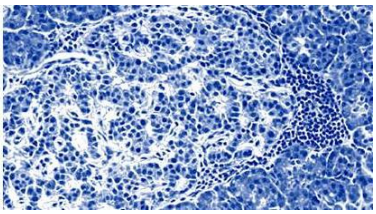
nPOD
Network for Pancreatic Organ Donors with Diabetes



Preproinsulin-specific cytotoxic T lymphocytes reside in healthy pancreatic exocrine tissue and progressively accumulate in islets during the advancement of type 1 diabetes

AUTHORS

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PURPOSE

Type 1 diabetes (T1D) is a chronic autoimmune disease. Before the clinical onset of T1D, autoantibodies against several beta cell autoantigens are present. From then on, autoreactive T cells recognizing islet autoantigens are believed to infiltrate the pancreas and contribute to beta cell destruction. In this context, CD8 T cells are the principal T cell type infiltrating the islets in T1D patients. Preproinsulin (PPI) is a key islet autoantigen in T1D patients but is also recognized by peripheral blood CD8+ T cells from healthy blood donors. While, due to limited access to human pancreas samples, autoreactive T cells have traditionally been studied in human blood, it remains unknown how quantitative information correlates to the target organ. In addition, little is known about the precise spatial distribution of autoreactive CD8+ T cells in the pancreas. Here, we precisely quantified PPI-specific CD8+ T cell frequencies across the exocrine and endocrine compartments of the pancreas at different stages of diabetes development.

METHODS

In situ immunofluorescence staining using the HLA-A2-restricted PPI_{15-24} tetramer in combination with CD8 and CD45RO was performed on frozen human pancreas section obtained from donors with diagnosed T1D, autoantibody-positive (‘at-risk’) and not-at-risk (autoantibody-negative) healthy controls. Sections were scanned using a Zeiss Axio.Scan Z1 slide scanner (Carl Zeiss). Afterwards, the same tissue sample was treated with hydrogen

peroxide to inactivate the fluorophores and re-stained for insulin, glucagon, and MHC class I. The analysis of the entire tissue section was done manually using the ZEN 2.5 lite software (Zeiss).

SUMMARY OF RESULTS

In non-at-risk controls, we found that almost half (45.3±3.7%) of all CD8+ T cells in the exocrine tissue recognized PPI_{15-24}, which was about 40-fold higher than previously described in peripheral blood. This high frequency remained comparable in at-risk and diagnosed T1D disease stages, while overall cellular infiltration increased by about 2-fold. Within or close to islets, already in autoantibody positive donors, 1.7-2.1 fold more PPI specific T cells were found and CD8 infiltration was substantially enhanced. Interestingly, in donors with diagnosed T1D, PPI_{15-24}-specific CD8+ T cells were solely found within insulin-containing islets, but not islets devoid of insulin (significantly increased within islets up to 7.8-fold and within the proximity of insulin containing islets up to 3.9 fold), irrespective of diabetes duration. This emphasizes a dominant role of PPI and a crucial role of autoreactive CD8+ T cells reactively targeting insulin-containing islets.

CONCLUSIONS

This is the first spatial quantitation of autoreactive CD8+ T cells in the human pancreas. In contrast to their previously-documented presence in the blood of healthy human donors in rather low numbers, we here report that PPI-reactive CD8+ T cells are abundantly present in the non-diabetic pancreas. Our findings question the dogma that T1D is primordially a disease caused by defective thymic deletion or systemic immune dysregulation since high numbers of autoreactive cells can be found in exocrine pancreata of healthy individuals. Rather, type 1 diabetes appears to depend on beta cell-specific factors (for example up-regulation of MHC class I and accumulation of pro-insulin, both hallmarks of human T1D), which attract autoreactive cells already in at-risk individuals to their antigenic site, the insulin-containing pancreatic islets, leading to beta-cell dysfunction and loss.