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B cells and type 1 diabetes ...in mice and men

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Abstract

Nearly 70% of newly produced B cells express autoreactive antigen receptors and must be silenced to prevent autoimmunity. Failure of silencing mechanisms is apparent in type 1 diabetes (T1D), where islet antigen-specific B cells appear critical for development of disease. Evidence for a B cell role in T1D includes success of B cell targeted anti-CD20 therapy, which delays T1D progression in both NOD mice and new onset patients. Demonstrating the importance of specificity, NOD mice whose B cell repertoire is biased toward insulin reactivity show increased disease development, while bias away from insulin reactivity largely prevents disease. Finally, though not required for illness, high affinity insulin autoantibodies are often the first harbingers of T1D. B cell cytokine production and auto-antigen presentation to self-reactive T cells are likely important in pathogenesis. Here we review B cell function, as described above, in T1D in humans and the non-obese diabetic (NOD) mouse. We will discuss recent broad-based B cell depletion studies and how they may provide the basis for refinement of future treatments for the disorder.

Keywords

Type 1 diabetes (T1D); B lymphocytes; Autoimmunity

Introduction

Type 1 diabetes (T1D) has traditionally been considered a T cell-mediated autoimmune disorder. It is certainly true that the pathogenic destruction of insulin-secreting pancreatic beta cells occurs via direct interaction with autoreactive T cells. However, an earlier breach in B cell tolerance or ignorance is also a major contributor to disease. This conclusion is based in part on the success of anti-CD20 (Rituximab) therapy, which by broadly depleting B cells delays disease progression, preserving beta cell function in both non-obese diabetic (NOD) mice and new onset human patients [1–3]. In T1D, B cells have been shown to promote autoimmunity through a number of mechanisms including, but not limited to: autoantibody production [4,5], antigen presentation [6–9], and the secretion of pro-inflammatory cytokines [10,11].

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Here, we review the extant literature related to B cell function in T1D in humans and the non-obese diabetic (NOD) mouse. We discuss what can be learned from the broad based B cell depletion studies that have been completed in mouse and man, and speculate as to how these findings may refine future curative and preventative strategies for the disorder.

2. The paradox of B cell requirements for development of T1D

The importance of B cells in the spontaneous development of autoimmune diabetes (T1D) in NOD mice was clearly established using NOD. I μ null mice that lack B cells [12]. These mice are resistant to T1D, but disease develops after reconstitution of a polyclonal B cell compartment [13]. Similarly, B cell depletion by chronic treatment with anti-IgM antibodies protects from disease development [14]. Further indicative of a requirement for B cell specificity are findings that biasing the B cell repertoire with an immunoglobulin heavy chain transgene that raises the frequency of insulin-specific B cells to a few percent greatly increases the penetrance and decreases the time of onset of disease [15]. Repertoire bias away from insulin reactivity largely prevents disease development. Thus islet antigen specificity appears key to B cell participation in T1D. It is somewhat paradoxical that in some cases modest disease is seen in the absence of B cells, and islet antigen-specific T cells can transfer disease to NOD. *scid* recipients lacking B cells [16,17]. Why is this? Development of disease following transfer of TCR transgenic T cells may simply reflect the ability of nonphysiologically high numbers of islet antigen-specific T cells with pathogenic potential to compensate for inefficient antigen presentation resulting from the absence of B cells. Furthermore, lymphopenic conditions in *scid* that promote rapid homeostatic expansion of transferred autoreactive T cells may also contribute to disease development.

In humans, this paradox is seen in the oft-cited study of a T1D patient that was deficient in B cells due to a Bruton's tyrosine kinase (Btk) mutation causing X-linked agammaglobulinemia (XLA) [18]. It is noteworthy that the particular Btk mutation seen in this patient usually does not result in complete loss of B cells [19]. Keep in mind, as noted above, that B cell depleting therapy has efficacy in human T1D [3]. Thus, on balance, evidence indicates the B cells are important in development of T1D in both mouse and man.

3. B cells intrinsic defects in NOD contribute to breach in tolerance

At least three mechanisms: clonal deletion, receptor editing, and anergy normally function to eliminate and/or inactivate potentially dangerous auto-reactive B cells. Among these, available evidence suggests anergy is the most prominent silencing mechanism [20]. Anergic B cells survive in the periphery for a brief period of time, yet, despite continued availability of unoccupied antigen receptors (BCR), they are unresponsive to antigen stimulation [21,22]. The anergic state is labile, as it is dependent on continuous occupancy of antigen receptors and their transduction of inhibitory signals [23]. It is important to note that B cells bearing BCR with high autoantigen affinity can also persist in a naïve state in the periphery if they recognize only low avidity antigens. These cells are termed "ignorant" because despite binding autoantigen, BCR signaling does not occur due to lack of receptor aggregation. Since most ambient insulin is monomeric it seems likely that under normal circumstances insulin-specific B cells should be naïve by virtue of ignorance unless they

have the ability to recognize insulin bound to its receptor. Nonetheless, there is evidence that in T1D-resistant mouse backgrounds many insulin-specific B cells are eliminated by receptor editing or clonal deletion, while others are anergic (Hinman and Cambier, manuscript in preparation).

B cell participation in T1D in NOD may result from a break in tolerance or ignorance. Supporting the possibility that anergy is faulty in NOD mice are studies that explore the integrity of MD4 anti-HEL tolerance. In the NOD genetic background, MD4 anti-hen egg lysozyme (HEL) B cells undergo efficient clonal deletion upon encounter with membrane bound HEL. Deletion is comparable to that seen in autoimmunity resistant C57BL/6 mice. In mice expressing soluble HEL antigen, anti-HEL B cells are normally silenced primarily by anergy. However, in the NOD background the anergic status of HEL-specific B cells appears unstable [24]. This is true whether soluble antigen is expressed systemically, or limited to the pancreas by use of the insulin promoter [25]. We have explored the status of insulin specific B cells in NOD, and found that a large proportion of these cells become activated prior to onset of diabetes, and present antigen to CD4 T cells (Hinman and Cambier, manuscript in preparation). Thus B cell intrinsic mechanisms that normally function to confer and maintain B cell anergy are defective in NOD mice.

At least twenty-five genetic loci contribute to T1D in NOD. Cox et al. have mapped aspects of defective B cell anergy to loci on chromosome 1 (Idd5) and 4 (Idd9/11) [25]. In addition to faulty B cell tolerance, NOD mice have enlarged populations of marginal zone B cells [26]. This trait is shared by other models of autoimmunity, most notably lupus in (NZB × NZW)F₁ [27]. Marginal zone B cells have a distinct phenotype, described as innate-like and weakly auto-reactive. Increased surface levels of IgM and the complement receptor CD21 may lower their activation threshold [28]. MZ B cells are efficient presenters of antigen to naïve CD4⁺ T cells [29]. Over-representation of this B cell subpopulation occurs independent of disease progression, and has been mapped to chromosome 4 (Idd9/11) [26]. It has been suggested the MZ enlargement in NOD is the result of defects in B cell migration within lymphoid tissues [26]. Although its genetic basis and disease relevance is unknown, CD19 expression is also modestly increased on NOD B cells [30]. CD19 is an important BCR co-receptor and accessory signal transducer that participates in Lyn tyrosine kinase and PI3-kinase activation following antigen stimulation. Heightened CD19 levels have been linked previously to B cell hyper-responsive and autoimmunity [31].

4. In NOD mice, B cells contribute to T1D development via mechanisms distinct from autoantibody production

Although a role for transplacentally acquired anti-insulin antibodies in T1D development in NOD mice has been suggested, it is unlikely that these autoantigenic antibodies make a major contribution to pathogenesis [32]. NOD mice in which B cells express membrane-bound but not secreted IgM develop T1D with penetrance comparable to wild-type NOD [33]. However, Silva et al. showed that anti-islet antibodies are capable, admittedly under very contrived circumstances, of supporting the survival and proliferation of islet-reactive CD4⁺ T cells. Diabetes resistant B10.BR mice were bred with a TCR transgene from clone

3A9, recognizing a dominant HEL peptide. Mice were also transgenic for the HEL transgene, with expression of soluble HEL driven by the gene promoter. Spontaneous T1D incidence, with increased thymic production of islet reactive CD4⁺ T cells recognizing a single experimentally expressed autoantigen, was low (<25%). Passive transfer of anti-HEL autoantibodies raised disease incidence (>70%). Increased proliferation of CFSE-labeled islet-reactive CD4⁺ T cells was also observed (80% CFSE diluted as opposed to 20% in the control) [34]. Despite this demonstration of principle, it seems most likely that in NOD mice B cells promote beta cell destruction and disease progression primarily through presentation of antigen to auto-reactive T cells and the secretion of pro-inflammatory cytokines.

B cells capture beta cell autoantigen via their BCR, then process and present it in peptide form to T cells. B cell-specific deletion of the MHC class II I-Ag7 prevents T1D in NOD [35]. In mixed bone marrow chimeras where only B cells are MHC class I deficient, disease development was prevented [36]. Thus, B cells likely play a role in T1D pathogenesis via interactions with both CD4⁺ and CD8⁺ T cells. Pancreas infiltrating B cells have elevated levels MHC I and II. B7-1 and B7-2 are also up regulated [37]. These B cells are primed for destructive APC function within the niche [36].

5. Antigenic targets in NOD T1D

Although antibodies reactive with multiple islet antigens can be detected in the serum of patients at high risk for T1D development [4,38,39], NOD mice have thus far been shown to only produce anti-insulin autoantibodies [40]. It is unlikely that these antibodies make a major contribution to disease [33], though their existence is indicative of a breach in B cell tolerance and ongoing antigen-specific autoimmune response. Moreover, NOD mice that express an irrelevant B cell receptor by transgenesis are resistant to T1D [9].

In NOD, insulin autoantibodies are detectable from the earliest stages of insulinitis, 4 to 8 weeks of age [41]. In mice transgenic for the 125Tg heavy chain (VH125.NOD) 1–2 percent of B cells are insulin specific and transgenesis results in development of T1D at an accelerated rate. Conversely, VH281.NOD mice, whose transgenic immunoglobulin heavy chain biases the repertoire against insulin reactivity, are largely resistant to disease [15]. In our colony disease develops in <1% of VH281.NOD, and disease is only seen in mice >30 weeks of age. Disease in these mice is associated with loss of heavy chain exclusion consistent with appearance of insulin-specific B cells expressing endogenous heavy chains (unpublished observations). These B cells may thus drive disease. Indeed, *in vitro*, Kendall et al. showed that insulin-binding 125Tg B cells are more competent APC than their non insulin-binding counterparts [42]. Finally, the very fact that insulin-specific T cells transfer disease, implicates function of antigen-presenting insulin specific B cells in pathogenesis.

Transgenic T and B cell models specific for other pancreatic antigen targets have yet to be developed. Stadinski et al. have identified chromogranin A (ChgA) as the antigen for several highly diabetogenic T cell clones [43]. Islet amyloid polypeptide is likewise a known target antigen for disease inducing CD4⁺ T cells [44]. B cells specific for either of these autoantigens in the NOD have yet to be identified. Their existence, or lack thereof, would shed further light on the role of other islet antigens in disease development.

6. Efficacy of B cell-targeted therapies in NOD

Multiple approaches have been taken to prevent or treat T1D by targeting B cells. These have included targeting of B cells for destruction using antibodies recognizing CD20 [1,2] or CD22 [45], and starvation from the critical survival factor BAFF using BCMA-Fc [46] or anti-BAFF mAb [47]. In all cases, some degree of disease prevention or reversal was achieved, but results were dependent upon timing and kinetics of B cell depletion, as well as the subsets targeted. In new onset diabetic NOD, long term disease reversal was observed in a third of mice treated with anti-CD20 [1]. However, a potential pitfall of the drug was discovered. Murine B cells down-regulate surface expression of CD20 upon entering the pancreas [48]. Thus the most pathogenic B cells may survive and continue to provide cognate help to self-reactive effector CD4+ T cells. Whether this is true of the pancreas infiltrating B cells in human T1D remains to be established.

The efficacy of B cell depleting strategies in NOD depended largely on disease state at the time of treatment. When therapy was initiated prior to the onset of insulinitis disease was delayed, but not prevented with both anti-CD20 and anti-BAFF mAb [1,47]. After treatment the B cell compartment was replenished, and disease ensued. However, if treatment was begun after initiation of insulinitis, in the period of elevated blood glucose (160–200 mg/dl) before overt disease (>250 mg/dl), long-lasting protection was achieved using either BCMA-Fc or anti-BAFF mAb. Disease was disrupted for the duration of each of these studies [46,47].

Pan-B cell targeted therapeutic approaches are unlikely to be accepted for general use in the clinic because of their greater risk relative to current alternatives. However, specific targeting of B cells that recognize islet antigens may be useful. To model such approaches Henry et al. attempted to affect disease development in the VH125.NOD model by treatment of animals with anti-insulin mAbs that recognize an epitope that is not sterically hindered by antigen binding to receptors containing the VH125 heavy chain [15]. The study explored whether such antibodies would, by depleting insulin auto-reactive B cells, prevent disease development. Findings suggested this strategy could be effective despite the fact that in this study the treatment did not lead to complete depletion of the insulin-binding B cells. It is noteworthy that anecdotal evidence from many laboratories has shown that BCR-targeted antibodies are poor inducers of B cell depletion *in vivo*, likely because these receptors recycle rapidly, internalizing their cargo, thus preventing ADCC or complement mediated killing.

7. Efficacy of Rituximab therapy in T1D in man

Rituximab, an anti-human CD20 monoclonal antibody that depletes most B cells in the body, was initially approved for the treatment of non-Hodgkin's lymphoma [49]. Subsequently it been used to treat over 18 autoimmune disorders, including T1D [50]. In a TrialNet phase II clinical study, four injections of Rituximab were given to T1D patients within 3 months of diagnosis [3]. In patients given the drug, beta cell function was preserved at one year following initiation of treatment as measured by increased C-peptide levels and reduced requirement for insulin [3]. Despite the fact that significant improvement was not

seen two years after treatment, this study demonstrates a role for B cells in the pathogenesis of T1D and the potential therapeutic utility of targeting of B cells. In addition, timing may be crucial for targeting of these cells, since by the time patients are hyperglycaemic, B cells may have likely already undertaken their deleterious acts as APCs in the pancreas and pancreatic lymph nodes.

8. T1D autoantigens in man

The majority of target antigens in human T1D have been identified by analysis of specificity of antibodies produced by patients. Four major autoantigens have been discovered using this approach, including: insulin, glutamic acid decarboxylase 65 (GAD65), insulinoma-associated antigen 2 (I-A2, ICA512), and the more recently described zinc transporter 8 (ZnT8) [4,51]. Although antibodies to these autoantigens are likely non-pathogenic, they have great value for identification of high-risk individuals. Anti-insulin antibodies are typically first to develop during the course of disease and occur in 90% of patients 5 years of age and younger who develop T1D [39,52,53]. In addition, half of the T cells isolated from pancreatic lymph nodes from diabetic patients recognize insulin A chain epitopes [54]. Although insulin specific B cells may play a predominant role, the tolerance/ignorance of GAD65, I-A2 and ZnT8 must also be subverted during disease development.

9. Phenotypic changes in the B cells associated with T1D in the human

The mechanism(s) by which B cells participate in development of T1D in man is relatively unstudied. However, it has been hypothesized that, as in NOD, B cells promote pathogenesis by antigen presentation. DR3/4-DQ2/8 alleles of HLA class II genes confer greatest risk of T1D development, indicating a critical role for antigen presentation to CD4 T cells [55]. HLA Class I alleles rank second among risk alleles. Interestingly, among lymphoid cells in pancreata of newly diagnosed T1D patients B cells occur in frequency second only to CD8 T cells [56]. It is tempting to speculate that these B cells function as the primary APCs for CD8 T cells, as has been shown in NOD mice [36]. These B cells must have escaped normal silencing mechanisms and responded to antigen. In this context, it has been reported that both T1D and SLE patients have decreased recombining sequence (RS) rearrangements in λ^+ B cells compared to healthy controls, indicating that reduced receptor editing occurs in these autoimmune patients [57]. These findings suggest that autoimmune patients may have a higher autoreactivity threshold for initiation of RS rearrangements, which may allow more autoreactive B cells to enter the periphery. In this context it was recently shown that the Ptpn22 T1D risk allele acts in a B cell intrinsic fashion to allow autoreactive B cells to enter the periphery [58,59]. Thus circumstantial evidence supports the possibility that in human T1D islet antigen reactive B cells escape tolerance and enter the periphery, localizing in the pancreas where they promote disease by presenting antigen to CD4 and CD8 T cells.

In our own studies, we have begun to analyze the status of insulin-autoreactive B cells along the continuum of T1D development. We found that all pre-diabetic and newly diagnosed T1D patients, and some first degree relatives, exhibit reduced frequencies of both insulin-specific and total anergic BND B cells in their peripheral blood compared to long term diabetics and healthy controls (Smith and Cambier, submitted for publication). BND anergic

B cells were first defined in healthy individuals as having normal levels of surface IgD but reduced levels of IgM, and were termed BND cells [60]. Greater than 75% of BND cells are autoreactive and these cells are unresponsive to BCR stimulation. These studies suggest that anergic B are transiently lost prior to development of T1D, a likely consequence of injury and/or infection. Alternatively or additionally, this loss of anergic B cells from blood may indicate that these cells have entered tissues rich in autoantigen, such as the pancreas, where they can become activated by antigen and present antigen to T cells.

10. Conclusion

Compelling evidence documents the important role for islet antigen-reactive B cells in development of type I diabetes in mouse models and in man. However, therapeutic strategies that deplete all B cells, while effective, are unlikely to find utility in the clinic due to risk considerations. Rituxan elimination of naive B cells required to mount protective immune responses to newly encountered pathogens can render patients vulnerable to infection for many months [50,61]. Targeted depletion of islet antigen, especially insulin reactive B cell may provide an effective and low risk alternative.

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