Disruption of normal beta cell phenotypes precedes Type 1 diabetes diagnosis.

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Abstract

Purpose: The current dearth of successful immune therapies to prevent Type 1 Diabetes (T1D) is partly due to a lack of knowledge of the concurrent changes in the beta cell phenotype. It has been previously reported that beta cells of adult human islets, but not those of postnatal human or of mouse islets, are populated by cells that express both proinsulin (ProIN) and variable levels of proprotein convertase 1/3(1/3)(1), the main enzyme involved in insulin production. Islets also contain ProIN+PC1/3- and ProIN-PC1/3+ cell types(2). The current study sought to determine whether these three beta cell types persist in islets of donors harboring circulating autoantibodies (AA+) and /or in T1D donors.

Methods: sections of human pancreas from donors control, AA+ and T1[provided by the network of pancreatic organ donors (nPOD)] were immunostained for ProIN, PC1/3 and insulin and were examined using Leica TCS SP5 confocal laser scanning microscope system at 40x magnification. The same confocal microscope settings were used to obtain all images. The fluorescent intensity (FI), determined using the Image J, was calculated as FI/islet area. Two regions of the pancreas per donor and 10-25 islets per region were evaluated.

Summary of results: Beta cells of islets of T1D and AA+ express a uniform, albeit abnormal, phenotype. In some AA+ donors, the alteration in PC1/3 and/or ProIN expression differed between regions of the pancreas. In contrast, in other AA+ donors both pancreatic regions examined showed similar defects in the expression of each marker. In these later group, the alteration/s was observed in all beta cells of a subset of islets of one region and of all islets of the contiguous region. The regional difference in beta cell phenotype was also found in T1D islets. Notably, the FI for insulin was similar for both pancreatic regions of AA+ and T1D donors.

Conclusions- This analysis revealed that most islets of AA+ and all islets of T1D donors lacked the three beta cell subtypes found in normal islets. Rather, they were populated by beta cells displaying identical, but aberrant, expression of PC1/3 and/or ProIN that was region-specific. These observations suggest that different signals regulate the expression of each of those molecules. Conceivably, factors inducing the alterations of the beta cell phenotypes play an important role in the progression of the disease.

Objective

It has been previously reported that pancreatic islets of human adult , but not those of postnatal human or of mouse, are populated by insulin cells that express proinsulin(ProIN) and variable levels of Proprotein Convertase (PC1/3), the main enzyme involved in insulin synthesis(1). Islets also contain ProIN+PC1/3 cells and ProIN-PC1/3 insulin cell types(2). It has been proposed that the three beta cell types interconvert and represent different functional states (2). The current study sought to determine whether the three beta cell types, reflecting the heterogeneous nature of PC1/3 expression, persist in islets of donors harboring circulating autoantibodies (AA) and /or inT1D donors.

Materials and Methods

Histological sections of pancreas from 8 AA+ and 5 T1D donors were provided by the network of pancreatic organ donors (nPOD). List of donors examined and of autoantibodies are indicated in Table 1. Pancreas from two anatomical regions of human pancreas were examined. Sections were immunostained for ProIN (GS-9A8, DSHB) that labels non-processed proinsulin, PC1/3 and insulin. Bound antibodies visualized using secondary antibodies that fluoresce at different wavelengths as previously described (2). The antibody to PC1/3 recognizes the two active (74 and 66-kDa) forms of the enzyme (3). Stained sections were examined using Leica TCS SP5 confocal laser scanning microscope system at 40x and 60x magnification using sequential scanning for each wavelength. The same confocal microscope settings were used to obtain all images.
Table1 indicates the Autoantibodies of each donor examined.

Table 1

	GAD	IA2	mIAA	ZnT8
6505	+	-	+	-
6424	+	-	+	-
6314	+	-	-	-
6450	+	-	-	+
6517	+	-	-	-
6123	+	-	-	-
6397	+	-	-	-
6429	+	-	+	-
6520	+	+	-	+
6362	+	-	-	-
6325	+	+	+	-
6484	+	+	-	-
5000	+	-	+	-
6436	-	+	+	-

Tyrosine phosphatase-like insulinoma antigen (IA-2), glutamic acid decarboxylase-65 (GAD65), zinc transporter protein 8 (ZnT8),insulin (micro mIAA). Underlined: T1D

Results

1- Expression of three functional markers of the beta cells is altered prior to T1D diagnosis.

Table 2

	ProIn	PC1/3	IN
Control	22.1±1.3	17.1±0.6	38.4±2
6505PB	30.2±1.8	9.47±0.6	34.4±0.3
6505PT	16.5±1.2	11.9±1.3	38.3±2
6424 PB	11.8±0.3.	11.7±0.6.	27.8±0.8
6424PT.	9.6±1.0	8.5±0.7	26.0±1.7
6314PB	17.8±1	11.8±0.8	34±1.3
6314PT	18.1±0.9	26.7±0.9	38.1±1.8
6450PH	14.6±0.8	5.54±0.5	36.6±2.2
6450PB	15±1.0	12.8±1.2	32.0±2.0
6517PB	21.3±1.4	11.7±0.6	33.4±1.2
6517PT	19.3±1.3	13.3±1.0	39.3±2.0
6123PB	23.8±1.1.	27.6±1.0	44.2±1.4
6123PT.	21.3±0.3.	29.2±1.0.	39.6±1.4
6397PB	13.3±0.9.	18.9±1.8	29.2±1.9
6397PT.	16.5±0.7.	14.3±0.6	29.5±0.9
6429PB	15.1±1.0	18.1±1.8.	39.0±1.7
6429PT.	14.9±1.0.	20.1±1.4	38.5±2.0
6520PB	12.6±2.3	7.9±0.7	43.8±2.3
6362PT	8.7± 1.0	29.6±2.4	45.2±2.0
6362PB.	2.4 ± 0.8.	15.8 ± 1.6.	XX
6325 (PT+PB)	11.1 ± 0.72	18.8 ± 1.0	36.3(3 islets)

X= p<0.005 X= p<0.05 . Underlined: T1D

Table 2- **Results indicate the mean values of the fluorescent intensity islets/ region of pancreas/ donor**. For AA+ tissues, results (± SE) illustrate the mean of 10-20 islets per region of pancreas. Fewer islets of T1D donors were scanned because of their scarcity. Islets were delineated using the freehand tool of Image J and the fluorescent intensity (FI) for each wavelength determined as FI/Area. PT: Pancreas Tail; PH: Pancreas Head; PB: Pancreas body. Note the presence of differences in each marker between donors. Moreover, values for each marker were either similar or different for the two regions examined. These observations highlight the need to examine changes in individual markers for each islet in a region. Also note that no correlation was found between the Autoantibodies present in each donor (Table 1) and the values of the different markers (Table 2).

2- PC1/3 Heterogeneous expression is lost in T1D

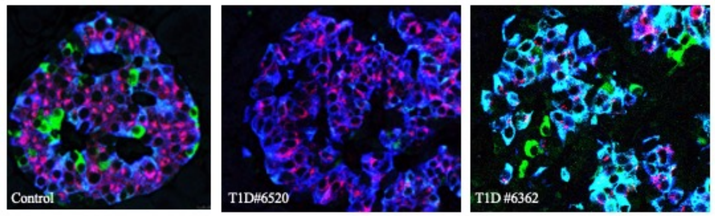


Figure 1- Islets of control and of T1D1# 6520 and 3632 stained for visualization of insulin, PC1/3 and proinsulin. Note that beta cells of control have different levels of PC1/3, which, when combined with insulin (blue) is seen as cyan. In contrast to control, most beta cells of #6520 islets have very low levels of PC1/3, appearing blue, while the level of the enzyme in cells of #6362 is uniformly high in all cells.

3- Pancreas of all AA+ have a subset of islets with abnormal PC1/3 expression

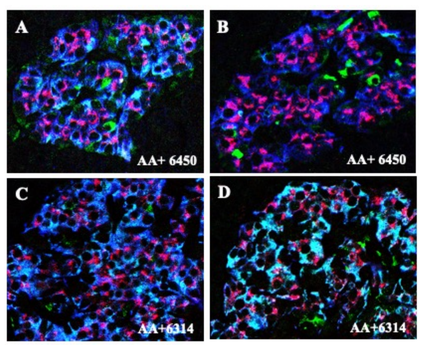


Fig 2- Illustrates pancreatic islets of AA+ donors immunostained for visualization of insulin, PC1/3 and proinsulin. Each pancreata contained a mixture of normal islets (Fig 2 A,C) containing the three described beta cell types expressing different PC1/3 levels and abnormal islets. The latter group are comprised of beta cells expressing similar either low (Fig 2B) or high PC1/3 (Fig 2D) levels. Green cells are somatostatin cells (1).

4-PC1/3 expression defines two subgroups of AA+ and T1D

Table 3

Donors with increased PC1/3 expression in beta cells		Donors with decreased PC1/3 PC1/3 expression in beta cells
6123 PT,PB		6450 PH, PT
6397 PT		6424 PT,PB
6429 PT,PB		6505 PB
6450 PB		6517 PB
6325 PB,PT		6520 PB,PT
6362 PB,PT	Underlined:T1D	5000 PT,PB
6484		

Donors with heterogeneous PC1/3 expression in beta cells (as in controls)

6314 PB; 6397 PT; 6505PT; 6517PT

Conclusions

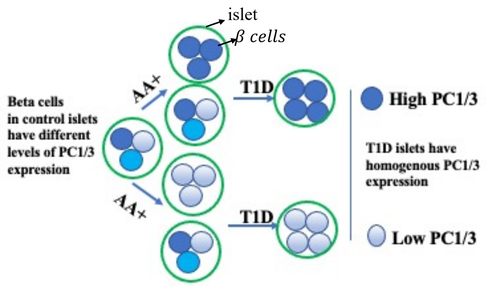
These studies indicate:

1- A trait of control beta cells is the presence of heterogeneous expression of PC1/3. This characteristic is lost in T1D. Thus, all beta cells of each pancreas express either high or low levels of the enzyme.

2- All beta cells of a subset of AA+ islets show either high or low PC1/3 levels, suggesting a gradual appearance of the abnormality.

3- These observations indicate that the loss of normal PC1/3 expression is a marker of disease progression in beta cells and that this defect appears prior to diagnosis.

Graphic summary of findings-



PC1/3 expression defines two subgroups of AA+ and T1D donors

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