COMMENTARY

Bait and Trap: Enriching Autoreactive T Cells With β-Cell Antigen-Loading Biomaterial Scaffolds for Early Detection of Type 1 Diabetes

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Developing effective immune therapies for type 1 diabetes (T1D) remains an immense challenge. To date, results from most of the clinical trials have been largely disappointing, with only temporary improvement and limited efficacy (1,2). One of the major obstacles is the heterogeneous and multifactorial nature of the disease. Developing a personalized immune intervention protocol that can attenuate the specific anti-islet autoimmune responses in high-risk individuals or new-onset patients would be a more productive approach.

It is well established that autoreactive T lymphocytes that recognize pancreatic β-cell antigens (Ags) (e.g., insulin, GAD65, and Znt8) play central roles in mediating the anti-islet autoimmunity in T1D (3–7). Recent studies demonstrated the presence of a variety of islet-infiltrating, β-cell Ag-reactive T cells in T1D patients (8). Characterizing these autoreactive T cells, including their frequencies, phenotypes, predominant Ag targets, cytokine production, and T-cell receptor specificities, in high-risk individuals or new-onset patients will help to monitor the disease progression and develop the right regimens for immune intervention. However, unlike the well-established autoantibody assays that can detect the presence of the autoantibodies against β-cell antigens with high sensitivity and specificity, detecting autoreactive T cells is more challenging (9). Tissue biopsy is clinically prohibitive and unjustifiable, as the procedure is dangerously invasive and the immune cell–infiltrated islets are likely distributed irregularly within the pancreas. Liquid biopsy is the primary tool to assess autoreactive T cells in T1D patients. Regrettably, autoreactive T cells are extremely rare and hard to detect in peripheral blood, even with recent advances of the highly sensitive tetramer- and multimer-based technologies (10,11). In addition, the limited amount of blood that can be sampled provides only a narrow snapshot of any given time. Moreover, whether the circulating β-cell Ag-reactive T cells share the same properties and phenotypes as the islet-infiltrating ones remains to be determined.

In this issue of *Diabetes*, Thelin et al. (12) describe the development of a novel tool to enrich and isolate rare autoreactive T cells in T1D, using biomaterial scaffolds loaded with β-cell Ags. Because of their biodegradable and biocompatible nature, synthetic poly(D,L-lactide-co-glycolide) (PLG) polymers have been widely used in commercial biomedical devices, such as sutures, implants, and other prosthetic products (13). Under normal physiological conditions, PLG polymers undergo hydrolysis to produce monomers of lactic acid and glycolic acid; both are by-products of various metabolic pathways in the body. The degradation rate of PLG matrices can be tailored by fine-tuning the ratio of the two monomers to allow localized, sustained drug delivery (14). Taking advantage of these properties, the group has previously formulated an antitumor vaccine in which tumor Ags are incorporated into the PLG matrices in conjunction with granulocyte-macrophage colony-stimulating factor (as the proinflammatory signal) and CpG-rich oligonucleotides (as the immune dangerous signal) (15). When implanted into mice engrafted with tumors, the biomaterial scaffolds can effectively activate dendritic cells to present tumor Ags and prompt the expansion of tumor-targeting cytotoxic T lymphocytes for an extended period of time, resulting in a dramatic increase of antitumor immune response.

The observations that tumor Ag-specific cytotoxic T lymphocytes preferentially trafficked to the PLG scaffolds loaded with tumor lysates prompted them to investigate the possibility of using biomaterial scaffolds to trap autoreactive T cells. Using ovalbumin (OVA) as a model Ag, they were able to demonstrate the proof of principle that both $CD8^+$ (OT-I) and $CD4^+$ (OT-II) T cells specific to OVA peptides are preferentially recruited to the OVA-loaded PLG scaffolds in comparison with empty PLG controls. Remarkably, an ~500-fold enrichment of OT-II T cells was

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observed in the OVA scaffolds compared with those in the spleens of OT-II transgenic mice. The enrichment of Ag-specific T cells are primarily attributed to Ag-mediated recruitment (not intrascaffold proliferation), as OT-II T cells adoptively transferred to B6 mice proliferated vigorously in the drainage lymph nodes but not within the scaffolds.

Next, they examined whether the Ag-loaded scaffolds can recruit autoreactive T cells under autoimmune conditions. PLG matrices loaded with lysates of NIT-I cells, a pancreatic β-cell line, were implanted in prediabetic NOD mice, the gold standard rodent model for T1D. Two weeks after the implantation, significantly higher percentages of β-cell Ag-reactive T cells were recruited in the β-cell scaffolds in comparison with those of the spleen or the control scaffolds (0.5% vs. 0.2% vs. 0%). Consistent with these findings, the adoptive transfer of matrix-infiltrating T cells to NOD.SCID mice elicit autoimmune diabetes, with kinetics similar to those transplanted with splenocytes. In contrast, recipients adoptively transferred with T cells harvested from control scaffolds remained euglycemic throughout the experiments, suggesting that the infiltration of islet-reactive, pathogenic T cells was driven by β-cell Ags (not by the PLG polymers). Indeed, clonotyping analysis of Ag-scaffold infiltrating T cells revealed more restricted T-cell receptor repertoires with higher clonality scores than those isolated from the control scaffolds, pancreata, or spleens. Moreover, overlapping of highly represented clones was only found between Ag scaffolds and the pancreata, demonstrating further that β-cell reactive, pathogenic T cells can be enriched and isolated by subcutaneous implantation of β-cell Ag-loaded scaffolds in prediabetic NOD mice.

Although more preclinical works need to be done before the novel technology developed by Thelin et al. (12) can be translated into clinical applications, it clearly has many potentials. These include, but are not limited to, identifying and characterizing rare, pathogenic T cells for developing therapeutic regimen; monitoring the T1D progression in high-risk individuals; and evaluating the efficacy of the immune intervention. One major concern is whether the Ag-loaded scaffolds can stimulate the activation and proliferation of islet Ag-reactive T cells and thereby provoke and/or accelerate the autoimmune responses against islet β-cells. To address this critical safety issue, the authors implanted β-cell Ag-loaded scaffolds into a small cohort ($n = 10$) of 10-week-old prediabetic NOD mice and followed their diabetes onsets for 30 weeks. Although no acceleration of T1D onset was observed, further characterization of the β-cell Ag-reactive T cells within the scaffolds and the drainage lymph nodes need to be performed. Will the autoreactive T cells activated in the drainage lymph nodes become effector memory cells and migrate to the pancreatic islets to accelerate the destruction of β-cells?

Another critical question is whether the Ag-loaded PLG scaffolds will recruit β-cell reactive T cells that are innocuous or even tolerogenic. Recent studies have shown that β-cell Ag-reactive $CD4^+$ T cells (e.g., GAD65 and preproinsulin) are also present in healthy donors (16,17). Most of these cells display a more regulatory phenotype (e.g., production of IL-10) in contrast to those present in T1D patients, which are primarily polarized to secret proinflammatory cytokines (e.g., IFN-γ and IL-17). Recruitment of these β-cell Ag-specific, but innocuous, T cells to the PLG scaffolds will complicate the detection of pathogenic T cells in high-risk individuals. Recent studies have shown that interactions between the infiltrating macrophages and biomaterials can influence the local immune response, skewing it to either immunity or tolerance. As the PLG scaffolds are heavily infiltrated with $MAC\text{-}1^+$ cells, including macrophages and neutrophils, the microenvironments of the scaffolds might alter the properties and phenotypes of the infiltrating T cells, which will further complicate the interpretation of the results. On the bright side, the biomaterial scaffolds loaded with β-cell extracts can be potentially modified to adopt a tolerogenic milieu, promoting the expansion of β-cell–specific regulatory T cells to induce islet tolerance (18).

In summary, Thelin et al. (12) described the fabrication of a novel biocompatible scaffold that can be potentially used to enrich and isolate the rare population of β-cell Ag-reactive pathogenic T cells in high-risk individuals with prediabetes. The device is easily implantable and retrievable and can be conditioned to support prolonged Ag release. Conceivably, the same matrix platform can be applied to other organ-specific autoimmune diseases when loaded with their cognate Ags. Just as the saying goes: "Good things come to those who bait."

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