or significantly postpone T1D in mouse models, but indicate that, as in other autoimmune applications, combination therapies will probably be required to ablate disease.

Depletion of B cells by anti-CD22/cal prevents and reverses disease

Anti-CD22/calchaemicin-conjugated monoclonal antibody (CD22/cal) depletion in NOD mice showed significantly greater B cell depletion than an unconjugated anti-CD22 antibody, with exceptional results in disease prevention and reversal [37*]. Anti-CD22/cal mAb significantly delayed diabetes onset compared with untreated controls, and 50% of the mice were protected long-term from disease. Further, rapid reversal of hyperglycemia was achieved by anti-CD22/cal treatment of newly diabetic (glucose levels more than 250 mg/dl for 3 consecutive days) female mice. Although 100% of the treated mice immediately normalized their blood glucose, only 60% remained normoglycemic in the long term (>100 days), indicating variable efficacy in long-term remission of disease [37*].

In-vivo neutralization of B lymphocyte stimulator/B cell-activating factor suppresses non-obese diabetic autoimmune diabetes

B lymphocyte stimulator (BlyS) [B cell-activating factor (BAFF)] is a critical survival-promoting factor for which peripheral B cells compete [40,41]. BAFF overexpressing mice develop autoimmunity, highlighting the importance of a limited B cell lifespan in prevention of autoimmune disease [42]. A recent study utilized anti-BLyS mAb therapy in NOD mice to examine whether BLyS neutralization would inhibit diabetes onset [43*]. NOD mice treated with long-term anti-BLyS therapy had selective depletion of marginal zone and follicular B cells, which correlated with a significant delay in autoimmune diabetes onset, and reduced IAA titers. On the basis of these findings, anti-BLyS might be effective in conjunction with existing therapies to modulate T1D.

The efficacy of rituximab in T1D patients and the future of novel B cell depletion therapies in disease

There is currently only a single B cell targeted drug (rituximab) that is approved for use in humans. Rituximab is a chimeric murine/human monoclonal IgG₁ kappa antibody that selectively targets and depletes human CD20-expressing B cells. It is currently indicated for non-Hodgkins lymphoma and for rheumatoid arthritis. Side effects of treatment include infusion reactions, increased incidence of infection, cardiac events, serum sickness or lupus-like syndromes, and prolonged cytopenias. Its use has been reported for other immune-mediated conditions such as systemic sclerosis, Sjogren's syndrome and several hematologic disorders. A phase II double-blinded trial in relapsing-remitting multiple sclerosis demonstrated that a single course of rituximab reduced inflammatory brain lesions and clinical relapses for 48 weeks, providing further evidence of B cells in a broad spectrum of autoimmunities [44**].

A clinical trial by TrialNet involving 15 centers and entitled 'The effects of Rituximab on the progression of type 1 diabetes in new onset subjects' is now fully recruited. This study is investigating anti-CD20 treatment in diabetics within 3 months of diagnosis, between the ages of 8 and 45 years. All individuals were required to have evidence of residual β cell function by preserved C-peptide. Preliminary analysis suggests that this type of B cell treatment may have an effect on preserving C-peptide. If this trial demonstrates that rituximab is efficacious in T1D, it may be worthwhile to explore more antigen-specific B cell targeted therapies in future studies.

An alternate strategy for B cell depletion in humans is targeting CD19, a pan-B cell surface receptor expressed from early stages of pre-B cell development until its downregulation following terminal differentiation into plasma cells. XmAb5574, a humanized Fc-engineered anti-CD19 antibody is efficacious in primate models of lymphoma and leukemia, and demonstrates cytolytic activity [45*], and therefore could be a consideration for future B cell targeting in autoimmune disease.

Lastly, in a murine lupus model, targeting CD79 α (Ig α) and CD79 β (Ig β) has been shown to effectively deplete B cells [46**]. Ig α and Ig β comprise the heterodimeric signaling component of the B cell receptor. In addition to the potential for depletion, anti-CD79 therapy may have other immunomodulatory effects due to its role in cell signaling, and may be an effective target for the treatment of autoimmunity. In addition, drugs coupled to antihuman CD79 are being explored in lymphoma, and might provide the basis for this therapy in autoimmune diseases in the future [47].

Conclusion

Although type 1 diabetes has traditionally been considered a T cell mediated autoimmune disorder, evidence in murine studies and humans clearly indicate an important role for B cells in this disease. Although total B cell ablation is currently being studied as a way to mitigate T1D, antigen-specific B cell therapies may be possible to design and implement in future trials, based on individual autoimmune profiles of patients. The development and evaluation of additional B cell targeted therapies, in addition to forthcoming studies on the mechanisms by which B cells contribute to type 1 diabetes, will provide the basis for design of next-generation B cell targeted therapies.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

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Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

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