How benign autoimmunity becomes detrimental in type 1 diabetes

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Central questions for organ-specific autoimmune diseases such as type 1 diabetes (T1D) are, What are the relevant self-antigens of the destructive processes and Why does the immune system become interested in them? In PNAS, Anderson et al. (1) provide more evidence that insulin and its precursors constitute a key antigen in T1D. Nakayama, Eisenbarth, and coworkers had previously demonstrated that autoimmune diabetes in the NOD mouse model does not develop without immune targeting of insulin (2, 3). Now, Nakayama and her team take advantage of T1D pancreas procurement programs to address insulin reactivity of $CD8^+$ T cells found around the islets from seven patients with T1D. They sequenced the T cell receptors (TCRs) of islet $CD8^+$ T cells and successfully expressed 142 clonotypes to examine their specificity against preproinsulin (PPI). This enormous undertaking found that 24 (17%) of the TCR clonotypes reacted against PPI peptides and a further three responded to peptides from defective ribosomal products of PPI messenger RNA (DRiP). Clonotypes responsive to PPI peptides were observed in all patients, although at varying frequencies, and peptides across the whole PPI molecule were targeted. $CD8^+$ T cell clonotypes were present in at least two cells, suggesting that they had undergone local rounds of expansion. In islets from nondiabetic donors, reactivity to PPI or DRiP peptides was weaker and infrequently observed.

The findings of Anderson et al. (1) are supported by several other studies. Peakman and coworkers isolated PPI-specific $CD8^+$ T cell clones from the blood of patients with T1D (4), and Kent et al. isolated insulin-specific $CD4^+$ T cells from pancreatic lymph nodes of patients (5). PPI-reactive $CD8^+$ T cells were present in human islets during T1D pathogenesis (6) and also in the exocrine pancreas of healthy individuals, from where they appear to become attracted and migrate to islets during disease development (7). With the present study and the recent study of Rodriguez-Calvo et al. (8) we now learn that a good proportion of the $CD8^+$ T cells in islets of organ donors with T1D do indeed recognize and respond to PPI or DRiP peptides. Moreover, while HLA A2 was also frequently used to present PPI peptides in the study of Anderson et al. (1), many of the TCRs were specific for PPI peptides presented by other HLA class I alleles and molecules. These observations are in line with studies from Mallone and coworkers, which found a preponderance of secretory pathway and PPI-specific reactivity presented by a range of HLA class I molecules in $CD8^+$ T cells isolated from blood of T1D patients (9). Collectively, the work of Anderson et al. and other groups make a strong case for a rather universal prevalence of PPI reactivity in human T1D.

What Does This Mean Mechanistically?

Insulin and PPI are present in relative abundance locally in the islets of Langerhans, and it is conceivable that they would constitute eminent autoantigens. Insulin reactivity in the form of autoantibodies precedes development of clinical diabetes in the majority of childhood cases and can, together with other autoantibodies, be used as a biomarker for the risk of developing T1D (10). Interestingly, T and B cell autoreactivity appear to target different PPI regions. The study of Anderson et al. (1) and other studies found relatively abundant $CD8^+$ and $CD4^+$ T cells reactive against peptides found only within the nonprocessed PPI protein or modified PPI, including hybrid peptides (11). In contrast, autoantibodies are against intact, unmodified insulin and few PPI-specific antibodies are found (12). Nothing is known of the specificity of human islet-infiltrating B lymphocytes and whether they may also target PPI-specific epitopes and therefore corroborate a PPI-preponderant autoimmune attack. A potential drawback of findings from human islet-derived T cells is that they are almost always from patients after the

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onset of type 1 diabetes when multiple daily insulin injections may affect numbers and specificity of insulin-specific T cells. It is also possible that the antibody response to insulin provides the clue that insulin is primary as an antigen and that spreading of the T cell response to PPI epitopes is a critical part of disease progression. This will require more detailed analyses of T cells at the time of insulin autoantibody seroconversion.

An obvious unresolved question in the work of Anderson et al. (1) is what the specificities of the remaining (majority) $CD8^+$ T cells are. Mallone and coworkers previously reported reactivity to other known islet autoantigens such as ZnT8 (13). Anderson et al. looked for and found no reactivity against other previously described islet autoantigen peptides or glucagon peptides. One possibility is that they are against posttranslationally modified neoantigens or hybrid peptides often derived from PPI, as suggested for $CD4^+$ T cells. Alternatively, they may be against viral proteins (14). A second question is whether there are specificities that contribute to accelerating the disease course. Anderson et al. do not present evidence for pathogenicity. While PPI-specific $CD8^+$ T cells are in relative abundance, we do not know if they are the ones that orchestrate the damage. We might learn from antiviral immunity, where we can sometimes observe single specificities that remain "in the driver's seat," or much broader responses, both of which can achieve viral control (15). In both scenarios, the numbers of T cells correlate with the degree of protection.

Another key question preoccupying the field for decades is why and how autoreactivity comes about. We can take some clues from studies that have shown physiological autoreactivity—notably also in the form of PPI $CD8^+$ T cells in healthy individuals (16, 17). Indeed, PPI-reactive $CD8^+$ T cells are also found in exocrine pancreata of healthy individuals, albeit in lower numbers than in T1D patients and in lower numbers than in T1D islets (7). This physiological "benign" autoreactivity might be important for immune surveillance and homeostasis. While the physiological relevance of these autoreactive cells is debatable, their presence certainly tells us that some of the checkpoints such as thymic selection that are thought to keep autoreactivity under control are "leaky." Considering the clinical manifestation of organ-specific autoimmune diseases such as T1D, it appears likely that, with exceptions in patients who develop multiple autoimmune manifestations, there may be a (preferentially) local rather than a systemic dysregulation of the immune response. We, therefore, need to look closer at what occurs during T1D pathogenesis on the organ level to understand why cells that recognize PPI turn pathogenic and expand. Here the answer might lie with the beta cell itself (18, 19). Through the study of pancreata from human organ donors, we have learned that beta cells exhibit abnormalities during T1D pathogenesis consistent with an "unmasking" process to the immune system (Fig. 1). Pathognomic features seen in T1D prior to immune infiltration are the up-regulation of major histocompatibility complex (MHC) class I, a processing defect of proinsulin to insulin, and later in disease potential expression of MHC class II as well as secretion of inflammatory cytokines by beta cells themselves. While we cannot be sure what leads to these pathological changes (viral infections, other forms of beta cell stress, or changes in innervation signals), we can be more certain that beta cells expressing higher levels of MHC class I and containing a relative increase in PPI are more likely to be seen by autoreactive PPI-specific $CD8^+$ T cells.

What Does This Mean for Biomarkers?

Prospective studies have shown that insulin together with other autoantibodies occur early and are effective in identifying children at risk for developing T1D. Although similarly extensive presymptomatic disease-stage studies have not been performed on autoreactive T cells, there is consensus that autoantibody seroconversion rather than $CD8^+$ T cell accumulation constitutes the earliest detectable manifestation of autoreactivity in T1D. Anderson et al. (1) indirectly approached whether their islet $CD8^+$ T cell findings could impact what could be gleaned from peripheral blood. Using interferon-γ ELISpot assays, they searched for peripheral $CD8^+$ T cell responses to PPI and DRiP peptides. Some response was variably observed in patients soon after the onset of diabetes. Responses were to similar PPI regions

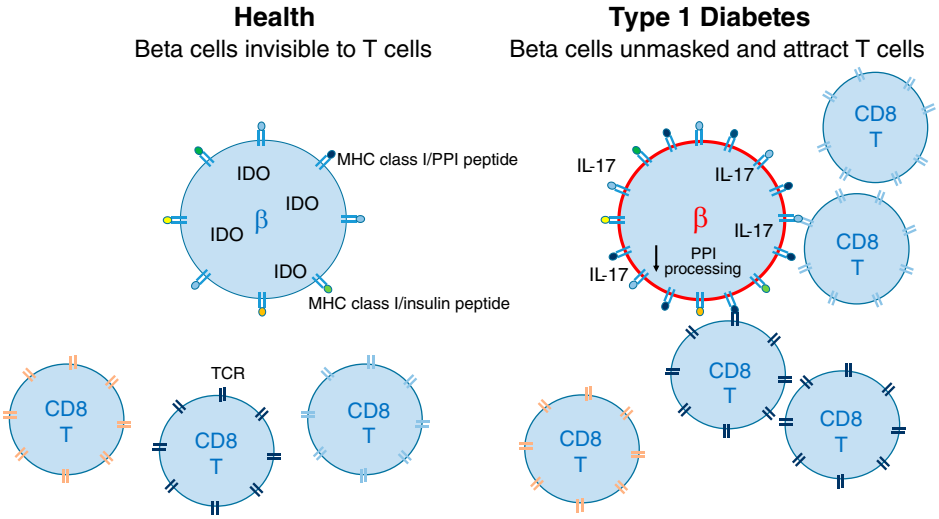

Fig. 1. How benign physiological autoreactivity turns pathogenic in T1D: a beta cell-centric view. There are several published hallmarks of T1D, some of which clearly precede islet infiltration by autoreactive T cells, most notably overexpression of MHC I (21, 22), a processing defect in PPI to insulin (23), and, later on, overexpression of MHC II and interleukin (IL)-17 and loss of indoleamine-pyrrole 2,3-dioxygenase (IDO) expression by beta cells (24). This would result in an unmasking of the beta cell to the immune system where PPI autoreactive T cells are suddenly capable of recognizing beta cells.

recognized by the islet T cells, thereby providing some confirmation that the cells are also circulating. However, the picture for biomarkers remained bleak. Responses were observed in less than half of the patients and were also observed in healthy control subjects. Others have also found responses in control subjects (17), and although the quality of the response may differ in patients, variation and difficulties in detection of these cells in blood and the fact that they appear sequestered in the pancreas make it difficult to use their frequencies in blood to define individual trajectories toward disease (20). Moreover, little is known of the dynamics of PPI-specific $CD8^+$ T cell movement between blood, peripheral lymphoid organs, and the pancreas in humans, so even if we were able to accurately measure changes in these cells we are unlikely to interpret what they mean with respect to islet infiltration and damage.

Are There Therapeutic Implications?

A possible implication of the findings of Anderson et al. (1) is that PPI-targeting $CD8^+$ T cells contribute to T1D development. If true, then it would be therapeutically effective to specifically reduce or control these cells. Antigen-specific therapies (AST) have been successful in modulating allergies, but these therapies have not yet made a positive impact in other immune disorders. Nevertheless, there remain several paths that could be successful. One is the direct induction of PPI or islet-specific Tregs that could home to the pancreas and/or draining lymph nodes where they directly or "bystander" suppress the $CD8^+$ T cells described by Anderson et al. Treg induction with insulin, PPI peptides, or other islet antigens has been attempted, but results have so far been negative, inconclusive, or still "in progress." The findings of Anderson et al. indicate that many of the islet $CD8^+$ T cells are against peptides found in PPI, but not insulin, and that PPI may, therefore, be a preferred antigen to use for AST. Second, it is theoretically possible to divert the $CD8^+$ T cells away from islets through ectopic (nonpancreatic) depots of PPI autoantigen. Coupling this to local therapies that exhaust or delete these cells may reduce numbers. Third, one could consider blocking or masking certain MHC molecules associated with PPI autoreactivity. In view of the limited effects observed for AST in autoimmune diseases so far, it seems likely that antigen alone will be insufficient to reverse autoimmune destruction of beta cells and that combination therapies will be needed. Such combinations include the concept that systemic immune modulation could be used as a short-term induction therapy to then allow for more efficacious antigen-specific tolerance. One could also envision including beta-cell-specific agents that help recloak the beta cells from the immune system, for example by reducing apoptosis and beta cell stress with drugs such as GLP1s or Ca^{2+} channel blockers.

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