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Invasion of the killer B's in type 1 diabetes

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1. ABSTRACT

Type 1 Diabetes (T1D) is an autoimmune disease requiring contributions from effectors in both $CD4^+$ and $CD8^+$ T cell compartments in order to destroy insulin producing pancreatic β cells. Autoantibodies specific for islet cell proteins are also often generated during the prodromal stages of T1D development. While providing excellent prognostic markers of future disease risk, it has generally been believed that the induction of autoantibody secretion by B cells was a secondary consequence of the ongoing autoreactive T cell response. However, studies in the NOD mouse model of disease have demonstrated that B cells play a key function during T1D development by serving as a subpopulation of antigen presenting cell (APC) which can most efficiently support the expansion of diabetogenic $CD4^+$ T cells. Furthermore, studies utilizing this model have indicated that autoantibodies may play an important role in initiating an early phase of pancreatic β cell destruction ultimately leading to overt T1D. This review will focus on the under appreciated role B cells play in T1D development not only in NOD mice, but also potentially in humans.

2. INTRODUCTION

Type 1 diabetes (T1D) results from autoimmune destruction of insulin producing pancreatic β cells (1). While multiple susceptibility genes contribute to T1D development, the primary risk factor is provided by particular major histocompatibility complex (MHC) haplotypes (2-4). The primary function of MHC molecules is to bind peptides derived from either pathogens or endogenous "self" proteins forming antigenic complexes that can be specifically recognized by T cells through the engagement of clonally distributed T-cell receptor (TCR) molecules (3). Depending upon the context in which antigen presentation occurs, following engagement of a peptide/MHC complex a T cell can either be triggered to elicit immunological effector activities, or undergo an abortive response leading to apoptotic death or the induction of functional anergy (5). These latter types of responses are considered to be "tolerogenic" since they normally delete or functionally inactivate T cells that recognize self-peptide/MHC complexes. Given these important roles of MHC molecules in triggering both immunological effector responses and tolerance induction,

and the fact that particular variants represent the greatest risk factor for T1D, it is not surprising T cells are the primary mediators of pancreatic β cell destruction during disease development. The NOD inbred mouse strain spontaneously develops a high incidence of T1D which shares many similarities with the human disease and thus serves as a useful model (2, 3). Studies in NOD mice have shown that T1D development requires contributions from T cells in both the $CD4^+$ and $CD8^+$ compartments that respectively recognize pancreatic β cell autoantigens presented by MHC class II and class I molecules (2, 3). However, there is now compelling evidence from the NOD mouse model that at least the $CD4^+$ T cell responses contributing to T1D development are largely dependent on functions provided by the B cell component of the immune system. This review will focus on the pathogenic role of B cells in T-cell mediated autoimmune T1D.

3. AN IMPORTANT ROLE FOR B CELLS IN T1D

Given the clear pathogenic role of T cells in mediating pancreatic β cell destruction during T1D development, it was initially surprising to find strong disease resistance was elicited in NOD mice made B cell deficient either by antibody treatment, or introduction of a functionally inactivated immunoglobulin μ heavy chain gene (designated NOD.$Ig\mu^{null}$ mice) (6-9). Furthermore, B cell reconstitution of NOD.$Ig\mu^{null}$ mice abrogated their T1D resistance (10). These collective results established that at least in NOD mice, B cells play an important pathogenic role in T1D development.

In contrast to NOD mice, the role of B cells in humans with T1D remains uncertain, largely due to the lack of relevant experimental data, but also the report of one patient who developed T1D despite suffering from congenital X-linked agammaglobulinemia (XLA), a disease that results in markedly impaired development of B cells (11). Based on this single case study, the authors concluded neither autoantibodies nor B cell function is critically involved in the pathogenesis of T1D, and immunotherapy directed specifically toward B cells or autoantibodies would not be effective in preventing disease. However, while this case study clearly demonstrates that B cells are not an absolute requirement for T1D development in every single human patient, it should not be used to summarily dismiss a role for B cells in the development of all T1D in humans. Especially since it is not known if the patient examined in the above case study had a typical autoimmune presentation or an exceptional pathogenic basis of T1D development. A parallel could be drawn to B cell deficient NOD.$Ig\mu^{null}$ mice, which in most cases do not develop T1D, but there is an occasional exception (~5% disease incidence in females held at The Jackson Laboratory). Moreover, it should also be considered that unlike inbred NOD mice, the genetic and environmental diversity that characterizes humans developing T1D is likely to result in different sets of contributory factors being involved in individual cases of disease. For these reasons, coupled with their clear pathogenic role in NOD mice, it remains likely that B cells may also serve as an important component of T1D development in a significant proportion of human patients.

4. WHAT IS THE ROLE OF B CELLS IN T1D?

4.1. B cells function as a diabetogenic APC

As discussed earlier, certain MHC haplotypes represent the primary risk factor for the development of autoimmune T1D in humans and NOD mice. It has been well established that T1D development in NOD mice is dependent upon contributions from both autoreactive $CD8^+$ and $CD4^+$ T-cells that respectively recognize pancreatic β cell peptides bound to the class I molecules (K^d, D^b) or the sole class II variant (A^{g7}) encoded by the $H2^{g7}$ MHC haplotype characterizing this strain (3). Like most other cell types, pancreatic β cells express MHC class I molecules, and hence can be directly recognized by diabetogenic autoreactive $CD8^+$ T cells (12). On the other hand, MHC class II expression is largely restricted to specialized hematopoietically derived antigen presenting cells (APC) that are comprised of B cells, macrophages, and dendritic cells. APC take up extra-cellular proteins and degrade them into peptide fragments that are then bound to MHC class II molecules for presentation to $CD4^+$ T cells (13). Thus, the autoreactive $CD4^+$ T cells contributing to T1D in NOD mice indirectly recognize pancreatic β cell derived antigens displayed by APC. However, a normally important function of APC, particularly the dendritic cell and macrophage subsets, is to present both MHC class I and class II bound peptides derived from self-proteins in a tolerogenic fashion. Tolerance is maintained through the deletion or inactivation of these potentially autoreactive T cells either during their differentiation in the thymus, or after they have seeded the periphery (14, 15). A key component of T1D pathogenesis in NOD mice are defects controlled by diabetes susceptibility (*Idd*) genetic loci that impair the ability of dendritic cells and macrophages to present self antigens to T-cells in a tolerogenic fashion (16-22).

Subsequent to the tolerance induction defects that allow for the initial development of autoreactive diabetogenic T cells in NOD mice, the question then becomes what APC subset must they then interact with to exert their pathogenic effector functions? In the case of diabetogenic $CD4^+$ T cells in NOD mice, it appears their functional activity is most efficiently supported when B cells serve as APC. An important role for B cells as diabetogenic APC was revealed when NOD.$Ig\mu^{null}$ mice were found to produce poor $CD4^+$ T cell responses to candidate T1D autoantigens including GAD, (pro)insulin, and IA-2, despite mounting adequate responses to other nominal antigens (10, 23, 24). Additional evidence supporting the role of B cells as diabetogenic APC was provided when NOD mice that were engineered to express the A^{g7} MHC class II molecule on all APC except B-cells, remained T1D resistant (25).

4.2. Specific Ig-mediated antigen capture allows B-cells to be preferential diabetogenic APC

There are several possible explanations for why B cells represent the subset of APC that most efficiently activates MHC class II restricted diabetogenic T cell responses in NOD mice. Unlike other APC, B cells express specific immunoglobulin (Ig; antibody) molecules on their

surface that can efficiently capture and internalize antigens from which peptides are subsequently generated and bound to MHC class II molecules for presentation to $CD4^+$ T cells (26). Hence, an ability to efficiently take up low abundance pancreatic β cell proteins through such an Ig mediated capture mechanism could account for why B cells have a greater capacity than other APC subtypes to propagate diabetogenic T cell responses. On the other hand, while it represents a less efficient mechanism than Ig mediated capture, B cells share an ability with other APC subtypes to present $CD4^+$ T cells with MHC class II bound peptides derived from proteins they had originally taken up by pinocytosis (27). Furthermore, regardless of the mechanisms by which they initially capture and internalize the native protein, variations in processing pathways enable B cells to generate different MHC class II bound peptides for presentation to $CD4^+$ T cells than those produced from the same protein by other APC subtypes (28, 29). Thus, while key populations of diabetogenic $CD4^+$ T cells in NOD mice clearly recognize MHC class II bound pancreatic β cell peptides that are preferentially generated in B lymphocytes, it is also possible the native proteins from which they are derived do not have to be initially taken up by an Ig mediated capture mechanism.

To distinguish between the mechanistic possibilities described above, we analyzed the extent of T1D development in a stock of NOD mice that can only produce B cells expressing an Ig specific for the pathologically irrelevant antigen hen egg lysozyme (HEL). Such NOD.*IgHEL.Igμ*^{null} mice proved to be T1D resistant (30). Conversely, T1D development was significantly accelerated in a NOD stock engineered to have an increased frequency of B-cells expressing an insulin specific Ig (31). Both results indicated the ability of B cells to serve as a preferential subpopulation of diabetogenic APC in NOD mice is due to their ability to take up β cell autoantigens through an Ig mediated capture mechanism.

4.3. Do secreted antibodies also contribute to T1D?

The initial detection in T1D patients of B cell derived circulating autoantibodies that could recognize pancreatic islet cells provided early evidence this disease has an autoimmune etiology (32). Such islet specific autoantibodies can be utilized as highly effective markers for predicting progression to T1D (32). β cell antigens most frequently targeted by autoantibodies in T1D susceptible humans include insulin, glutamic acid decarboxylase (GAD) and the tyrosine phosphatase-like molecules IA-2/IA-2β (32). In contrast, to date only insulin has been identified to be a *bona fide* target of β cell specific autoantibodies in NOD mice (33). However, antibodies that bind GAD and IA-2 in a non-specific fashion are also present in NOD mice (33). While the presence of autoantibodies provides an excellent diagnostic marker of humans at future risk of T1D (32), and can identify particular NOD mice that are closest to progressing to overt hyperglycemia (34), their ability to mediate pancreatic β cell damage *in vivo* has been a matter of contention.

It has been reported that maternally transmitted autoantibodies can be an important component of T1D pathogenesis in NOD mice (35). It is not known whether these antibodies can directly mediate β cell pathogenesis, or alternatively, form immune complexes with β cell proteins that enhance their ability to be taken up through an Fc receptor mediated mechanism by dendritic cells and macrophages which then process peptides from these antigens for presentation by MHC molecules to T cells. However, the specificity of these maternal autoantibodies remains a mystery based on a finding that T1D development was not affected in NOD mice regardless of whether they had or had not received maternally transmitted insulin autoantibodies (36). Other studies found that NOD.*Igμ*^{null} mice chronically infused with antibodies from overtly diabetic NOD females or born to B cell intact (autoantibody positive) NOD.*Igμ*$^{null/+}$ heterozygous mothers remained resistant to T1D (8, 10). Collectively, these studies indicate that while autoantibodies may contribute to T1D development in NOD mice, their production does not represent the sole pathogenic role of B cells in this disease. Whether maternal autoantibodies play a role in human T1D is questionable given that their transmission from diabetic human mothers to their offspring appear to be, if anything, mildly protective against disease (36). It should however be noted that the human studies analyzed the effect of maternal antibodies produced by diabetic mothers undergoing insulin-treatment, whereas in the murine studies, autoantibodies were transferred from pre-diabetic mothers.

5. DO B-CELL TOLERANCE DEFECTS UNDERLIE THE DEVELOPMENT OF DIABETOGENIC APC?

5.1. Mechanisms of B cell tolerance induction

An implication of the findings that Ig specificity is an important determinant of T1D susceptibility is that in addition to impaired T cell tolerance induction mechanisms, another disease predisposing factor in NOD mice may be defects in tolerogenic processes that normally prevent the development or activation of B cells expressing autoreactive specificities. NOD mice produce autoantibodies to a diverse range of antigens on different tissues including pancreatic ß cells (37), suggesting the presence of a generic defect in B cell tolerance induction. Interestingly, similar defects may also characterize many humans with T1D since autoantibodies to multiple tissue targets are commonly observed in individuals with disease and their relatives (38-40).

The transgenic expression in non-autoimmune prone mouse strains of an Ig specific for a native or nominal self antigen has provided significant insights to the processes that normally prevent the development or functional activation of autoreactive B cells (41). Such studies have demonstrated that the avidity of interaction (combination of affinity and valency) between the Ig and self-antigen determines which mechanisms of B cell tolerance operate in order to prevent development or activation of autoreactive clones (41). High avidity interactions result in the apoptotic deletion of B cells early in their development within the bone marrow (BM), unless self-reactivity is lost by re-arranging alternative light chains (termed receptor editing). On the other hand, B cells that undergo low avidity interactions with self-antigen can

emigrate from the BM, but are either deleted during the transitional stage in the spleen, or are rendered functionally anergic with a greatly reduced lifespan.

5.2. B cell tolerance induction defects in NOD mice

NOD mice were found not to be defective in the deletion of B cells expressing Ig transgenes that could undergo high avidity interactions with multivalent membrane-bound forms of HEL or H-2K^b class I MHC molecules expressed as neo-self antigens (42). In contrast, tolerance mechanisms induced by low avidity interactions with soluble HEL were defective in NOD mice as manifest by impaired deletion and anergy of self-reactive B cells compared to non-autoimmune prone B6 mice. This impaired ability to trigger tolerogenic responses after undergoing an initial low avidity interaction with a soluble self-antigen could underlie the development in NOD mice of functional β cell specific B cells that serve as diabetogenic APC. Acaveda-Suarez *et. al.* (43) addressed this question through the alternative approach of introducing an insulin-specific Ig transgene into NOD and B6 mice. Surprisingly few differences were observed between the NOD and B6 backgrounds in the tolerogenic characteristics of B cells transgenically expressing the particular insulin reactive Ig analyzed in this study. Nevertheless, the capacity of the Ig transgenic insulin-specific B cells to act as diabetogenic APC in NOD mice was not impeded by these tolerance mechanisms (43). It should be remembered that standard NOD mice produce insulin autoantibodies (33), indicating the presence of non-anergic B cells specific for this β cell autoantigen. At first glance, this might appear to be contradictory to the results reported by Acaveda-Suarez *et. al.* However, one possible reconciliation is that the insulin reactive B cells which remain functional in standard NOD mice bypass tolerance induction because the Ig they express have a lower functional avidity for antigen than the transgenic variant studied by Acaveda-Suarez *et. al.* Indeed, a recent study addressed this question using NOD and B6 background mice expressing only the heavy-chain of the transgenic insulin-specific Ig described above (44). Such a strategy permits generation of insulin-specific B cells of varying affinities due to random pairing of the transgenic heavy chain with endogenous Ig light chains. A small proportion of insulin-specific B cells were only detected in the peripheral lymphoid organs of NOD but not B6 genetic background mice (44). While allelic polymorphisms in Igκ light chain genes may be partially responsible for this difference (44), it is also possible that these autoreactive clones survive in NOD mice due to the avidity threshold for activating tolerogenic mechanisms against insulin specific B cells being set higher than in non-autoimmune prone B6 mice.

Alterations in the availability of co-stimulatory signals may also alter the threshold for tolerance induction in NOD mice. In particular, NOD mice exhibit increased expression of co-stimulatory molecules such as CD40, CD72, CD80 and CD86 and hyper-sensitive NF-kB activation (24, 45, 46). These molecular differences may act to lower the threshold for activation of NOD B cells that have escaped deletion due to deficiencies in central tolerance induction. Thus once potential autoreactive clones have entered the periphery, their enhanced capacity to interpret exposure to self antigen in a pro-inflammatory context may contribute further to the breakdown of tolerance in this strain.

6. B CELLS AMPLIFY, RATHER THAN INITIATE DIABETOGENIC CD4$^+$ T CELL RESPONSES IN NOD MICE

6.1. Circumstances that bypass B-cell contributions to T1D

Despite their normally strong resistance to disease, β cell autoreactive T cell responses that on rare occasions reach a level sufficient to induce T1D are generated in B cell deficient NOD.*Igμ*^{null} mice. This is partly illustrated by the fact their pancreatic islets are characterized by mild levels of leukocytic infiltration (insulitis) (47), which as discussed earlier, can occasionally progress to overt T1D. The implication of these observations is that diabetogenic T cell responses can be initiated in NOD.*Igμ*^{null} mice (probably through their interactions with dendritic cells and/or macrophages), but are usually not expanded to the levels required to cause overt disease in the absence of B cells. Interestingly, under lymphopenic conditions T cells from NOD.*Igμ*^{null} mice show an enhanced capacity to destroy β cells (47, 48). This suggests that lymphopenia-induced homeostatic expansion can serve as a partial surrogate for the role normally played by antigen presenting B cells in amplifying β cell autoreactive T cell responses to the point necessary for T1D development. The normal need for B cells to elicit efficient development of T1D in NOD mice is also overridden by other manipulations that allow for non-physiologic expansion of β cell autoreactive CD4$^+$ T cells in NOD mice. This would include the B cell independent development of T1D in NOD stocks transgenically expressing the TCR from β cell autoreactive CD4$^+$ T cell clones (49, 50). In addition to mediating the expansion of pathogenic T cells through antigen presentation, B cells may also promote T1D development in other ways. For instance, B-T cell interactions within germinal centers of lymphoid organs also serve to facilitate affinity maturation and epitope spreading (51), which could represent another means whereby B cells can enhance CD4$^+$ T cell responses against ß cells.

6.2. Why are other NOD APC subtypes inefficient at activating diabetogenic T-cells?

B cells (with perhaps the exception of marginal zone (MZ) B cells; see below) must undergo cognate interactions with T cells before they can become competent APC for other naïve CD4$^+$ T cells (51). Because of this, B cells are unlikely to be the primary APC for initially activating diabetogenic CD4$^+$ T cells. However, once B cells have received the necessary co-stimulation by T cells, this APC subpopulation becomes an important player in the expansion of CD4$^+$ T cell responses (51). Nevertheless, the question remains as to why dendritic cells or macrophages are insufficient to drive the autoimmune CD4$^+$ T cell response in NOD mice. Macrophages and dendritic cells in NOD mice are characterized by various developmental

defects that diminish their capacity to provide co-stimulatory signals to T cells (52-55). Perhaps this impaired co-stimulatory capacity of their other APC subtypes could be one reason why NOD mice depend on B cells to support the expansion of diabetogenic $CD4^+$ T-cell responses. Indeed, switching the preference of APC from B cells to macrophages by TGF-β1 treatment was shown to protect NOD mice from T1D (56). Also consistent with this notion is a report that while macrophages/dendritic cells from non-autoimmune prone B6 mice could provide the co-stimulatory activity needed to support the maximal anti-CD3 stimulated proliferation of $CD4^+$ T cells, the only subtype of APC from the NOD strain that could mediate this process was B cells (47, 57). Similarly, the diabetogenic $CD4^+$ T cell clone BDC2.5 only proliferates maximally when presented with its cognate autoantigen in pancreatic lymph nodes (PLN) of B cell sufficient, but not deficient, NOD mice (47). Interestingly, many, but not all, humans with T1D are characterized by defects in the development and co-stimulatory function of dendritic cells and macrophages similar to those observed in NOD mice (58-60). It is in this majority of patients that we posit B cells play an important role as APC in the development of T1D. On the other hand, the need for B cells in the development of disease may be by-passed in the minority group of T1D patients where dendritic cells and macrophages develop normally. Perhaps, compensatory dendritic cell and/or macrophage function may characterize the diabetic subject with XLA described by Martin et. al. (11).

6.3. Potential sites of interactions between diabetogenic B and T-cells

Given that their excision significantly retards T1D development in NOD mice (61), PLNs represent one site where primary interactions between diabetogenic B cells and $CD4^+$ T cells may occur. Another, non-mutually exclusive site is the spleen, which provides the largest reservoir of diabetogenic T cells (62). However, during the early prodromal stages of T1D development in NOD mice, B cells also infiltrate the pancreas (63, 64), where they are capable of participating in the formation of lymphoid follicular structures (with distinct B and T cell zones) surrounding the islets (65, 66). These pancreatic lymphoid structures have a critical bearing on T1D development in NOD mice as their dissolution by lymphotoxin signaling blockade has a strongly protective effect, even at a very late prodromal stage of disease (67) . Due to greater access to autoantigens, pancreatic lymphoid follicles may serve as secondary sites of B-T interaction, leading to additional amplification and diversification of diabetogenic $CD4^+$ T cell responses.

7. DIABETOGENIC B-CELL ACTIVITY IS CONTROLLED BY *Idd* GENES

7.1. *Idd* genes within the $H2^{g7}$ MHC haplotype contribute to diabetogenic B cell activity

It is now clear that an important pathogenic component of T1D development in NOD mice is the presence of B cells capable of taking up pancreatic β cell antigens through Ig mediated capture for subsequent MHC class II mediated presentation to autoreactive $CD4^+$ T cells. Hence, it seems likely that the pathogenic function of some Idd genes is to allow for the development and functional activation of such B cells. Multiple genes within the $H2^{g7}$ MHC haplotype interactively contribute to the Idd1 locus effect in NOD mice (3). This includes a failure to express H2-E MHC class II molecules on APC, illustrated by the fact that transgenic reversal of this defect imparts strong T1D resistance (3). Part of the diabetogenic effects elicited by the absence of H2-E expression in NOD mice is manifest by B cells. This was demonstrated by a finding that while the T1D resistance of NOD.Igμ^{null} mice was abrogated when repopulated with standard NOD B cells, those repopulated with B cells transgenically expressing H2-E MHC class II molecules could not develop disease (68). However, when even a small fraction of the total B cells used to repopulate NOD.Igμ^{null} mice were of standard NOD (H2-E negative) origin, this was sufficient to support T1D development. This indicated that H2-E expression on B cells does not exert an immunosuppressive effect on T cells, but rather leads to the loss of some pathogenic function by these APC (68).

7.2. Non-MHC *Idd* genes contribute to defects in B cell tolerance induction

Some non-MHC Idd genes also exert pathogenic effects at the B cell level. This is most amply demonstrated by our initial analyses of B cells from the NOR strain, that despite sharing ~88% of their genome, including the $H2^{g7}$ MHC haplotype with NOD, are T1D resistant (69, 70). Resistance variants of non-MHC Idd loci in NOR mice have been mapped to regions of chromosomes 2 (Idd13), 1 (Idd5), 4 (Idd9/11), and possibly 11 (Idd4) (69-71). In preliminary studies, we have found that while they repopulate NOD.Igμnull recipients to an equivalent extent, B cells from the NOR parental strain support a significantly lower level of T1D development than those of NOD origin (P.A.S. and D.V.S. unpublished observation). This indicates the function of the resistance variant of some Idd gene(s) in the NOR strain is to inhibit the development or function of B cells that can serve as pathogenic APC. As a first step in mapping and functionally characterizing such a gene(s), we have been assessing the diabetogenic activity of B cells from NOD stocks that are congenic for chromosomal segments containing NOR derived Idd13, Idd5, or Idd9/11 resistance loci (69, 71). As assessed by reconstitution of NOD.Igμnull recipients, B cells from the stock congenic for the NOR derived Idd9/11 resistance locus are significantly less diabetogenic than those from standard NOD donors (P.A.S. and D.V.S. unpublished observation). Other preliminary data indicate the presence of the NOR derived Idd9/11 resistance locus corrects the defective ability of autoreactive B cells in NOD mice to be rendered functionally anergic after engaging a low avidity soluble self antigen during their development (P.A.S. and D.V.S. unpublished observation).

8. WHICH B CELL SUBSETS CONTRIBUTE TO T1D?

8.1. Follicular B cells

Follicular (FO) B cells are good candidates for serving as a preferential subpopulation of diabetogenic PC in NOD mice. In particular their anatomical location facilitates interaction and germinal centre formation with autoreactive $CD4^+$ T cells from the peri-arteriolar lymphoid sheath (PALS) in lymphoid organs (72). Another important feature of FO that may allow them to contribute to T1D development is their ability to circulate through lymph nodes, with those draining the pancreas reported to be a critical site for the pathogenic activation of diabetogenic $CD4^+$ T cells (61).

8.2. Marginal Zone B cells

NOD mice also have an unusually large number of MZ B cells within the spleen (42, 73, 74). MZ B cells have been shown to have the capacity to serve as potent APC for activating naïve $CD4^+$ T-cells (75). Compared to other subsets, the MZ compartment contains a larger proportion of B cells with self-reactive specificities, and their aberrant expansion and migration is associated with various autoimmune pathologies in both humans and mice (75, 76). Interestingly, depletion of marginal zone B cells using CD21/35 specific antibodies can protect NOD mice from cyclophosphamide-induced diabetes (73). Collectively, these characteristics suggest the B-cells that serve as a preferential subset of APC for activating diabetogenic $CD4^+$ T cells may also reside within the MZ compartment.

8.3. Transitional 1 (T1) B cells

In non-autoimmune strains, B cells at the T1 developmental stage either undergo apoptosis or are anergized upon self antigen engagement (77, 78). Those that survive proceed onto the T2 stage, where they begin to acquire protection from deletion due to increased expression of anti-apoptotic genes and a decrease in pro-apoptotic products (79). T2 B cells are believed to be the precursors that give rise to FO and MZ B cells. Interestingly, NOD mice contain significantly lower numbers of T1 B cells, but enlarged numbers of T2 B cells in their spleens compared to non-autoimmune prone strains (42). This skewing of immature splenic populations suggested that NOD T1 B cells were not being properly deleted leading to an accumulation of autoreactive clones at the T2 stage, and ultimately in the FO and MZ populations. This was confirmed upon finding that T1 B cells from NOD mice were more resistant to deletion upon IgM cross-linking than those of non-autoimmune prone B6 mice (42). Therefore, defects at the T1 stage of development may have an important role to play in the development of autoreactive B cells capable of acting as diabetogenic APC.

8.4. B-1 B cells

B-1 cells are a subset that make up a substantial fraction of B cells in the peritoneal and pleural cavities and produce antibodies of limited diversity whose specificity is skewed towards self-reactivity (80). This subset normally produces natural autoantibodies but also enables responses to many T-independent antigens. A role for peritoneal B-1 B cells in T1D has been proposed due to a highly active natural autoantibody repertoire in NOD mice and the ability of B-1 B cells to infiltrate the pancreas during the prodromal stages of disease (81, 82). Furthermore, temporary depletion of B-1 B cells in the peritoneal cavity could both decrease insulin-specific antibodies in the sera and partially protect/delay NOD mice from developing T1D (81).

9. CONCLUSION: IMPLICATIONS FOR THE ROLE OF B CELLS IN T1D

It is now clear that in addition to $CD4^+$ and $CD8^+$ T cells which are final mediators of autoimmune pancreatic β cell destruction, B cells expressing autoreactive Ig molecules are also an important contributor to T1D development in NOD mice, and by inference in most human patients. There are a number of critical junctures where B cells may contribute to the development of T1D that are illustrated in Figure 1. While secreted autoantibodies may play a yet to be defined early pathogenic role in disease, it is the expression of self-reactive Ig on the plasma membrane of B cells that allows them to efficiently capture and take up self antigens and thus serve as a subpopulation of APC that preferentially mediates the expansion of diabetogenic $CD4^+$ T cell responses. It is tempting to speculate that in susceptible human individuals, the production of ß cell specific autoantibodies which are highly predictive of disease represents an important checkpoint in the development of T1D, where B cells have been activated and recruited as APC for expansion of the autoreactive $CD4^+$ T cell population. Interestingly, key roles for B cells as APC have also been described in various other autoimmune diseases, in which they are critical for bringing about the expansion, affinity maturation and epitope spreading of autoreactive $CD4^+$ T cell responses (83-85). The engagement of self-reactive B cells in an APC capacity may therefore represent a common factor integral for the precipitation of autoimmune diseases targeting many different tissues.

In designing potential intervention protocols for the prevention of T1D, it should therefore be considered that in addition to the well-studied impairments in T cell development, pathogenesis may also entail defects in tolerance mechanisms which give rise to autoreactive B cells that contribute to disease as APC. Rather than making the prospects more bleak, the finding that T1D entails interactive defects in B cell as well as T cell selection may actually improve the chances of ultimately developing a successful intervention protocol, as normalizing tolerance induction processes in either lineage could block disease progression.

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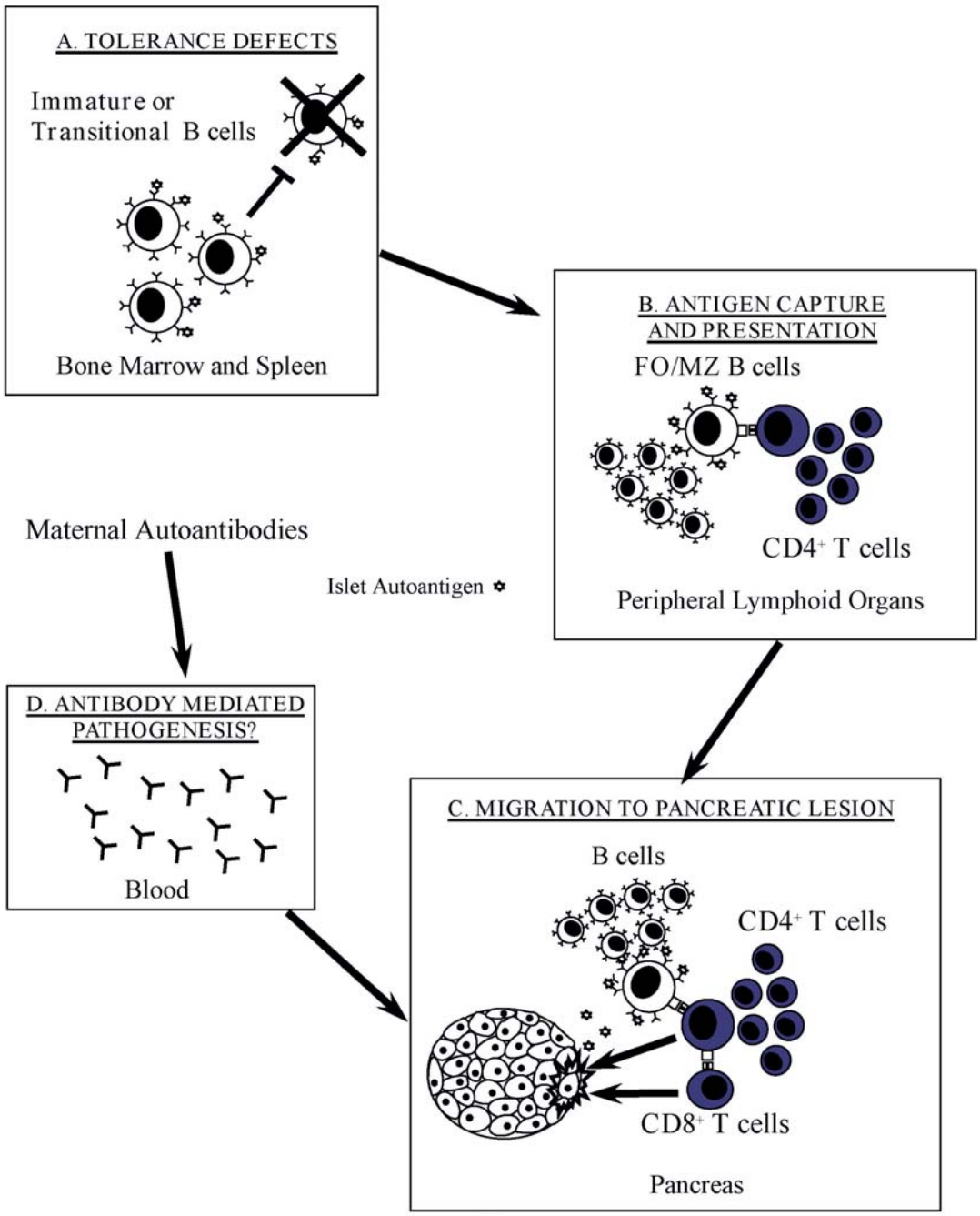

Figure 1. Different stages of participation of B cells in the development of T1D. A. A fundamental component in T1D development is the existence of self reactive B and T-cells. Breakdowns in tolerance mechanisms that normally leads to deletion or anergy of self reactive B-cells recognizing low avidity antigens results in the survival of β cell specific clones. B. By virtue of their ability to efficiently capture autoantigens through surface Ig, self-reactive FO and MZ B cells in NOD mice can act as preferential APC for MHC class II restricted β cell specific $CD4^+$ T-cells. In turn, activated T cells provide 'help' to FO and MZ B-cell populations, thereby further exacerbating impaired B-cell tolerance and anti-self hyperactivity. Collaboration of β cell specific B and T-cells results in the expansion of both these populations and also stimulates affinity maturation and epitope spreading of the autoimmune response. C. Activated B cells may also cause direct tissue pathology by migrating to the pancreatic lesions where they can help organize lymphoid structures and undergo successive waves of direct cognate interactions with autoreactive T-cells resulting in further amplification and diversification of the β cell specific response. D. B cells may also contribute to disease pathology via alternate mechanisms including modification of the fetal environment through maternally derived autoantibodies.

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