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Evidence for Residual and Partly Reparable Insulin Secretory Function and Maintained β-Cell Gene Expression in Islets From Patients With Type 1 Diabetes

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It is commonly accepted that type 1 diabetes (T1D) is an autoimmune and inflammatory disease that results from the wholesale yet surprisingly selective killing of the insulin-secreting β-cells of pancreatic islets (1–4). While the relative importance of reduced β-cell function versus reduced β-cell mass is currently debated in type 2 diabetes (5), T1D has been considered the classic example where diabetes results from reduced β-cell mass secondary to autoimmune attack (1,3,4,6). However, this view has recently been challenged (2,6–9).

In this issue of *Diabetes*, Krogvold et al. (10) compare residual glucose-dependent insulin secretion and whole-genome RNA sequencing of islet tissue from donors with and without diabetes. Islets were obtained from adult T1D subjects soon after the onset of the disease. Recent studies have shown that residual plasma C-peptide levels are present long after the onset of diabetes in patients with T1D (1,6,11–14). However, it is unknown whether this low level of insulin production represents secretion from β-cells that have somehow survived autoimmune attack, perhaps because the autoimmune process was halted or because not all of the β-cells were equivalently affected, or whether they represent a newly regenerated β-cell subpopulation (2). A third possibility is that the surviving β-cells, being in the minority, may simply represent the tail end of the normal distribution of islets originally residing in the pancreas (15). Finding residual C-peptide secretion is correlated with a more favorable long-term clinical outcome, including better metabolic control and a lower risk for micro- and macrovascular complications, as reviewed by VanBuecken and Greenbaum (12).

In five of six adult subjects with T1D, where islets could be successfully isolated from pancreatic tissue (the tissue came from surgical resections not from brain-dead donors), residual glucose-dependent insulin secretion could be observed in vitro, and culturing the T1D islets for 1–6 days under euglycemic conditions tended to improve overall secretory function and sometimes even restored first-phase insulin secretion (Fig. 1) (10). While culturing the islets for 3 days in vitro was found to be optimum, in all cases glucose-induced secretion never increased to that seen in control subjects and thus remained rather low. While there have been previous reports of β-cell function persisting in islets from T1D patients, these have been few and far between as human T1D islets are rarely isolated for study. In addition, previous studies resulted in generally discrepant data regarding the ability of culture to improve function (11,16–19). The current data set is more extensive than those of the few previous studies, and the relative rarity of this kind of research adds considerably to the significance of this work to the field, even if the data are understandably incomplete.

In the article by Krogvold et al. (10), measurements of RNA abundances were also carried out using islets from control and T1D donors and were quantified by the widely used reads per kilobase per million (RPKM) measurement (20). The different groups showed similar gene expression patterns, as determined by an agglomerative clustering method. This approach presents the distance between RPKM values for all genes expressed between the pairs of subjects within a group as a phylogenetic tree. In this analysis, the RPKM of the insulin gene pathway for T1D subjects was split into a different clustering group than that for the control subjects.

Whole transcriptome sequencing using 362 million reads provided some notable surprises; for example, all

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COMMENTARY

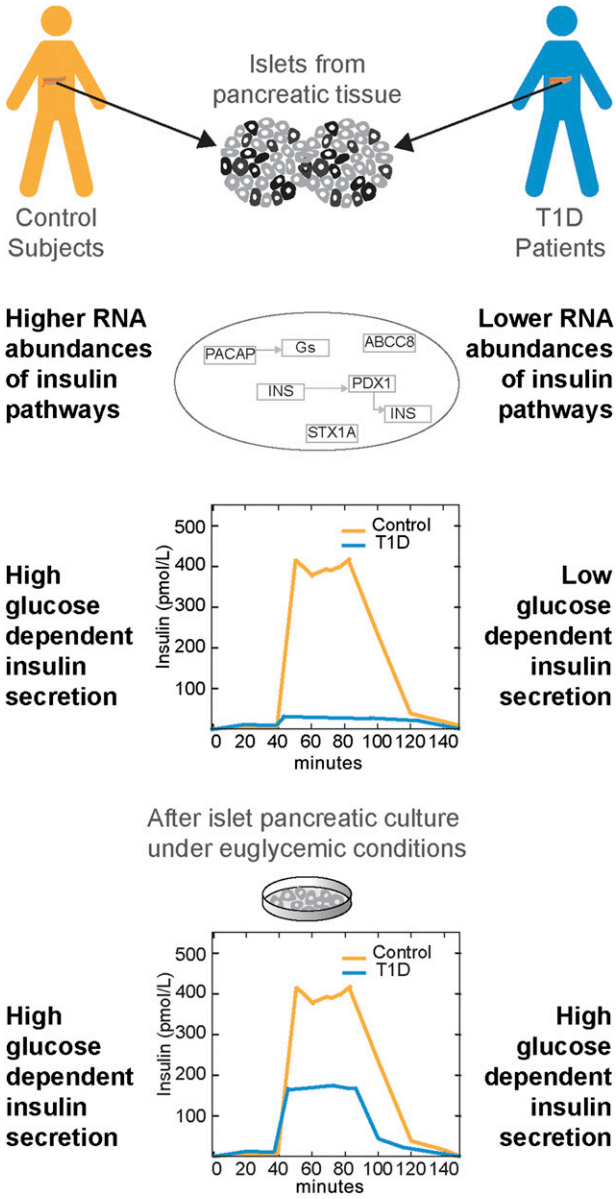

Figure 1—Schematic representation of results obtained by Krogvold et al. (10). Islets were isolated from control and T1D human subjects for analysis. In vitro measurements of glucose-induced insulin secretion revealed residual secretion at low levels in most T1D islets vs. control islets, but secretion was improved after several days in tissue culture under euglycemic conditions. RNA analysis revealed retention of key β-cell genes involved in insulin secretion but reduced expression of these genes in T1D islets. G_s, the alpha subunit of G_s; STX1A, Syntaxin-1A.

samples from T1D subjects expressed all of the genes of the insulin secretory pathway and the characteristic gene profile expected of β-cells, albeit at lower levels of relative expression compared with the control subjects (10). One caveat regarding the reported reduced expression of these β-cell genes is that there is no way to exclude an alternative possibility that the results reflect a reduction in the relative number of β-cells compared with other islet cells. RPKM measurements could thus be biased if they are not normalized by the number of cells, the length of the RNA species assayed, or the sequencing depth of the samples (20). In addition, the cell composition (i.e., the ratio of β-cell vs. other islet cell types) is very likely to be different in T1D islets versus normal islets, which could influence the transcriptional profiling.

What does this all mean? While the results are tantalizing, a major limitation in the study by Krogvold et al. (10) is the small number of samples that were available for study. In part, this occurred because of the complications in the biopsy procedure. Another limitation with the results is that the agglomerative clustering algorithm was designed to study large data sets and assumes that the distance between clusters is additive (an assumption that is rarely satisfied, particularly when there are only a small number of samples [21]). Small sample sizes tend to show substantial variation in *P* values (22). Also, somewhat surprisingly, the authors did not test whether β-cell gene expression was restored by euglycemic culture of T1D islets.

Despite these potential limitations, however, the results of the study by Krogvold et al. (10) are important for two major reasons. First, decreased islet function as well as decreased β-cell mass should be considered as possible contributors to the etiology of T1D. The data show that the β-cells of T1D islets retain their glucose responsiveness (although again, the level of glucose-induced insulin secretion was generally quite low compared with islets from normal subjects) and retain their normal set of characteristic genes. Second, early therapy to rapidly restore euglycemia and/or diminish exposure to injurious cytokines may, in turn, preserve or improve β-cell mass and function in T1D patients (23) (see also 11,24). Whether pharmacological or gene therapy approaches could restore full-strength gene expression to T1D β-cells in patients remains to be seen but is an enticing possibility.

The authors' methods may also stimulate important research in type 1 diabetes. The availability of islets from T1D patients should facilitate studies of the relative importance of autoimmune destruction versus intrinsic deleterious β-cell processes (the so-called "homicide vs. suicide" question) (23,25,26), which might collaboratively result in the death of β-cells in T1D (6). Thus, it seems that we still have much to learn about T1D!

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