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Beta-cell destruction and preservation in childhood and adult onset type 1 diabetes

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Abstract

Previous studies describing the symptomatic onset of type 1 diabetes (T1D) and rate of beta-cell loss (C-peptide) support the notion that childhood onset T1D exhibits more severe beta-cell depletion compared to adult onset T1D. To test this notion, we performed whole pancreas analyses in two T1D cases; one of childhood onset (7-yr old, onset at 1.5-yr) along with an adult onset case (43-yr old with onset at 27-yr). Both cases were matched for age and gender with control subjects. Striking regional differences in beta-cell loss were observed in both T1D cases, with severity of loss in the order of tail>body>head regions. In contrast, pancreatic alpha- and delta-cell mass was similar in controls and T1D patients. In the childhood onset T1D case, no intra-islet beta-cells were detected while in the adult onset case, beta-cell containing islets were found, exclusively in the head region. In the latter case, considerable numbers of small cellular clusters negative for three major endocrine hormones were observed, in islets with or without beta-cells. Ultrastructural analysis suggests these cells correspond to degenerating beta-cells, with empty granular membranes and abnormal morphology of nuclei with intranuclear pseudoinclusions, adjacent to healthy alpha- and delta-cells. These results support a hypothesis that during T1D development in childhood, beta-cells are more susceptible to autoimmune destruction or immune attack is more severe, while beta-cell death in the adult onset T1D may be more protracted and incomplete. In addition, T1D may be associated with the formation of “empty” beta-cells; an interesting population of cells that may represent a key facet to the disorder's pathogenesis.

Keywords

type 1 diabetes; childhood onset; adult onset; pancreatic islets; beta-cells

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Introduction

Type 1 diabetes (T1D) is characterized by a progressive immune-associated destruction of insulin-secreting pancreatic beta-cells. T1D is one of the most common chronic diseases of childhood, with onset peaking between 5-7 years of age as well as near puberty (1). The prevalence of T1D in the US population under 20 years of age has increased by 30% between 2001-2009 (2). The clinical diagnosis of adult-onset autoimmune diabetes is complex and these patients are often initially diagnosed as having type 2 diabetes (T2D). However, the spectrum of autoimmune diabetes extends across all ages (3).

Previous studies have demonstrated young age at onset as one of high risk factors for T1D progression along with human leukocyte antigen (HLA) risk alleles and a presence of multiple autoantibodies (4-9). In children, T1D is often presented with more severe insulin deficiency and ketoacidosis at disease onset compared to adult onset T1D. A review by Pipeleers et al. found that T1D progression, as assessed by C-peptide secretion, varied with age at onset, with depletion of beta-cells more severe in young children, defined as onset under age 7 (10). Studies of Diabetes Prevention Trial-1 (DPT-1) reported the age/growth-related increase in basal C-peptide responses, which may account for higher levels of C-peptide observed in adults with newly diagnosed T1D compared with those in children and adolescents (7).

In terms of histology, the presence of residual beta-cells at different stages of T1D has been reported by a number of investigators (4, 11-13). Recent studies demonstrated that a large fraction of patients with T1D had insulin-producing cells unrelated to duration of disease or age at death (14). Residual beta-cells were found in islets or individually scattered in the duct and the exocrine pancreas. Yet, the effects of age at diagnosis on pathogenesis of T1D remain unsettled.

Here we report large-scale quantitative analysis of the whole pancreas from an early childhood and an adult onset of T1D, which includes immunohistochemical and electron microscopic analyses as well as detailed mathematical analysis. The present study demonstrates a unique approach to extract the maximal information from donor pancreatic tissues for a better understanding of the pathogenesis of T1D.

Materials and Methods

Human pancreas specimens

Human pancreata were generously provided by the Gift of Hope Organ Procurement Organization in Chicago. The use of human tissues in the study was approved by the Institutional Review Board at the University of Chicago.

Donor clinical information

Patient with a childhood onset T1D: 7-yr old male, body mass index (BMI) 17.6, onset at 1.5-yr of age, on insulin pump for 4 yr, cause of death (COD) anoxia, pancreas weight 27g, HLA: Class I - A1, A3, B7, B8, BW6, CW7, Class II - DR4, DR17, DR52, DQ2, DQ8, DR53. Control: 6-yr male, BMI 13.9, COD cerebrovascular/stroke, pancreas weight 27g,

HLA: Class I – A2, A68, B7, B49, BW4, BW6, CW7, Class II – DR15, DR17, DR51, DR17, DR52, DQ2, DQ6. Patient with an adult onset T1D: 43-yr old male, BMI 26.6, onset at 27-yr of age, COD head trauma, pancreas weight 55g, HLA: Class I – A2, A68, B27, B53, BW4, CW2, CW4, Class II – DR4, DR13, DR52, DR53, DQ6, DQ8. Control: 44-yr old male, BMI 36, COD anoxia, pancreas weight 77g, HLA: Class I – A1, A3, B8, B61, BW6, CW2, CW7, Class II – DR8, DR11, DR52, DQ4, DQ7.

Immunohistochemistry

Paraffin-embedded sections (5 μ m) were stained with the following primary antibodies (all 1:500): polyclonal guinea pig anti-porcine insulin (DAKO, Carpinteria, CA), mouse monoclonal anti-human glucagon (Sigma-Aldrich, St. Louis, MO), polyclonal goat anti-somatostatin (Santa Cruz, Santa Cruz, CA), monoclonal mouse anti-human CD45 (DAKO), and DAPI (Invitrogen, Carlsbad, CA). The primary antibodies were detected using a combination of DyLight 488, 549, and 649-conjugated secondary antibodies (1:200, Jackson ImmunoResearch Laboratory, West Grove, PA).

Image capture and quantification

Microscopic images were taken with an Olympus IX8 DSU spinning disk confocal microscope (Melville, NY) with imaging software StereoInvestigator (SI, MicroBrightField, Williston, VT). A modified method of “virtual slice capture” was used (15-17). Briefly, the SI controls a XYZ-motorized stage and acquires consecutive images, which creates a high-resolution montage composed of images obtained from multiple microscopic fields of view. The entire tissue section was captured as “a virtual slice” using a 10 $\times$ objective. Each virtual slice taken at four fluorescent channels were further merged into one composite. Quantification of cellular composition (i.e. each area of beta-, alpha- and delta-cell populations, and islet area by automated contouring of each islet) and measurement of coordinates of each endocrine cell type within an islet were carried out using custom-written scripts for Fiji/ImageJ (<http://rsbweb.nih.gov/ij/>). MATLAB (MathWorks, Natick, MA) was used for mathematical analyses.

Mathematical analysis of islet architecture

To quantitatively examine the regional specificity of different cellular arrangements, graphs/networks consisting of vertices and edges were created for each islet. The vertices were located at the given (x-y) coordinates of each cell and edges were placed between cells that were within a 15 mm distance of each other. Then, the nearest neighbor correlation (NNC) was calculated for alpha-alpha, alpha-delta, and delta-delta edges. Positive NNC values correspond to endocrine cell types that are attracted to each other, whereas negative values depict repulsion, which values were used to distinguish between two types of islet architecture with either mixed or segregated endocrine cells.

Transmission electron microscopy

Microdissected pancreas tissues were fixed with 4% paraformaldehyde and 0.02% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) and embedded in resin. Ultrathin

sections (80 nm) were stained with uranyl acetate and lead citrate. Images were taken using a Tecnai F30 microscope (FEI, Hillsboro, Oregon).

Statistical analysis

Data are expressed as mean $\pm$ SEM. Statistical analyses were performed using paired Student's *t* test. Differences were considered to be significant at $P < 0.05$.

Results

Whole pancreas analysis of endocrine cell mass in a childhood and an adult onset T1D patients

Pancreata were divided into consecutive tissue blocks (~ 5 mm in thickness) with preparation alternating between fresh-frozen and paraffin-embedding. The latter set of tissue blocks was used in this study. Regional distribution of the total endocrine cell area per tissue section was examined for the three major endocrine cells (beta-, alpha- and delta-cells) in a childhood onset T1D (7-yr old male) and an age and sex matched control (6-yr old male; Fig. 1A and 1B, respectively) and an adult onset T1D and a control (43-yr old male and 44-yr old male; Fig. 1C and 1D, respectively). Absolute numbers of each cell type are summarized in Table 1, which demonstrates the large scale of our measurement. Regional differences in alpha- and delta-cell mass were observed in all cases. This mass gradually increased from the head through the body and toward the tail region (Fig. 1), as we have previously shown in the human pancreas including patients with T2D (18, 19). Both T1D patients lost the majority of beta-cell population but retained a similar mass of alpha- and delta-cells compared to controls (all $P > 0.1$). Regional analysis of residual beta-cells in T1D patients showed severe beta-cell depletion in the body and tail region. Considering the fact that the tail region contains ~ 2 -fold beta-cell mass compared to the head and body region (18, 19), massive destruction of beta-cells appears to have occurred in the tail region.

Quantitative analysis of individual islet size distribution and cellular composition

Relative ratios of endocrine cell composition are plotted in relation to islet size distribution from single cells to large islets in each region (Fig. 2). Note that islet area/size is presented as a logarithmic scale considering the high number of singlets/small clusters and the low number of large islets. The conversion between logarithmic islet area and effective diameter (μm) is provided. In both T1D cases, the loss of beta-cells resulted in the reduction of the overall islet size distribution compared to the controls (the range depicted in the X-axis). Note that in these T1D cases with severe depletion of beta-cells, the remaining “islets” are largely composed of alpha- and delta-cells, which led to the marked “shrinkage” of islet size. We propose to term such islets as “pseudo-atropic islets”. This also reflected increased ratios of alpha-cells, however the number of alpha-cells was not increased in T1D patients. Statistical analysis was carried out first by converting each islet area (in various shapes) to a perfect circle and compared each case-control pair regionally using an effective diameter (Table 2). Islet size distribution was significantly reduced in both T1D patients compared to the controls in all pancreatic regions.

Immunohistochemical analysis of residual beta-cells in T1D

Based on the large-scale analysis of residual beta-cells in the whole pancreas of the childhood and adult onset T1D cases, we examined their spatial distribution. In the childhood onset T1D, we failed to detect any insulin-positive cells in islets, but residual beta-cells were singly scattered in the exocrine pancreas (Fig. 3A). In the 6-yr old control subject, islet architecture was relatively similar to rodents with a core of beta-cells and alpha- and delta-cells in the periphery (Fig. 3B). Beta-cell containing islets were observed in the adult onset T1D patient, which were found exclusively in the head region (Fig. 3C and 3D). Figure 3E depicts representative large islet structure in the 44-yr old control, which is typically observed in the adult human pancreas (20-22). In islets with or without beta-cells (Fig. 3F.a and b, respectively), clusters of non-endocrine hormone positive cells (i.e. negative for insulin, glucagon and somatostatin) were identified (asterisks in Fig. 3F). These clusters were not immune cells as CD45-positive cells were rarely found in islets but were widely distributed in the exocrine pancreas (Fig. 3G.a.b.) Regional quantification of CD45-positive cells in the pancreas showed no differences between the T1D patients and the controls (Table 3).

While the majority of residual pseudo-atrophic islets was devoid of beta-cells and comprised with intermingled alpha- and delta-cells in the head and tail region (Fig. 3H), interestingly in the body region, remaining islets appeared to be segregated either with alpha-cells or delta-cells (Fig. 3I). To quantitatively analyze this distinct architectural difference, edge graphs were constructed between closely located cells. We then computed the nearest neighbor correlation (NNC) of alpha-alpha, alpha-delta, and delta-delta edges (Fig. 3J and 3K). The NNC quantitatively illustrates whether two cell types are attracted (positive NNC) or repulsed (negative NNC). The islets were grouped by head ($n = 1592$: 1425 small and 167 large islets), body ($n = 1741$: 1481 small and 260 large islets) and tail ($n = 3940$: 3277 small and 663 large islets) regions and we calculated 2-sided t-tests for these grouped islet edge ratios. We found a statistically significant difference between the body and head/tail regions, but the difference was not significant between the head and tail for each edge type. In the head and, to a lesser extent, tail regions, cells are attracted to the opposite cell type and repulsed by the same cell type similar to mixed cell-type islet. However in the body region, cells of the same type are attracted to each other similarly to a segregated cell-type islet. Thus alpha- and delta-cells in islets in the body of the pancreas are more likely to be separated than islets in the head or the tail regions.

Ultrastructural analysis of residual beta-cells in T1D

Ultrastructure of beta-cells in the young control subject shows insulin granules of varying electron density with halos containing polymorphic granular structures including rhomboidal or crystalline shapes (Fig. 4A.a). Exocrine acinar cells contain dilated endoplasmic reticulum (ER) and large zymogen granules (Fig. 4A.b). Glucagon granules in alpha-cells show dense cores without a halo, and somatostatin granules in delta-cells share similar electron density but markedly smaller in size (Fig. 4A.b). In the childhood onset T1D patient, alpha-, delta- and pancreatic polypeptide (PP)-cells appear normal, however, we observed some blood vessels with layered basal lamina, which is one of the characteristics of diabetic conditions (23) (Fig. 4B.a). Some acinar cells contained multiple

autophagosomes (Fig. 4B.b). In the adult onset T1D, alpha- and PP-cells appeared normal (Fig. 4C.a), however, a cluster of beta-cells with normal appearance of nuclei were largely granule-depleted, where a small number of insulin granules remained (Fig. 4C.b). A cluster of degenerating beta-cells, with an appearance of a multinucleated cell, exhibit multiple intranuclear holes which are likely to be pseudoinclusions of cytoplasm (Fig. 4C.c). Another cluster of cells filled with mitochondria also had intranuclear holes (Fig. 4C.d). These clusters of degenerating cells presumably correspond to non-endocrine hormone positive cells observed by immunohistochemistry (asterisk in Fig. 3F.a.b). Blood vessels often had abnormally thick walls composed of basal lamina (Fig. 4C.e.f). Acinar cells appeared to be normal (Fig. 4C.f). Normal beta-cell and acinar cell structures in the adult control subject are shown in Fig. 4D.

Discussion

In the early childhood onset T1D (a 7-yr old male, disease onset at 1.5-yr, on pump for 4 yr), residual beta-cells were singly scattered in the exocrine pancreas but not in the islets throughout the pancreas. This severe depletion of intra-islet beta-cells suggests that beta-cells during early development are more susceptible to autoimmune destruction and that islets are the primary target as well as the driver of the disease (24). Beta-cells during development and growth (i.e. in children and adolescents) may be more vulnerable to cell death (7, 25), or autoimmune destruction may be more aggressive in young age onset T1D. It is also noted that no decrease or increase was observed in alpha-and delta-cell mass in this young patient throughout the pancreas.

In contrast, the adult onset T1D patient (a 43-yr old male with the onset at 27-yr of age) preserved insulin-positive cells in islets exclusively in the head region, despite the long duration with a high HbA1c level of 7.5 % (9.4 mM/L) at the time of death. While the majority of remaining pseudo-atrophic islets were devoid of insulin-positive cells and mainly consisted of alpha- and delta-cells, we observed clusters of cells that were negative for three major endocrine hormones. The considerable amount of these intra-islet cell clusters excludes a possibility of other endocrine cell types such as pancreatic polypeptide- or epsilon-cells (13, 26). Furthermore, robust intra-islet immune cell infiltration, which is characteristic of non-obese diabetic (NOD) mice, was not observed, consisting with the past reports on human T1D patients (27, 28). Ultrastructural analysis of this donor pancreas has revealed that these cell clusters appear to correspond to degenerating beta-cells with little or no insulin granules but filled with empty granular membranes and abnormal morphology of nuclei with multiple intranuclear holes.

It was rather unexpected that a considerable number of degenerating beta-cells were still spared within islets in the adult onset T1D patient for as long as 16 yr since the onset of the disease. The cell death of adult beta-cells is apparently a slower process than it has been previously considered, where a complete loss of insulin granules in beta-cells did not trigger immediate apoptosis. By definition, apoptosis is induced by signals from cells that are not properly functioning. The question is, what signals are the final determinants of beta-cell death? We have shown that it is not a physical loss of insulin granules in beta-cells in the case of adult-onset T1D. It is not a loss of the ability of insulin secretion as it has been

demonstrated in various types of diabetes. We and others have shown that a large fraction of insulin positive beta-cells are preserved in patients with T2D (14, 29-32). The preservation of non-functional beta-cells over years has been most clearly demonstrated in cases of neonatal diabetes with activating mutations in the genes encoding the two subunits of the ATP-sensitive potassium channel (K_{ATP}), *KCNJ11* and *ABCC8* (33). These patients can be treated with oral sulfonylureas in lieu of insulin injections. Sherry et al. reported such functional recovery of pre-existing beta-cells in response to immune therapy in a mouse model of T1D (34).

Our observation further includes regional heterogeneity in beta-cell loss and preservation, as well as possible remodeling of the remaining islets in the body region in the adult onset T1D as the closest neighborhood analysis demonstrated. To the best of our knowledge, no histological studies on T1D were conducted in the scale of the whole pancreas from individual patients, where the regional differences in endocrine cell mass is the intrinsic feature of the human pancreas (17). Interestingly, while we have shown preferential beta-cell loss in the head region in T2D (18), the present study on both childhood and adult onset T1D cases demonstrated 5-fold higher preservation of beta-cells in the head of the pancreas.

Besides the possible sampling bias due to random selection of the pancreatic regions for comparison in the past studies as described above, the major concern is the accuracy of a clinical diagnosis of T1D in donors that have been studied. Dimorphic histopathology of long-standing childhood onset diabetes has been reported, where 30% of patients had numerous insulin-positive cells (35). Such heterogeneity could stem from misdiagnosis of early-onset diabetes due to single gene mutations as T1D, neonatal diabetes and maturity onset diabetes of the young (MODY). The SEARCH for Diabetes in Youth Study Group reported that in cases of neonatal diabetes (i.e. onset before 6 months of age), the majority (66.7%) had a clinical diagnosis of T1D (36). Another study of youth with diabetes diagnosed at age younger than 20 years, the SEARCH identified 47 MODY cases out of 586 diabetic youth, where only 3 had a clinical diagnosis of MODY and the majority was treated with insulin (37).

In this study, despite the small number of specimens with limited clinical information, we presented our unique approach to quantitatively analyze histological changes associated with T1D. Studies through cohort analyses of additional cases will likely aid our understanding of the pancreatic pathology. With the identification of more T1D-associated biomarkers, together with advanced knowledge of beta-cell/islet/pancreas/immune system development in the pediatric population, to which currently very little is known, should lead to “reverse scientific approach” that investigators could evaluate disease progression of T1D by morphology and histology.

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Fig. 1. Whole pancreas analysis of the endocrine cell distribution

A: Early childhood onset T1D. A 7-yr old male. **B:** A 6-yr old non-diabetic male. **C:** Late onset T1D. A 43-yr male. **D:** A 44-yr old non-diabetic male.

Fig. 2. Quantitative analysis of individual islet size distribution and cellular composition
 Relative frequency of islet size (gray bar) and ratios of beta (green), alpha (red) and delta (blue) cells within islets in the head, body and tail regions are plotted against islet size; means $\pm$ SEM. **A.** 7- yr old male, early childhood onset T1D. **B.** 6-yr old non-diabetic male. **C.** 43-yr male, late onset T1D. **D.** 44-yr old non-diabetic male.

Fig. 3. Immunohistochemical analysis

A. Representative view of the pancreas from the early childhood onset T1D. Beta-cells (green), alpha-cells (red), delta-cells (white) and nuclei (blue). Inset showing singly scattered beta-cells. **B.** Islets from 6-yr old control subject. **C.** Beta-cell rich small islets in the adult-onset T1D. **D.** Beta-cell rich large islets in the adult-onset T1D. **E.** Large islet from 44-yr old control subject. **F-K:** the adult onset T1D. **F.a.** Beta-cell containing islet. Note clusters of non-endocrine hormone positive cells (*) within an islet. **b.** Non-endocrine hormone positive cells (*) in islet with no beta-cells. **G.a.b.** Alpha-cells (white). CD45-positive cells (red). **H.** Islets in the tail region. Inset showing islets with alpha- and delta-cells intermingled. **I.** Islets in the body region. Inset showing islets segregated with either alpha- or delta-dells. **J.** Schematic representation of edge graphs for computing nearest neighbor correlations (NNC) of edges between different cell types. Mixed morphology islets have negative alpha-alpha and delta-delta values compared to segregated morphologies. Alpha-cells (red), delta-cells (blue), alpha-alpha edge (red), alpha-delta edge (pink), delta-delta edge (blue). **K.** Bar graph showing NNC between alpha-alpha (aa), alpha-delta (ad) and delta-delta (dd) cells for islets in the head (blue), body (red) and tail (green) regions of the pancreas. Positive values represent edge types that appear more than random, i.e. cell

types that are attracted to each other, whereas negative values appear less or are repulsive.
(*: $p < 0.05$; **: $p < 0.01$, ***: $p < 0.001$).

Fig. 4. Ultrastructural analysis

A. 6-yr old control. **a.** Cluster of beta-cells. **b.** Exocrine acinar cell (e). Endoplasmic reticulum (ER). Alpha-cells (a). Delta-cell (d). Autophagic vacuole (arrow head). **B.** Childhood onset T1D. **a.** Alpha-cells (a). Delta-cell (d). PP-cell (P). Blood vessel with layered basal lamina (bl). **b.** Exocrine acinar cell (e). Endoplasmic reticulum (ER). Alpha-cells (a). Autophagosomes (arrow heads). **C.** Adult-onset T1D. **a.** Alpha-cells (a). PP-cell (P). **b.** A cluster of granule-depleted beta-cells. Remaining insulin granules labeled as b. **c.** Degenerating beta-cells with multiple intranuclear holes (*). **d.** A cluster of beta-cells (*) filled with mitochondria (m). **e.** Alpha-cells (a). Thick basal lamina (bl). **f.** Exocrine acinar cell (e). Blood vessel with abnormally thick basal lamina (bl). **D.** 44-yr old control. **a.** Beta-cell (b) and acinar cell (e). **b.** High-power image of an insulin containing beta-cell.

Table 1
Absolute number of each endocrine cell type (total, mean $\pm$ SEM)

	Cell type	Head	Body	Tail
Childhood onset T1D	Beta	67; 22.3 $\pm$ 23.1	11; 1.8 $\pm$ 1.0	13; 2.6 $\pm$ 1.8
	Alpha	6,194; 2,064.7 $\pm$ 315.3	9,498; 1,583.0 $\pm$ 281.8	17,693; 3,538.6 $\pm$ 710.4
	Delta	5,561; 1,853.7 $\pm$ 254.3	5,271; 878.5 $\pm$ 124.3	11,200; 2,240 $\pm$ 266.5
Control	Beta	19,587; 6,529 $\pm$ 1836.8	66,152; 11,025.3 $\pm$ 1204.4	95,809; 15,968.2 $\pm$ 2733.0
	Alpha	7,041; 2,347 $\pm$ 913.0	16,547; 2,757.8 $\pm$ 278.9	41,579; 6,929.8 $\pm$ 1,005.5
	Delta	4,771; 1,590.3 $\pm$ 712.0	8,313; 1,385.5 $\pm$ 197.3	12,732; 2,122 $\pm$ 485.6
Adult onset T1D	Beta	867; 216.8 $\pm$ 173.2	120; 15.0 $\pm$ 6.8	1,313; 164.1 $\pm$ 167.5
	Alpha	6,878; 1,719.5 $\pm$ 134.0	13,019; 1,627.4 $\pm$ 286.8	32,242; 4,030.3 $\pm$ 1023.8
	Delta	3,486; 871.5 $\pm$ 193.6	4,694; 586.8 $\pm$ 161.6	10,458; 1,307.3 $\pm$ 473.8
Control	Beta	28,122; 7,030.5 $\pm$ 1,704.1	64,932; 8,116.5 $\pm$ 1,385.5	103,807; 14,829.6 $\pm$ 1,862.8
	Alpha	16,455; 4,113.8 $\pm$ 1,499.0	42,214; 5,276 $\pm$ 1,123.0	101,942; 14,563.2 $\pm$ 2,010.1
	Delta	5,073; 1,268.3 $\pm$ 291.8	10,513; 1,314.1 $\pm$ 205.8	23,735; 3,390.7 $\pm$ 480.9

Table 2
Comparison of islet size distribution (Average effective diameter of islets $\pm$ SEM)

	Head (μm)	Body (μm)	Tail (μm)
Childhood-onset T1D	23.2 $\pm$ 0.4	26.2 $\pm$ 0.5	26.9 $\pm$ 0.4
Control	31.5 $\pm$ 0.5	46.4 $\pm$ 0.7	48.9 $\pm$ 0.6
P value	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Adult-onset T1D	19.3 $\pm$ 0.5	24.4 $\pm$ 0.5	22.8 $\pm$ 0.4
Control	37.6 $\pm$ 0.4	43.2 $\pm$ 0.4	45.7 $\pm$ 0.3
P value	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$

Table 3
Comparison of CD45-positive cells in the pancreas (cell number/cm²)

	Head	Body	Tail
Childhood onset T1D	1,631.7 ± 202.6	1,529.8 ± 347.7	1,183.6 ± 101.1
Control	1,109.5 ± 474.6	1,429.1 ± 111.6	1,121.8 ± 395.1
P value	0.74	0.79	0.20
Adult onset T1D	1,277.3±358.9	1,635.2±488.4	1,414.9±494.3
Control	1,710.3±498.2	1,583.1±219.0	1,282.9±169.6
P value	0.30	0.37	0.17