Review

# B cell-directed therapies in type 1 diabetes

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**B cells play a pathogenic role as antigen-presenting cells and autoantibody secretors in the lead up to T cell-mediated autoimmune destruction of insulin-producing β cells in type 1 diabetes (T1D). This has led to significant interest in the use of B cell depletion therapies as a treatment for T1D. In this review, we compare results from five recent studies that used distinct B cell-depleting agents and protocols to successfully prevent and even reverse T1D in the non-obese diabetic (NOD) mouse model. We discuss how information gained from animal studies could be used to improve on the positive outcomes of a completed phase II clinical trial of the B cell-depleting drug rituximab in humans with recent-onset T1D.**

## B cells in type 1 diabetes (T1D)

The contribution of B cells to autoimmune disease has recently received invigorated interest because of the demonstration that this lymphocyte population is important not only for autoantibody-mediated diseases, but also for those that are ultimately T cell-mediated [1]. This has been particularly evident for T1D [2], a disease resulting from the specific destruction of β cells within pancreatic islets by autoreactive $CD4^+$ and $CD8^+$ T cells. A pathogenic role for B cells in this disease was first demonstrated in the non-obese diabetic (NOD) mouse model (Box 1), in which mice rendered deficient in B cells through mutation of the gene (*Ighm*) encoding immunoglobulin (Ig) μ-chain [3] or chronic treatment with anti-IgM antibodies [4] were strongly protected from the onset of T1D. Since these landmark studies, it has become evident that various subsets of B cells contribute to the etiology of T1D as antigen-presenting cells (APC) [5–10], autoantibody secretors [11,12] and modulators of the pancreatic microenvironment [13] (Figure 1). B cells are also capable of serving regulatory functions that prevent T1D [14,15]. However, two independent studies showed that short-term B cell depletion via monoclonal antibodies (mAb) targeting CD20 could achieve long-term prevention, and, in some cases, reversal from the onset of hyperglycemia in NOD mice [16,17]. These observations established the primary contribution of B cells to T1D as promoters of β cell destruction. These studies set the stage for a phase II multicenter clinical trial in which newly diagnosed human T1D subjects were treated with the anti-CD20 B cell-depleting antibody, rituximab [18]. Even at this advanced stage of disease, short-term B cell depletion was effective in delaying the decay of β cell function in patients, confirming that B cells are also important drivers of human T1D. With the identification of new B cell targeting strategies capable of mediating strong protection from T1D in NOD mice [19–21], B cell depletion as a method for treating T1D has become a hot topic for discussion. Here, we review all of the recent B cell depletion studies performed in NOD mice, and discuss the strengths and weaknesses of each strategy as a potential treatment for human T1D.

## New therapeutics for treating T1D by targeting B cells

B cell depletion for the treatment of T1D in NOD mice has been achieved via direct strategies that used mAb to directly target the B cell surface proteins CD22 [19] or CD20, expressed by a human transgene (hCD20) [16] or the endogenous mouse gene (mCD20) [17]. Alternatively, indirect B cell depletion was also trialed by preventing B cell access to trophic support provided by the B cell activating factor belonging to the TNF family (BAFF) and a proliferation induced ligand (APRIL) system. This was achieved via mAb-mediated blockade of BAFF [20], or administration of a B cell maturation protein (BCMA)-receptor fusion protein (BCMA-Fc) capable of blocking BAFF and APRIL [21]. The functional features and expression levels of CD20, CD22 and the BAFF-APRIL system are detailed in Box 2 and Table 1, respectively. A summary of the dosing regimens and outcomes of each B cell depletion study are presented in Table 2. Major considerations when assessing B cell depletion agents as therapeutics are the difference in kinetics of depletion, the B cell subsets targeted, the efficacy for prevention and reversal of T1D, as well as the mechanisms of protection. These are compared below.

### *Kinetics of B cell depletion*

BCMA-Fc [21] and anti-BAFF mAb [20] inhibit B cell access to survival signals by blocking soluble or superficial transmembrane BAFF. In NOD mice, both agents caused >90% B cell depletion in blood within ~2 weeks of commencing treatment. The protracted kinetics of B cell depletion by these agents relates to the time taken for B cell development to slow past the transitional 1 (T1) stage of development, in the face of diminishing levels of BAFF and turnover rates of existent mature B cells (Box 2). In contrast, mAb targeting CD20 coat the surface of B cells and rapidly activate Fc receptors on monocytes, which in turn, subject them to antibody-mediated cellular cytotoxicity [22]. As a result, anti-CD20 mAb depletes >70% of B cells

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**Box 1. NOD mouse model of T1D**

The NOD inbred mouse strain is a well established and widely used spontaneous model of autoimmune T1D [55]. In this model, immune infiltrates begin to infiltrate pancreatic islets by 4–5 weeks of age in a process termed insulitis. Similar to humans [56], the infiltrate consists mostly of B cells, $CD4^+$ and $CD8^+$ T cells, but also contain dendritic cells, natural killer cells and macrophages [57]. As infiltration proceeds, insulin-producing β cells are destroyed progressively within islets. Mice begin to show signs of hyperglycemia (>250 mg/dl glucose in blood) after 10 weeks of age, marking the onset of clinical diabetes. Although both male and female NOD mice show evidence of insulitis, females are more susceptible to destructive insulitis and diabetes, resulting in an incidence of 60–90% compared with 20–60% in males at 30 weeks. The model shares various features with human T1D. For instance, predisposition to T1D in both species is conferred by a complex interplay between multiple 'susceptibility' genes and the environment [55,58]. MHC class II alleles provide the strongest susceptibility component, with the antigen-binding groove of the T1D-predisposing MHC class II proteins in humans and NOD mice sharing uncanny structural similarities [59]. As a result, similar autoantigens [including insulin, glutamic acid decarboxylase (GAD), heat shock protein (HSP) and islet-specific glucose 6-phosphatase catalytic subunit-related protein (IGRP)] are targeted in both types of disease [55]. However, in addition to species-specific variations in genetics and physiology, there are differences in the development of T1D in NOD mice and humans that should be considered when using it as a model of disease [60,61]. Most importantly from a clinical perspective, the inbred nature of the NOD strain means that it potentially only represents a single case of T1D. In contrast, T1D in humans is more heterogeneous in terms of genetics, environment and timing, meaning that the groups of factors involved in the pathogenesis of disease are likely to vary between individuals [58]. Nevertheless, the ability to genetically and therapeutically manipulate NOD mice has provided an unrivalled model for studying T1D, which has offered great insight into the pathological mechanisms as well as potential targets for the treatment of disease.

in blood as early as 1 h post-administration and >90% within 4 days [16]. mAb targeting CD22 are normally poor B cell depleters because of the rapid internalization of antibody-bound CD22 molecules, but instead possess the ability to impair B cell function by inducing the inhibitory function of CD22 (Box 2) [23]. However, for the study in NOD mice, the depleting capacity of the anti-CD22 mAb was improved significantly by attaching it to the cytotoxic antibiotic calicheamicin (cal) [19]. The kinetics of depletion with anti-CD22-cal mAb was intermediate to that of BAFF-blockade and CD20, such that >90% B cells were depleted within 1 week of starting treatment. In most short-term treatment strategies in NOD mice, B cell numbers in blood were restored 7–10 weeks after commencing treatment [16,17,19–21].

### *B cell populations targeted*

As illustrated in Figure 1, different B cell populations play distinct roles during the development of T1D. The potential therapeutic efficacy of each B cell-depleting agent could therefore relate to how well they target different populations. Consistent with the broad level of CD20 expression (Table 1), anti-CD20 mAb are effective in depleting B cells from immature populations in the bone marrow (BM), and transitional and mature follicular (FO) subsets in the spleen, blood and lymph nodes (LN) [24,25]. Anti-CD20 mAb are also effective in eliminating memory B cells [26]. Interestingly, depletion of germinal centre and marginal zone (MZ) B cells was effective in studies where endogenous mCD20 was targeted [17,25], but not hCD20 in transgenic mice [16,24]. This could be explained by the distinct isotypes of the mAb (Table 2) or the epitopes they recognize, which might induce divergent effector mechanisms. Both types of anti-CD20 mAb are poor depleters of B1 B cells and plasma cells [24–26], which both play a specialized role in T1D pathogenesis (Figure 1). Resistance of B cells to anti-CD20 mAb can be attributed to poor CD20 expression [i.e. plasma cells (Table 1)], or the protective environmental niches occupied by these cells (i.e. B1 B cells) [24–26].

Treatment with anti-CD22-cal mAb is effective at depleting mature populations of $CD22^+$ FO B cells in blood, spleen and LN [19,27]. In contrast to anti-CD20 mAb, anti-CD22-cal mAb are unlikely to be effective in depleting immature/transitional B cells in the BM and spleen because of the scarcity of CD22 surface expression at these stages (Table 1) [28]. This might permit autoreactive B cells to pass through early stages of self-tolerance [29]. Whether anti-CD22/cal mAb can deplete MZ or B1 B cells has not been examined directly. However, high expression of CD22 in these subsets (Table 1) [30], and its important role in their maintenance and/or function [31,32] suggest that it might be able to do so. CD22 was shown to be expressed modestly on a small proportion of splenic plasma cells in NOD mice [19], but has not been detected on long-lived plasma cells of the BM [31]. The effect of CD22/cal mAb on plasma cell populations is thus likely to be minimal.

Consistent with the phenotype of BAFF-deficient mice [33], BCMA-Fc and anti-BAFF mAb blocked B cell development beyond the transitional stage in NOD mice, preventing the emergence of mature B cells [20,21]. This resulted in the transient reduction of MZ B cell precursors (MZP) as well as mature FO and MZ B cells from blood, spleen, pancreatic and mesenteric LN. Interestingly, BCMA-Fc and anti-BAFF mAb differed in the magnitude to which FO versus MZ B cell subpopulations were depleted, which could be of relevance to their relative efficacy for treating T1D. Anti-BAFF mAb was more effective at depleting MZ than FO B cells (~8 vs ~4-fold reduction, respectively), whereas BCMA-Fc was more effective at depleting FO B cells rather than MZ B cells (~10 vs ~4-fold reduction, respectively). This suggests that MZ B cells are highly BAFF-dependent, whereas FO B cells might be more APRIL-dependent. Survival of long-lived plasma cells in BM are not affected by inhibition of BAFF alone, but are dependent on the combination of BAFF and APRIL, which activate BCMA on these cells [34,35]. By virtue of its ability to sequester both BAFF and APRIL, only BCMA-Fc would be expected to affect long-lived plasma cells, thereby contributing to its efficacy in the prevention of T1D. Despite the expression of BAFF and TACI receptors by B1 and memory B cells (Table 1), these subsets are unlikely to be affected by anti-BAFF mAb or BCMA-Fc treatments, since both populations have been shown previously to be impervious to BAFF and APRIL blockade [35,36].

## Efficacy for the treatment of T1D

### *Disease prevention*

In prevention studies where B cell depletion treatment was commenced after initiation of insulitis, but before the onset

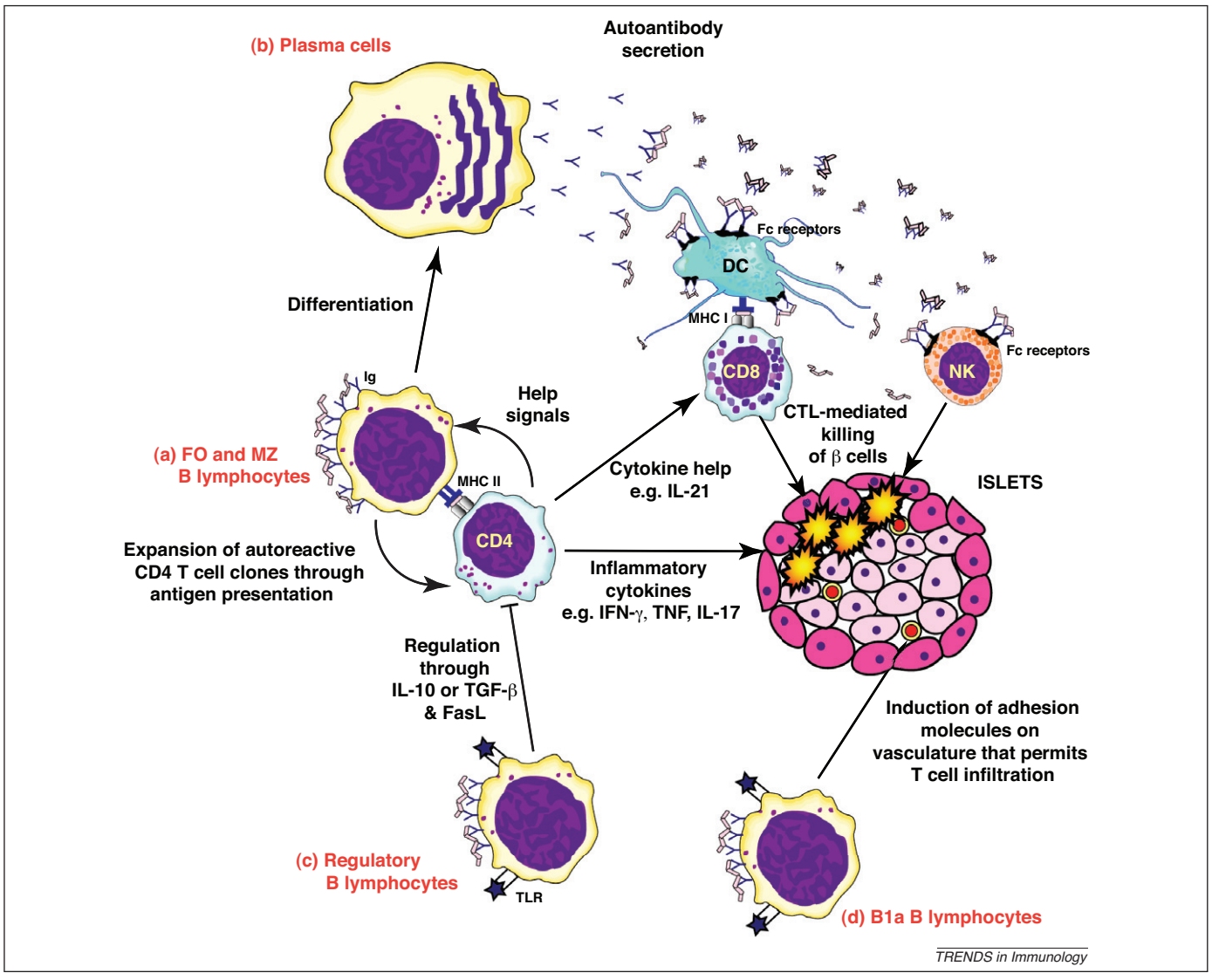



**Figure 1**. **(a)** By capturing autoantigens through surface immunoglobulin, FO and MZ B cells subsets act as APC that expand β cell-reactive $CD4^+$ T cells [5–10]. Expanded autoreactive $CD4^+$ T cell clones contribute to T1D through secretion of inflammatory cytokines that mediate β cell killing [68–70] as well as enhancing survival and differentiation of autoreactive $CD8^+$ T cells [38]. In turn, activated $CD4^+$ T cells provide 'help signals' to B cell populations, further exacerbating impaired B cell tolerance [40] and their differentiation into germinal centre and memory B cells as well as plasma cells [29]. **(b)** Islet-specific antibodies secreted by plasma cells, results in the generation of autoantigen–autoantibody complexes that bind to activating Fcγ receptors (FcγR) on other immune cells. This promotes β cell pathogenesis by activating the effector cytotoxic functions of natural killer (NK) cells, as well as promoting the uptake of autoantigens that dendritic cells (DC) use to activate both $CD4^+$ and $CD8^+$ T cells [11,71]. **(c)** Regulatory B cells activated through surface Ig or toll-like receptor (TLR)-4 have been shown to dampen β cell pathogenesis through the secretion of immunomodulatory cytokines (IL-10 or TGF-β), or expression of Fas ligand (FasL) that mediates the apoptosis of autoreactive T cells [14,15]. **(d)** Peritoneal B1a ($CD5^+$) B cells infiltrate the pancreas during early stages of disease and induce the expression of adhesion molecules such as V-CAM on pancreatic vasculature by secreting particular cytokines [e.g. tumor necrosis factor (TNF)] [13,72]. This permits pancreatic infiltration by circulating, islet-specific T-lymphocytes expressing appropriate partner integrins (e.g. $\alpha_4\beta_1$).

of overt diabetes in NOD mice, BCMA-Fc treatment achieved the highest efficacy with 100% protection from diabetes [21]. This was followed by anti-CD20 mAb [16,17] and anti-CD22/cal mAb [19], which provided ~35–55% and 40% protection, respectively (Table 2). NOD mice treated with anti-BAFF mAb also exhibited strong protection (~70%) in the reported prevention study [20]. However, the anti-BAFF mAb treatment regime, however, differed substantially from other studies in that it was given as a maintenance therapy throughout a large part of the 40-week diabetes incidence study after an initial short-course of high-dose anti-BAFF mAb treatment (Table 2). On the contrary, anti-CD20, anti-CD22/cal and BCMA-Fc treatments were given only as a short-course, thus making the anti-BAFF treatment difficult to compare in this setting. All B cell depletion agents were also capable of retarding the progression of insulitis to some degree [16,17,19–21]. It is worthwhile mentioning two studies that performed a short-term depletion of B cells using anti-CD20 or anti-BAFF in a timeframe where insulitis is marginal in NOD mice (4–6 and 6–8 weeks of age, respectively; Table 2) [16,20]. These treatments resulted in a delay, but not protection from the onset of T1D in NOD mice. Hence, depleting B cells too early in the disease process could provide sufficient time for B cells to regenerate and recommence their pathogenic activity, whereas B cell depletion at a later stage of disease appears to cause a breakdown in the disease process that is irreversible.

### *Disease reversal*

Significant within a clinical context, anti-CD20 and anti-CD22/cal mAb treatments showed efficacy in conferring

**Box 2. Targets of B cell depletion**

CD20 and CD22 are membrane-bound proteins primarily expressed on the surface of B cells whose structure, function and expression are well conserved between humans and mice [28,62]. Expression of both proteins starts at the late pre-B cell stage in the BM (although CD22 is restricted to the cytoplasm at immature stages), is maintained at high levels on the surface of mature B cells in peripheral lymphoid organs, and is extinguished upon differentiation into plasma cells (Table 1). The function of CD20 is not fully elucidated, but it appears to contribute to B cell activation, division and apoptosis through its ability to regulate transmembrane calcium transport [62,63]. CD22 acts as a co-inhibitory molecule due to ITIM domains in its intracellular region that attenuate BCR signaling through the recruitment of the tyrosine phosphatase, SHP-1 [28]. Recent studies have shown that both CD20 and CD22 could be particularly important for the positive and negative regulation of responses towards T-independent antigens in B cells, respectively [64,65].

APRIL (TNFSF13) and BAFF (also known as BLyS and TNFSF13b) are tumor necrosis family (TNF) molecules primarily produced by the innate immune system. These factors exert powerful effects on B cell development, survival and function through their ability to bind three receptors: BAFF receptor (BAFF-R, also known as BR3 or TNFRSF13c); transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI, also known as TNFRSF13b); and BCMA (also known as TNFRSF17) [33]. BAFF-R is expressed widely on B cells (Table 1), and specifically binds BAFF. This interaction leads to the activation of the NF-κB pathway in a way that is crucial for the survival of the B2 lineage B cells beyond the first transitional stage (T1) of maturation in the periphery [33]. In contrast, both BAFF and APRIL share the capacity to bind TACI and BCMA receptors [33]. These receptors display a more restricted expression pattern in B cells (Table 1), with both being present on germinal center B cells, plasmablasts and plasma cells, whereas TACI is also expressed on activated FO B cells, as well as B1 and MZ B cell subsets. The functions of BCMA and TACI remain enigmatic. Although the generation of knockout mice for these receptors has suggested a role for BCMA in the survival of plasma cells in BM, while TACI seems to be important for the generation of T-independent antibody responses, but might also act as an inhibitory receptor for the general B cell population [66,67].

**Table 1. Surface expression of molecules targeted by B cell depletion therapies.**

| Marker | Bone Marrow | | | Periphery | | | | | Antigen Response | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pro | Pre | Imm | T1 | T2 | FO | MZ | B1 | Act | GC | Mem | PB | PC |
| CD20 | – | –/+ | ++++ | ++++ | ++++ | +++ | +++ | +++ | +++ | +++ | +++ | – | – |
| CD22 | – | – [a] | – [a] | + | ++++ | ++++ | ++++ | ++++ | ++++ | ++ | +++ | –/+ [b] | –/+ [b] |
| BAFF-R | + | + | ++ | ++ | +++ | +++ | ++++ | +++ | ++ | ++ | ++ | ++ | – |
| TACI | – | – | – | – | – | – | ++++ | ++++ | ++ | + | + | + | ++ |
| BCMA | – | – | – | – | – | – | – | – | – | + | + | ++ | ++ |

Abbreviations: Imm, immature; T1/T2, transitional 1/2; Act, activated; GC, germinal center; Mem, memory; PB, plasmablast; PC, plasma cell.

[a]Restricted to the cytoplasm.

[b]Only on a proportion of splenic PB and PC, but not BMPC.

long-term reversal of disease in a third and two-thirds of new-onset diabetic NOD mice, respectively (Table 2) [16,19]. Assuming that B cells provide cognate help to self-reactive effector $CD4^+$ T cells [2], these data suggest that because of their ability to cause rapid B cell depletion, administration of anti-CD20 and anti-CD22/cal mAb can mediate the reversal of T1D by collapsing the T cell-mediated attack against islets. Studies with the anti-CD22/cal mAb found that treatment was more effective if B cell depletion was commenced 3 days, rather than 5 days, after the onset of hyperglycemia [19]. Thus, it was perhaps surprising that anti-BAFF mAb was also a capable reversal agent, despite its slow B cell depletion kinetics [20]. Indeed, it proved to be the most potent antibody with regards to long-term reversal in the NOD model (Table 2). One potential explanation for this protection was that treatment in this study was initiated when mice first showed signs of elevated blood glucose (160–200 mg/dl blood glucose, i.e. the 'honeymoon' phase), rather than when they were overtly diabetic (>250 mg/dl). In addition, BAFF blockade might also degrade the ability of B cells to act as APC that drive the activation of effector $CD4^+$ T cells well before B cell reduction [37].

## Mechanisms of protection mediated by B cell depletion

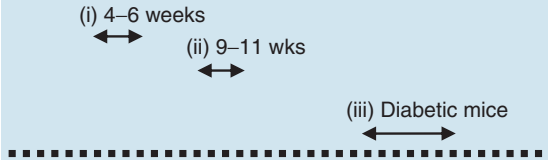
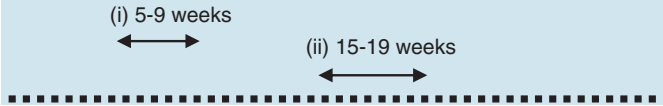
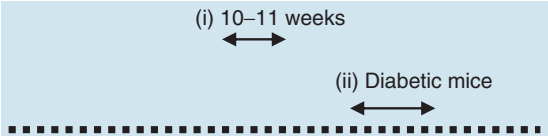
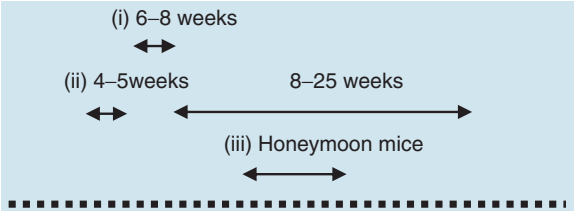
### *Decreased antigen presentation capacity*

B cell depletion might confer protection from T1D by a number of complex, but possibly intertwined mechanisms. Consistent across all B cell depletion studies in NOD mice was the suppression of the self-reactive $CD4^+$ [16,17,19–21], and in some cases $CD8^+$ [17,21], T cell responses. These results agree with the notion that B cells: (i) support self-reactive $CD4^+$ T activation and expansion through direct cognate interactions [5–10]; (ii) provide support for $CD8^+$ T cell differentiation and survival [21,38], most likely through the release of helper cytokines such as IL-21 by activated $CD4^+$ T cells (Figure 1) [39]. It is curious that B cell depletion initiated before the onset of insulitis delayed the disease, but was unable to provide significant protection [16,20]. This could indicate that the involvement of B cells as APC for T cells is more significant at a stage before the transition from subclinical insulitis to hyperglycemia, but not during the early stages of insulitis.

### *Improved self-tolerance*

B cell depletion could also dampen autoimmunity by restoring normal B cell tolerance. Several studies have demonstrated NOD mice to have various defects in self-tolerance mechanisms that permit the survival and function of autoreactive B cells [40–43]. The ability of B cell depletion to correct such defects was evident in the particular study examining the effect of B cell depletion via administration of anti-BAFF mAb, where protection was associated with the restoration of normal B cell negative selection at the transitional to the FO stage of differentiation [20]. This would be predicted to decrease the frequency of self-reactive B cell clones that can contribute to the autoimmune process. Consistent with this observation,

**Table 2. B cell targeting agents and protocols used to prevent or reverse diabetes in NOD mice.**

| Agent | Treatment protocol [a] (Weeks of Age) | Dose regimen | Outcome [b] | Refs |
|---|---|---|---|---|
| Anti-hCD20<br>Murine anti-human<br>CD20 (2H7, IgG1) mAb |  | Female *hCD20*-transgenic NOD mice received 4 i.v. injections (1st 500 μg, then 250 μg) in 3-day intervals. | (i) Delay.<br>(ii) ~35% protection<br>(iii) Hyperglycemia reversed in 36% of newly diabetic mice for 130 days. | [16] |
| Anti-mCD20<br>Murine anti-mouse<br>CD20 (MB20-11, IgG2c)<br>mAb |  | Female NOD mice received 3 i.v. injections (250 μg) in 2-week intervals. | (i) ~55% protection.<br>(ii) ~35% protection. | [17] |
| Anti-CD22/cal mAb<br>Murine anti-CD22<br>(Cy34.1, IgG1) mAb<br>attached to calicheamicin |  | Female NOD mice received 2 i.p. injections (160 μg/kg) 5 days apart. | (i) ~40% protection.<br>(ii) Hyperglycemia reversed in 60% of newly diabetic mice for >100 days. | [19] |
| Anti-BAFF<br>Hamster anti-mouse<br>BAFF (10F4, IgG) mAb |  | Female NOD mice received 2 i.p. injections (100 μg) 5 days apart. In protocol ii, this was followed by a maintenance dose (15 μg bi-weekly) from 8 to 25 weeks of age. In protocol iii, 3 i.p. injections (100 μg) were followed with 50 μg weekly maintenance dose. | (i) Delay.<br>(ii) ~50% protection.<br>(iii) 100% honeymoon mice (160–200 mg/dl blood glucose) prevented from progressing to diabetes (>250 mg/dl). | [20] |
| BCMA-Fc<br>Soluble fusion protein |  | Female NOD Mice received 2 i.p. injections of BCMA-Fc (150 μg) weekly for 6 weeks. | (i) 100% protection. | [21] |

[a] Arrows represent the initiation time and duration of the treatment. Dotted lines represent length of the incidence study.

[b] Roman numerals correspond to those listed in the treatment protocol column. Percentage protection in comparison to the control Ig treated group calculated from the original manuscripts.

treatment with anti-BAFF and anti-CD20 mAb were shown to decrease the production of anti-insulin autoantibodies after the regeneration of the B cell compartment in NOD mice [16,20].

*Enhanced regulatory mechanisms*

As well as dampening the activation of self-reactive $CD4^+$ T cells, B cell depletion also affords protection through activation of regulatory mechanisms (Figure 1). Indeed, BCMA-Fc, anti-hCD20 and anti-CD22/cal treatment resulted in the accumulation of regulatory $CD4^+$ $CD25^+$ $Foxp3^+$ T cells that, in the case of BCMA-Fc, was shown to be responsible for restoring long-term tolerance to islet antigens [16,19,21]. These observations were consistent with regulatory T cells in B cell-deficient NOD.$Ig\mu^{null}$ mice, being crucial for maintaining resistance to T1D [21]. Together, the aforementioned studies would suggest that in the absence of B cells, presentation of autoantigens by other APC (i.e. dendritic cells and macrophages) leads to a balanced generation of regulatory and effector T cells, whereas participation of self-reactive B cells as APC might tip the scales in favor of effector T cells. Interestingly, B cell depletion by anti-BAFF or mCD20 mAb did not increase regulatory T cells [17,20]. Whether this difference is related to the type of B cell populations targeted by these agents, or alternatively, the ability to block BAFF but not APRIL, requires further investigation.

In addition to the induction of regulatory T cells, anti-hCD20 and anti-CD22/cal mAb treatment of NOD mice also enhanced the reconstitution of a B cell subpopulation with features reminiscent of regulatory B cells [16,19] that exert immunomodulatory functions in other autoimmune disease models [44]. These might also overlap with undefined populations of regulatory B cells found in the spleen of NOD mice that have the capacity to dampen β cell autoimmunity, after being subjected to *in vitro* activation with anti-IgM cross-linking antibodies or lipopolysaccharide (LPS) [14,15]. Regulatory functions of NOD B cells in these studies were mediated by the expression of IL-10 (after anti-IgM activation) or TGF-β and Fas ligand (after LPS activation). It is possible that the expansion of a regulatory B cell subset after depletion could be explained by the increased availability of BAFF, which has recently been shown to be potent at inducing the production of such cells [45].

## Clinical development of B cell-depleting agents

With this background, it is worthwhile considering where we are in terms of the clinical development of these compounds. Although BCMA-Fc has not yet been used in human trials, other variants of BAFF inhibitors have. Indeed, human anti-BAFF mAb LymphoStat-B® (belimumab) by Human Genome Sciences, Inc., is being tested in a phase III clinical trial for systemic lupus erythematosus (SLE) [46,47]. TACI-Ig (atacicept) developed by ZymoGenetics, Inc. (which can also block BAFF and APRIL), is under clinical trials in SLE and rheumatoid arthritis (RA) patients [48,49]. The anti-CD20 mAb rituximab (Biogen Idec/Genentech) has been used for the treatment of human B cell malignancies and autoimmune diseases such as RA, idiopathic thrombocytopenic purpura, hemolytic anemia, SLE and pemphigus vulgaris [50]. A phase IIb clinical trial for the treatment of SLE is now underway to evaluate the effects of a CD22 mAb (epratuzumab, IMMU-103; Immunomedics, Inc.) [51]. Of these agents, only rituximab has been tested in T1D subjects [18].

## Concluding remarks: B cell depletion as therapy for human T1D

Studies in NOD mice have resulted in B cells becoming promising therapeutic targets for the prevention and reversal of T1D in humans. Indeed, T1D patients treated with a four-dose course of rituximab within 80 days of clinical diagnosis showed partially preserved β cell function over a period of 1 year [18]. The positive results of this study support the concept that targeting B cells, with one or more of the newly emerging agents, could have therapeutic benefit for preventing and reversing T1D. In human trials, one might predict that faster acting depleting agents, such as anti-CD20 and anti-CD22/cal mAb, would be more efficacious in the setting of new-onset diabetes, or perhaps during the so-called 'honeymoon' phase. This is not entirely supported by the current animal studies, as anti-BAFF mAb was also able to reverse the onset of hyperglycemia [20]. By contrast, one would expect all these proposed treatments, that is mAb directed against B cell surface proteins as well as BAFF and APRIL inhibitors, to be beneficial when used in prevention strategies. Nevertheless, it is important to note that these interpretations are based on results in NOD mice. It remains to be seen how the relatively fast tempo of disease progression in the NOD model relates to the tempo of disease progression in humans. Indeed, given the broad 'honeymoon' period observed clinically, one might predict that the therapeutic window in humans would be greater than that observed in NOD mice. However, extended regimens of B cell depletion might be required to achieve long-term protection, an observation supported by the discussion of results in the rituximab clinical trial [18]. Although at this time only rituximab has been trialed in humans, the NOD studies support the testing of other agents alone, and possibly in combination. For instance, the rapid B cell depletion achieved with anti-CD20 mAb-based approaches could be coupled with the ability of BCMA-Fc to restore immune tolerance to islet antigens. It is also of interest to consider whether B cell depletion might be combined with other short-term therapies such as T cell ablation. T cell directed therapies using anti-CD3 mAb shown promise for the reversal of T1D in trials [52,53]. Combining B cell depletion to prevent B cell antigen presentation to self-reactive T cells, with T cell depletion to directly ablate existent T effector cells, might provide a powerful therapeutic combination. Finally, the demonstration that B cell depletion induces regulatory populations, such as that achieved with BCMA-Fc, offers potential new avenues to restore tolerance to islet antigens. This property could be exploited by combining B cell depletion and immunization with insulin or other autoantigens [54] to enhance antigen-specific tolerance. The wealth of emerging B cell-depleting agents with clinical potential now sets the stage for an exciting new era in curative and preventative strategies for T1D.

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