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B cell depletion in autoimmune diabetes: insights from murine models

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Abstract

Introduction—The incidence of type 1 diabetes (T1D) is rising for reasons that largely elude us. New strategies aimed at halting the disease process are needed. One type of immune cell thought to contribute to T1D is the B lymphocyte. The first Phase II trial of B cell depletion in new onset T1D patients indicated that this slowed the destruction of insulin-producing pancreatic beta cells. The mechanistic basis of the beneficial effects remains unclear.

Areas covered—Studies of B cell depletion and deficiency in animal models of T1D. How B cells can influence T cell-dependent autoimmune diabetes in animal models. The heterogeneity of B cell populations and current evidence for the potential contribution of specific B cell subsets to diabetes, with emphasis on marginal zone B cells and B1 B cells.

Expert opinion—B cells can influence the T cell response to islet antigens and B cell depletion or genetic deficiency is associated with decreased insulinitis in animal models. New evidence suggests that B1 cells may contribute to diabetes pathogenesis. A better understanding of the roles of individual B cell subsets in disease will permit fine-tuning of therapeutic strategies to modify these populations.

Keywords

autoimmunity; B cells; diabetes; rituximab

1. Introduction

Individuals diagnosed with type 1 diabetes (T1D) require life-long insulin therapy and remain at risk from numerous complications including heart disease, kidney failure and blindness. While islet transplantation techniques continue to improve, this option is complicated by the requirement for ongoing immunosuppression to permit acceptance of

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Declaration of interest

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grafted tissue. Overall the current approach to T1D tends towards disease management rather than disease elimination. Although advances in patient management and insulin delivery have generally led to improved glucose control and reduced risk of complications, strategies aimed at halting the disease process are still urgently needed.

The pathogenesis of T1D involves the selective destruction of the insulin-producing beta cells in the pancreas. T cells are thought to be the primary mediators of disease [1,2] and targeting these cells with a non-depleting anti-CD3 antibody has produced encouraging results [3,4]. However recent attention has focused on the role of B cells in mediating various types of autoimmune pathology. The primary reagent employed has been Rituximab, a mouse-human chimeric antibody that binds to B-cell-expressed CD20 and mediates destruction by antibody-dependent cellular cytotoxicity utilising Fc γ receptors (Fc γ R) [5]. Flow cytometric analysis suggests that CD20 expression is restricted to the B cell lineage in mouse and man [6], but is poorly expressed on plasma cells, the fully differentiated antibody producers. Depletion of B cells has been met with varying degrees of success in numerous autoimmune conditions including rheumatoid arthritis, pemphigus vulgaris and multiple sclerosis [7-9]. Recently attention has turned to the application of B cell depletion strategies in T1D, with the early results showing promise. However, the mechanistic contribution of the B cell compartment to diabetes pathogenesis remains poorly defined and a better understanding of the role of this population in disease promotion is necessary to refine future treatment regimens. In this review we discuss the latest insight that has been gained from B cell manipulation in animal models of diabetes.

2. B cells at the scene of the crime

The presence of B cells within the pancreatic islet infiltrates of diabetes-prone animals was first observed over 20 years ago, and holds true in multiple models. These include the non-obese diabetic (NOD) mouse [10,11], the BioBreeding rat [12,13] and the more recently developed DO11 \times RIP-mOVA mouse [14,15] in which OVA-specific T cells respond to pancreas-expressed OVA consequently triggering insulinitis, autoantibody production and loss of blood glucose homeostasis (Figure 1). Despite the distinct origins of these three models, the presence of B cells within pancreatic infiltrates is a feature common to all of them. Similarly, histopathological evidence from isolated cases in which patients with type 1 diabetes have died shortly after diagnosis have indicated the presence of B cells in the islets [16]. Recently a more comprehensive analysis of pancreatic biopsies from new onset T1D patients has shed further light on the nature of the immune cell infiltration [17]. This analysis revealed the B cell to be a significant contributor to islet inflammation, being almost as numerous as the CD8⁺ T cells that represented the predominant population. Thus there is no question that B cells can be found 'at the scene of the crime' during the autoimmune attack in T1D (Figure 1).

3. B cell deficiency in animal models of T1D

Does the positioning of B cells within the pancreatic infiltrate indicate culpability? The contribution of B cells to the immune response that destroys pancreatic beta cells has been a highly controversial topic. It is clear that diabetes can be induced in experimental animal

models in the absence of B cells. Such models frequently involve the adoptive transfer of purified T cell populations into lymphopenic recipients, a scenario in which T cell responsiveness may be heightened by the lack of competition. Typically the recipient animals are NOD/SCID [18,19], but the same principle holds true for RIP-OVA/rag^{-/-} recipients [15,20]. It has been suggested that the increased numbers of monocytes in NOD/SCID mice (compared with the lymphocyte-replete NOD strain) could contribute to the ability of these animals to support diabetes in the absence of B cells [18]. Regardless of the mechanistic explanation, the data clearly indicate that the presence of B cells is not an absolute requirement for initiation and progression of diabetes.

However, what is equally clear is that genetic deficiency in B cells inhibits diabetes in animal models, although the extent of protection varies from study to study. Table 1 shows collated data from published studies of B-cell-deficient NOD mice, illustrating the effects on diabetes incidence and insulinitis [21-25]. While the majority of studies have used the NOD model, it has recently been demonstrated that B cell deficiency also results in a small but significant delay in diabetes onset in the DO11 × rip-mOVA model and again this is associated with a marked decrease in insulinitis [15]. Importantly, not every B-cell-deficient animal is resistant to diabetes (since protection from disease is not always 100% in the studies described in Table 1), emphasising that disease is not absolutely dependent on B cells. This is consistent with an isolated report of T1D in a patient lacking B cells [26].

4. B cell depletion in T1D

4.1 Mouse

One of the earliest strategies employed to target B cells was the use of an anti-IgM antibody that was injected into NOD mice continuously from birth. Such mice were significantly protected from diabetes and showed strikingly reduced insulinitis [27,28]. More recent studies have exploited either various B cell-specific markers such as CD20 [29] or CD22 [30], or used transgenic mice expressing humanized forms of the CD20 molecule [31]. Others have targeted B cells indirectly using Fc fusion proteins directed against B cell activating factor (BAFF) and a proliferation-inducing ligand (APRIL) [32].

All studies reported a significant benefit of depleting B cells both in delaying onset and in reducing the incidence of T1D. Success ranged from 50 to 100% of mice remaining diabetes-free following treatment. This was largely dependent on the treatment modality itself, and the timing of the intervention. B cell depletion clearly reduced insulinitis in these animal models, although at least some peri-insulinitis remained in each study (Table 2) [29-32].

Several of the studies also examined the possibility of reversing established disease. Outcomes ranged from no reversal [29] to 36% of mice reverting to normoglycaemia for at least 2 months [31], to 100% in newly glycaemic mice, which persisted in 60% of mice for up to 100 days [30]. This clearly highlights that timing of the intervention is critical for effective treatment of disease.

4.2 Man

Support for a role of B cells in the pathogenesis of T1D in humans comes from a clinical Phase II analysis of anti-CD20 (Rituximab) [33]. Recent onset T1D patients aged between 8 and 40 years received 4 doses over 22 days of either Rituximab (49 patients) or placebo (29 patients), with the primary outcome measure being stimulated C-peptide response during a mixed-meal tolerance test conducted after 1 year. Initial results suggest a clear benefit of B cell depletion in T1D in terms of promoting C peptide levels (0.56 pmol/ml (Rituximab) versus 0.47 pmol/ml (placebo)), reducing glycosylated haemoglobin levels (6.76 versus 7.00%) and reducing insulin dose ($0.39\text{U} \pm 0.22/\text{kg}$ of body weight versus $0.48\text{U} \pm 0.23/\text{kg}$). The preserved C-peptide levels and decreased insulin requirement indicated a protective effect of Rituximab on beta cell function. While this study does show a clear benefit early after treatment, the initial improvement was short-lived, and investigators observed resumption in the decline of C peptide levels. However the fact that the insulin requirement was still significantly lower in the Rituximab-treated group even 1 year after the 3 week treatment is encouraging. It should be noted that B cell numbers (as assessed by CD19 staining) remained effectively suppressed at 6 months post treatment (below 20% of the number in control-treated individuals) but had returned to approximately 60% of control numbers by 12 months post treatment. This suggests scope for improvement if a more sustained depletion of B cells could be achieved, for example by repeated administration of Rituximab.

5. Contribution of B cells to T1D

5.1 Role of autoantibody

The beneficial effects of B cell depletion on diabetes development in mice and humans raises the issue of what B cells might actually do to promote disease. The archetypal function of B lymphocytes is the production of circulating immunoglobulin (antibody). Serum levels of islet-specific autoantibodies precede T1D and indeed are predictive of disease onset [34,35] and possibly islet graft failure [36]. Autoantibodies against islet antigens are also a feature of the NOD and DO11 \times rip-OVA diabetic mouse strains, although certain diabetes-resistant NOD congenic strains have high levels of insulin autoantibodies yet remain disease-free [37].

Elegant studies in the rip-mOVA transgenic mouse have shown that antibodies to islet antigens can augment the cross-presentation of such antigens to CD8⁺ T cells [38]. The specificity of the B cell population is known to be important in diabetes, with many studies focusing on insulin recognition. One notable study used transgenic expression of V_H genes that conferred either good (VH125) or very poor (VH281) insulin binding to show that the former could accelerate diabetes in NOD mice, whereas the latter imparted disease protection [39]. There is evidence that cross-placental transfer of maternal autoantibody can promote diabetes in the NOD mouse. Accordingly, B-cell-deficient offspring surgically implanted into NOD mothers experienced an increased incidence of disease [40], although this approach does not rule out other environmental influences of the NOD uterus.

Despite the above, a number of findings have suggested that the role of B cells in T1D extends beyond autoantibody production. Reconstitution of serum antibody in NOD or DO11 × rip-mOVA mice that lack B cells is insufficient to trigger insulinitis or diabetes [41,42] indicating that B cells drive these processes by doing more than just providing autoantibody. Intriguingly the beneficial effects of B cell depletion in autoimmune settings are frequently uncoupled from changes in autoantibody production [43,44], again arguing for an additional contribution of B cells to pathology. Furthermore, elicitation of anti-insulin antibodies in NOD mice before or during pregnancy did not augment diabetes development in offspring [45] and evidence suggests that T1D is transmitted less frequently to the offspring of diabetic women than to those of diabetic men [46]. These findings further support the concept that production of autoantibodies does not necessarily explain the ability of B cells to promote diabetes.

In a landmark set of experiments it was shown that B cells that are unable to support antibody production are still capable of promoting diabetes in the NOD mouse model [47]. This provided the first definitive evidence that B cell functions, other than antibody secretion, could contribute to the pathogenesis of diabetes.

5.2 Role of B cells in antigen presentation

When considering how B cells might contribute to autoimmune diabetes without production of circulating antibody, an obvious possibility is that they participate in antigen presentation. For an antigen, or autoantigen, to be perceived by the T cell immune system, it is required that the antigen be presented in the context of the appropriate MHC (murine) or HLA (human) molecule. With respect to CD8⁺ T cells, the relevant MHC molecules (class I) are expressed on virtually all nucleated cells in the body. However, presentation to CD4⁺ T cells is more stringent, requiring expression of MHC class II molecules, that are restricted to 'professional' antigen presenting cells (APC) such as dendritic cells (DC), B cells and macrophages. B cells are generally believed to be the least efficient of these three APC types, partly as a result of their poor constitutive expression of costimulatory ligands. Nevertheless B cells are capable of taking up antigens, either by pinocytosis or by binding to surface immunoglobulin, and presenting these to CD4⁺ T cells in the context of MHC class II molecules. A series of careful studies have provided experimental evidence for the uptake and presentation of antigen by B cells [48-52]. Elegant models using transgenic B cells indicate that B cells specific for autoantigen may serve as important APCs early on, even before DCs take on this role [53]. In addition, it has been shown that B cells do not have to bind antigen with their B cell receptors in order to acquire and present it [54], emphasising the role of non-antigen-specific uptake mechanisms such as pinocytosis, Toll-like receptors (TLRs) or complement receptors [52].

With respect to diabetes, B-cell-deficient NOD mice have been shown to exhibit impaired T cell responses to islet antigens such as glutamic acid decarboxylase (GAD) and heat-shock protein 60 (HSP60) [41,55,56]. Diabetes progression was also interrupted in NOD mice whose B cells lacked the diabetes-associated I-Ag7 MHC class II antigen [57] although this experiment is complicated by an additional change in MHC class I antigens (see [58]). A direct effect of B cell depletion on T cell responses in the pancreatic lymph nodes was

provided by the work of Tedder and colleagues who showed that islet-specific CD4⁺ T cells divided less in NOD recipients that were treated with anti-CD20 [6]. Thus B cells can clearly participate in the presentation of islet antigens, which could represent an important contribution to diabetes progression (Figure 2).

5.3 Role of B cells in tertiary lymphoid structures

The formation of lymphoid cell aggregates at sites of chronic inflammation shares many features with secondary lymphoid organ development. Such structures are often characterised by the organisation of different cell types into distinct regions, reminiscent of the cellular organisation observed in lymph nodes and spleen. In NOD mice, immune cells infiltrating the pancreas have been shown to organise into lymphoid structures capable of supporting germinal centre formation [59] although it is not clear to what extent this happens in other animal models of diabetes or in patients. Since B cells are early participants in pancreatic infiltration in diabetes models, this raises the possibility that these cells might in some way contribute to the development or organisation of lymphoid aggregates at this site.

A number of studies have suggested a possible role for B cells in lymphoid tissue development or organisation. B-cell-deficient mice have impaired development of Peyer's patches [60] with a lack of the specialised M cells that allow antigen uptake from the gut lumen. The B cell population is also required for the development of mature follicular dendritic cell (FDC) networks in the spleen [61,62]. Similarly in the absence of B cells the architecture of the spleen is disrupted, with decreased expression of the chemokine CCL21 and a reduction in stromal cells bearing the T zone stromal marker, glycoprotein gp38 [63]. B cells could also potentially contribute to the structure and stability of lymphoid aggregates by promoting the survival of particular cell types. Importantly it has been demonstrated that B cells enhance the survival of cytotoxic T lymphocytes (CTL) within inflamed islets in a transgenic mouse model of diabetes [64]. Thus although further work is needed in this area, it is possible that B cells contribute to diabetes by influencing the development and organisation of pancreatic lymphoid aggregates and the survival of particular cell types within these structures (Figure 2).

6. Role of marginal zone B cells and B1 B cells in T1D

The beneficial effects of genetic or experimental removal of B cells in the NOD and DO11 × rip-mOVA models of diabetes begs the question: which type of B cell is responsible for promoting disease? Although conventional B2 cells are the numerically dominant B cell population within lymphoid tissues, marginal zone (MZ) B cells and B1 B cells play important roles in immune defence and may also contribute to autoimmune responses. Accordingly the repertoires of both MZ and B1 B cell populations have been shown to contain some autoreactive clones [65,66]. The putative role of these B cell populations in T1D is considered below.

6.1 Marginal zone B cells

MZ B cells exhibit a characteristic surface phenotype featuring the complement receptors CD21 (CR1) and CD35 (CR2) in addition to high levels of IgM and low levels of IgD and CD23. They are typically located at the outer limit of the splenic white pulp, bordered by the marginal zone sinus on the inside and the red pulp on the outside [67]. MZ B cells are known to be highly efficient presenters of antigen to T cells [68] and more recently an active role for MZ B cells in transporting antigen to FDCs in B cell follicles has been identified [69].

In the steady state, MZ B cells are considered to be a spleen-resident population without a blood-borne component, although they derive from a recirculating precursor population [70]. However in the diseased state, MZ B cells have been detected at additional sites. For example B cell populations infiltrating the thyroid in Graves disease or the salivary glands in a mouse model of Sjogren's disease have been shown to have a MZ phenotype [71,72]. With respect to autoimmune diabetes, it has been shown that numbers of MZ B cells are significantly higher in NOD mice than in non-autoimmune prone strains [73-75]. Indeed the increase in the MZ B cell population has been suggested to map to the insulin dependent diabetes susceptibility 11 (*Idd11*) region on chromosome 4 [73] suggesting the possibility of a causal link between MZ B cell numbers and disease. However subsequent analysis revealed that NOD mice bearing an *Idd11* locus derived from C57/B16 [76] or NOR [77] mice were resistant to diabetes, despite maintaining an augmented MZ B cell population.

In addition to numerical differences, MZ B cells in NOD mice exhibit heightened sensitivity to CD40 engagement, IL-4 and TLR9 ligands and are capable of presenting autoantigens to T cells [75]. The latter study identified B cells with a MZ phenotype both in the pancreatic lymph nodes and within the pancreatic lesion itself. In contrast, a study by Kendall and colleagues failed to find MZ B cells within the inflamed NOD pancreas [78]. Analysis of pancreas-infiltrating populations in the DO11 × rip-OVA diabetes model also failed to identify significant numbers of MZ B cells at any stage of disease [42]. Thus although changes in MZ B cell number and function are reported in some studies, definitive evidence for a role in disease pathogenesis is currently lacking.

6.2 B1 B cells

B1 cells are the principal B cell in the body cavities and also make up approximately 5% of splenic B cells [79]. Like MZ B cells, B1 cells are capable of mounting responses in a T cell independent manner and the natural antibodies they elicit provide a critical defence against encapsulated bacteria. Although no single surface antigen defines the B1 lineage, a combination of markers can be used to identify this population, including IgM^{hi}IgD^{lo}CD11b⁺B220^{lo}. The B1 cell compartment can be further subdivided into B1a cells, which are distinguished by expression of CD5, and B1b cells, which lack this marker [80]. The B1 subset has been linked historically with autoimmunity in man [81,82] and mouse [66,83] and this has been fuelled by the appreciation that B1 cells recognise self antigens in addition to common bacterial antigens [84]. In addition, overproduction of B1a cells as a result of Shp1 deficiency can trigger tissue infiltration and autoimmunity [85]. In

contrast, B1 cells can also exhibit regulatory function in certain settings, often via their production of the cytokine IL-10 [86].

The involvement of the B1 cell subset in autoimmune diabetes has been controversial, with evidence both for and against a role in disease pathogenesis. In the NOD model, cells with a B1a phenotype have been identified amongst the pancreas-infiltrating lymphocytes in some studies [11,78] but not others [87]. Insulin autoantibodies in NOD mice have the characteristics of B1-cell-derived natural antibodies, bearing unmutated V gene regions and lacking N segment additions [88]. Furthermore NOD mice deficient in Bruton's tyrosine kinase (BTK), in which B1 cell development is profoundly impaired, are protected from diabetes [89].

Our own work using the DO11 × rip-mOVA diabetes model has identified a role for B1 cells in promoting pancreas infiltration by islet-reactive CD4⁺ T cells [42]. In an intriguing prior study it was shown that depletion of the peritoneal B1 population by hypotonic lysis had a marked effect on pancreatic islet infiltration and diabetes onset in NOD mice [78]. In this study the authors took advantage of the fact that B1 cells self-renew within the peritoneal cavity and reconstitute poorly from bone marrow: thus short-term hypotonic treatment of the peritoneal cavity affects B1 cells much more severely than bone-marrow-derived populations. Loss of peritoneal B1 cells with this protocol was associated with a decrease in pancreas infiltration by both B1 and B2 cells [78]. Taken together with our own study, this suggests that B1 cells can influence the ability of both B2 cells and T cells to enter the pancreas in diabetes-prone animals.

Their location in the peritoneal cavity makes B1 cells one of the first populations to encounter gut-associated material (enteric pathogens, commensals, dietary antigens) if the intestinal barrier becomes compromised. In this regard, there is evidence for increased intestinal permeability in T1D [90,91] and the association between T1D and celiac disease is well established [92]. Evidence that diet can modify T1D in animal models [93,94] strengthens the link between the intestinal tract and diabetes pathogenesis and raises the possibility that B1 cells are exposed to antigens that could modify disease.

Connections between the peritoneal cavity and the pancreatic lymph nodes are only beginning to be fully appreciated but represent a key area for future investigation. In a landmark study it was demonstrated that a defined trafficking route between peritoneal cavity and pancreatic lymph nodes exists for both lymphocytes and antigens [95]. Interestingly, it has been reported that B1 cells in NOD mice exhibit enhanced trafficking from the peritoneal cavity to the pancreatic lymph nodes when compared with those from other mouse strains. The increased migration apparently reflects a relative insensitivity to sphingosine-1-phosphate (S1P) that would normally retain B1 cells in the peritoneal cavity. Once in the pancreatic lymph node the B1 cells are thought to be capable of presenting insulin autoantigen to T cells [96]. Egress of B1 cells from the peritoneal cavity can be prompted by TLR ligation [97] providing a potential mechanism by which infection might lead to increased B1 cell traffic to pancreatic LN [96] or pancreas [42] with consequent effects on antigen presentation or tissue infiltration (Figure 2).

Overall there is emerging evidence to support a role for B1 cells in promoting diabetes in animal models. Such a role might include the presentation of islet autoantigens to T cells, and the initiation or augmentation of insulinitis. We speculate that the anatomical location of the B1 cell population might allow them to integrate cues from diet and gut microbiota in a manner that could influence diabetes onset. Notably, the phenotype of the B1 cell population in humans has recently been elucidated [98]. The inability to phenotypically define B1 cells in humans has until now prevented meaningful analysis of this population. However, the long-awaited description of robust markers for human B1 cells will now permit enumeration and localisation of these cells such that their role in T1D can be explored.

7. B cells and immune regulation: what about Breg and Treg?

One issue surrounding the therapeutic depletion of B cells is whether this will have adverse effects on immune regulation, either by altering the homeostasis of regulatory T cells (Treg) or by depleting B cell populations that have regulatory function. With regard to the former possibility, it seems that if anything B cell depletion augments regulatory T cell populations. Following B cell targeting in NOD mice, increases in the number of forkhead box p3 (Foxp3⁺) Treg cells were reported [30,31] although this was not universally observed [29]. Likewise, an increase in Treg was also observed in mice in which B cells were depleted by injection of B cell maturation antigen (BCMA)-Fc which blocks BAFF and APRIL signalling [32]. Enhanced protection by Treg in the absence of B cells has previously been reported in an autoimmune thyroiditis model [99].

While not the focus of this review, it is now firmly established that certain B cell populations can exhibit immune regulatory function [100]. The phenotype of such B cells is the subject of ongoing debate and indeed Breg cells may not exist as a definitive lineage as eloquently argued by Gray *et al.* [101]. Rather the ability of B cells at different developmental stages to elicit regulation may depend on contextual cues. Despite the potential fear that B cell depletion could remove a potentially beneficial population, experimental evidence for this scenario is sparse. Indeed some have suggested a 'reprogramming' of the re-emerging B cells following depletion that may contribute to the beneficial effects of B cell depletion [30]. Along these lines, the B cells that repopulated animals following B cell depletion in one study were able to mediate dominant suppression of diabetes when adoptively transferred into recipient animals [31]. Therefore, although the capacity of certain B cells to elicit immune regulation is now beyond doubt, it appears that the net effect of pan B cell depletion is to decrease autoimmune pathology, possibly by encouraging such regulatory populations to emerge.

8. Expert opinion

Targeting of B cells with reagents like Rituximab provides the potential to dampen autoimmune responses while retaining a degree of humoral immunity due to the relative sparing of plasma cells.

In terms of how safe such a strategy is, results to date are encouraging. As reported in trials of anti-lymphocyte antibodies in general, Rituximab triggers an initial transient increase in inflammatory cytokines in particular IL-6 [102], although unlike in trials of CD3-specific

antibody, IFN- γ is not induced [103]. Despite the valid concern that immunosuppression associated with B cell depletion could lead to increased infection rates, this does not seem to have been borne out by evidence to date.

It is clear that the effectiveness of B cell depletion varies according to both B cell sub-type and B cell location. Bone-marrow-resident B cells and MZ B cells have been shown to be readily depleted, even by low concentrations of anti-CD20 [104]. Within the spleen B1a cells were less sensitive to depletion than B2 cells although the majority of both were depleted (70% depletion for B1a versus 95% for B2 cells) [104]. In our own study, using toxin-conjugated anti-CD22 antibody [42], we also observed that B1 cells were less readily eliminated than B2 cells; one factor that contributed to this was the rapid recovery of the B1 population due to their capacity for self-renewal. However repeated injections at high dose achieved good depletion of both populations. B cell depletion in T1D patients has provided some encouraging data thus far. However a precise understanding of which B cell populations contribute to diabetes is currently lacking. As more information on this topic becomes available, there will be opportunities to select the most relevant B cell depletion agents based on their relative efficacy against different B cell populations. The development of additional reagents targeting different B cell antigens is an important development in this regard [105]. It will be important to confirm that targeted B cell epitopes are expressed on the B cells present at diseased sites. In this regard recent analysis has confirmed CD20 expression on B cells in the pancreas of T1D patients [17], and mouse models certainly suggest that CD19 is also expressed at this site.

Fc γ R-mediated cytotoxicity appears to be a major mechanism of B cell loss after anti-CD20 treatment [106]. The engineering of B cell depletion agents to allow enhanced antibody-dependent cytotoxicity [105] represents an important step forward in maximising the clinical potential of this treatment strategy. It is of note that the known defect in Fc γ Rs in the NOD strain makes it important to also test depletion regimens in additional mouse strains [29]

A clear prediction of the murine studies is that B cell depletion will be more effective if administered early in the disease course. In addition, patient age may be a factor in shaping responsiveness to therapy. In the Phase II trial of Rituximab in T1D, there was a hint that those aged over 18 responded less well than those in the 8 – 12 or 13 – 17 age groups [33]. Analysis of a larger treatment group is required to assess the significance and predictive power of this observation. Given that early intervention favours efficacy in mouse models, that B cells may influence the earliest events in diabetes development (i.e., initial T cell pancreas invasion) [42,78], and that younger patients may respond better [33], it is tempting to make a case for B cell depletion in children at risk of developing diabetes (i.e., those positive for multiple autoantibodies). Longer-term follow up of Rituximab-treated individuals is probably required before this option could be effectively explored. With the development of novel pancreas-imaging techniques [107] it may eventually be possible to detect the presence of insulinitis in at-risk individuals, allowing treatment regimens to be targeted to this patient group.

In summary, the major findings from mouse models indicate that in the context of T1D, B cells play important roles in antigen presentation and in potentiating insulinitis. In certain

settings it can be demonstrated that B1 cells as well as B2 cells contribute to disease pathogenesis, although the capacity of many B cell subsets (including B1 cells) to elicit regulatory function should be borne in mind. A better understanding of the roles of individual B cell subsets in disease will ultimately permit fine-tuning of therapeutic strategies to modify these populations.

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Article highlights

- B cells can be detected within the inflamed pancreatic islets in animal models of diabetes and in patients with type 1 diabetes (T1D).
- Genetic deficiency or depletion of B cells reduces insulinitis and diabetes in animal models.
- Depletion of B cells in new-onset T1D patients decreases insulin requirement.
- B cells may contribute to diabetes pathogenesis by presenting islet antigens and promoting pancreas infiltration.
- Data from animal models indicate that B1 cells may contribute to diabetes pathogenesis in certain settings; more research is needed to explore the role of this population in humans.

This box summarizes key points contained in the article.

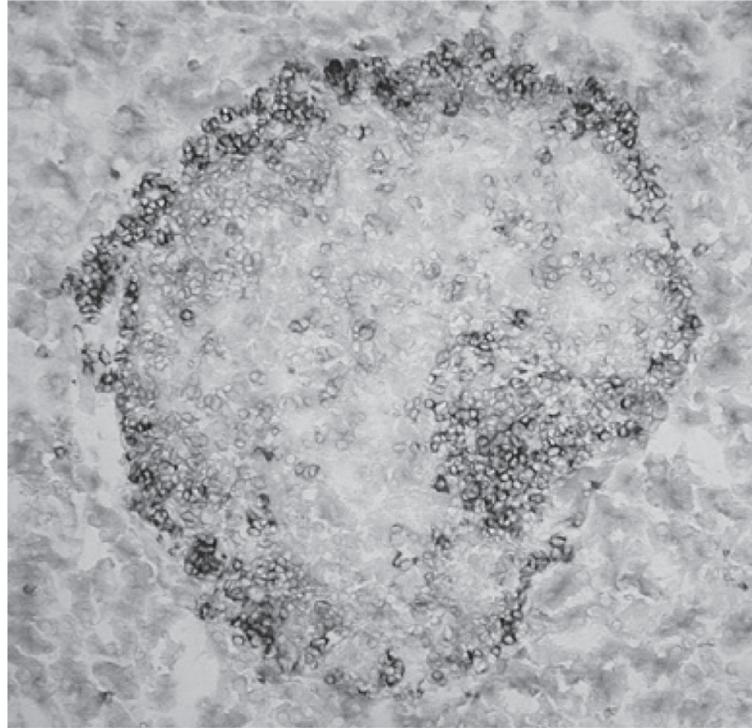


Figure 1. B cells infiltrate the pancreatic islets along with T cells in the DO11 × rip-mOVA mouse model

Pancreas sections from an 8 week old DO11 × rip-mOVA mouse were stained for the presence of DO11 T cells (KJ126; Gold) and B cells (B220; Blue). The figure shows a single islet; lymphocytes can be seen both at the islet perimeter and penetrating the islet mass.

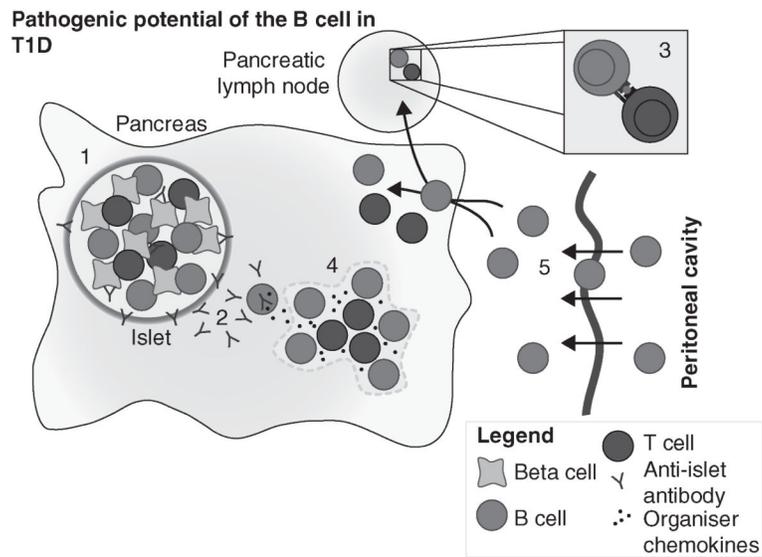


Figure 2. The pathogenic potential of B cells in T1D

B cell function is critical for efficient immune defence against infection. However, in autoimmunity, these same functions can potentially contribute to pathology. In T1D, it is clear that B cells are present at the site of immune attack, that is the pancreatic islet (1). B cells may also generate islet-specific antibodies that could amplify immune responses against islet antigens (2). Numerous studies have inferred a role for the B cell in presenting islet antigen to autoreactive T cells in the draining pancreatic lymph node (3), paving the way for targeted destruction of the islet. B cells may also have a role in organising the infiltrating lymphocytes within the islet (4) and contributing to their survival, and thus the ongoing pathology. A specific B cell subset (B1 B cells) may have a distinct role in promoting entry of autoreactive T cells into the pancreas (5).

Table 1
B cell knockout studies in the NOD mouse

Study	Incidence of diabetes (B cell knockout versus wild type)	Effect on insulinitis (B cell knockout versus wild type)
[21]	0/8 versus 7/10	'Virtually free of insulinitis'
[22]	0/13 versus 43/49	'Insulinitis significantly suppressed' 18% of 300 versus 73.3% of 300 islets scored > 0
[23]	29% of 7 versus 70% of 8	N/A
[24]	0% of 13 versus 20% of 56	'Mild insulinitis'
[41]	0/8	Low levels of insulinitis
[56]	0/19	'Mild insulinitis' 25.8 versus 72.5% of islets inflamed per mouse
[25]	20% of 18 versus 90% of 24	Lower insulinitis scores compared with NOD mice
[47]	1/120 versus 32/40	'Milder insulinitis compared with NOD mice'

Incidence of diabetes and effect on insulinitis in B-cell-deficient NOD mice compared to their B cell sufficient counterparts.

Table 2
B cell depletion studies in the NOD mouse

Study	B cell depletion strategy	Diabetes onset (Treated versus controls)	Diabetes reversal
[31]	Anti-hCD20 in hCD20/NOD transgenic mice	4 week old: 80% diabetes-free versus 20% diabetes-free 9 week old: 70% diabetes-free versus 30% diabetes-free	5/14 (36%) demonstrated reversal of hyperglycaemia
[30]	Anti-CD22-Calicheamicin	10/20 (50%) protected versus 2/20 (10%) protected	Newly hyperglycaemic mice: 10/10 (100%) demonstrated reversal of hyperglycaemia 6/10 (60%) remained normoglycaemic for > 100 days Treated 5 days after hyperglycaemia: 5/6 (83%) normoglycaemic for 20 days. All reverted to hyperglycaemia
[29]	Anti-CD20	5 week old: 7/11 (64%) diabetes-free versus 0/11 diabetes-free	No reversal
[32]	Indirect using BCMA-Fc	10/10 (100%) diabetes-free versus 0/10 diabetes-free	Not applicable

Effect of B cell depletion (by the indicated treatment regimen) on the onset of diabetes and on diabetes reversal.

BCMA: B cell maturation antigen.