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To B or not to B — pathogenic and regulatory B cells in autoimmune diabetes

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B cells have a vitally important function to produce antibodies which are directly pathogenic in some autoimmune diseases. However, it is clear that a number of other B cell functions are also critical in the pathogenesis of organ-specific autoimmune diseases that were previously thought to be mainly T cell mediated. Therapeutic agents that target B cells and their functions may therefore be of considerable importance in these autoimmune diseases. In this review, we will focus on B cell characteristics and functions that contribute to type 1 diabetes (T1D) and discuss why anti-B cell treatment may be effective in T1D, a disease that was previously considered to be primarily T cell mediated.

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Introduction

Many organ-specific autoimmune diseases have, in the past, been considered to be primarily T cell mediated. In type 1 diabetes, although T cells are major pathogenic cellular players, B cells have an integrally linked role in the development of pathogenic T cells, beyond that of producing autoantibodies as markers of disease. This has come to the forefront since anti-B cell therapy has shown benefit in restoring normoglycemia after diabetes onset in animal models [1[•],2[•]] and had beneficial outcomes, shown at one year after treatment when trialed in type 1 diabetes in humans [3[•]]. These studies will be further discussed in this review.

What role do B cells play in diabetes?

Autoantibodies are very good predictive markers for the development of type 1 diabetes in humans [4,5]. Studies in the non-obese diabetic (NOD) mouse model

of autoimmune diabetes showed that autoantibodies are not themselves pathogenic and disease cannot be transferred passively by autoantibodies alone [6]. However, in studies where B cell sufficient offspring born to B cell deficient mothers [7], as well as embryo transfer experiments of NOD embryos to non-diabetes prone foster mothers, investigators showed early insulinitis in the islets but a reduction in diabetes [7,8]. While it was suggested that maternally transmitted antibodies from NOD mice could influence the development of disease, and transmitted insulin autoantibodies were clearly measurable in the offspring [7], other studies have not supported this view as no insulin autoantibodies were measurable in embryo-transferred mice at the time of the early insulinitis [8]. In neither of these studies were antibodies to islet autoantigens other than insulin measured. Another study, using cross-fostering experiments to examine the effect of autoantibodies transferred in maternal milk, showed that autoantibodies in milk were not required for the development of diabetes [9]. Thus, although evidence indicates that autoantibodies have a role in the development of disease in the NOD mouse, the precise part that they play is yet to be elucidated. In humans, much earlier studies showed that when diabetes occurred in both parents and children, there were a significantly greater number of diabetic fathers, arguing against maternally transmitted antibodies playing a major role in pathogenesis [10,11]. More recently, in the German BabyDiab study, offspring of mothers who had anti-islet autoantibodies were followed for the development of autoantibodies and diabetes for up to 12.5 years after birth. There was, in fact, a decreased risk of developing diabetes in offspring exposed to IA-2 and GAD autoantibodies [12]. In this study, the majority of the offspring also had antibodies to exogenously administered insulin at birth, and this also did not affect the risk of developing diabetes-associated antibodies or diabetes [12]. Thus, at this time, the available evidence indicates that it is unlikely that maternally transmitted antibodies play a role in the development of diabetes in humans and it is still unknown if autoantibodies developing after birth play any pathogenic role in the development of human diabetes.

The ability of B cells to produce antibodies is not required for diabetes to occur as shown by studies using B cells that have antigen presenting function dissociated from the ability to produce antibody [13]. However, the specificity of immunoglobulins that the B cells express is important, as mice which only express a limited repertoire of immunoglobulin have a reduced incidence

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of diabetes [14]. Furthermore, when most B cells in NOD mice express an anti-insulin BCR, diabetes is accelerated [15]. B cell signaling through the B cell receptor is modulated by Bruton's tyrosine kinase (*btk*). NOD mice genetically deficient in *btk* have considerably reduced populations of B1 cells [16], a subset of cells that may be particularly important for diabetes development [17–19], although this population has not been clearly defined in humans. In keeping with the block in maturation of B cells, mature B cells were also reduced in *btk* deficient mice although T cell numbers were maintained. The mice have reduced insulin-specific B cells and a much reduced incidence of diabetes. Interestingly, restoration of insulin-specific B cells in these *btk*-deficient mice restored diabetes [16].

Overall, the data in mice indicate that B cells, particularly autoreactive B cells that recognize insulin, may be crucial cells in propagating the autoimmune responses leading to diabetes, as further discussed in the next section.

What are the characteristics of B cells in autoimmune diabetes?

B cell tolerance in T1D

B cells are normally tolerized throughout life via a number of mechanisms which include B cell receptor editing, clonal anergy, clonal deletion, and ignorance. Much evidence suggests that anergy, defined as a state where B cells that have reduced lifespan, changed patterns of migration and location, together with an inability to interact with CD4 helper T cells, is a major mechanism by which autoreactive B cells are silenced [20•]. However, cells may 'escape' and demonstrate some but not all features of anergy. Acevedo-Suarez *et al.* showed that anti-insulin reactive B cells are anergic in terms of reduced proliferation to both T cell-dependent (anti-CD40) and T cell-independent (anti-IgM or lipopolysaccharide (LPS)) signals [21]. However, these insulin-reactive B cells still developed to become mature follicular and particularly marginal zone B cells and could increase the expression of CD86 on stimulation with anti-CD40, IgM or LPS in spite of reduced proliferation. It was therefore suggested that although the cells displayed some aspects of anergy, other B cell functions, such as increase of costimulation were intact. Costimulation is a major part of effective antigen presentation, and contributes to T cell-mediated autoimmune disease [21].

The other major mechanism of B cell tolerance that may be defective in autoimmune diabetes is receptor editing, a process whereby B cells are able to continuously rearrange genes encoding the antibody receptor, thus removing autoreactive B cells. Using an assay developed for examining recombining sequence (RS) rearrangement, as an estimate of light chain receptor editing, it was shown that B cells in NOD mice have an inappro-

priately low level of RS rearrangement [22•]. Patients with systemic lupus erythematosus (SLE) and type 1 diabetes also have decreased RS rearrangements in λ + B cells compared with control subjects. It was suggested that patients with autoimmunity may have different 'set points' for RS rearrangement [22•]. More studies are required to demonstrate if these cells with lower RS rearrangements could be used as a marker for those at risk of developing autoimmune disease and whether cells with a low level of RS rearrangement are indeed the most autoreactive B cells.

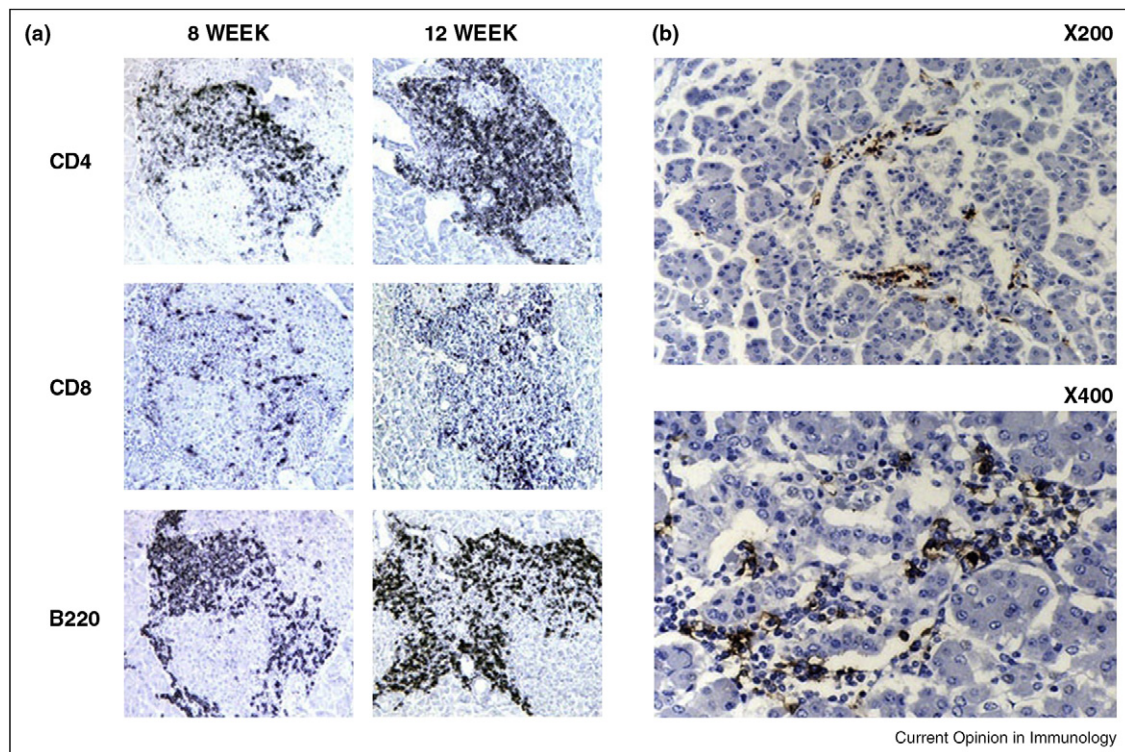
B cells are highly effective antigen-presenting cells to CD4 T cells

B cells express antigen-specific receptors and this endows them with the ability to present peptides from these specific antigens highly efficiently, thus enhancing antigen presentation many fold [23]. In line with this, B cells may be particularly important antigen-presenting cells when amounts of antigen are low [24]. Diabetes in NOD mice that were B cell deficient, either by genetic targeting or depletion early in life using anti-IgM, was largely prevented, indicating the importance of B cells in autoimmune diabetes development as reviewed in [25,26]. The early studies suggested that a major role for B cells in diabetes was in antigen presentation [6,27,28].

Recent studies have provided further information as to which subset of B cells present islet autoantigens in T1D. Marginal zone B cells (CD21^{hi}CD23^{lo} IgM^{hi}, CD1d, CD9^{hi}) are found in the spleen and may have an important role in priming naïve CD4 T cells [29]. NOD mice have increased marginal zone (MZ) B cells [30,31], with increased numbers detectable at five weeks of age compared to B6 mice [31]. In addition to the expected location in the spleen, MZ B cells in NOD mice could be found in both the pancreatic lymph nodes (PLNs) and the pancreas [32]. These MZ B cells were able to process and present insulin, and it was suggested that they may be key autoantigen-presenting cells in the PLN, supporting an earlier study indicating that antigen presentation by B cells in the PLNs is particularly important [33].

However, B cells are undoubtedly also present in abundance in the insulitis lesions of the islets of NOD mice, usually in close association with CD4 T cells (Figure 1). In recent islet histology studies in humans, B cells were found in insulitis within the islets examined in 2/62 organ donors who were antibody positive, and therefore may have had pre-diabetes [34]. Postmortem specimens from newly diagnosed patients with type 1 diabetes have also shown infiltration with B cells within insulitis, in addition to CD8 T cells, CD68+ macrophages and CD4 T cells [35••]. The B cells were the second most abundant cell type after CD8 T cells in the insulitis and were mostly present only if CD8 T cells were also seen [35••].

Figure 1



Insulinitis in NOD mouse and human islets. **(a)** NOD mouse islets from 8-week-old and 12-week-old pre-diabetic mice have been stained (black) with anti-CD4, anti-CD8 and anti-B220 (B cells) monoclonal antibodies. **(b)** Sections from human postmortem pancreas stained (brown) with anti-CD20 (B cells) at two different magnifications (images for (b) kindly provided by A Willcox, A Foulis and N Morgan).

A study focusing on the infiltrating B cells within the islets of NOD mice has shown that the B cells are activated and can also stimulate T cell activation [36]. The infiltrated B cells within the pancreatic islets are organized into tertiary lymphoid structures (TLS) [37]. However, this architecture does not appear to be strictly necessary for diabetes onset, as the diversity of B cell receptors and diabetes development in NOD mice were not affected in mice deficient in CXCL13 that disrupts TLS organization [38]. The TLS have germinal centers and B cells here have different light chain usage. This is a unique feature of B cells to hone the immune response and increase avidity for their antigen, giving rise to a different repertoire of B cells within the islets compared with pancreatic draining lymph nodes. These findings provide evidence for T–B cell interactions within the pancreatic islets beyond earlier interactions in the lymph nodes. Interestingly, many of the receptors identified were insulin-specific [37]. Other studies have indicated that insulin is not the only antigen recognized by these islet infiltrating B cells which have reactivity against peripherin [36,39], a protein found in peripheral nerves that has also been identified as a T cell autoantigen in diabetes. Together, these studies indicate that there is

ongoing interaction between B cells and T cells within the islets that may be necessary to maintain CD4 T cell activation and pathogenicity toward islet components leading to disease.

The other aspect of antigen presentation that is particularly important for B cells is their role in diversification of the epitopes presented and the concept of antigen spreading in autoimmune disease [40]. A hierarchy of antigen epitopes has been shown with both intramolecular and intermolecular antigen spreading, with reactivity to different epitopes appearing over time. This has been best demonstrated in diabetes for the autoantigen glutamic acid decarboxylase (GAD). In diabetes, diversification of antigen epitopes may be uniquely related to the ability of B cells to express costimulatory molecules on binding to antigen and hence activate a variety of T cells [41]. In two-way communication, these T cells may further activate antigen-specific B cells to generate a number of epitopes that dendritic cells or macrophages may not display when processing these antigens non-specifically [40]. As shown for MHC class II restricted peptides of tetanus toxoid, the binding of an antibody to an antigen may hinder the processing and presentation of

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the epitope bound by the antibody and increase the processing and presentation of other epitopes [42]. Indeed this has been shown for GAD where B cell hybridomas, generated from a DRB1*0401-positive patient, presented GAD epitopes to mouse DRB1*0401 restricted T cell hybridomas. When the T cell epitope recognized by the hybridomas was outside the GAD antibody binding site the responses were increased but they were suppressed when the epitope recognized was within the binding site [43]. In another important autoantigen in T1D, insulinoma associated antigen-2 (I-A2), amino acids 831–836 are part of an antibody epitope that is adjacent to amino acids 841–860, a region of the protein that contains T cell epitopes. T cells which recognize a peptide in the 841–860 region produce IL-10 in patients with type 1 diabetes who are I-A2 antibody positive [44].

Most functional studies in B cells have been carried out in association with CD4 T cells, either investigating antibody production or testing antigen presentation including the outcome of epitope spreading. It is noteworthy that B cells also have an intimate relationship with CD8 T cells and this will be discussed below.

B cell effects on CD8 T cells

Although less well recognized, B cells may have important effects on maintaining autoimmune CD8 T cell responses. In studies where B cells are deficient in NOD mice, not only has CD4 T cell infiltration been reduced, but CD8 T cells are also reduced within the insulinitic lesions [13]. Whether this is a consequence of the effects of B cells on CD4 T cells which then affect CD8 T cells or a direct effect on CD8 T cells remains to be established. TNF- α has different effects on the development of insulinitis and diabetes, dependent on the stage of disease. However, studies using RIP-TNF- α transgenic NOD mice showed that these mice develop accelerated spontaneous diabetes with more synchronized timing. CD8 T cells are the predominant pathogenic T cells in this model system [45[•]]. When RIP-TNF- α transgenic NOD mice were crossed onto a B cell deficient background, there was a reduction of CD8 T cells in the insulinitis in the pancreas, implying that B cells were important for the CD8 T cell accumulation within the islets [45[•]]. Further investigation showed that the role of B cells here did not relate to antigen presentation to CD4 or CD8 T cells but rather promoted CD8 T cell survival. In the absence of B cells, there was increased apoptosis of CD8 T cells within the islets in this model. This therefore increased the ability to damage islet β cells and cause diabetes and highlighted a novel role for B cells in the process of diabetes development [45[•]]. This is particularly interesting in the light of the observation mentioned earlier that B cells in human insulinitis are usually found in islets that also have infiltrating CD8 T cells [35^{••}].

B cells produce a diversity of cytokines

Like T cells, B cells can produce a variety of cytokines and can be functionally divided into different subsets depending on the cytokine profile [46]. The production of pro-inflammatory cytokines could well contribute to ongoing immune responses. However, there is also considerable evidence that regulatory subsets of B cells exist that produce TGF- β and IL-10 [47[•]]. This concept is not new as B cells producing IL-10 protected against arthritis and were also able to reverse disease in animal models [48]. Similarly, IL-10 produced by B cells has been shown to be important in recovery from experimental autoimmune encephalomyelitis (EAE) [49]. Immature B cells, likely to be transitional B cells (B220^{hi}, CD21⁺, CD23⁺), when injected with pathogenic T cells, can suppress diabetes development in a co-adoptive transfer experimental system in NOD.SCID mice [1^{••}]. These transitional B cells express high levels of IL-10 and IL-10 receptor (Hu and Wen, unpublished data). Recently, another IL-10 producing B cell subset has been characterized as expressing CD1d^{hi}CD5⁺ [50[•]], and this subset of B cells can modulate the onset of EAE [51,52]. LPS stimulated B cells can protect NOD mice against diabetes and the protection is mediated by TGF- β [53]. Transfer of NOD spleen cells activated through the BCR, delayed the development of diabetes in prediabetic NOD mice, and the retardation was dependent on IL-10 production after BCR ligation [54]. Whether there is a defect in number or function of regulatory B cell subsets in autoimmune diabetes, and whether the CD1d^{hi}CD5⁺ population will protect against diabetes remains to be established. A recent study has indicated that the B cells from patients with diabetes may express more pro-inflammatory cytokines and release less IL-10 when stimulated by toll-like receptor agonists [55]. Much emphasis has recently been placed on the studies of regulatory T cells and their therapeutic potential in autoimmunity. However, it is clear that the possibility arises of utilizing protective B cell subsets to control autoimmunity.

Thus, the role of B cells in diabetes, as in other autoimmune diseases such as EAE, is complex and determined by different subsets of B cells. On the one hand, effector B cells are important in a variety of ways in the pathogenesis of autoimmune diabetes while on the other hand, regulatory subsets modulate this activity. The studies described in the next section suggest that therapeutic interventions that increase this regulatory subset of B cells may be effective and thus a very important focus for further investigation.

Effect of B cell depletion in diabetes

Remarkably, five studies were recently reported where B cells were targeted by different methods, all of which were efficacious in delaying or reversing autoimmune diabetes in mice [1^{••},2[•],56[•],57[•],58^{••}]. This was particularly exciting for the prospects for therapy for type 1

diabetes as many treatments in the NOD mouse may potentially be useful for the prevention of disease, but few strategies other than anti-CD3 treatment have been shown to restore normoglycemia. These findings have considerably extended the interest in the role of B cells in the pathogenesis of diabetes as they highlight a possible therapeutic strategy. The effects on disease are dependent on the agent used and the stages of disease at which the anti-B cell agents are administered as discussed below. These are also summarized in Table 1.

Anti-B cell therapy has been used in non-Hodgkins lymphoma for some years and following on from this has been trialed in a number of autoimmune diseases. CD20 is a widely expressed B-cell-specific protein involved in the development and differentiation of B cells. It is however, not expressed in the earliest stages of B cell development, nor on plasma cells. Rituximab, a human/murine chimeric anti-human CD20 monoclonal antibody that depletes most B cells, has been the most commonly tested anti-B cell agent in human autoimmune disease. It is approved by the FDA for treating rheumatoid arthritis [59] and trials have been conducted in a variety of non-rheumatoid arthritic diseases [60]. We developed transgenic NOD mice that expressed human CD20 (hCD20), which allowed the timed depletion of B cells using a reagent similar to Rituximab that targets the same epitope [1^{••}]. Depletion of B cells at 4 and 9 weeks of age delayed and prevented the onset of diabetes in a proportion of mice. Furthermore, when administered after the onset of disease, diabetes was reversed in ~35% of the mice which remained disease free for a prolonged period of time. The B cell depletion led to the generation of regulatory B and T cells that were both increased after treatment and were able to control the destructive autoimmune responses [1^{••}]. A similar delay was seen when a mouse anti-CD20 was given at early time points although no increase in regulatory T cells was seen and this treatment was not effective in restoring normoglycemia after diabetes onset [57[•]]. Targeting CD22, a widely expressed B cell surface protein that functions as a negative regulator of B cell signaling, using an anti-CD22 monoclonal antibody conjugated to the toxin calicheamicin also effectively depleted mature B cells. This treatment delayed/prevented diabetes in NOD mice when given at 10 weeks of age and was also able to restore normoglycemia in mice treated early after the onset of hyperglycemia [2[•]]. B cell activating factor (BAFF), a B lymphocyte regulatory factor involved in B cell development, function, and survival, has also been effectively targeted using an anti-BAFF antibody [56[•]]. BAFF and a proliferation-inducing ligand (APRIL) bind to B cell maturation antigen (BCMA) and thus, the BAFF pathway can alternatively be inhibited using B cell maturation antigen Fc reagent (BCMA-Fc) [58^{••}]. The targeting of B cells using BCMA-Fc was particularly effective in completely preventing diabetes when given in later

Table 1
Summary of B cell depletion studies in NOD mice.

Mouse	Reagent used	Target cells	Treatment protocol	Repopulation characteristics	Effect on Treg	Islet auto-antibody	Treatment age	Diabetes onset	Reference
Human CD20 transgenic NOD	Mouse anti-human CD20 (2H7, IgG2b)	CD20+ B cells (immature and mature B cells)	4 × 250 µg i.v., 3d interval	↑ T2 subset	↑ Foxp3+ and CTLA4+ two types of Treg	↓ anti-insulin antibody	4–5 wk 9–10 wk Diabetic mice	→ and ↓ ↓ 36% reversal	[1 ^{••}]
NOD	Hamster anti-mouse BLyS (10F4, hamster IgG)	FO, MZ B cells	2 × 100 µg i.p., 5d interval, then 15 µg biweekly	↑ transitional B cell	↑ Foxp3+ Treg	↓ anti-insulin antibody	4 wk	→ and ↓	[56 [•]]
NOD	Mouse anti-mouse CD20 (MB20-11, IgG2c)	CD20+ B cells (immature and mature B cells)	3 × 250 µg (i.v., 2-wk interval)		No effect	N.D.	6–8 wk 5 wk	→ and ↓ → and ↓	[57 [•]]
NOD	Calicheamicin-conjugated mouse anti-mouse CD22 (Cy34.1, IgG1)	CD22+ B cells	2 × 160 µg/kg i.p., 5d interval)	↑ anergic B cell (T3 cells)	↑ Foxp3+ Treg	N.D.	15 wk 10 wk	→ and ↓ ↓	[2 [•]]
NOD	BCMA-huFc fusion protein	FO and MZ B cells	12 × 150 µg i.p., twice/wk)		↑ Foxp3+ Treg	N.D.	Diabetic mice 9–15 wk	100% reversal 100% protection	[58 ^{••}]

↑: increase; ↓: decrease; →: delay. N.D. Not done.

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prediabetic stages of 9–15 weeks and this report highlighted the fact that on B cell depletion, regulatory CD4⁺CD25⁺ cells were increased [58^{••}]. CD19 which affects Src family kinase activity and thus regulates signal transduction, is another B cell surface antigen which is more widely expressed than CD20 in early pre-B cells as well as on more differentiated B cells except terminally differentiated plasma cells. A hCD19 and hCD20 double transgenic mouse model which allows much more complete depletion of B cells than has hitherto been achieved in autoimmune diseases [61], is a new addition to the tools available to investigate the role of B cells. Use of this model will add important information about depletion and regeneration of the lymphocyte repertoire.

As shown in Table 1, in the mouse studies, a number of subsets of B cells are affected by anti-B cell treatments and interestingly, both regulatory B cells and regulatory T cells are altered in the reconstitution process. This allows not only for the reduction of pathogenic B cells by the depletion process but also for the possibility of harnessing increased numbers of regulatory cell types to control the regenerating immune response. Synthesizing the information from all these studies may also provide important information about the interaction of B cells on the development of regulatory T cells and reciprocally the effect of T cells on the generation of regulatory type B cells.

In parallel with these efforts in animal models, a TrialNet initiated phase II clinical trial using four infusions of the anti-CD20 monoclonal antibody, Rituximab in newly diagnosed patients with type 1 diabetes was also carried out [3[•]]. Interestingly, similar results were obtained when compared with some of the studies in the animal models. After the first year, there was a delay in the loss of islet β cells as shown by the preservation of C-peptide after mixed meal stimulation, a standard measure of production of endogenous insulin. In addition, patients required less insulin and had better overall blood glucose control in the first year [3[•]]. The second year results were recently reported at the American Diabetes Association annual conference where it appeared that significant differences in various parameters compared to control groups may be less sustained but the full reported results for this are awaited. It is possible that the autoimmune destruction of islet beta cells will resume upon the return of the B cells since the effect of a single treatment may not have a sustained therapeutic effect. However, it is also important that we aim for treatment that will be immunomodulatory but not immunosuppressant in the long term. Anti-B cell treatment appears to be safe and B cell depletion has now been carried out for low-grade B cell lymphoma in adult patients for as long as 10 years and there is little, if any, infectious liability, evidently due to the preservation of long-lived plasma cells and perhaps some residual B cells. It is not known at this point

whether the risks of induction of even mild long-term immune compromise would be sufficiently balanced against the benefits of possibly alleviating long-term diabetes and its complications. Thus, it will not necessarily be desirable to have multiple or long-term B cell depletion. It is also possible that added benefit may be obtained by combining this type of single treatment to deplete B cells with strategies to increase the production of regulatory cytokines from B cells. Adjunct antigen-specific treatment may also improve the efficacy of anti-B cell therapy.

The fact that B cell depletion can modulate the development of diabetes and other autoimmune diseases gives important information about the role of B cells in these diseases. However, the depletion is, of necessity, temporary and this is vital as normal B cells are important for the maintenance of adaptive immunity to infection.

In addition to knowledge of the B cell immunobiology, the depletion of B cells using these agents has also given very valuable information about the effects of B cell reconstitution after treatment. Some of the therapeutic effects result from alteration of the balance of cellular subsets. In humans, the reconstitution is heterogeneous and differs in some aspects in different diseases and is also dependent on the different reagents used. The kinetics for the use of anti-CD20 in autoimmune diseases has been reviewed by Pers *et al.* [62] and, in the context of diabetes, it will be important to study effects of depletion in the absence of other immune toxic drugs, that are used in hematological malignancies and other autoimmune diseases, as these could influence depletion and repopulation. As mentioned earlier, in the studies using anti-hCD20 [1^{••}] and anti-CD22 [2[•]], when B cells returned, there was an increase in potentially regulatory B cell populations as well as populations of regulatory T cells. These altered dynamics may have effects quite separate from the actual effects in depletion of the B cells. In addition this repopulation of cells could cause changes in cellular composition that would not normally be seen in a mature immune system, at a time point long after early development. This ‘resetting’ of the immune system, if it could recreate a ‘benign pre-diabetic’ immune status, may allow for reprogramming of potentially autoreactive T cells using strategies that are able to deviate pathogenic immune responses to those of a more regulatory type.

Conclusion

In conclusion, there is evidence that B cell tolerance, particularly receptor editing may be defective in T1D. Autoreactive B cells escaping from tolerance will increase the likelihood of B cells playing a role in the development of diabetes. As in other autoimmune diseases, the role of B cells is complex. Pathogenic B cells are likely to be involved, but regulatory B cell subsets

also modulate the development of disease. The recent studies targeting B cells using a variety of agents including anti-CD20, toxin-conjugated anti-CD22, anti-BAFF and BCMA-Fc in mice as well as anti-CD20 in humans, have shown that this may be an important therapeutic maneuver. The efficacy of these agents may not only be related to targeting of B cell subsets and decrease of specific pathogenic cells, but perhaps more importantly, the effect of increasing regulatory B and T cell subsets on reconstitution after treatment. Although the early evidence so far obtained in the human trial has indicated some early efficacy for B cell targeting treatment in diabetes, it is likely that other additional modalities of treatment will be required. However, it is clear that as therapeutic trials continue, important information will be gained not only about therapeutic possibilities but also about the use of these agents to reshape and reset the immune system. This may suggest a rationale for the treatment of autoimmune diseases with non-specific therapies, followed by more specific directly targeted treatment.

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