Direct Analysis of Insulin-Specific T Cells Provides New Insights

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Type 1 diabetes (T1D) is an autoimmune disease in which destruction of insulin-producing β-cells leads to dependence on exogenous insulin (1). T1D progression involves progressive loss of tolerance to β-cell antigens, but this has been challenging to observe in human disease. Loss of self-tolerance is most evident through development of islet cell antibodies, among which insulin antibodies typically appear first (2). Given strong genetic association of T1D with HLA-DQ8, DQ8-restricted $CD4^+$ T cells activated by self-peptides are thought to play a crucial role in disease. Key evidence from the nonobese diabetic (NOD) mouse model supports a primary role for recognition of an epitope within the insulin B chain amino acids 9–23 ($InsB_{9–23}$) peptide in disease initiation. The majority of $CD4^+$ T cells isolated from pancreatic islets of NOD mice were shown to respond to this peptide (3). Furthermore, expression of an insulin transgene with a substitution within this peptide abrogated diabetes development (4). Efforts to directly visualize and study insulin-specific T cells in human subjects have been hampered by the biochemical instability of the HLA-DQ8 protein (5). However, recent reports highlight the development of HLA-DQ8 tetramer reagents and their application to study T1D, affirming that $InsB_{11–23}$ peptide is analogously presented by IA^{g7} and HLA-DQ8 (6,7). In addition, two recent reports documented the presence of $InsB_{9–23}$-specific T cells within pancreatic infiltrates of cadaveric donors with T1D (8,9). Nevertheless, key attributes of InsB-specific responses have remained unstudied.

In this issue of *Diabetes*, Spanier et al. (10) report a characterization of DQ8/$InsB_{10–23}$-specific $CD4^+$ T cells in subjects with T1D. Their results support the relevance of such T cells and demonstrate an intriguing correlation between their frequency and insulin antibody titers. After first validating the performance of their DQ8 tetramer by staining of T cells from peptide-immunized and control HLA-DQ8 transgenic mice and peptide-stimulated peripheral blood mononuclear cells (PBMCs), Spanier et al. determined the ex vivo frequency of DQ8/InsB tetramer binding cells within PBMCs obtained from human subjects, detecting more than twofold higher frequencies in subjects with T1D than in control subjects. Although the insulin B chain is present in exogenous insulin, they observed higher frequencies in patients with a disease duration of <5 years. Furthermore, DQ8/$InsB_{10–23}$-specific T cells were present within 15 days of diagnosis, suggesting that T cells were not merely expanded in patients because of daily insulin injections. Based on direct surface marker analysis, DQ8/$InsB_{10–23}$-specific T cells were antigen experienced ($CD45RO^+$) and exhibited markers indicative of T helper 1 (CXCR3) and T follicular helper (CXCR5) lineages. Finally, frequencies of DQ8/$InsB_{10–23}$-specific T cells were positively correlated with insulin antibody titers, implying a possible causal relationship.

Spanier et al. (10) observed that DQ8/$InsB_{10–23}$-specific T cells were detectable in approximately half of the T1D patients tested and were rarely detected in control subjects. Their results affirm disease relevance of these T cells but also bring into question the central role of this epitope in human T1D that had been postulated based on observations in NOD mice. As summarized in Table 1, the evidence supporting the preeminence of DQ8/$InsB_{10–23}$-specific T cells is somewhat disparate in mouse and man. Although DQ8/$InsB_{10–23}$-specific T cells were seen at higher frequencies in subjects with T1D, observed frequencies were 20-fold lower than DQ8/$GAD65_{250–266}$-specific T cell frequencies that were observed in another study (6). Spanier et al. did not characterize the cytokine profiles of DQ8/$InsB_{10–23}$-specific T cells. However, their observation that a significant proportion were $CXCR3^+$ memory T cells is in agreement with a prior study that demonstrated that DQ8/$InsB_{10–23}$-specific T cell clones isolated from subjects with T1D predominantly produced IFN-γ, IL-2, and TNF-α (7).

Despite their novel observations, Spanier et al. (10) leave some compelling questions unanswered. In their in vitro tetramer studies, Yang et al. (7) noted that subjects who

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Table 1—Insulin autoimmunity in mouse and man

	Murine diabetes (reference)	Human diabetes (reference)
Insulin Ab appear early in disease	Yes (17)	Yes (2)
$InsB_{9–23}$ has low affinity binding register	Yes (12)	Yes (7)
$InsB_{9–23}$-specific T cells present in islets	Yes (3)	Yes (8,9)
$InsB_{9–23}$-specific T cells predominant	Yes (3)	No (10)
$InsB_{9–23}$ linked to insulin Ab	Yes (18)	Yes (10)
Insulin-specific T cells increase with duration	Unknown	Yes (10)

Ab, antibody.

had detectable responses to $InsB_{11–23}$ were highly enriched for the susceptible VNTR genotype (7). Given the influence of this variant on levels of thymic insulin expression and T cell selection (11), it would be of interest to know whether the susceptible genotype is correlated with higher frequencies of DQ8/$InsB_{10–23}$-specific T cells. Recent studies verify that $InsB_{10–23}$ is presented by IA^{g7} and DQ8 in an unconventional weak binding register (7,12) such that truncation of the peptide results in an improved ligand. Given recent observations that hybrid insulin peptides are important antigens relevant in human disease (8,13), it could be speculated that a hybrid peptide could generate a more potent agonist. However, rigorous experimental work would be needed to corroborate that idea. Although interesting, the significance of the observed correlation between DQ8/$InsB_{10–23}$-specific T cells and higher titers of insulin autoantibodies remains unclear. In particular, it is not discernable whether insulin antibody titer was measured from serum samples drawn at the same time as PBMCs that were subjected to tetramer analysis or whether antibody data were obtained closer to onset. To date, relatively few studies have correlated autoreactive T cell and antibody responses. McLaughlin et al. (14) observed modest correlations between T cell responses to IA-$2_{841–860}$ and IA-2 juxtamembrane domain antibodies. Acevedo-Calado et al. (15) identified T cell and antibody responses to newly described antigenic determinants within the amino terminus of IA-2. However, responses were not assessed in the same subjects. Consequently, the new data provided by Spanier et al. (10) represents a useful contribution to the field.

Although confirming key findings in a larger patient cohort and/or interrogating a longitudinal sample set would be advisable, these results have the potential to refine our understanding of the contribution and role of DQ8/$InsB_{10–23}$-specific T cells in human disease. It should be noted that another recent study (16) used HLA-DQ8 tetramers to explore the potential of inducing HLA-DQ8–restricted insulin-specific $Foxp3^+$ regulatory T cells. Their findings indicated that DQ8/insulin-specific regulatory T cells were associated with delayed progression, suggesting that the DQ8/$InsB_{10–23}$ epitope would be applicable to elicit antigen-specific tolerance. Clearly, DQ8/$InsB_{10–23}$ tetramer assays will be crucial to facilitate such studies and to address remaining questions about the role of InsB-specific responses in T1D.

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