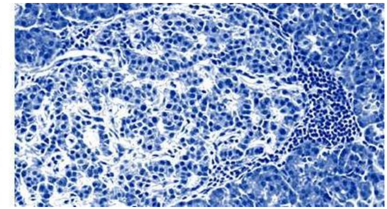
nPOD
Network for Pancreatic Organ Donors with Diabetes



Investigating type 1 diabetes pathogenesis with the live pancreas tissue slice platform

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PURPOSE

A goal of type 1 diabetes (T1D) research is to understand the complex islet microenvironment to determine the mechanisms of autoimmune-mediated beta cell dysfunction and loss. Here, we investigate the use of live pancreas tissue slices as a genuine and native three-dimensional microenvironment in the study of the islet-immune cell interface within the context of type 1 diabetes. In this system, exogenous, engineered islet-reactive T cells can be introduced into histocompatible non-diabetic human pancreas tissue to simulate the beta cell destruction observed in T1D. Further, endogenous tissue-resident or pathological immune cells can be studied in slices to identify translatable differences between donors at varying levels of T1D risk. Additionally, islet functionality can be assessed through calcium imaging and insulin perifusion traces. This allows for an overview of islet health and function under various insulitic conditions and can provide insight into disease progression.

METHODS

Live human pancreas tissue slices were prepared from non-diabetic and autoantibody-positive donors co-developed by the nPOD/OPPC at the University of Florida and Dresden University. Engineered human $CD8^+$ T cell avatars were introduced into live pancreas slices and imaged by time-lapse confocal microscopy on a Leica SP8 confocal equipped with an automated stage and incubation system. The T cell avatars express eGFP and the alpha and beta chains of a TCR recognizing the beta cell antigen islet antigen glucose-6-phosphatase catalytic subunit 2 (G6PC2, also known as IGRP) in the context of HLA-A*0201 (A2), a globally common human MHC class I allele. Cell viability/apoptosis in exocrine and islet tissue was dynamically tracked by SYTOX Blue dead cell stain and NucView 530 Caspase-3 substrate. Exogenous T cell motility was tracked by eGFP and the position of islets/beta cells by reflected light.

Endogenous T cells were stained and tracked in live tissue slices with anti-CD3-APC and HLA-multimers. Beta cell functionality was assessed through Ca^{2+} fluxes.

Live mouse pancreas tissue slices were prepared from NOD-*Rag1*$^{-/-}$ and NOD-*Rag1*$^{-/-}$-Ai4α/β TCR transgenic (Ai4) mice. Endogenous T cells were stained with anti-CD3-APC and anti-CD8-APC and tracked in the tissue. Ca^{2+} fluxes were recorded using Fluo-4 calcium indicator dye. Perifusion was also performed on the slices and insulin traces will be obtained from insulin ELISAs. In additional studies, splenocytes will be isolated from Ai4 mice and introduced on to control NOD-*Rag1*$^{-/-}$ mice pancreatic tissue slices and imaged through time-lapse confocal microscopy to determine the mechanisms and effects of early-stage insulitis.

SUMMARY OF RESULTS

The motility of exogenous T cell avatars in human pancreas slice tissue was tracked over 15 hours in nondiabetic donors (n = 2). Lacking any applied chemotactic stimulus, the T cells' motility qualitatively appeared to be directionally random, pending a more detailed cell tracking analysis. However, individual T cells did occasionally infiltrate periphery and core of islets and slow their migration speed. This is consistent with published results reporting random diabetic T cell migration in NOD mouse pancreas until cognate antigen is encountered. The beta cells continued to produce calcium fluxes in response to changes in glucose concentration. The rate of endocrine and exocrine cell apoptosis was quantified and compared to negative controls without T cells, or positive controls where apoptosis was artificially introduced by staurosporin. IGRP and GAD reactive T cells were found in or near islets from slices of recent onset human T1D organ donors. When endogenous $CD3^+$ cells were examined in live pancreas slices from a GAD autoantibody-positive donor (n = 1), one islet was found with >18 $CD3^+$ T cells forming an apparent focal insulitis. To our knowledge, this is the first image ever taken of live, endogenous human immune cells attacking live insulin-producing beta cells *in situ*. Using single cell RNAseq, autoreactive T cell receptors were identified from these slices.

In the mouse pancreas tissue studies, islets experiencing heavy insulitis no longer fluxed calcium in response to high glucose concentrations. These same islets also were found to have lost all reflectivity. Conversely, islets from AI4 mice with few to no T cells continued to have calcium responses to both high glucose and potassium chloride.

CONCLUSIONS

Using live human pancreas slices, engineered T cells or T cell lines are able to be exogenously introduced and their interactions, migration, and effector functions within the human pancreas tissue can be visually tracked dynamically. Features such as in situ islets, peri-islet basement membrane, vasculature, and endogenous immune cells remain intact. Furthermore, the slice model allows for the continued functionality of the tissue allowing for the assessment of beta cell functionality under various conditions. Using the Ai4 mouse model with consistent early islet inflammation, we can observe differences in islet calcium fluxes that are dependent on insulitis. These initial successes are guiding our development of pancreas slices for further detailed three-dimensional studies of islet—immune cell interactions relevant to human T1D. This technology has strong potential as a fully human platform for understanding the etiopathology of T1D and testing interventional therapies that act in the local islet immune microenvironment.