Changes in insulin and proinsulin expression, beta and alpha cell islet composition, islet cellularity, and endocrine infiltration are evident before T1D onset

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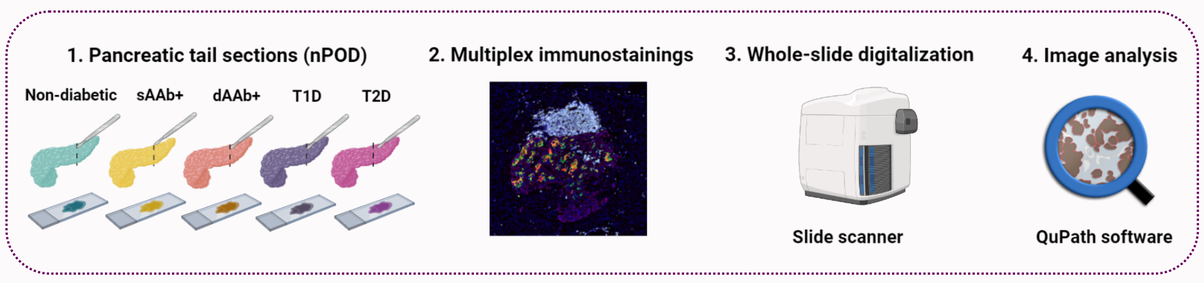
Purpose

Type 1 diabetes (T1D) is a complex disease in which genetic and environmental factors play an important role. The interplay between beta cells and the immune system has recently become a major research focus. Beta cells might be triggering their own demise, in part due to increased cellular stress, which could contribute to the aberrant processing and accumulation of proteins, triggering or potentiating beta cell specific immune responses. Individuals at risk of developing T1D, such as autoantibody positive donors (AAb+) have an increase in proinsulin levels in the serum, months to years before clinical diagnosis. Moreover, recent studies have shown an increase in pancreatic insulin and proinsulin content and increased proinsulin-to-insulin ratio in these individuals. Despite intensive research in the field, there are still unanswered questions regarding the expression and localization of proinsulin and insulin, how it changes with disease progression, and if it might correlate with immune infiltration. For this purpose, we employed our recently published whole-slide image analysis workflow, to extract quantitative data about the endocrine and exocrine cellular composition and immune infiltration of the pancreas during type 1 diabetes progression.

Methods

A. Summary



B. Donor characteristics

	Males	Females	Age	BMI	Duration	nPOD IDs
Non-diabetic (n=6)	2	4	19,1	25,7	N/A	6060, 6178, 6227, 6232, 6234, 6271
sAAb+ (n=4)	2	2	29,3	23,6	N/A	6123, 6147, 6151, 6156
dAAb+ (n=4)	3	1	30,6	26,9	N/A	6158, 6167, 6197, 6267
Short duration T1D (n=6)	4	2	22,0	27,2	3,7	6211, 6212, 6306, 6325, 6362, 6367
Long duration T1D (n=1)	-	1	22,6	21,6	7,0	6070
T2D (n=3)	1	2	41,7	38,5	1,8	6114, 6139, 6273

C. QuPath analysis

Procedure

- Tissues and islets were detected with threshold-ing/pixel classification
- Endocrine and immune cells were classified with machine learning
- Islets consisting of ≥ 30 cells were included
- Triple positive Ins+/PI+/Gluc+ and double positive Ins+/Gluc+ and PI+/Gluc+ were excluded

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Abbreviations & Definitions

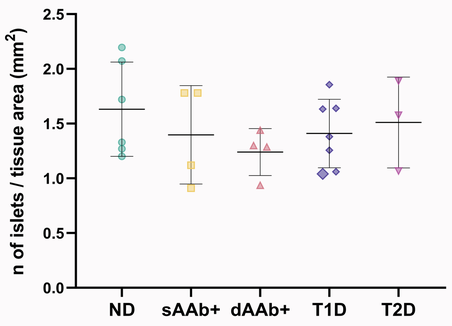
- ND=non-diabetic, sAAb+/dAAb+=single/double autoantibody positive, T1D/T2D=type1/2 diabetes
- Beta cells = Ins+, Proins+, Ins+/Proins+
- Alpha cells = Gluc+
- Insulin-deficient islets - IDIs = 0 % beta cells
- Poor insulin-containing islets (pICIs) = >0 - ≤ 10 % beta cells
- Insulin-containing islets - ICIs= >10% beta cells

Summary of results

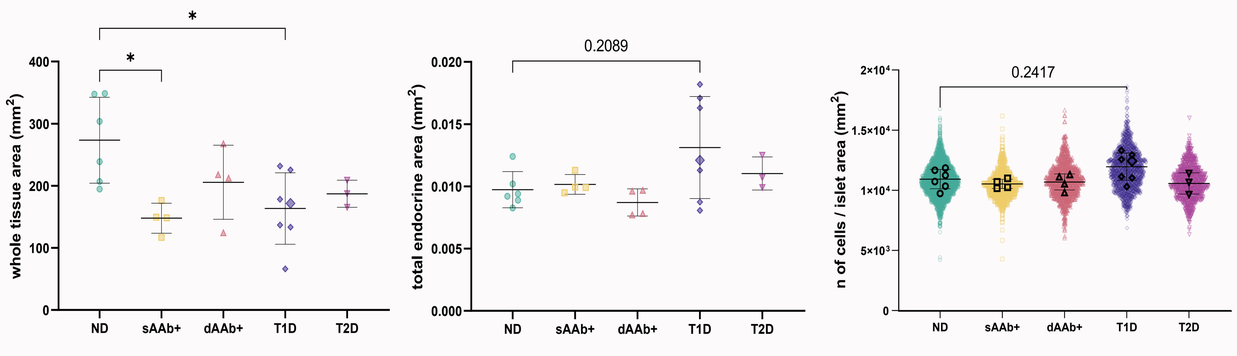
We generated quantitative data for islet density, cellularity and cellular density (number of cells per islet, or per islet area), cellular composition, islet phenotype and immune cell infiltration during the development of T1D. Specifically, we found that islet density decreases with disease progression. In dAAb+ individuals, the proportion and density of beta cells increases, while the proportion and density of alpha cells decreases. This is followed by a significant increase in both, proportion and density of alpha cells in T1D. Following a similar trend, insulin- and proinsulin-positive cells and Proinsulin-to-Insulin ratio increase with disease progression, but decrease in T1D. Furthermore, we classified the islets into 3 major islet types: insulin-deficient (IDIs, 0% of beta cells), poor insulin-containing (pICIs, >0 - ≤ 10 % beta cells), and insulin-containing islets (ICIs, >10% beta cells). We found IDIs, pICIs, and ICIs in all disease stages. However, IDIs and pICIs are more frequent in dAAb+ and T1D individuals. As expected, ICI density dramatically decreases in T1D. The number of cells per islet (islet cellularity) tends to decrease in ICIs compared to IDIs in all donor categories. Interestingly, T1D individuals have the highest cellularity in all islet types, compared to the rest of the donor groups. Analysis of immune infiltration revealed that high CD45+ cell density in endocrine and exocrine compartments is a defining feature during disease progression, being ICIs the islet type with the highest degree of infiltration. Of note, some dAAb+ individuals had the highest endocrine CD45+ density, indicating that immune infiltration is already present at early stages of the disease.

1. Islet numbers and density decrease with disease progression

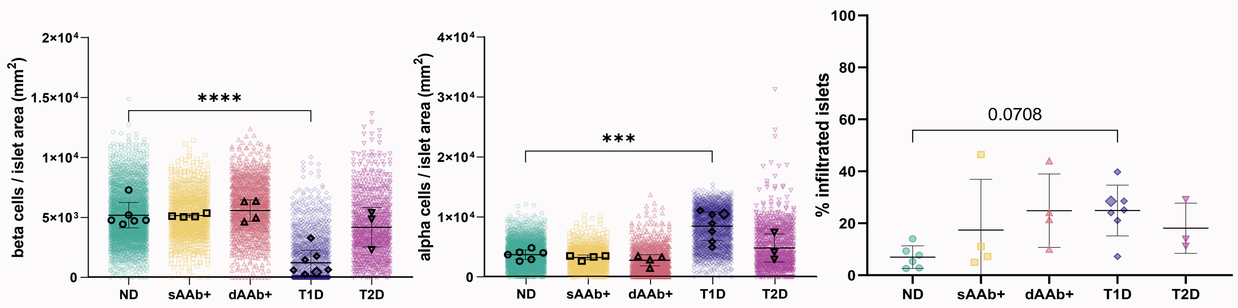
Donor type	n of donors	Total n of islets	Average n of islets per donor
ND	6	2526	421
sAAb+	4	800	200
dAAb+	4	1020	255
T1D	7	1613	230,4
T2D	3	830	276,6



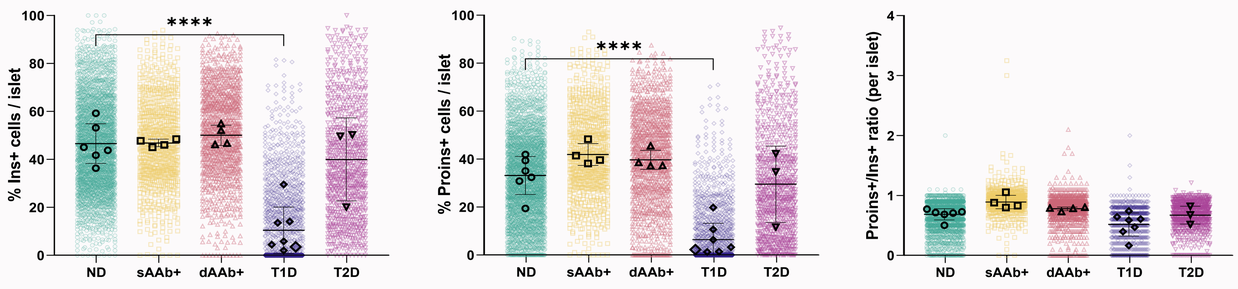
2. Tissue area decreases, whereas islet area and cellular density increases in T1D



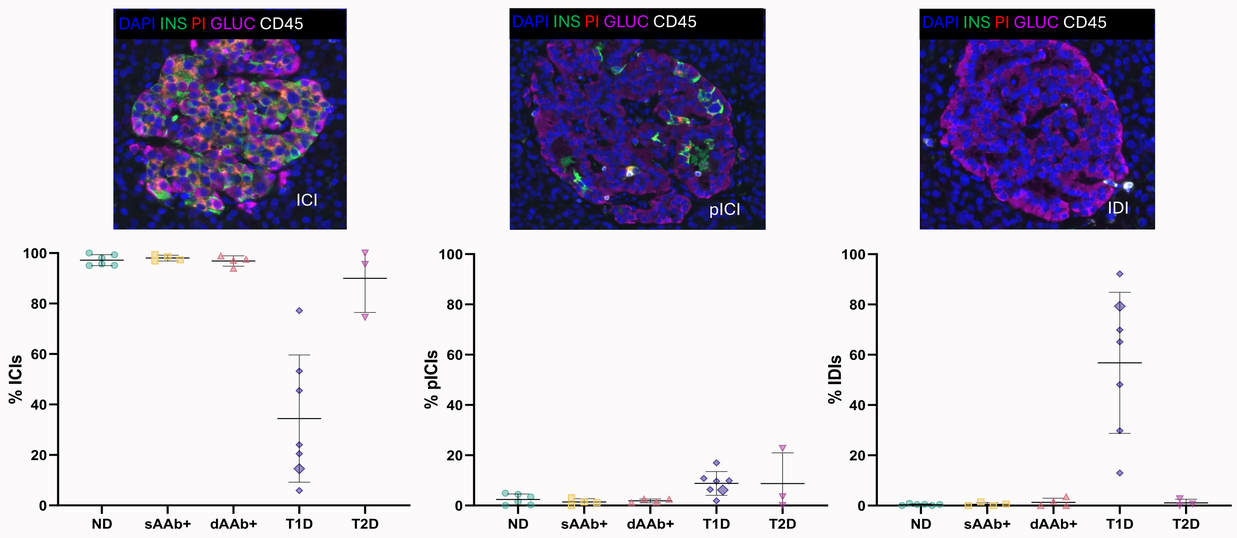
3. Islet beta cell density decreases, whereas alpha cell density and immune infiltration increase with disease progression



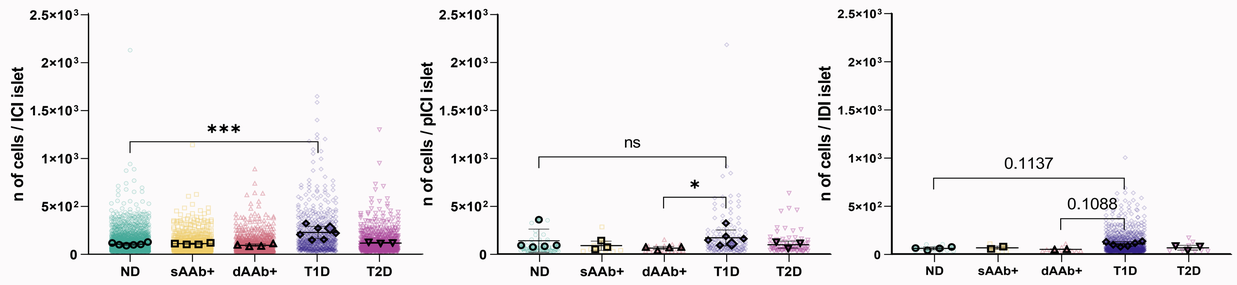
4. Insulin and proinsulin positive cells, and proinsulin-to-insulin ratio increase in prediabetes, but decrease substantially in T1D



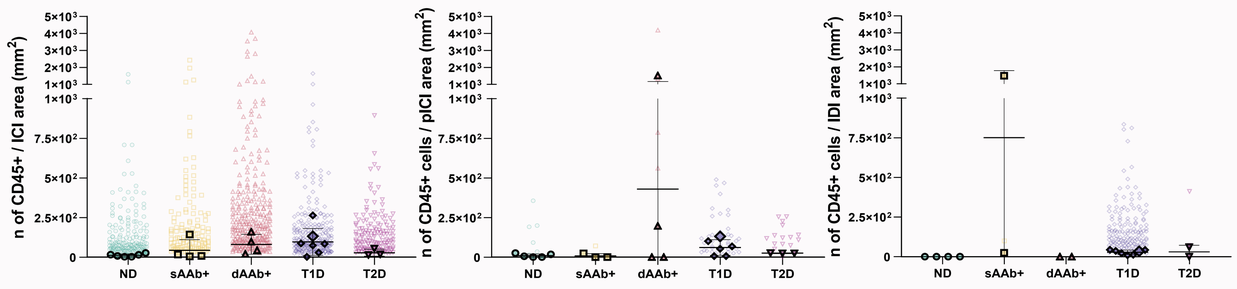
5. ICIs decrease and IDIs increase in T1D



6. Cellularity increases in all islet types in T1D



7. Immune infiltration impacts primarily ICIs



Conclusions

Our results show distinct differences in insulin and proinsulin expression, beta and alpha cell islet composition, islet cellularity, and endocrine infiltration during T1D progression. These changes are already observed in dAAb+, indicating that alterations in beta cell function (insulin and proinsulin expression) and immune infiltration (CD45+ cell density) occur before clinical onset. This information is critical to understand disease pathogenesis and progression, and to inform clinical trials aiming to preserve beta cell function and to stop the immune attack. Ongoing analysis on the same donors will allow us to further categorize islets according to their HLA-I content, and CD3- and CD8-positive infiltration, providing a better understanding of the events leading to T1D.

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