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REVIEW ARTICLE

Interactions between B-Lymphocytes and Type 1 NKT Cells in Autoimmune Diabetes

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Type 1 diabetes is one of the most prevalent autoimmune conditions that develops during childhood and has an increasing incidence worldwide. The disease results from the destruction of pancreatic β cells mediated by autoreactive T-lymphocytes. In order to develop preventive therapies, the cellular mechanisms responsible for the generation and activation of β cell-specific T-lymphocytes need to be characterized. Recent studies in the NOD mouse model of autoimmune diabetes suggest that the MHC Class II presentation of β cell-derived antigens by B-lymphocytes could support the development and activity of autoreactive $CD4^+$ T-lymphocytes in this disease. Interestingly, B-lymphocytes are also the most frequent antigen presenting cells expressing the MHC Class I like molecule, CD1d, the restriction molecule responsible for presentation of lipid and glycolipid antigens to Type 1 NKT cells. Splenic marginal zone B-lymphocytes, which express CD1d at particularly high levels, seem poised to signal to Type 1 NKT cells. In contrast to the disease-promoting role of conventional $CD4^+$ T-lymphocytes, several lines of evidence have shown that Type 1 NKT cells are involved in the prevention of Type 1 Diabetes. This review will analyze current knowledge on the roles of B-lymphocytes and Type 1 NKT cells in the onset of Type 1 Diabetes and explore possible outcomes of their interactions in relation to disease.

Keywords B-lymphocytes, Type 1 NKT cells, autoimmune diabetes

TYPE 1 NKT CELLS: A UNIQUE SUBPOPULATION OF T-LYMPHOCYTES

Type 1 NKT cells are a conserved subset of T-lymphocytes that express a semi-invariant TCR characterized by a monomorphic TCRα-chain, Vα14-Jα18 in mice and Vα24-Jα18 in humans, and a restricted set of TCRβ-chains comprising Vβ8.2, Vβ2, or Vβ7 in mice (Fowlkes et al., 1987; Lantz and Bendelac, 1994) and Vβ11 in humans (Dellabona et al., 1994). As is suggested by their name, Type 1 NKT cells also share characteristics with natural killer (NK) cells, expressing markers specific to the NK cells such as members of the Ly49 family and CD161 (also called NK1.1 in mice) (Sköld et al., 2003; Godfrey et al., 2004). Although expres-sion of NK1.1 is not uniform among the population (Pellicci et al., 2002) and is not expressed at all in some genetic backgrounds (e.g., NOD mice; Godfrey et al., 2000; Poulton et al., 2001).

The ontogeny of Type 1 NKT cells occurs in the thymus and involves a positive selection event at the double positive stage mediated by the monomorphic, β2-microglobulin-associated, major histocompatibility complex (MHC) Class I-like molecule, CD1d (Godfrey et al., 2000; Godfrey and Berzins, 2007). In contrast to the classical MHC molecules that present peptide antigens, CD1d is involved in the presentation of lipid and glycolipid antigens (Brigl and Brenner, 2004; Cardell, 2005). The origin of the natural antigens presented to Type 1 NKT cells in the context of CD1d and responsible for their maturation and activation remains disputed. CD1d is expressed by a range of cells including dendritic cells, monocytes, B- and T-lymphocytes, keratinocytes, hepatocytes, thymic epithelial cells, and thymocytes (Porcelli and Modlin, 1999; Brigl and Brenner, 2004), which all have potential to activate Type 1 NKT cells *in vivo*.

Murine mature Type 1 NKT cells may be divided into two major subsets; the $CD4^+CD8^-$ subset, expressing CD4 at intermediate levels, and the double negative ($CD4^-CD8^-$) subset (Cardell et al., 1995), They are mostly found in the liver, bone marrow, thymus, spleen, pancreatic and mesenteric lymph nodes (Laloux et al., 2002). When activated, Type 1 NKT cells promptly secrete large amounts of several cytokines, including interleukin (IL)-4, -2, -5, -10, and -13, and γ-interferon (Pellicci et al., 2002; Kronenberg and Gapin, 2002; Linsen et al., 2005).

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Due to these unique properties, Type 1 NKT cells are considered to be an immunoregulatory population of T lymphocytes, playing a role in both innate and acquired immunity through the production of chemokines and cytokines associated with immune deviation after interaction with antigen presenting cells (APC) via CD1d. Despite their very low proportions in the immune system at steady state (0.2% of peripheral blood T-lymphocytes in humans), they are involved in the immune responses to fungal, bacterial, viral and parasitic infections (Sköld and Behar, 2003), tumors (Smyth et al., 2002), transplants, and to self tissues in autoimmune diseases (Godfrey and Kronenberg, 2004; Linsen et al., 2005).

Restoration of Type 1 NKT Cell Number or Function in NOD Mice and Protection from Autoimmune Diabetes

NOD mice are characterized by a deficiency in Type 1 NKT cells (Gombert et al., 1996; Baxter et al., 1997; Godfrey et al., 1997). Type 1 NKT cells in this strain are also deficient in their IL-4 production on a per cell basis (Poulton et al., 2001) suggesting an additional functional deficiency.

Restoring Type 1 NKT cell numbers in NOD mice through adoptive transfer (Hammond et al., 1998; Poulton et al., 2001) or transgenic over-expression of the invariant TCRα-chain of Type 1 NKT cells (Lehuen et al., 1998) results in a significant reduction in the diabetes incidence. On the contrary, targeted mutant NOD mice deficient for CD1d, and as such lacking the entire NKT cell population, develop diabetes at a higher incidence than wild type mice (Shi et al., 2001). The location of CD1d-restricted antigen presentation also has an important bearing on the Type 1 NKT cell-mediated protection against Type 1 diabetes. Falcone and colleagues (2004) demonstrated this in a study where they engineered transgenic NOD mice expressing CD1d on the islets of Langerhans under the control of the human insulin promoter. The resulting enhanced activity of Type 1 NKT cells at the site of insulitis was sufficient to protect NOD mice from developing diabetes.

Diabetes can also be prevented in NOD mice by α-galactosylceramide (αGalCer)-mediated stimulation of Type 1 NKT cells (Hong et al., 2001; Sharif et al., 2001). αGalCer is a glycolipid derived from the *Agelas mauritanius* marine sponge that can activate Type 1 NKT cells when presented in the context of CD1d, for which the molecule possesses strong affinity (Shimosaka, 2002; Crowe et al. 2003). This protection is dependent on IL-4 (Mi et al., 2004), confirming the idea that Type 1 NKT cell-mediated resistance to Type 1 diabetes is associated with a T_H2 response of the immune system (Burdin et al., 1999; Singh et al., 1999; Laloux et al., 2001). Importantly, treatment with αGalCer protected diabetes development even in mice where insulitis was already initiated (Sharif et al., 2001).

The protection mediated by Type 1 NKT cell activation – via CD1d presentation of αGalCer – was found to be, at least in part, mediated through recruitment of tolerogenic dendritic cells to pancreatic lymph nodes (Naumov et al., 2001; Chen et al., 2005). Indeed flow cytometric analysis of αGalCer protected NOD mice revealed an accumulation of myeloid dendritic cells and Type 1 NKT cells in the pancreatic lymph nodes (Naumov et al., 2001). NOD mice exhibit several abnormalities in dendritic cell subsets, the most striking being a deficiency of the CD8α^+ DC population (Vasquez et al., 2004). As this dendritic cell subset expresses high levels of CD1d (Naumov et al., 2001) and may be involved in tolerance induction (Shortman and Liu, 2002), the defective immunoregulatory function of Type 1 NKT cells in NOD mice may be due to (1) a deficiency in NKT cell numbers and (2) impaired presentation of CD1d restricted antigen presentation by dendritic cells.

Genetic Control of NKT Cell Numbers in NOD Mice

Esteban and colleagues (2003) performed a linkage analysis on mice resulting from a backcross between C57BL/6 and NOD.*Nkrp1*^{b} mice. Two loci were found to control Type 1 NKT cell number: the *Nkt1* locus on distal chromosome 1 and the *Nkt2* locus on distal chromosome. *Nkt1* and *Nkt2* regions co-localize with the lupus susceptibility gene *Babs2*/*Bana3* and with the diabetes susceptibility gene *Idd13*, respectively (Esteban et al., 2003). The ability of both loci to control Type 1 NKT cell numbers and function have been confirmed in NOD congenic lines containing C57BL/6 variants of *Nkt1* or *Nkt2* (Rocha-Campos et al., 2006; Jordan et al., 2007; Fletcher et al., submitted). A similar increase in Type 1 NKT cells was also found in NOD mice containing a congenic region encompassing the *Idd13* locus from the diabetes resistant NOR strain (Chen et al., 2007).

Application of microarray technology to compare gene expression in thymi from NOD.*Nkrp1*^{b}.*Nkt1*^{b} versus NOD.*Nkrp1*^{b} mice identified *Slamf1*, encoding the signaling activation molecule (SLAM), as the most likely candidate gene controlling Type 1 NKT cell numbers within *Nkt1* (Jordan et al., 2007). Of only 28 locatable highly differentially expressed genes, *Slamf1* and *Slamf6* were remarkable in being dependent on SLAM-associated protein (SAP) for signaling; SAP deficient mice and humans have no NKT cells (Chung et al., 2005; Nichols et al., 2005; Pasquier et al., 2005). Flow cytometric analysis of thymic SLAM expression revealed that in NOD mice, SLAM expression was delayed in thymic ontogeny, so that the levels expressed on DP thymocytes were greatly reduced compared to C57BL/6 mice. This phenotype was corrected in the NOD.*Nkrp1*^{b}.*Nkt1*^{b} congenic line, consistent with Bendelac's model of SLAM-dependent NKT cell positive selection by DP thymocytes (Griewank et al., 2007).

Diabetogenic Roles of B-lymphocytes in NOD Mice

B-Lymphocyte-derived autoantibodies that target molecules expressed by β cells are found in the sera of most pre-diabetic NOD mice and humans, and are commonly used to predict progression to disease (Miao et al., 2007). B-Lymphocytes are also among the first cells to invade the islets and make up a significant proportion of the eventual infiltrate (Jarpe et al., 1991; Fox et al., 1998). Both of these characteristics of diabetes were previously

considered a consequence of T-lymphocyte-mediated autoimmunity rather than a causative agent. However, an important pathogenic role for B-lymphocytes in the development of Type 1 diabetes became apparent when NOD mice rendered deficient in these lymphocytes by chronic treatment with anti-immunoglobulin (Ig) antibodies (Forsgren et al., 1991; Noorchashm et al., 1997) or by gene-targeted mutation of the Igμ heavy chain gene (Serreze et al., 1996; Akashi et al., 1997; Yang et al., 1997) were revealed to be highly resistant to development of diabetes.

Susceptibility to diabetes in NOD.$Ig\mu^{null}$ mice could be fully restored by reconstituting them with NOD B-lymphocytes, but not with chronic infusion of autoantibodies from diabetic donors (Serreze et al., 1998). Therefore, secretion of autoantibodies is not the sole mechanism by which B-lymphocytes contribute to disease. This is not to say that autoantibodies do not play a role in disease. Indeed, Greeley and colleagues (2002) have shown that maternally-transmitted autoantibodies could increase susceptibility of NOD progeny to diabetes. The mechanism underlying this contribution has not yet been determined. It is possible that autoantibodies could damage pancreatic tissues through antibody-mediated cytotoxicity, which has been demonstrated *in vitro* (Dobersen et al., 1980; Eisenbarth et al., 1981; Kanatsuna et al., 1981), but not *in vivo*. Autoantibodies may also enhance the early uptake of β cell antigens by dendritic cells through F_c receptors (Inoue et al., 2007), which initiates activation of autoreactive $CD4^+$ and $CD8^+$ T-lymphocytes.

If instead of NOD B-lymphocytes, NOD.$Ig\mu^{null}$ mice were reconstituted with B-lymphocytes from NOD congenic mice containing an entire $H2^{nb1}$ diabetes resistance MHC haplotype from the NON strain, recipient mice showed significant protection from diabetes (Chen et al., 2007). Analogous protection from diabetes was conferred by NOD B-lymphocytes transgenically expressing the *H2-E* MHC Class II diabetes resistance gene that is normally absent in the NOD strain (Johnson et al., 2001). Thus, as expected, MHC diabetes susceptibility genes contribute to the diabetogenic activity of B-lymphocytes. If, on the other hand, NOD.$Ig\mu^{null}$ mice were reconstituted with B-lymphocytes from NOR mice, a diabetes-resistant strain sharing 88% of its genome with NOD mice including the $H2^{g7}$ MHC haplotype, recipients only regained half their susceptibility to disease (Silveira et al., 2006).

This result indicated that some non-MHC *Idd* genes also mediate their effects through B-lymphocytes. To identify non-MHC *Idd* genes contributing to the diabetogenic activity of B-lymphocytes, NOD.$Ig\mu^{null}$ mice were reconstituted with B-lymphocytes from various NOD congenic lines containing *Idd* resistance loci (Silveira et al., 2006). Only NOD B-lymphocytes expressing *Idd5* or *Idd9* resistance alleles (on Chromosome 1 and 4, respectively) could confer protection from disease in NOD.$Ig\mu^{null}$ mice, similar to NOR B-lymphocytes. Both *Idd5* and *Idd9* resistance loci were found to correct B-lymphocyte tolerance to soluble self-antigens in NOD mice, which probably represents one mechanism by which these loci confer protection against diabetes.

NOD B-Lymphocytes Enhance Diabetes Susceptibility by Presenting Self-antigen to Effector T-lymphocytes

B-Lymphocytes are one of three specialized APC for T-lymphocytes in the immune system, the two others being dendritic cells and macrophages. B-Lymphocytes are unique in that they are able to recognize specific antigens via the variable region of Ig molecules on their plasma membrane (B-cell receptor; BCR). Antigens captured by BCR are endocytosed, processed and presented at relatively high concentrations on the surface as peptides in the context of MHC Class II molecules. Using this mechanism, B-lymphocytes are highly effective in presenting low abundance antigens to T-cell receptors (TCR) on CD4 T-lymphocytes, induc-ing specific clonal expansion and activation of their effector function (Constant et al., 1995).

Analysis of CD4 T-lymphocyte responses in NOD.$Ig\mu^{null}$ revealed that those specific for various diabetes autoantigens (GAD, pro-insulin, and IA-2) were greatly diminished, while these mice remained capable of forming adequate responses to certain foreign antigens (Falcone et al., 1998; Serreze et al., 1998; Wheat et al., 2004). These data suggested that diabetes resistance shown by NOD.$Ig\mu^{null}$ mice is caused by inadequate auto-antigen presentation to autoreactive CD4 T-lymphocytes, which is normally performed by B-lymphocytes. This was confirmed in a study where bone marrow from NOD.$Ig\mu^{null}$ and NOD.CIITA were mixed to reconstitute NOD.$Ig\mu^{null}$ mice, thus producing NOD mice specifically lacking MHC Class II on all B-lymphocytes (Noorchashm et al., 1999). These mice were rendered resistant to diabetes despite expressing MHC Class II on other APC. The ability of NOD B-lymphocytes to act as preferential APC for β cell reactive CD4 T-lymphocytes also depends on the generation of autoreactive clones able to capture β cell antigens through BCR, since transgenic NOD mice capable of only producing B-lymphocytes expressing Ig specific for hen egg lysozyme, a protein irrelevant to disease, showed diminished CD4 T-lymphocyte auto-reactivity and protection from diabetes (Silveira et al., 2002).

In a recent study, Brodie and colleagues (2008) introduced the $Ig\mu^{null}$ mutation into RIP-TNFα transgenic NOD mice, a diabetes model where CD8 T-lymphocytes mediate β cell destruction independently of CD4 T-lymphocytes. B-Lymphocyte deficiency in this model caused decreased expansion and prevented generation of an effector phenotype in auto-reactive CD8 T-lymphocytes in the pancreatic lymph nodes. In the islets, the absence of B-lymphocytes rendered cytotoxic CD8 T-lymphocytes more susceptible to apoptosis. This resulted in a significant delay of disease compared to B-lymphocyte-sufficient controls. The rate of diabetes in these mice was restored by re-introducing normal B-lymphocytes as well as B-lymphocytes incapable of secreting Ig. Hence, in a yet to be defined mechanism that is independent of autoantibodies, B-lymphocytes are involved in promoting CD8 T-lymphocyte destruction of β cells.

B-Lymphocyte Diabetogenic APC Function Appears to be Required in the Later Stages of Autoreactive T-Lymphocyte Activation

Temporary depletion of B-lymphocytes using anti-CD20 monoclonal antibodies in NOD mice transgenically expressing human CD20 on B-lymphocytes or BAFF antagonists in wild type NOD mice were found to confer greater protection against diabetes if mice were treated later in the disease process (9–12 weeks of age) rather than early (6 weeks of age) (Marino et al., 2005; Hu et al., 2007). Anti-CD20 antibodies were even effective at reversing disease in a third of animals that already developed diabetes. These data suggest that the B-lymphocyte contribution as a diabetogenic APC is probably most critical in the later stages of the T-lymphocyte response against β cells. This would explain why NOD.$Ig\mu^{null}$ mice, despite their strong resistance to diabetes, still show early signs of β cell specific T-lymphocyte reactivity in the form of mild insulitis (Greeley et al., 2001). Autoreactive T-lymphocytes generated in NOD.$Ig\mu^{null}$ mice are capable of causing diabetes if placed in an environment where they can undergo homeostatic expansion, such as in lymphopenic hosts (Chiu et al., 2001).

Similarly, in diabetes models where β cell-specific CD4 T-lymphocytes are artificially expanded using TCR transgenes, B-lymphocytes are also not required for the development of diabetes. These studies imply that development and targeting of diabetogenic T-lymphocytes can be initiated in the absence of B-lymphocytes; however, their ability to be expanded to the patho-genic levels required to cause disease in the later stages of disease appears greatly diminished. In addition to the expansion of the autoreactive CD4 T-lymphocyte response, Tian et al. (2006) have demonstrated that the absence of B-lymphocytes abrogates spreading of CD4 T-lymphocyte autoreactive response to different antigenic determinants on β cells, an event that also occurs in the late stages of disease. Finally, Kendall and colleagues (2007) have also shown that B-lymphocytes are central for the generation of tertiary lymphoid structures surrounding islets, which form late in the development of diabetes and are predicted to be an important site for the conversion of a benign into a destructive T-lymphocyte response against pancreatic β cells.

NOD Mice Develop an Enlarged Marginal Zone B-Lymphocyte Pool

NOD mice exhibit a considerably enlarged splenic marginal zone (MZ) B-lymphocyte pool (Silveira et al., 2004; Rolf et al., 2005). MZ B-lymphocytes exhibit unique characteristics not shared by follicular (Fo) or B-1 B-lymphocytes; *viz*. they are able to produce rapid antibody responses to blood borne antigens independently of T-lymphocyte help (Balazs et al., 2002), they transport opsonized antigens from the marginal zone to the lymphoid follicle (Cinamon et al., 2007) and lastly, they have been shown to be the most potent B-lymphocyte subset for activating naïve CD4 T-lymphocytes (Attanavanich and Kearney, 2004).

They are distinguishable by high expression levels for CR1/2 (CD21/35) complement receptors used for antigen uptake and transporting antigens into lymphoid follicles (Gray et al., 1984; Cinamon et al., 2008), low expression of the $F_{c\varepsilon}$RII (CD23) (Waldschmidt et al., 1991), and high expression of CD9 (Won and Kearney, 2002), a molecule involved in membrane clustering of MHC Class II molecules (Unternaehrer et al., 2007) and which may account for their enhanced ability to activate naïve CD4 T-lymphocytes. Finally, MZ B-lymphocytes are also differentiated from other B-lymphocytes through their high expression of the CD1d molecule (Amano et al., 1998; Roark et al., 1998), which suggests that they are an important APC for NKT cells.

Apart from the increased numbers of these cells in NOD mice, various other data implicate MZ B-lymphocytes in the pathogenesis of Type 1 diabetes:

(1) Prior to development of diabetes in NOD mice, marginal zone B-lymphocytes were found to aberrantly migrate from the spleen into the pancreatic lymph nodes as well as the pancreatic infiltrate, which are sites critical for the activation of β cell-reactive T-lymphocytes (Marino et al. 2008).
(2) B-Lymphocytes expressing a transgenic Ig specific for the diabetes autoantigen insulin show an increased capacity for developing into marginal zone B-lymphocytes compared to wild-type B-lymphocytes in both NOD and B6 genetic backgrounds (Acavedo-Suarez et al., 2005).
(3) Marginal zone B-lymphocytes from wild-type NOD mice were shown to be able to capture and present insulin autoantigen in a form that can activate autoreactive CD4 T-lymphocytes in NOD mice (Marino et al., 2008).
(4) Depletion of MZ B-lymphocytes in NOD mice using anti-CD21/35 antibodies conferred protection against cyclophosphamide-induced Type 1 Diabetes (Noorchashm et al., 1999).

To determine the genetic basis of MZ B-lymphocyte expansion in NOD mice, linkage analysis was carried out in an NOD x C57BL/6 F2 cross (Rolf et al., 2005). This study revealed a significant linkage peak controlling MZ B-lymphocyte numbers in a segment co-localizing with the *Idd11* susceptibility region on Chromosome 4. However, a congenic NOD line carrying the B6 *Idd11* region, which was developed by an independent group, failed to confirm the control of NOD MZ B-lymphocyte phenotype by this chromosomal segment (Brodnicki et al., 2006). Nevertheless, it remains possible that this trait may be under epigenetic control by genes inside and outside the *Idd11* interval.

Effect of Type 1 NKT Cells Signaling on B-Lymphocyte Response

The earliest evidence of a link between Type 1 NKT cell activity and B-lymphocyte activity was given by a study carried out in 1995 showing that treatment of mice with α-GalCer induced

secretion of IgE (Yoshimoto et al., 1995). Further analysis of the effect of human Type 1 NKT cells on B-lymphocytes, carried out by Galli and colleagues (2003), revealed CD1d-dependent B-lymphocyte proliferation and Ig production *in vitro*. The B-lymphocyte response induced by Type 1 NKT cells was equivalent to that induced by CD4 T-lymphocytes and could be stimulated without α-GalCer, albeit at a lower level, suggesting that some B-lymphocytes present an endogenous ligand to Type 1 NKT cells via CD1d (Galli et al., 2003).

A more recent study carried out by the same group (Galli et al., 2007) demonstrated that the antibody production elicited by α-GalCer *in vivo* was comparable to those induced by the adjuvants CpG, Complete Freund's adjuvant and Alum. This B-lymphocyte response was entirely dependent on interactions with Type 1 NKT cells, as it did not occur in Type 1 NKT cell-deficient mice. Furthermore the secretion of IgG was CD4 T-lymphocyte-independent, suggesting that α-GalCer-induced B-lymphocyte response is dependent on Type 1 NKT cell signaling only. Galli and colleagues also injected influenza A virus-derived H3N2 antigen into mice lacking Type 1 NKT cells ($J\alpha18^{-/-}$) or wild type mice ($J\alpha18^{+/+}$), and they observed a faster decay of H3N2-specific antibody in the serum in the absence of Type 1 NKT cells, thereby revealing that Type 1 NKT are required to sustain serological memory. Furthermore, immunization of mice to H3N2 combined with α-GalCer-mediated activation of Type 1 NKT cells increased the rate of anti-body production in response to secondary injection of H3N2. Thus, enhanced Type 1 NKT cell signaling to B-lymphocytes upon activation contributes to the development and maintenance of an effective B-lymphocyte memory.

The production of anti-double-strand DNA antibodies by NZB/W mice, that spontane-ously develop systemic lupus erythematosus (SLE), is also enhanced by activation of Type 1 NKT cells by α-GalCer (Zeng et al., 2003). In this strain, not only did purified NKT cells increase spontaneous secretion of IgM anti-dsDNA autoantibodies by B-1 and marginal zone cells, but they also facilitated secretion of IgG anti-dsDNA autoantibodies by B1-B-lymphocytes. In contrast, conventional T-lymphocytes failed to provide helper activity to any B-lymphocyte subset (Takahashi and Strober, 2008). These data are consistent with an interaction between innate B-lymphocytes and Type 1 NKT cells contributing to autoimmunity in this model.

In contrast, the treatment of NOD mice with α-GalCer reduced serum levels of anti-GAD antibodies and deviated the T_H1-like response displayed by these mice to one characterized by switching the autoantibody isotype from IgG_{2c} to IgG_1, and prevention of disease (Sharif et al., 2001). The differences in the outcome of Type 1 NKT cell activation on MZ B-lymphocyte activity in these two animal models may reflect differential cytokine dependencies in each case. Certainly, MZ B-lymphocytes appear to mediate the generation of auto-reactive T-lymphocytes in NOD mice and any role for NKT cells in this process remains to be determined.

Effect of B-Lymphocyte Signaling on NKT Cell Response

In addition to the recently discovered help provided by Type 1 NKT cells to B-lymphocytes, parallel studies have suggested that Type 1 NKT-B-lymphocyte interaction can also enhance Type 1 NKT cell function. Bezbradica and colleagues (2005) studied the role of dendritic cells and B-lymphocytes in Type 1 NKT cell activation in NOD mice. They reported that, whereas the dendritic cell-mediated activation of Type 1 NKT cells is defective in these mice, B-lymphocytes could elicit the production of IL-4 by Type 1 NKT cells. The ability of B-lymphocytes to present α-GalCer to Type 1 NKT cells is increased by 100-1000 fold when their BCR is targeted by biotin conjugated α-GalCer (Lang et al., 2005), suggesting that B-lymphocytes, and especially $CD1d^{high}$ B-lymphocytes such as MZ B-lymphocytes, may recognize endogenous glycolipids through their BCR facilitating presentation of the CD1d-restricted antigens to Type 1 NKT cells.

In anterior chamber-associated immune deviation, the induction of regulatory T-lymphocytes capable of inhibiting localized T-lymphocyte-mediated inflammatory lesions (local adoptive transfer model) was dependent on the expression of CD1d on a subpopulation of MZ B-lymphocytes in the spleen (Sonoda and Stein-Streilein, 2002). NOD mice have relatively few regulatory T-lymphocytes (Salomon et al., 2000; Wu et al., 2002; Alard et al., 2006) despite enhanced thymic selection of this T-lymphocyte lineage (Feuerer et al., 2007), consistent with peripheral defects in their development or maintenance being responsible. The ability of B-lymphocytes to present glycolipid antigens to Type 1 NKT cells in a CD1d restricted manner, as well as downstream effects on regulatory T-lymphocyte circuits may therefore play a role in NKT cell-mediated prevention of T1D in NOD mice.

As B-lymphocyte-deficient mice do not exhibit any particular NKT cell phenotype (Lang et al, 2008) and NKT cell deficient $B2m^{-/-}$ targeted mutant mice display normal B-lymphocyte development (Amano et al., 1998), the development of these two lymphocyte subsets is likely to be relatively independent, and their interaction restricted to modulating their functions.

CONCLUSIONS

Type 1 NKT cells and B-lymphocytes have contrasting roles in the development of diabetes. Type 1 NKT cells play an important part in preventing autoimmunity, but their deficient numbers and function in NOD mice mean they are unable to restrain T-lymphocyte-mediated β cell destruction unless artificially expanded or activated. On the other hand, B-lymphocytes promote the development of diabetes by being preferential APC for the expansion and differentiation of β cell-specific CD4 T-lymphocytes and CD8 T-lymphocytes. Moreover, autoantibodies produced by activated B-lymphocytes may also serve to enhance the presentation of β cell autoantigens by dendritic cells and macrophages.

Given that B-lymphocytes are one of the most frequent APC cell types expressing CD1d, it is likely that they present (or try to present) antigens to NKT cells during the development of Type 1 diabetes. This raises the question of whether the deficiency of NKT cells in NOD mice might be a factor that contributes to the pathogenic behavior of B-lymphocytes, and that of whether activation of NKT cells through α-GalCer could alter the pathogenic function of B-lymphocytes. These questions would especially be relevant for MZ subset of B-lymphocytes, which various lines of evidence suggests may be an important player in the development of Type 1 diabetes, since they comprise one of the highest expression levels of the NKT cell restriction element CD1d. Understanding the interaction between these two cell types may provide new avenues of treatment for preventing the development of Type 1 diabetes.

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