Use of a state-of-the-art digital pathology platform to analyze insulitis in pancreas from young people with recent-onset type 1 diabetes

AUTHORS

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PURPOSE

Worldwide, fewer than 600 Type 1 diabetes pancreata have been described in the literature or are accessible within tissue biobanks. Less than 80 of these are from individuals who were <10y at symptomatic onset and had a short duration (<1y) of disease. Among these, the majority were collected >30 years ago and were gathered from autopsy cases, with only limited information regarding fixation and tissue processing. Due to welcome improvements in the diagnosis and clinical management of Type 1 diabetes, deaths close to diagnosis are now very rare in young children, highlighting the value and importance of these archival samples. In the present study, we examined pancreas sections from two different historical collections, the Exeter Archival Diabetes Biobank (EADB n=58) and Seattle Children Hospital (n=6). Specifically, we optimized a triple chromogen immunohistochemical staining method to assess these tissues for insulitis and beta cell mass in a blinded manner, using a state-of-the-art image analysis platform.

METHODS

Serial sections of pancreas from the two biobanks were triple-immunostained for insulin/glucagon/CD45; CD8/CD20/glucagon; and CD4/CD20/glucagon. Sections, which included Type 1 diabetes cases (n=56) and non-diabetic controls (n=8), were scanned using an Aperio CS2 system and analyzed in a blinded manner using Halo 2.1 image software with Cytonuclear and Tissue classifier modules. Sections were annotated to identify inflamed insulin-containing islets (ICIs; ≥15 CD45+ cells); ICIs with <15 CD45+ cells; inflamed insulin-deficient islets (IDIs; ≥15 CD45+ cells) and IDIs with <15 CD45+ cells. The average number of islets/mm^2 tissue and average islet area (μm^2) were calculated as well as the total numbers of CD45+, CD8+ T, CD4+ T and CD20+ B cells in each of the islet categories. The total number of CD45+ cells found within the acinar tissue (and not directly adjacent to the islets) was also quantified.

SUMMARY OF RESULTS

Staining for CD45, insulin and glucagon was successful in 56 Type 1 diabetes cases and 8 non-diabetic controls, allowing for assessment of CD45 positive immune cells in a total of 7646 and 3508 islets, respectively. Of these 2646 and 3505 (34.6% and 99.9%) islets contained insulin and 14% and 0.03% were defined as insulitic, respectively. The numbers of CD20+ B cells, CD4 and CD8+ T cells were calculated in 35 Type 1 diabetes cases, which had not been post-fixed in mercuric chloride, and in all 8 controls. High numbers of CD20+ B cells were identified in a subset of these cases. An average of <3 B cells/ICI (CD20Lo) versus >3 B cells/ICI (CD20Hi) was used as a criterion to divide the cases into two groups. CD20Lo cases (n=15) had a median age at diagnosis of 15.5y (range 4-40y) and a median of 32.9% residual ICIs in the sections studied (range 0-90.5%), of which 10.8% (range 0–52.2%) were defined as insulitic. The mean number of CD45+ cells and CD20+ B cells/ICI were 8.48±2.0 and 0.20±0.0.4, respectively. By contrast, subjects defined as CD20Hi (n=20), had a median age at diagnosis of 6y (range 0.92-17y) and were all under 11y, except 1 case (17y), which had extensive evidence of pancreatitis. The group had a median of 10.3% residual ICIs (range 0-49.5%) with 77.9% (range 18.5-100.0%) defined as insulitic. The mean number of CD45+ cells and CD20+ B cells were 63.7±9.3 and 28.1±7, respectively. CD20Hi donors differed significantly from CD20Lo donors in all of these criteria ($p<0.05$).The average number of islets/mm^2 of tissue was reduced in all T1D cases (whether CD20Hi or CD20Lo) compared with controls ($p=0.0005$ and $p<0.0001$, respectively). A reduction in islet number/mm^2 was also evident in CD20Lo donors compared with CD20Hi cases ($p=0.09$). The area occupied by insulin positive cells was correlated with chronological age in controls and with age at onset in Type 1 diabetes cases ($r=-0.55$, $p=0.16$ and $r=0.33$, $p=0.07$, respectively). Fewer CD45+ cells were present within the pancreatic parenchyma (remote from islets) in controls and CD20Lo cases compared with those defined as CD20Hi.

CONCLUSIONS

We demonstrate that pancreata collected 30-40 years ago from two independent sources can be utilized to investigate the immunopathology of T1D using state-of-the-art image analysis platforms. Importantly, this dataset strengthens and expands previous observations that individuals with young onset Type 1 diabetes (<13y) display a different profile of insulitis from those diagnosed after the age of 13y. Among the youngest children, this is characterized by infiltration of the majority of residual ICIs with high numbers of CD8+ T and CD20+ B cells and the retention of lower numbers of residual ICIs at diagnosis. Preliminary evidence also suggests that Type 1 diabetes pancreata may have fewer islets/mm^2 of tissue compared with controls. This reduced islet mass may contribute to the early loss of glucose tolerance during islet inflammation in Type 1 diabetes.