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Emerging roles for B lymphocytes in Type 1 diabetes

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Self-reactive B lymphocytes play two main pathological roles in autoimmune diseases: as secretors of autoantibodies and as specialized antigen-presenting cells that present self-components to autoreactive T lymphocytes. In recognition of these roles, recent clinical trials have utilized B-lymphocyte-depleting monoclonal antibodies to treat various autoimmune diseases, with encouraging results in those where humoral autoimmunity is clearly important. Surprisingly, recent results in animal models suggest that B-lymphocyte depletion may also be effective in the treatment of T-lymphocyte-mediated autoimmune diseases, such as Type 1 diabetes (T1D). This article reviews the experimental evidence that has uncovered pathogenic as well as regulatory roles for B lymphocytes in the prodrome of T1D and how this information is being used to develop novel therapeutic strategies to treat the disease.

Keywords: antigen-presenting cell • autoantibody • autoimmunity • B cell • immune tolerance • NOD mouse • susceptibility gene • Type 1 diabetes

Type 1 diabetes (T1D) is caused by immune infiltration of the pancreatic islets of Langerhans (referred to as insulitis) followed by specific autoimmune-mediated destruction of the insulin-secreting β-cells within them [1]. Predisposition to T1D depends on the interaction of multiple disease-susceptibility genes and unspecified environmental factors [2]. While regular insulin administration can temporarily halt the fatal outcome of this disease, the diabetic patient remains predisposed to long-term and potentially life-threatening complications involving the vascular and nervous systems, eyes and kidneys [3]. It is therefore essential that new therapeutic strategies are developed that prevent the immune system from attacking the β-cells, thereby averting the onset of T1D in susceptible individuals or reversing disease in newly diagnosed patients who still have remaining β-cell activity.

Lack of access to relevant tissue samples from patients at high risk of developing T1D or those with recent-onset disease have made animal models essential for understanding the pathogenesis of this disease. The most useful of these has been the nonobese diabetic (NOD) inbred mouse strain, which spontaneously develops a naturally high incidence of T1D of between 10 and 30 weeks of age and shares many similarities with the human disease [4,5]. In humans and NOD mice developing T1D, autoreactivity towards the same islet autoantigens can be detected in both T- and B-lymphocyte compartments [6], with both cell types also constituting significant parts of the insulitic lesions within the pancreas [7,8]. Adoptive-transfer experiments from diabetic into young prediabetic or immunodeficient NOD mice clearly indicate that $CD4^+$ and $CD8^+$ subsets of T lymphocytes, rather than autoantibodies, are directly responsible for β-cell destruction in T1D [9–12]. As a consequence, the presence of activated autoreactive B lymphocytes producing β-cell-specific autoantibodies was believed to be a secondary phenomenon of T-lymphocyte activation, with little or no effect on T1D. However, while B lymphocytes and their products are not directly pathogenic to β-cells, emerging evidence in the NOD mouse model has revealed that they play important accessory roles in the development of T1D.

Evidence of a pathogenic role for B lymphocytes in T1D

A role for B lymphocytes in the development of T1D became evident when a mutation in the *immunoglobulin (Ig)μ* gene abrogating their production was backcrossed onto the NOD

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background, rendering the resulting animals (termed NOD.*Igμ*^{null} mice) strongly T1D resistant [13,14]. Mice in these studies were virtually free of insulitis at the end of the study (20 weeks), suggesting that disease was not merely delayed, but that destructive T-lymphocyte responses to β-cells were not being initiated. Reconstitution of NOD.*Igμ*^{null} mice with syngeneic bone marrow (BM) together with B lymphocytes completely restored their susceptibility to T1D, confirming the pathogenic role of B lymphocytes [12]. Similar observations were made by Noorchashm and colleagues, who used polyclonal anti-Igμ antibodies to deplete NOD mice of B lymphocytes [15]. Chronic *in vivo* B-lymphocyte depletion from birth until 30 weeks of age resulted in complete abrogation of insulitis in NOD mice. Cessation of anti-Igμ antibody treatment at 8 weeks of age led to full reconstitution of the B-lymphocyte pool and reappearance of insulitis after 10 weeks but, interestingly, none of these mice developed T1D.

Compared with the strong resistance to insulitis and T1D shown by B-lymphocyte-deficient NOD mice in the above studies, Yang and colleagues described another independent line of NOD.*Igμ*^{null} mice that developed a moderate incidence of disease (29%), although this was still significantly lower than their B-lymphocyte-sufficient littermates (70%) [16]. Other more recent studies have also detected a low incidence of T1D (1–17%) in NOD.*Igμ*^{null} mice used as controls [17,18]. These results point to the existence of certain environmental conditions and/or genetic variations that, on rare occasions, allow B lymphocytes to be bypassed during the development of T1D. Thus, while B lymphocytes appear to play an important diabetogenic role in NOD mice, they are not always critical for T1D development.

Pathogenic roles for B lymphocytes in T1D

Secretion of autoantibodies

Autoantibodies specific for β-cell proteins, including insulin, glutamic acid decarboxylase (GAD), tyrosine phosphatase IA-2 and the zinc transporter 8 are regularly detected in human subjects who eventually develop T1D and, together with major histocompatibility complex (MHC) haplotypes, currently serve as the best prognostic indicators of disease onset [19–22]. Greater numbers of these autoantibody specificities are associated with an increased risk of diabetes development [22,23]. Similar observations have been made in NOD mice [24–27].

It is unlikely that autoantibodies themselves have direct pathogenic effects on β-cells *in vivo*, given that chronic infusion of IgG from diabetic NOD donors between 8 and 20 weeks of age failed to confer insulitis or T1D susceptibility in NOD.*Igμ*^{null} mice [12]. However, autoantibodies do appear to play an indirect role in the development of disease, given that the incidence of T1D in NOD mice lacking expression of activating Fcγ receptors (FcγRs) was significantly reduced [28]. The diabetogenic effects resulting from the binding of antibody–antigen complexes to activating FcγRs were found to be mediated by dendritic cells (DCs) and natural killer (NK) cells, since adoptive transfer of wild-type forms of either cell type restored full diabetes susceptibility in FcγR-deficient NOD mice. Activating FcγRs promoted disease by triggering antibody-dependent effector functions of NK cells, as well as enhancing the uptake of autoantigens that DCs can process for presentation to T lymphocytes. Of therapeutic interest, blocking activating FcγRs through treatment with intravenous polyspecific γ-globulin (IVIg) was found to significantly delay and prevent diabetes onset in NOD mice [28], as well as demonstrating beneficial effects in a subset of children with recent-onset T1D [29].

In 2002, Greeley and colleagues conducted a fascinating study showing that transmission of autoantibodies from NOD mothers could confer a significantly increased risk of T1D in their progeny [30]. A follow-up study revealed that transmission of pathogenic autoantibodies occurred during the prenatal period, as T1D susceptibility did not differ in pups fostered onto B-lymphocyte-deficient or -sufficient NOD dams after birth [31]. Whether or not this mechanism of pathogenesis is relevant to humans is unclear, given that:

- Children of diabetic fathers are more susceptible to T1D than those with diabetic mothers [32];
- Neonates with transiently high levels of β-cell autoantibodies were not rendered more susceptible to T1D [33];
- In a study where a large cohort of T1D-susceptible children were monitored over several years for disease development, autoantibody transfer from diabetic mothers to their children was actually found to marginally decrease the risk of developing T1D [34].

Antigen presentation to MHC class II-restricted CD4$^+$ T lymphocytes

Along with DCs and macrophages, B lymphocytes have the capacity to act as antigen-presenting cells (APCs) for MHC class II-restricted CD4$^+$ T lymphocytes. The fact that self-antigens targeted by autoantibodies in NOD mice and T1D-prone humans are also those targeted by autoreactive T lymphocytes [6] implies that productive interactions between B and T lymphocytes occur in the development of disease. Whether this interaction was causative or secondary to disease pathology was not clear until subsequent studies of NOD.*Igμ*^{null} mice revealed poor CD4$^+$ T-lymphocyte responses to various T1D autoantigens, including GAD, proinsulin and IA-2, compared with B-lymphocyte-sufficient NOD mice, despite showing comparable responses to certain foreign antigens [12,35,36]. Autoreactive CD4$^+$ T-lymphocyte responses and, consequently, the development of insulitis and T1D could all be restored by reconstituting NOD.*Igμ*^{null} mice with syngeneic BM and NOD B lymphocytes, but not autoantibodies, highlighting the important antigen-presenting role of B lymphocytes in this model [12]. Further support for their key diabetogenic role as APCs for CD4$^+$ T lymphocytes was provided in a study showing strong protection from T1D in NOD mice whose B lymphocytes (but not other APCs) were selectively rendered deficient in MHC class II molecules [37].

All APC subsets can internalize exogenous protein by endocytosis for presentation of the resulting peptides via the MHC class II pathway [38]. However, only B lymphocytes are capable of specific capture of proteins through surface immunoglobulins that comprise B-cell receptors (BCRs) [39]. This mechanism increases presentation of captured antigens on MHC class II molecules by

up to three orders of magnitude compared with endocytosis [40]. To examine if capture of autoantigens by BCRs was necessary for B lymphocytes to act as diabetogenic APCs, NOD mice were generated expressing a transgenic Ig (termed *IgHEL*) specific for the T1D-irrelevant protein hen egg lysozyme (HEL) in combination with the *Igμ*^{null} mutation. This combination rendered all B lymphocytes in NOD mice specific for HEL and thus incapable of taking up β-cell autoantigens through BCRs [17]. Similar to control B-lymphocyte-deficient NOD.*Igμ*^{null} mice, NOD.*IgHEL.Igμ*^{null} mice mounted poor CD4$^+$ T-lymphocyte responses to β-cell autoantigens and remained mostly T1D resistant. Conversely, Hulbert and colleagues demonstrated that introduction of an Ig heavy-chain transgene (*VH125*) into NOD mice, which increased the frequency of B lymphocytes recognizing the T1D insulin autoantigen, resulted in accelerated onset of T1D [41]. Another study showed that transgenic NOD mice containing B lymphocytes able to produce membrane-bound BCRs but not secreted antibodies could still develop insulitis and T1D at increased levels compared with B-lymphocyte-deficient control mice [18]. Together, these studies demonstrate that the role of B lymphocytes as APCs for autoreactive CD4$^+$ T lymphocytes relies on the unique ability of certain clones to specifically capture β-cell autoantigens through membrane-bound BCRs, raising the prospect that T1D susceptibility is caused by defects in self tolerance at the level of B as well as T lymphocytes.

Mild insulitic infiltrates eventually develop after 30 weeks of age in NOD.*Igμ*^{null} mice [42] (i.e., after the vast majority of NOD mice have already developed T1D). Hence, in the absence of B lymphocytes, T lymphocytes with the capacity to target β-cells can be generated and primed, but in the majority of cases fail to cause destruction. T lymphocytes from strongly T1D-resistant colonies of NOD.*Igμ*^{null} mice could be induced to cause disease if transferred into T- and B-lymphocyte-deficient severe combined immunodeficiency (SCID) mutant mice, albeit at a reduced incidence compared with T lymphocytes from wild-type NOD mice [43]. Antigen presentation by B lymphocytes could therefore be bypassed if β-cell-reactive T lymphocytes were allowed to undergo homeostatic expansion induced by lymphopenic conditions. This may also explain why various T-cell receptor (TCR) transgenic models of T1D, which have artificially expanded levels of CD4$^+$ β-cell-specific T lymphocytes, can progress to overt T1D without requiring the presence of B lymphocytes [44–46]. Once autoreactive CD4$^+$ T lymphocytes have been expanded in B-lymphocyte-sufficient hosts, their activation and proliferation can be maintained *in vitro* by other APCs, whereas this was not the case for CD4$^+$ T lymphocytes derived from NOD.*Igμ*^{null} mice [12]. Collectively, these observations suggest that while other APCs (e.g., DCs and macrophages) are capable of activating autoreactive CD4$^+$ T lymphocytes, B lymphocytes appear to be necessary for mediating their expansion to the pathogenic levels required for β-cell destruction. The reliance on B lymphocytes as the dominant APC for the expansion of CD4$^+$ T lymphocytes in NOD mice may be partly attributed to developmental and maturation defects in DCs and macrophages in this strain [47–50]. These defects have been shown to diminish the capacity of NOD mice DCs and macrophages to provide appropriate costimulatory stimuli for T lymphocytes, while not affecting the capacity of B lymphocytes [51]. Interestingly, similar defects in DCs and macrophages are also a feature in many humans diabetics [52–55].

In addition to their expansion, B lymphocytes are also involved in 'spreading' of the CD4$^+$ T-lymphocyte responses to different β-cell antigens in NOD mice. This was demonstrated in NOD.*scid* and NOD.*Igμ*^{null} mice reconstituted with T and B lymphocytes, which spontaneously formed successive Th1 responses to GAD, heat-shock protein (HSP) 277 and insulin β-chain autoantigens, whereas mice reconstituted with only T lymphocytes did not undergo antigen spreading, even after the animals were immunized with the individual autoantigens [56].

Promotion of cytotoxic T-lymphocyte effector functions

Nonobese diabetic mice expressing a TNF transgene under the rat insulin promoter (*RIP-TNF*) develop an accelerated form of T1D that is dependent on CD8$^+$ but not CD4$^+$ T lymphocytes [57]. Brodie *et al.* discovered that introduction of an *Igμ*^{null} mutation significantly delayed T1D onset in this model [58], indicating that B lymphocytes could promote CD8$^+$ T-lymphocyte-mediated β-cell destruction. This function is independent of antibody secretion, given that NOD.*RIP-TNF* mice containing transgenic B lymphocytes capable of only expressing membrane-bound Ig restored accelerated disease kinetics. Comparison of *Igμ*-sufficient and -deficient NOD.*RIP-TNF* mice revealed that B lymphocytes support the expansion and differentiation of CD8$^+$ T lymphocytes into cytotoxic T lymphocytes (CTLs) in the pancreatic lymph nodes (PLNs), while increasing survival of CTLs in the intraislet environment. Whether these effects are mediated by direct (e.g., antigen presentation) or indirect (e.g., cytokines or regulation of other APCs) mechanisms remains to be resolved.

Organization of secondary lymphoid organs & tertiary lymphoid structures within the pancreas

Through their production of key factors such as lymphotoxin (LT)-$\alpha_1\beta_2$, B lymphocytes play a critical role in the in the organization of secondary lymphoid tissues [59,60]. Of these, PLNs are a critical site for the activation and expansion of diabetogenic CD4$^+$ T lymphocytes in the early prodromal stages of T1D in NOD mice [61]. B lymphocytes have been shown to be essential for the proliferation of diabetogenic CD4$^+$ T lymphocytes at this site [42]. The spleen may also pose as a site for early β-cell reactive CD4$^+$ T-lymphocyte expansion via B lymphocytes, given the increased capacity of NOD splenocytes to transfer disease compared with PLN cells [62]. However, neither the spleen nor PLNs are required during the late effector phase of T1D, as excision of either at 10 weeks of age did not halt progression of disease [61]. Alternative lymphoid structures must therefore take over as primary sites of B- and T-lymphocyte interactions.

B lymphocytes are one of the earliest cell types to infiltrate the pancreas during the initial stages of T1D development in NOD mice [63,64]. Here, LT-$\alpha_1\beta_2$ and TNF superfamily member (TNFSF) are secreted and bind to lymphotoxin β-receptors (LTβRs) on pancreatic stromal cells, causing the production of key chemokines (i.e., CCL21 and CCL19) and adhesion molecules

(e.g., ICAM, VCAM and MadCAM-1). Collectively, these factors potentiate the recruitment of lymphocytes, DCs and follicular DCs (FDCs) to the insulitic lesions, and orchestrate their organization into distinct T- and B-lymphocyte zones, resembling those of secondary lymphoid organs [65,66]. Such tertiary lymphoid structures permit maximum exposure of T and B lymphocytes to highly concentrated and diversified β-cell antigens, which enhances their activation, interaction in the form of germinal centers and affinity maturation. Indeed, analysis of the B-lymphocyte repertoire within tertiary lymphoid structures indicated that they differed markedly from draining PLNs or spleens, and contained distinct shared mutations in Ig light-chain genes indicative of antigen selection and clonal expansion [7]. Detection of these organized structures was associated with progression from a benign to an invasive form of insulitis associated with β-cell destruction [7]. Tertiary lymphoid structures appear to have a critical bearing on T1D development in NOD mice, as their dissolution by the lymphotoxin signaling blockade was found to exert a strongly protective effect, even at a very late prodromal stage of the disease [67,68]. Conversely, artificial generation of these structures through the transgenic expression of TNFSF14 or LT by β-cells gave rise to accelerated development of T1D in NOD mice and the development of insulitis in nonautoimmune-prone strains, respectively [68,69].

Self-tolerance defects that give rise to diabetogenic B lymphocytes

The findings described above pointed to activation of self-reactive B lymphocytes in T1D-prone NOD mice. However, they do not provide a direct answer to the question of whether self tolerance is defective at the level of B lymphocytes. Transgenic expression of Ig specific for native or neoself antigens has provided significant insights into the mechanisms of self-tolerance that normally prevent the development or functional activation of autoreactive B lymphocytes in nonautoimmune-prone mouse strains (reviewed in [70]). In order to identify the nature of any putative defects in B-lymphocyte tolerance in NOD mice, we introduced transgenes expressing high-avidity multivalent membrane-bound hen egg-white lysozyme (mHEL) or lower-avidity oligovalent-soluble (sHEL) forms of HEL as ubiquitous neoself antigens into NOD.*IgHEL* mice that predominantly produce HEL-specific B lymphocytes [17,71]. In comparison to nonautoimmune-prone C57BL/6 (B6) mice expressing identical transgenes, NOD mice were found to be equally adept at deleting or receptor editing B lymphocytes that recognized mHEL within the BM [71]. By contrast, the presence of sHEL rendered NOD *IgHEL* immature B lymphocytes less susceptible to deletion than their B6 counterparts. Furthermore, while self-reactive NOD and B6 B lymphocytes surviving deletion displayed features of functional anergy, including downregulation of surface IgM, loss of marginal zone (MZ) B lymphocytes and decreased production of antibodies, this state was readily reversed in the former, but not in the latter, by stimulation of BCR and CD40 receptors. Thus, impaired partial-deletion and anergy mechanisms could account for the breakdown of tolerance in NOD B lymphocytes when exposed to low-avidity (i.e., soluble) self antigen(s).

To determine the relevance of these defects to the generation of autoreactive B lymphocytes specific for pancreatic β-cell antigens, we recently developed a model whereby NOD and B6 HEL-specific B lymphocytes were transferred into hosts expressing the neoself antigen mHEL under an insulin promoter (*insHEL*). Although primarily restricted to β-cells of the pancreas, the HEL antigen in this model can also be found in the circulation at similar levels to those of insulin [72]. On both backgrounds, transgenic B lymphocytes transferred into *insHEL* transgenic hosts showed characteristics consistent with anergy. However, similar to our previous observations, B lymphocytes from NOD but not B6 mice could be rescued from anergy upon provision of *in vivo* T-lymphocyte help, resulting in equivalent levels of survival and activation as those transferred into wild-type hosts (Cox SL, Silveira PA, Unpublished Data). Acevedo-Suarez and colleagues have also examined B-lymphocyte tolerance to native β-cell antigens using an alternative approach of introducing insulin-specific Ig heavy- and light-chain transgenes (*125Tg*) into NOD and B6 mice [73]. *125Tg* B lymphocytes were rendered anergic on both NOD and B6 backgrounds, but on this occasion responded equally to signals delivered through BCR, TLR4 and CD40. Moreover, *125Tg* B lymphocytes on both backgrounds failed to respond to T-lymphocyte immunization and produced no spontaneous anti-insulin antibodies. Compared with the B-lymphocyte anergic state observed in other Ig transgenic models, *125Tg* B lymphocytes on both backgrounds were not developmentally arrested and exhibited a twofold increase, rather than a decrease, in the MZ resident population. These findings seemingly contradict observations in nontransgenic animals, where NOD mice produce significantly elevated levels of anti-insulin autoantibodies compared with B6 mice [74], which is indicative of faulty B-lymphocyte tolerance to this autoantigen. However, it is possible that such discrepancies result from differences in specificity or affinity of the *125Tg* clones compared with the autoimmune clones normally targeting insulin in NOD mice, especially since the B-lymphocyte clone expressing the original *125Tg* specificity was derived from a nonautoimmune-prone strain immunized with human insulin. Interestingly, utilizing only the heavy-chain Ig of the *125Tg*, thus allowing random pairing with endogenous Ig light chains, B lymphocytes with insulin specificity were only detectable in NOD but not in B6 mice, indicating that tolerogenic mechanisms against insulin-specific B lymphocytes were indeed less stringent in the former strain [75].

Upon completing their differentiation in the BM, immature B lymphocytes migrate to the spleen in a transitional (TR) state, where they are subjected to a secondary checkpoint of tolerance to peripheral self antigens [76]. Survival of TR B lymphocytes and their maturation into either follicular (FO) or MZ subsets (see next section) is a competitive process that is dominantly regulated by a cytokine called the B-lymphocyte Activation Factor from the TNF Family (BAFF, also known as BLyS) [77]. Although BM genesis of B lymphocytes seems adequate in NOD mice, various defects have been ascribed to the TR stages of development in this strain, which is likely to be a nidus for the accumulation of autoreactive B lymphocytes. Firstly, the numbers of TR B lymphocytes able to migrate to the spleen in NOD mice are greatly diminished [71,78], thus reducing their competition for BAFF.

Greater access to this survival factor may underlie other defects in NOD TR B lymphocytes, including their increased resistance to deletion upon BCR stimulation [71], faulty negative selection of Igλ-expressing autoreactive clones and accelerated differentiation into more mature ($CD23^+$) TR stages that express higher levels of apoptosis-resistance proteins (e.g., Bcl-2) [78]. Compared with nonautoimmune-prone mouse strains, Quinn and colleagues found that the production rates of TR B lymphocytes were nearly identical to those of FO subsets in NOD mice, signifying that almost all TR B lymphocytes reach maturity and thereby avoid this key checkpoint of peripheral self tolerance [78].

Given the presence of various autoantibody specificities in T1D patients, it is likely that they also possess defects in B-lymphocyte tolerance. Whether the same tolerance defects identified in NOD mice are mirrored in humans developing T1D is only starting to be investigated. In one of the first studies to address this issue, Panigrahi and colleagues developed a novel technique that sensitively measured attempts of B lymphocytes to undergo additional Igκ or λ gene rearrangements associated with receptor editing [79]. Employing this technique, they could detect deficiencies in receptor editing of polyclonal B lymphocytes from NOD compared with nonautoimmune-prone B6 mice. Importantly, they showed that approximately 30–45% of T1D patients also exhibited low levels of receptor editing in their B lymphocytes compared with 8–12% of healthy controls, which may partly explain the increased escape of self-reactive clones in humans with T1D [79]. Furthermore, the recent identification of a phenotype associated with anergy in the human polyclonal B-lymphocyte repertoire ($IgD^+IgM^-CD27^-$; termed the B_{ND} population) will undoubtedly allow future comparisons of this tolerance state in T1D patients versus healthy controls [80].

B-lymphocyte subsets participating in the pathogenesis of T1D

Follicular subset

Follicular B lymphocytes occupy follicular areas of lymphoid organs that are situated around the T-lymphocyte-enriched periarteriolar lymphoid sheath (PALS), and are thus in an ideal position to interact with diabetogenic T lymphocytes, resulting in the formation of self-reactive proliferative foci and germinal centers [81]. These lymphocytes also have the unique capacity to migrate between lymphoid and inflamed organs, such as PLNs and the pancreas, which are critical sites for priming autoreactive $CD4^+$ T lymphocytes in T1D [7,61,67,68].

MZ subset

A second major population of B lymphocytes (termed MZ B lymphocytes) is located within the marginal zones of the spleen and has also been shown to contain a potentially diabetogenic population in NOD mice. MZ B lymphocytes normally mount rapid antibody responses to blood-borne antigens and are essential for shuttling opsonized antigens into follicular areas for recognition by other B lymphocytes [82,83]. Although commonly associated with T-lymphocyte-independent antibody responses, MZ B lymphocytes can act as potent APCs for naive $CD4^+$ T lymphocytes [84]. This population harbors increased frequencies of B lymphocytes with self-reactive specificities and has been linked to numerous autoimmune conditions [77,83]. Of relevance to T1D, transgenic B-lymphocyte clones specific for the insulin autoantigen were shown to be preferentially selected into the MZ compartment in mice with NOD and B6 genetic backgrounds [73]. Compared with follicular B lymphocytes, this population also displays an enhanced ability to present insulin to autoreactive T lymphocytes and elicit a proliferative response [85]. NOD mice of all ages possess significantly expanded levels of MZ B lymphocytes compared with many nonautoimmune-prone strains [71,86], and these cells undergo a large secondary expansion prior to the onset of T1D [85]. Aberrant migration of activated MZ-like B lymphocytes into the PLNs and the pancreas of NOD mice can also be observed during the late stages of T1D development [85]. Finally, antibody-mediated depletion of this population with anti-CR1/CR2 antibodies resulted in a reduction in the incidence of ciclophosphamide-induced diabetes in NOD mice [87].

B-1 subset

The innate-like B-1 B-lymphocyte populations that reside within peritoneal and pleural cavities have also been implicated in the pathogenesis of T1D. This was initially implied by the high proportion of insulin-binding antibodies in NOD mice that have characteristics of the natural repertoire associated with B-1 B lymphocytes [88]. Furthermore, a significant proportion B lymphocytes infiltrating the pancreas express a phenotype ($CD5^+B220^{low}$) that is characteristic of the B-1 population [89]. Intraperitoneal hypotonic lysis to deplete the B-1-cell population resulted in a decrease in the B-lymphocyte component of pancreatic infiltrates, a substantial reduction in insulin-specific autoantibodies, and a delay in the onset of, and some protection from, T1D, underlining a potentially important contribution of peritoneal B-1 B lymphocytes to disease pathogenesis [89].

Genetic factors underlying the pathogenesis of B lymphocytes in T1D

Predisposition to T1D in NOD mice is determined by more than 20 genes (termed *Idd* genes) mapped to various locations throughout the genome [4]. The important role played by B lymphocytes in the development of T1D made it likely that some of these *Idd* genes should contribute to this pathogenic function. MHC alleles within the NOD $H2^{g7}$ haplotype (*Idd1*) serve as the most important susceptibility component contributing to T1D in these animals by permitting presentation of specific self peptides to $CD8^+$ and $CD4^+$ T lymphocytes. To confirm a role for MHC in regulating pathogenic B-lymphocyte functions, expression of whole MHC haplotypes ($H2^{nb1}$) or single MHC class II alleles ($H\text{-}2Ea^k$) known to be associated with diabetes resistance was restricted to B lymphocytes in NOD mice [90,91]. The result of this manoeuvre was significant protection from T1D. In addition, NOD.Igu^{null} mice reconstituted with B lymphocytes from nonobese resistant (NOR) mice (a T1D-resistant strain sharing ~88% genetic identity with NOD mice, including the $H2^{g7}$ MHC haplotype) showed a significantly lower T1D incidence than those reconstituted with NOD B lymphocytes [92], indicating that non-MHC *Idd* genes also contribute to the

diabetogenic function of B lymphocytes. T1D resistance in NOR mice is mainly mediated by genes located on chromosomes (Chr.) 1, 2 and 4 [93]. Only NOD B lymphocytes containing the NOR Chr. 4 *Idd* resistance region could reduce T1D incidence in NOD.*Igμ*^{null} recipients to a similar extent as NOR B lymphocytes [92]. This Chr. 4 region overlaps with previously identified *Idd9* and *Idd11* susceptibility loci originally identified in crosses between NOD mice and other nonautoimmune-prone strains [94,95]. We also discovered that a congenic region from nondiabetic C57BL/10 mice, encompassing three susceptibility loci on Chr. 1 (named *Idd5.1–5.3*), also reduced the diabetogenic activity of NOD mice B lymphocytes [92]. In order to identify the mechanism(s) by which *Idd5* and *Idd9/11* genes regulate the development of diabetogenic B lymphocytes, we compared various B-lymphocyte phenotypes in NOD mice versus NOD congenic mice containing resistance variants of these loci [92]. Although we noted no significant differences in B-lymphocyte subsets in different lymphoid organs, we did find that *Idd9/11*^{NOR} genes were associated with increased responsiveness of NOD B lymphocytes. More significantly, by comparing responsiveness to BCR plus CD40 stimulation in anergic B lymphocytes from mice expressing *IgHEL and sHEL* transgenes, we discovered that *Idd5* and *Idd9/11* resistance loci both corrected impaired B-lymphocyte anergy in NOD mice. These findings support the link between impaired B-lymphocyte tolerance and the development of T1D in NOD mice. Identification of the specific genes controlling diabetogenic B lymphocytes within these *Idd* loci is ongoing.

Genetic analysis has also suggested that aberrant production of MZ B lymphocytes in NOD mice was largely due to the *Idd11* susceptibility locus on Chr. 4 [86]. However, this was not confirmed in either our or others' NOD congenic mice containing T1D resistance genes at this region [92,96]. Given that suggestive evidence of linkage to the expanded MZ population was also detected on Chr. 1, 9 and 12, it remains to be determined whether this particular phenotype is under the epistatic control of products encoded inside and outside the *Idd11* region.

Evidence of regulatory roles for B lymphocytes in T1D

Regulatory B lymphocyte populations

Recently, evidence has been presented indicating that some B lymphocytes may act to inhibit, rather than to enhance, T1D development. A small regulatory subset of B lymphocytes (Bregs) has been identified in mice (and humans), which can be differentiated from other B lymphocytes by the coexpression of CD5 and CD1d and increased production of the immunoregulatory cytokine, IL-10 [97,98]. These cells share markers with MZ and B-1 B lymphocytes and may represent branches of a common lineage. However, compared with B-1 and MZ B lymphocytes, Bregs prevent T-lymphocyte-mediated inflammatory responses, possibly by inducing T regulatory (Treg) subsets [99]. Of particular interest, studies have shown that Bregs have the potential to protect animals from developing autoimmune diseases, including rheumatoid arthritis (RA) and experimental autoimmune encephalomyelitis (EAE) [100,101]. Although the functional status of the natural Breg population has not been characterized in NOD mice, one study demonstrated that NOD B lymphocytes activated *in vitro* with crosslinking anti-IgM antibodies could delay and inhibit the development of T1D when injected back into NOD mice at an early stage of disease [102]. Consistent with the involvement of Bregs, this protection was dependent on the production of IL-10 by transferred B lymphocytes. Similar protective effects were observed by another group who activated NOD B lymphocytes *in vitro* with lipopolysaccharides (LPS) binding to TLR-4 [103]. Compared with the previous study, the authors' data suggest that significantly increased expression of Fas ligand (FasL) and TGF-β by LPS-activated B lymphocytes led to the inhibition of β-cell pathology by inducing apoptosis of Th1-differentiated T lymphocytes and inhibiting general APC activity, respectively.

Activation of regulatory NK T lymphocytes

Natural killer (NK) T cells are an invariant population of T lymphocytes recognizing lipid and glycolipid antigens that share various markers with NK cells [104]. They play an important suppressive role in the development of autoimmunity by virtue of their ability to rapidly secrete large amounts of anti-inflammatory cytokines upon activation, including IL-4 and IL-10. Deficiencies in the numbers and regulatory functions of NK T cells in NOD mice have been established as T1D susceptibility factors, since augmentation of their numbers or activation state confers strong protection from disease [105]. B lymphocytes are the most frequent cell type expressing CD1d, a MHC class I-like molecule that serves as a restriction element necessary for the presentation of lipid and glycolipid antigens to NK T cells [106]. Capture of glycolipid antigens by BCRs can also increase the capacity of B lymphocytes to present to NK T cells by two-to-three orders of magnitude [107]. MZ B lymphocytes, which express high levels of CD1d, seem particularly adept at presenting antigens to NK T cells [106]. Bezbradica *et al.* investigated the capacity of different APCs to activate NK T cells, and found that in nonautoimmune-prone mice, DCs were most effective at activating NK T cells, resulting in the production of both Th1 (proinflammatory) and Th2 (anti-inflammatory) cytokines [108]. B lymphocytes, on the other hand, only stimulated Th2 cytokines, while suppressing DC presentation to NK T cells. In NOD mice, DCs were revealed to be defective in activating NK T cells, whereas B lymphocytes remained capable of eliciting secretion of Th2 cytokines from these cells [108]. Although B-lymphocyte-mediated activation of NK T cells may not be sufficient to prevent T1D in NOD mice owing to their normally diminished numbers, the accelerated kinetics of disease in NK T cell-deficient mice suggests that this interaction may delay β-cell pathogenesis [109]. Furthermore, given that treatment of NOD mice with the potent NK T cell activator glycolipid α-galactosylceramide provided significant protection from T1D through IL-4- and IL-10-dependent mechanisms [110], exploiting the interaction between B lymphocytes and NK T cells may provide new therapeutic avenues for disease prevention or amelioration.

Targeting B lymphocytes holds promise for the treatment of T1D

Given the studies showing strong T1D resistance in most B-lymphocyte-deficient NOD mice, several recent studies have sought to determine if a short-term B-lymphocyte depletion

strategy at a critical stage of disease could prove efficacious for the prevention or treatment of T1D. These studies were also necessary in order to identify stages of T1D development where B lymphocytes make either pathogenic or regulatory contributions. This was important in light of a study by Matsushita *et al.*, where antibody-mediated B-lymphocyte depletion was shown to confer exacerbation or protection from EAE if performed prior to or following disease induction, respectively, thus revealing contrasting roles for B lymphocytes at different stages of autoimmune pathology [100].

Hu and colleagues were the first to investigate this question, by generating NOD mice transgenically expressing human (h) CD20 on B lymphocytes [111]. This transgene did not alter the natural course of T1D in NOD mice but did allow selective targeting of murine B lymphocytes with an anti-hCD20 monoclonal antibody (mAb) specific for the same epitope as rituximab, an antibody clinically approved for the depletion of B lymphocytes in humans with certain lymphomas, which has more recently shown clear efficacy for treating patients with refractory RA as well as various other autoimmune syndromes [112–114]. Substantial B-lymphocyte depletion was achieved in NOD.*hCD20* mice with a single cycle of a treatment (four intravenous injections over 9 days), which lasted for approximately 3 weeks, with levels returning to normal over the next 9 weeks. Treatment was initiated at either 4 (pre-insulitic) or 9 (prediabetic) weeks of age, or immediately after onset of T1D in NOD.*hCD20* mice. Animals treated at 4 and 9 weeks of age displayed significantly delayed progression to T1D, whilst the final incidence of disease was partially reduced in mice treated at the latter time point. Of greater interest, anti-hCD20 antibody treatment could reverse hyperglycemia in a third of new-onset diabetic mice, with the majority remaining euglycemic for more than 130 days. B-lymphocyte depletion in these mice was associated with a decreased capacity of DCs and macrophages to present autoantigens to $CD4^+$ and $CD8^+$ T lymphocytes. The reason for the inhibition of antigen presentation by these other APCs after B-lymphocyte depletion was suggested to be due to an increase in the proportions of $CD4^+$ $FoxP3^+$ Treg lymphocytes and MZ precursor (MZP) B-lymphocyte subsets. Interestingly, this latter population has previously been shown to contain IL-10-producing Bregs with the ability to prevent RA in mice [115].

In comparison, Xiu *et al.* depleted B lymphocytes in wild-type NOD mice with a 6-week course of antimouse CD20 mAbs starting at early (5 weeks) or late (15 weeks) preclinical stages of T1D [116]. Full depletion with this regime lasted for at least 6 weeks and, when given early, resulted in a significant delay in the onset of, and protection from, T1D. Compared with the study of Hu *et al.*, mice treated at the later time point showed only a slightly delayed disease onset. While the ability to activate $CD4^+$ and $CD8^+$ T lymphocytes was impaired in B-lymphocyte-depleted mice, no differences were noted in the post-treatment levels of Tregs. Possible causes of the contrasting results in these studies may be due to variations in the course of treatment, isotypes of anti-CD20 antibodies (IgG2b vs IgG2c, respectively), epitopes targeted on human and mouse CD20 and possible differences in CD20 expression in transgenic versus wild-type NOD mice. Nevertheless, the fact that better results were obtained with an antibody similar to that of the clinically approved rituximab mAb is encouraging.

Antibodies targeting other B-lymphocyte-specific molecules have also been trialed for their ability to prevent T1D in NOD mice. These have included a mAb to CD22 that, in addition to being expressed on mature B lymphocytes, is also present on antibody-secreting plasma cells (unlike CD20). Short-course treatment (two injections 5 days apart causing B-lymphocyte depletion for 5–7 weeks) with the anti-CD22 antibody complexed to calicheamicin (a toxic agent that enhances target cell killing) in 10-week-old NOD female mice significantly delayed and partially prevented the onset of T1D [117]. Moreover, as occurred with the anti-CD20 treatment in NOD.*hCD20* mice, protection following B-lymphocyte depletion was associated with increased levels of Tregs and reduced autoreactive T-lymphocyte proliferation. Re-emerging B lymphocytes displayed a decreased capacity to induce proinflammatory cytokines from diabetogenic $CD4^+$ T-lymphocyte clones and also exhibited regulatory properties. Encouragingly, the anti-CD22 antibody complex seemed more effective at reversing hyperglycemia in NOD mice with recent-onset T1D than anti-hCD20 mAbs (two-thirds compared with a third of mice returned to normoglycemia in the long term, respectively). Importantly, the anti-CD22 antibody complex treatment was required very shortly after the onset of T1D in NOD mice (within 3 days of hyperglycemia) or else was not effective. This suggested that the anti-hCD20 treatment in NOD.*hCD20* mice, which was given within 6 days of diabetes onset, may also prove to be more effective at reversing disease if administered earlier [111].

Blocking the critical B-lymphocyte-survival factor BAFF could also provide distinct advantages over therapeutic strategies targeting CD20 and CD22, in that the latter treatments would allow elevated BAFF levels to persist after B-lymphocyte depletion. Thus, upon cessation of treatment, newly generated immature B lymphocytes exposed to high levels of BAFF would lead to re-emergence of self-reactive clones and autoimmunity. Theoretically, this situation might be averted by inducing B-lymphocyte depletion through BAFF neutralization. Furthermore, blocking BAFF has been shown to be more effective than the antibodies above at depleting potentially pathogenic MZ B lymphocytes. Indeed, a short course (two injections 5 days apart) of anti-BAFF mAbs at 6–8 weeks of age was found to significantly delay the onset of T1D in NOD mice [118]. Longer-term treatment consisting of two injections of a high dose of mAbs at 4 weeks of age (5 days apart), followed by lower doses given biweekly between 8 and 25 weeks of age, conferred significant protection from T1D and reduced the severity of insulitis. BAFF neutralization also prevented progression to full-blown diabetes in all mice showing early signs of hyperglycemia. As predicted, long-term BAFF neutralization restored tolerance induction of re-emerging immature B lymphocytes and abrogated production of anti-insulin auto-Abs. The authors did not report an increase in Treg lymphocytes in B-lymphocyte-depleted mice, but did observe disrupted $CD4^+$ T-lymphocyte activation resulting from this treatment.

Islet transplantation has recently gained favor as a treatment for controlling disease in T1D patients requiring other transplants or 'brittle' diabetics with difficulties in controlling their blood sugar levels. However, the recruitment of both allo- and auto-immune mechanisms of rejection in these patients has made long-term survival of islet grafts difficult to achieve [119]. Most immunosuppressive regimens following islet transplantation currently focus on T lymphocytes. However, using a simian model of islet allograft transplantation, Liu and colleagues, showed that B-lymphocyte depletion with an anti-CD20 mAb (i.e., rituximab) in combination with T-lymphocyte-depleting antibodies could vastly improve long-term survival of islets compared with T-lymphocyte depletion alone [120].

Taken together, the above studies show that depletion of B lymphocytes at various stages of disease can offer an effective treatment that either delays or halts T-lymphocyte-mediated β-cell destruction responsible for T1D in NOD mice (Table 1). As expected from previous studies, all treatments involving B-lymphocyte depletion resulted in a reduction of the ability to activate autoreactive $CD4^+$ T lymphocytes, emphasizing their critical pathogenic role as diabetogenic APCs. Unlike the EAE model [100], exacerbation of T1D due to depletion of Bregs was not observed in any of the treatment protocols in NOD mice. It was interesting, however, to note that two groups associated protection from T1D with an increase in the regulatory phenotype of re-emerging B lymphocytes after depletion, which also coincided with Treg expansion (Table 1). Understanding the basis of differences in protective mechanism(s) or efficacy provided by each mAb will allow future development of tailored treatment regimes, which can be optimized for patients at different stages of T1D or following islet transplantation.

Expert commentary & five-year view

A definitive case has been made for several roles of B lymphocytes in the development of T1D in NOD mice (Figure 1). Whether B lymphocytes play similar roles in the development of human T1D remains to be directly proven. Certainly, the strong association of isotype-switched, high-affinity autoantibodies against β-cell and islet proteins with susceptibility and progression to T1D suggests that autoreactive B lymphocytes are present, activated and interacting with T lymphocytes in the prodromal stages of disease. Given the data generated in NOD mice, we would speculate that detection of autoantibodies in T1D-susceptible humans represents a phase of disease whereby self-reactive B lymphocytes are being activated and recruited as APCs for expanding autoreactive $CD4^+$ T-lymphocyte populations. B- and T-lymphocyte interactions would lead to spreading of the cellular and humoral response to additional autoantigens, increasing β-cell pathogenesis and thus the risk of developing disease.

Skeptics of a role for B lymphocytes in human T1D have based their arguments on a single patient who developed the disease despite also suffering from X-linked agammaglobulinemia, a disease characterized by severely decreased numbers of functional B lymphocytes throughout life [121]. However, caution is necessary

Table 1. Summary of studies using B-lymphocyte depletion as therapy for Type 1 diabetes in nonobese diabetic mice.

Target in NOD mice (mAb isotype)	Treatment initiation* (weeks)	Duration of B-lymphocyte depletion (weeks)	Degree of T1D protection‡	Changes in re-emerging B lymphocytes	Effects on other cell types	Ref.
Human CD20§ (mouse IgG2b)	4 9 T1D onset	3 3 3	D D and ~30% P ~30% R	↑ T2-MZP cell ↑ Immunoregulatory properties (Bregs)	↑ Tregs ↓ Ag presentation function by DCs and macrophages	[111]
Mouse CD20 (mouse IgG2c)	5 15	6 6	~70% P D and 40% P	?	↓ Lymph node $CD4^+$ and $CD8^+$ T-cell activation	[116]
Mouse CD22 (mouse IgG1)	10 T1D onset	6 6	D and ~40% P 60% R	↑ Immunoregulatory properties (Bregs) ↓ APC capacity for diabetogenic $CD4^+$ T cells Altered transcriptional profile	↑ Tregs ↓ $CD4^+$ T-cell activation	[117]
Mouse BAFF (hamster IgG)	8 4 Honeymoon period	3 weeks >21 weeks ?	D D and ~50% P 100% P	↑ TR:FO subset ratio ↑ Self-tolerance at TR→FO subset transition	↓ $CD4^+$ T-cell activation	[118]

*T1D onset and honeymoon period defined as >250 mg/dl and 160–200 mg/dl blood glucose, respectively.
‡Delay, protection or reversal of T1D compared with control IgG-treated group.
§Treated NOD mice transgenically expressing human CD20.
APC: Antigen-presenting cell; BAFF: B lymphocyte activation factor for the TNF family; Breg: Regulatory B cell; D: Delay; FO: Follicular; mAb: Monoclonal antibody; MZP: Marginal zone precursor; NOD: Nonobese diabetic; P: Protection; R: Reversal; T1D: Type 1 diabetes; TR: Transitional; Treg: Regulatory T cell.

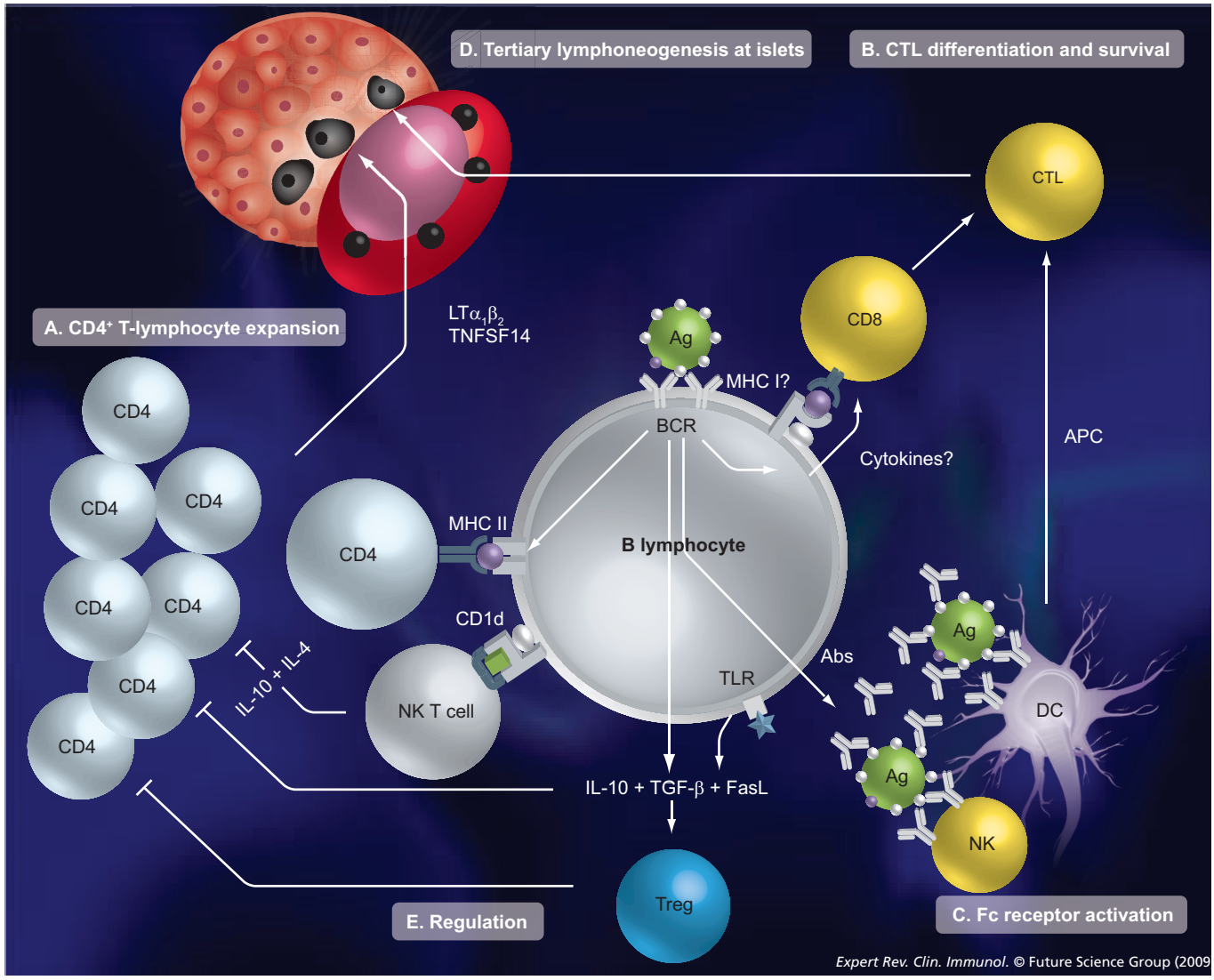

Figure 1. Emerging roles for B lymphocytes in the development of Type 1 diabetes (T1D). (A) The ability of B lymphocytes to efficiently capture autoantigens through surface BCRs make self-reactive B lymphocytes in nonobese diabetic (NOD) mice preferential APCs for the expansion of MHC class II-restricted β-cell-specific autoreactive CD4+ T lymphocytes. In turn, activated T lymphocytes provide 'help' to B-lymphocyte populations, further exacerbating impaired B-lymphocyte tolerance and anti-self-hyperactivity. Collaboration of β-cell-specific B and CD4+ T lymphocytes results in expansion of both populations, and also stimulates affinity maturation and Ag spreading of the autoimmune response. **(B)** Recent evidence has shown that B lymphocytes to also play a role in promoting differentiation of β-cell-specific CD8+ T lymphocytes into cytotoxic effector lymphocytes (CTLs), as well as enhancing their survival within insulitic lesions. Whether this role of B lymphocytes is dependent on MHC class I-restricted Ag presentation, or on the production of critical signals via cytokines or other molecules remains to be determined. **(C)** Secretion of β-cell-specific autoantibodies by B lymphocytes results in binding of autoantigen/autoantibody complexes to activating Fcγ receptors on other immune cells. This promotes T1D by enhancing effector functions of NK cells as well as promoting uptake of autoantigens that DCs can use to present to T lymphocytes. **(D)** Production of critical factors, including lymphotoxin-$\alpha_1\beta_2$ and TNFSF14 mean that B lymphocytes play a key role in the organization of tertiary lymphoid structures surrounding the islets. These permit maximum exposure of T and B lymphocytes to a diverse range of β-cell autoantigens, as well as enhancing their ability to form interactions and increasing their affinity for Ags through the generation of germinal centers. **(E)** B lymphocytes also have the capacity to play regulatory roles that inhibit T-lymphocyte-mediated β-cell destruction. Breg populations activated *in vitro* through BCR or TLRs were found to inhibit T1D development in NOD mice through their expression of IL-10 or TGF-β plus FasL, respectively. When specifically expanded or activated by recognition of lipids or glycolipids presented on CD1d by B lymphocytes, NK T cells can prevent the onset of T1D in nonobese diabetic mice through the rapid production of anti-inflammatory cytokines, including IL-4 and IL-10.

Ab: Antibody; Ag: Antigen; APC: Antigen-presenting cell; BCR: B-cell receptor; CTL: Cytotoxic T lymphocyte; DC: Dendritic cell; FasL: Fas ligand; NK: Natural killer; TLR: Toll-like receptor; TNFSF: TNF superfamily member; Treg: Regulatory T cell.

when extrapolating from single case reports. In light of the genetic and environmental diversity that exists among humans developing T1D, it is likely that different groups of factors are involved in the pathogenesis of disease in distinct individuals. Furthermore, even genetically identical NOD.*Igμ*^{null} mice, which are normally strongly protected from T1D, develop disease on rare occasions [16–18]. Hence, the fact that T1D can develop in the absence of B lymphocytes in one patient does not imply that they do not play an important role in other human subjects who are susceptible to disease.

Even though large numbers of compounds have been demonstrated to confer protection from T1D in NOD mice if administered in the preclinical phase of disease, very few are capable of reversing disease after the onset of hyperglycemia, as has been shown with B-lymphocyte-depleting mAbs [122]. The latter studies have prompted the setting up of large, multicenter Phase II clinical trials in recent-onset T1D patients with the aim of formally testing the therapeutic efficacy of B-lymphocyte depletion with the anti-CD20 mAb, rituximab (ClinicalTrials.gov Identifier: NCT00279305). Rituximab is also being employed in another clinical trial that will examine its ability to promote long-term survival of transplanted islet allografts in humans when used in conjunction with T-lymphocyte-depleting agents (ClinicalTrials.gov Identifier: NCT00468442). Both trials are currently ongoing and should, in the next 5 years, provide evidence on the significance of B lymphocytes in the late stages of human T1D. Antibodies targeting CD22 (e.g., epratuzumab) and BAFF (e.g., belimumab) are also currently approved for clinical use in humans and have shown clear therapeutic efficacy in treating B-lymphocyte lymphomas as well as other systemic autoimmune diseases, including RA and systemic lupus erythematosus [123,124]. Positive signs from clinical trials with rituximab in T1D patients are likely to lead to additional trials with epratuzumab and belimumab to determine their additional benefits either alone or in combination with rituximab.

Even though pan depletion of all B lymphocytes has shown promise for the treatment of T1D, this therapeutic strategy carries with it an increased risk of serious complications associated with immunosuppression (e.g., increased risk of infections and neoplasms) [125], especially if given over a prolonged period or combined with pan T-lymphocyte depletion. In the next 5 years, research in this field is likely to yield more information on the role played by certain B-lymphocyte subsets in the development of T1D, such as the MZ and B-1 populations, which may provide more selective depletion targets for the treatment of T1D. By contrast, determining ways to specifically stimulate or expand Breg populations or augment activation of NK T cells through B lymphocytes may also provide effective protection from T1D without the need to eliminate any B-lymphocyte populations. Finally, identification of diabetes-susceptibility genes that contribute to the development of diabetogenic B lymphocytes (such as those within the *Idd5* or *Idd9/11* loci) in NOD mice, may allow discovery of orthologous genes or molecular pathways in humans that contribute to the same phenotype. Identifying these susceptibility genes may not only lead to better selection of T1D patients who would be responsive to B-lymphocyte therapy (even before the onset of hyperglycemia) but also lead to the design of new 'magic-bullet' drugs that specifically target or even prevent the development of diabetogenic B-lymphocyte clones, while ensuring that humoral immunity to foreign pathogens and neoplasms remains intact.

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Key issues

- Autoimmune destruction of pancreatic β cells in Type 1 diabetes (T1D) is mediated by T lymphocytes.
- B lymphocytes play important accessory roles in the development of T1D, as revealed by the nonobese diabetic (NOD) mouse model of the disease.
- The major pathogenic contribution of B lymphocytes to T1D is as antigen-presenting cells (APCs) involved in the expansion of autoreactive CD4$^+$ T lymphocytes.
- The role of B lymphocytes as diabetogenic APCs is dependent on their unique ability to capture β-cell antigens through surface immunoglobulin molecules.
- Generation of self-reactive B lymphocytes contributing to T1D in NOD mice is due to various defects in tolerance mechanisms.
- Diabetes-susceptibility genes in NOD mice contribute to the pathogenic activity of B lymphocytes.
- Various B-lymphocyte subsets show evidence of contributing to T1D pathogenesis, while others have the capacity to play a regulatory role.
- Antibody-mediated depletion of B lymphocytes at various stages of disease was effective at preventing and/or delaying T1D onset in NOD mice.
- A role for B lymphocytes in human T1D is currently being investigated in ongoing clinical trials using the anti-CD20 monoclonal antibody rituximab in patients with recent-onset disease.
- Defining the pathogenic roles of B-lymphocyte subsets and the molecular bases underlying the generation of diabetogenic clones will provide more selective T1D therapies in the future.

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