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New Players in the Field of T1D: Anti-Peripherin B Lymphocytes

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COMMENTARY

B lymphocytes (B cells) play a key role in type 1A diabetes (T1D) via antigen presentation to T cells, but our understanding of their mechanism of action is still evolving (1–6). Pathogenic B cells recognize autoantigens, indicated by T1D-predictive autoantibodies against insulin, GAD65, transmembrane tyrosine phosphatase (IA-2), and zinc transporter 8 (ZnT8) (7). These autoantibodies are not thought to cause T1D but, rather, provide evidence of autoreactive T cell–B cell interactions. Each B cell recognizes a single antigenic site, or epitope, on folded proteins via the antibody variable (V) region of its B-cell receptors (BCRs), internalizes the protein, and processes it into peptides for presentation on MHC class II (MHCII) to T cells (Fig. 1). Thus, autoreactive B cells are uniquely suited to activate autoreactive T cells. Likewise, T cells provide help to B cells, driving antigen-specific germinal center (GC) formation and differentiation into plasma cells that secrete autoantibodies identical to the BCR. Transgenic models provide evidence that B cells must be autoantigen specific to support T1D: anti-insulin B cells present antigen, activate T cells, and promote T1D in nonobese diabetic (NOD) mice (5,6,8). Conversely, NOD mice with transgenic B cells that do not recognize an autoantigen fail to develop diabetes (5,9). While there are multiple T1D-related autoantibodies in humans, to date, insulin has been the only reliable B cell–related autoantigen in NOD mice. In this issue of *Diabetes*, Leeth et al. (10) report on a new transgenic model in which B cells recognize peripherin, a neuronal antigen, and are able to support diabetes development.

Peripherin is expressed in peri-insular areas of islets, and in central and peripheral nervous system cells, similar to the important human autoantigen GAD65. The BCR transgene, designated *PerIg*, was developed from a BCR cloned from islet-invading B cells in NOD mice (10–13). NOD.*PerIg* mice expressing the BCR heavy chain, the BCR light chain, or both develop diabetes at higher rates than wild-type NOD counterparts. As expected, disease outcome is T cell dependent.

PerIg B cells have many features that suggest that they escape central tolerance mechanisms in a functionally defective state termed anergy. They do not produce antibody or proliferate well when stimulated. They are significantly increased in number at the early transitional (T1) stage, which is a tolerance checkpoint, and have decreased mature naïve follicular numbers, suggesting that these cells are developmentally blocked and culled at the T1 stage. They are increased in the marginal zone compartment, which depends on low-affinity antigen-dependent selection and is enriched for autoreactive specificities, but do not populate the similar B1a compartment. *PerIg* B cells also have altered maturation profiles in the bone marrow and engraft poorly under a variety of conditions. Many of these characteristics are shared with anergic anti-insulin (125Tg) B cells, indicating that B-cell tolerance need not be broken for them to play this role (6). In fact, it suggests the possibility that the residual functions of anergic B cells may somehow be especially well suited to present antigen and support T cell–mediated T1D.

Despite having dysfunctional properties, *PerIg* B cells invade islets well, where they show evidence of proliferation ($Ki67^+$) and even differentiation into plasma cells ($CD138^+$). CD86 expression is higher on *PerIg* B cells in islets than on their WT NOD counterparts, suggesting increased capacity for T-cell activation. Consistent with this, GC reactions are increased in draining pancreatic lymph nodes (PLNs), as characterized by $Fas^+/GL7^+$ GC B cells and $CXCR5^+/ICOS^+$ T follicular helper cells. However, BCR sequencing from PLNs shows no somatic hypermutation, which normally increases affinity and is the final end point of GC reactions, and anti-peripherin antibody is not found in serum. This may reflect functional status of these cells or may simply be due to the unknown integration site of the transgene. Nevertheless, the presence of GCs in PLNs strongly suggests that anti-peripherin T cells are available to activate *PerIg* B cells and vice versa.

Of note, highly activated GC B cells do have increased ability to present antigens beyond those recognized by their BCRs, can cross present antigen to $CD8^+$ T cells, and also produce inflammatory cytokines (14,15). It is also possible, as stated by Leeth et al. (10), that the *PerIg* BCR cross-reacts

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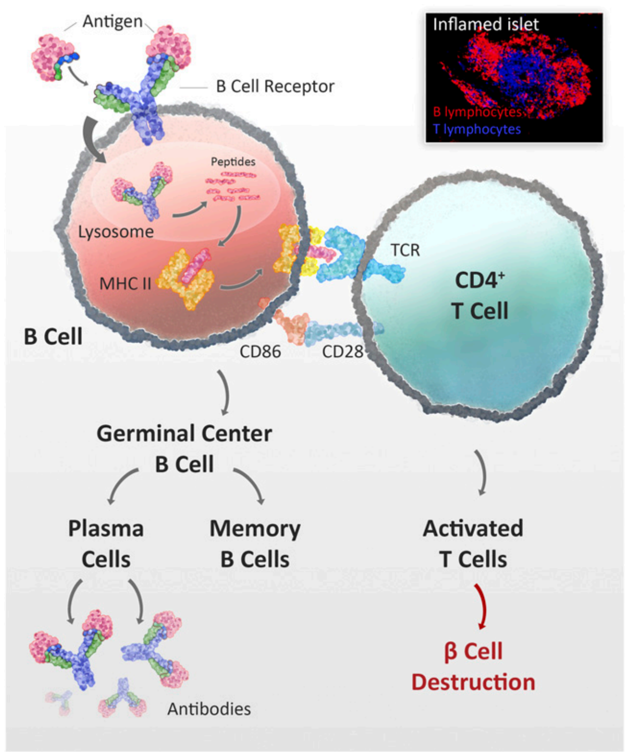

Figure 1—Autoreactive B cells present antigen and activate autoreactive T cells that recognize the same antigen. Each B cell recognizes a single antigen via the antibody component of BCRs. The BCR specifically binds its target autoantigen and then internalizes it into the B cell for processing. The protein antigen is cleaved into short peptides that are loaded into MHCII molecules. MHCII then moves to the surface of the B cell and presents the peptide to T cells that recognize the same antigen. The T cell further activates the B cell, driving it into germinal centers, where it differentiates into memory B cells and plasma cells that then secrete large amounts of antibody identical to the BCR. The antigen-presenting B cell also provides a critical costimulatory signal (CD86 to CD28) to activate the T cell. Activated autoreactive T cells destroy insulin-producing β-cells, while the autoantibodies serve as markers of autoimmune activity and can reveal the autoantigens that are recognized by pathogenic T cells. These processes may occur in draining pancreatic lymph nodes or in inflamed islets. The inset shows immunohistochemical staining of T cells (blue) and B cells (red) infiltrating an islet from an NOD mouse.

with β-cell antigens or that β-cell antigens form complexes with peripherin that are internalized by the anti-peripherin BCR. These scenarios would promote epitope-spreading and widespread β-cell destruction by T cells that recognize autoantigens beyond peripherin. This concept could be tested in the future by examining the frequency of T cells that respond to known epitopes (e.g., insulin B:9-23) or pairing *PerIg* with transgenic T cells that recognize insulin or chromogranin peptides (16,17).

There are other issues to be addressed as well. It is surprising that the *PerIg* BCR light chain alone confers disease. Understanding which BCR heavy chain(s) this light chain pairs with, and what antigen(s) are recognized, would be useful. Similarly, while BCR heavy chains alone sometimes confer specificity, they most often require a particular light chain. For example, V_H125, the anti-insulin heavy chain, combines with multiple Vκs, but only two support insulin-binding (18,19). The anti-insulin V genes are unrelated to those used by *PerIg*. Therefore, complementary pairing and antigen-binding profiles for both *PerIg* V_H and V_K are needed. Likewise, structural analyses of these V regions might clarify their potential to cross-react with multiple antigens. For example, large CDR3 loops sometimes bind multiple antigens with low affinity and could contribute to many of the features of *PerIg* B cells, such as altered bone marrow maturation and negative selection at the T1 stage.

PerIg joins anti-insulin 125Tg as the only two Ig transgenic models to fully support T1D development. These findings contrast with those of a recent similar report from Carrascal et al. (20) regarding another islet-derived transgene, 116C, which protects against T1D development relative to endogenous NOD B cells, indicating that islet invasion alone is not enough to determine pathogenicity of B cells. Importantly, this novel study by Leeth et al. (10) is the first to discover a B cell–related autoantigen beyond insulin important for diabetes in NOD mice and suggests that peripherin, like GAD65, may prove to be a key autoantigen.

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