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Endotypes in T1D: B lymphocytes and early onset

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Abstract

Purpose of the review: While Type 1 diabetes (T1D) is characterized by destruction of the pancreatic beta cells by self-reactive T cells, it has become increasingly evident that B cells also play a major role in disease development, likely functioning as antigen presenting cells. Here we review the biology of islet antigen-reactive B cells and their participation in autoimmune diabetes.

Recent findings: Relative to late onset, individuals who develop T1D at an early age display increased accumulation of insulin-reactive B cells in islets. This B cell signature is also associated with rapid progression of disease and responsiveness to B cell depletion therapy. Also suggestive of B cell participation in disease is loss of anergy in high affinity insulin-reactive B cells. Importantly, loss of anergy is seen in patient's healthy first degree relatives carrying certain T1D risk alleles, suggesting a role early in disease development.

Summary: Recent studies indicate that islet-reactive B cells may play a pathogenic role very early in T1D development in young patients, and suggest utility of therapies that target these cells.

Keywords

B cells; type 1 diabetes; islet antigen-reactive B cells; insulin-reactive B cells; anergy; autoimmunity

Introduction

Type 1 diabetes (T1D) is an autoimmune disorder characterized by destruction of the insulin producing beta cells of the pancreas by self-reactive lymphocytes, leading to hyperglycemia and requirement for lifelong finely-tuned administration of exogenous insulin. While it is well known that autoreactive T cells mediate destruction of the beta cells, the role of B cells is less well understood. However, recent findings suggest B cells may play a more pathogenic role in individuals who develop T1D at an early age. In this review we discuss recent findings regarding the phenotype and function of islet-reactive B cells in T1D, with particular emphasis on loss of anergy, genetic predisposition, and relationship to the age of

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onset, and conclude with discussion of therapeutic strategies that may be beneficial in particular age groups.

Evidence for failure of B cell tolerance in T1D

Studies indicate that as many as 70% of B cells that develop in the bone marrow are autoreactive (1). In healthy individuals these self-reactive lymphocytes are normally silenced by one of three tolerance mechanisms: 1) receptor editing, 2) clonal deletion, or 3) anergy. Both receptor editing and clonal deletion occur centrally in the bone marrow, whereas anergy typically occurs in the periphery. When immature B cells bind self-antigen with high avidity in the bone marrow, resultant strong antigen receptor (BCR) signals induce editing in which antigen receptor light chain gene usage changes, silencing one allele and expressing a second. If the new antigen receptor lacks self-reactivity, the B cell can continue development and populate the periphery as a naïve cell capable of responding to pathogenic insults (2, 3). For many B cells this process is successful, but when it is not, continuing strong BCR signals lead to death by clonal deletion/apoptosis. If the BCR has a moderate avidity for self-antigen, the B cell is able to exit the bone marrow and populate the periphery, but these cells reside in an unresponsive state termed anergy (4, 5). Anergic B cells are characterized by inability to respond to antigen stimulation by activation (6–10). Chronic stimulation by self-antigen is critical for induction and maintenance of anergy, and these signals impose unresponsiveness by downregulation of BCR, and activation of negative regulatory signaling circuitry involving inositol lipid and phosphotyrosine phosphatases. These regulatory phosphatases, which include SHP-1, PTPN22, PTEN, and SHIP-1, modulate antigen receptor signaling (11–13). Importantly, anergy is reversible if the autoantigen dissociates from the BCR. It is apparent that anergy can be compromised by genetic risk alleles that alter signaling thresholds, allowing B cells to become activated by BCR stimulation (discussed below).

It has been demonstrated that a breakdown in the tolerance mechanisms discussed above likely contribute to development of T1D. Menard et. al. found that self-reactive B cells, as defined by binding of their antibody to permeabilized HEp-2 cells, are increased among the new emigrant/transitional and mature naïve B cells in T1D patients, suggesting impairment of both central (receptor editing or clonal deletion) and peripheral (anergy) B cell tolerance (14). More recently the same group found that treatment with Rituximab failed to reset these impaired tolerance checkpoints, which may help explain why the benefits from Rituximab treatment in new onset patients were minimal one year after conclusion of therapy (15, 16). Another study analyzed the frequency of recombining sequence (RS) rearrangements in lambda positive B cells as a surrogate measure of receptor editing in T1D subjects compared to healthy controls. Results demonstrated T1D subjects have reduced RS rearrangements, indicating reduced receptor editing, which may allow increased numbers of autoreactive B cells to enter into the periphery (17).

More recently, groups, including our own, have analyzed whether T1D subjects demonstrate evidence of a breakdown in the tolerance mechanism anergy. In our studies we analyzed frequency of both insulin-reactive and total anergic B cells in the peripheral blood of individuals at risk for and previously diagnosed with T1D. We found that autoantibody

positive first-degree relatives and recently (< 1 year) diagnosed T1D subjects have a significant decrease in insulin-reactive and total anergic B cells compared to healthy controls and long standing T1D subjects (18). Interestingly, some autoantibody negative first-degree relatives displayed a similar loss of total and insulin-reactive anergic B cells in peripheral blood, suggesting that loss of anergy likely precedes activation and differentiation these cells autoantibody secreting cells. Loss of total anergic B cells in autoantibody positive first-degree relatives was recently confirmed by a different group as well (*19). To assess the genetic contribution to subversion of B cell anergy, we genotyped first degree relatives with normal or low levels of anergic B cells, and found that loss of B cell anergy was associated with the high risk T1D HLA class II DR4:DQ8 haplotype and risk-conferring polymorphisms in non-HLA loci including *INS, PTPN2, PTPN22*, and *IKZF3* (*20). Other studies have shown that expression of the *PTPN22* risk allele, which is the third highest risk contributor to T1D and functions to dampen B cell signaling, is associated with increased autoreactive B cells in the periphery of T1D patients (14, 21). The association of loss of B cell anergy with these risk alleles suggests T cell involvement in loss of B cell anergy, likely cooperating with failure of negative regulation of B cell signaling to drive activation of pathogenic cells (*20). Taken together, T1D patients show impairment in both central and peripheral B cell tolerance, likely due to both genetic susceptibility and environmental factors, which contribute to the pathogenesis of disease.

The role of B cells in T1D

A study often cited as evidence that B cells are irrelevant in T1D is a report of a person who developed T1D despite having a hereditary deficiency in B cells (22). However, previous studies have demonstrated that conditions of lymphopenia, either by genetic defects or pharmacologically induced, can support the accumulation and expansion of self-reactive T cells irrespective of the presence of B cells, and hence this could explain this individual's development of T1D (23, 24). On the other hand, studies have demonstrated a necessity for B cells using the non-obese diabetic (NOD) mouse model (25). Moreover, depletion of B cells using anti-CD20 or anti-CD22 prevents, and even reverses in some studies, diabetes in the NOD model (26–28), further demonstrating their importance in disease.

While it is well known that B cells are the source of islet autoantibodies in T1D, which serve as the best biomarkers for disease to date (29, 30), evidence suggests autoantibodies are dispensable for disease in the NOD mouse (31) and potentially in humans as well. Despite this fact, subjects who have high affinity anti-insulin antibodies, as evidenced by ECL assays, or antibodies to more than one islet antigen at screening are at very high risk of developing T1D (29, 32, 33). Moreover, recent studies have demonstrated an association of non-HLA risk alleles, including variants in *PTPN22* and *INS*, with development of particular islet autoantibodies (30, 34–36), suggesting genetic risk could act in either a B cell intrinsic fashion by compromising B cell tolerance mechanisms, or in a B cell extrinsic fashion by allowing more self-reactive T cells into the periphery, which in turn provide necessary T cell help to self-reactive B cells.

Aside from antibody production, B cells can also serve as regulatory cells, cytokine producers, and antigen presenting cells (37). Evidence thus far suggests the likely major

pathogenic role of B cells in T1D appears to be through potent antigen presentation to T cells (38–41). In classic studies in the NOD mouse model, inhibition of the ability of B cells to present antigen by either class I or class II prevents diabetes (38, 42). In addition, restricting the B cell repertoire to an irrelevant antigen, thus disallowing presentation of islet antigens, prevents diabetes (40). On the other hand, the VH125.NOD mouse, which is a BCR heavy chain transgenic with an increased frequency of insulin-reactive B cells in the periphery, is characterized by accelerated and increased rates of diabetes development (43). Recent studies using an immunoglobulin class-switching competent version of this mouse ($VH125^{SD}$.NOD) demonstrated insulin-reactive B cells enter all mature B cell subpopulations in the spleen and pancreatic lymph nodes and respond normally to stimulation *in vitro*, suggesting loss of B cell tolerance and capability to act as antigen presenting cells (44, 45). Studies in our own lab have demonstrated that high affinity insulin-reactive B cells from the VH125.NOD express the co-stimulatory molecule CD86, migrate to the pancreas and pancreatic lymph nodes, and are functionally responsive to BCR stimulation. On the other hand high affinity insulin-reactive B cells on the diabetes resistant C57BL/6 background (VH125.C57BL/6.$H2^{g7}$) fail to become activated, enter the pancreas, and are functionally anergic (**46). Similarly, a recent study found that islet infiltrating B cells from NOD-PerIg mice, which recognize the neuronal antigen peripherin and crossreact with an islet antigen, have increased expression of CD86, and mice exhibit accelerated rates of diabetes development, whereas B cells from 116C-NOD mice, which express an islet beta cell reactive BCR cloned from islet-infiltrating B cells of a diabetes resistant prone mouse, maintain an anergic phenotype and exhibit delayed onset of diabetes, and decreased disease incidence (47). Taken together, evidence suggests genetic determination of loss of B cell anergy, which allows some autoreactive B cells to become activated and present antigen to cognate T cells, leading to beta cell destruction. Further studies are needed to conclusively demonstrate this model in both mice and humans.

Phenotype and function of B cells in early onset T1D subjects

Although many studies over the last decades have analyzed the phenotype of B cells in long standing diabetics compared to healthy controls, more compelling studies would arguably compare differences in B cells in subjects at risk for and prior to disease onset, as well as at the time of T1D onset, when beta cell destruction and inflammation is ongoing. Thanks to studies such as the TrialNet Natural History/Pathway to Prevention study and programs like the Network for Pancreatic Organ Donation (nPOD), studies have determined important differences in the phenotype and function of B lymphocytes prior to and at disease onset compared to controls. For example, a recent study found that autoantibody positive FDRs who progressed to T1D had a decrease in fold change of phosphorylated PLCy2, a proximal BCR signaling molecule, compared to non-progressors, suggesting the BCR response is blunted as one progresses to clinical disease (*19). Moreover, another recent study found that total B cells from new onset T1D subjects exhibited decreased expression of PTEN, a negative regulator of the PI3-kinase pathway, compared to controls (*48). Studes have demonstrated that defects in regulation of the PI-3kinase pathway (i.e. gain-of-function (GOF) mutations) can lead to increased infections, cancer, and autoimmunity (49, 50). Hence one might speculate decreased expression of a negative regulator, such as PTEN, in

all B cells could lead to increased activation in all B cells. Further studies are needed to support this idea.

A groundbreaking study from Leete et al. analyzed B cells in the pancreas and pancreatic lymph nodes of cadaveric organ donors from nPOD. This group found two distinct patters of insulitis, designated CD20Hi (many B cells present) and CD20Lo (few B cells present), which distinguished T1D subjects based on age. Subjects who were diagnosed before the age of 7 always display the CD20Hi phenotype, while subjects diagnosed after 13 years of age always show the CD20Lo phenotype (51). Furthermore, they found that subjects who display the CD20Hi profile show loss of beta cell mass at a more rapid rate than those with the CD20Lo phenotype, suggesting the two forms are differentially aggressive (51). More recently this varying B phenotype based on age of onset was supported in a separate study that analyzed rate of C-peptide loss and variations in gene expression using whole blood RNA sequencing in new onset T1D subjects. This group found that only young subjects exhibited a rapid loss in beta cell function, as evidenced by C-peptide loss, which was associated with increased expression of B cell genes. The increased B cell gene signature correlated with increased frequency of CD19+ B cells in the subject's blood determined using flow cytometry (**52). Similarly, a third group recently developed a computational tool that could predict loss of insulin secretion two years following diagnosis. They identified a panel of immune markers that, in combination, highly associated with loss of insulin secretion. One of the major immune phenotypes associated with rapid progression was increased B cell activation, as tested by RNA-sequencing (*53). Hence recent studies over the past year implicate the presence of B cells with a more aggressive form of T1D, which is restricted to younger onset subjects.

Lessons from therapeutic trials

Given the role of B cells in the pathogenesis of T1D, a phase II clinical trial was conducted in new onset subjects using rituximab, a monoclonal antibody that binds CD20 expressed on the surface of most B cells, (16, 54). Rituximab targets B cells for destruction through antibody dependent cellular cytotoxicity (ADCC), thereby depleting peripheral B cells while presumably sparing autoantibody producing plasma cells that are CD20 negative. Results from the study showed that subjects treated with rituximab had reduced requirement for insulin and delayed C-peptide loss one year after treatment. However, two years following therapy treated subjects showed no clinical benefit over placebo treated subjects. One possible reason for this could be that at the time of treatment destruction of the beta cells was already sufficient to support hyperglycemia and the autoreactive B cells had already committed their crime. Interestingly, younger subjects responded better to treatment than older subjects, further highlighting an age-dependent pathogenic role for B cells in T1D (16, 54).

Recently Da Rosa et al. generated human CD20 (hCD20) transgenic NOD mice to facilitate treatment with human anti-CD20 depleting antibody to study its effect on the immune system and prevention of autoimmune diabetes. Interestingly, they found that early B cell depletion had a significant effect on pancreatic CD8 T cells, demonstrating lack of T cell activation and IFN-y production even long after B cell depletion and repopulation. These

results suggest a local effect of B cell depletion on the CD8 T cell population, which likely contributes to the early efficacy of anti-CD20 treatment (55). This same group recently crossed the hCD20.NOD mouse to the VH125, thus allowing identification and tracking of insulin-binding B cells in the pancreas upon repopulation following anti-CD20 treatment. They found that after treatment with anti-CD20, insulin-binding B cells repopulated the pancreatic islets earlier than non-insulin-binding B cells, and that a unique insulin-binding B cell population, designated by intermediate expression of the plasma cell marker CD138 and downregulation of CD19, was particularly enriched after B cell depletion, indicating another possibility why loss of C-peptide is only temporarily delayed following rituximab treatment in human T1D subjects (*56).

As mentioned earlier, total B cells from new onset T1D subjects exhibit reduced expression of PTEN, a negative regulator of PI3-kinase pathway and is important in maintenance of anergy (*48). Similarly, B cells from the VH125.NOD mouse have decreased PTEN expression compared to the diabetes-resistent VH125.C57BL/6.H2g7 (**46). In an effort to move towards more precision medicine based treatments, Franks et al. showed that low doseage of the highly specific PI3-kinase (PI3Kδ) inhibitor, idelalisib, blocks progression of autoimmune diabetes in the VH125.NOD mouse (*57). It remains to be shown whether treatment with this drug may be therapeutically effective in human T1D.

Previously a phase II clinical trial of abatacept (CTLA4Ig) in new onsets was conducted to determine whether inhibition of the costimulatory molecule CD28 on T cells presented or delayed C-peptide loss (58). Results indicated extensive heterogeneity in response to therapy with some individuals demonstrating significant and delayed C-peptide loss and others appearing resistant to therapy all together (58). Recently Linsley et al. investigated the possibility of an immune phenotype that associated with resistance to therapy. Using unbiased whole blood RNA-sequencing, they found that rapid progressors in both the abatacept and placebo treated groups were largely restricted to the younger early onset subjects and had elevated levels of B cells. Moreover, they found resistance to therapy was characterized by a transient increase in activated B cells, which bind to abatacept, and a reduced inhibition of anti-insulin antibodies (**59). Hence, it begs the question whether combination treatment with both abatacept and rituximab would be more beneficial to patients. A new clinical trial (TN25) will begin soon in which new onset subjects will be treated with rituximab followed by abatacept to assess the potential clinical benefit.

Conclusions

Despite the well-recognized role for autoreactive T cells in the pathogenesis of T1D, recent studies indicate the importance for B cell participation, likely through antigen presentation to T cells, in initiation of disease. Moreover, individuals who develop T1D at an early age have shown a unique immune cell signature in blood and pancreas characterized by increased numbers of activated B cells compared to later onset T1D patients. This young age-specific B cell signature is also associated with rapid progression of disease. Hence, studies indicate an aggressive pathogenic form of T1D, characterized by early onset and rapid progression, is likely due to increased numbers of B cells, thereby demonstrating a

likely need for combinational therapies and/or age-specific therapies for a more personalized treatment of disease.

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Key points

- In T1D autoreactive B cells undergo reduced central and peripheral tolerance.
- B cells likely act as antigen presenting cells in T1D.
- Loss of tolerance of islet reactive B cells is likely due to genetic polymorphisms and the environment.
- Early onset T1D is characterized by increased numbers of activated B cells and rapid progression of disease.
- Therapies targeting B cells are likely to be most beneficial in patients who develop T1D at a younger age.