β-cells Persist in Some T1DM Pancreata without Attempted Evidence of β-cell Turnover nor Insulin-Glucagon Co-expression

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Purpose: Regeneration of ß-cell function in T1DM is a fundamental research goal that requires improved knowledge of the lineage mechanisms of ß-cell growth and regeneration. ß-cell function is detectable in some T1DM patients, even some with longstanding disease. However, the developmental basis of persistent ß-cell function remains an utter mystery. Despite decades of research in animal models ranging from mouse to dog to pig, there is little quantitative data of ß-cell lifecycle in normal pancreata, in part due to the extremely low rates of endogenous ß-cell turnover in the basal state and the lack of robust high-throughput imaging methodologies. Importantly, ß-cell turnover has never been accurately measured in T1DM pancreata. We sought to establish such rates in both T1DM patients and controls across age groups with robust high-throughput methods.

Methods: We performed immunofluorescent staining of JDRF nPOD pancreata, quantifying total islet endocrine cells (Synaptophysin), ß-cells (insulin), and alpha cells (glucagon). To determine replication, we stained for Ki67. To measure death we used the TUNEL staining method combined with hormone specific markers. We used an automated X-Y stage with a motorized fluorescent microscope to obtain images from all visible islets from each section, which resulted in huge image files from each case comprising tens of thousands of individual nuclei. We then employed a proprietary image-processing algorithm written for the Perkin Elmer Volocity platform to analyze the images. We counted an average of 43,000 nuclei per sample per staining group across 41 samples (20 T1DMs and 21 controls). In total our findings summarize hormone expression data from upwards of 5.5 million individual nuclei, achieving unprecedented levels of quantitative rigor.

Summary of Results: Residual insulin producing ß-cells were detected in some (but not all) T1DM cases. Several T1DM cases had substantial numbers of ß-cells. However, T1DM pancreata with persistent ß-cells did not exhibit increased ß-cell or islet endocrine cell replication when compared to controls, or to T1DM cases without ß-cells,. Similarly, T1DM pancreata with persistent ß-cells had equivalent rates of ß-cell or islet endocrine cell death compared to controls, or to T1DM cases without ß-cells. As expected from previously published rodent studies, ß-cell and islet endocrine cell replication decreased with age in both control and T1DM pancreata. ß-cell death was infrequent in both control and T1DM pancreata, with no age specific associations. Islet endocrine cells that co-expressed insulin and glucagon were not detected in control pancreata, nor in T1DM pancreata.

Conclusions: A few ß-cells often persist in T1DM pancreata. However, there is no evidence of ß-cell regeneration in T1DM pancreata, except within a single unusual case (#6052). Human islet endocrine cell replication decreases with age. Human postnatal pancreatic ß-cells do not co-express insulin and glucagon, even in T1DM pancreata. Thus, longstanding ß-cell function in T1DM patients appears to be largely due to ß-cells that simply persist, without any evidence of attempted ß-cell regeneration nor trans-differentiation from other endocrine cell types. Future ß-cell regeneration therapies must overcome very low endogenous rates of ß-cell turnover in order to achieve substantial islet endocrine cell mass in T1DM patients.