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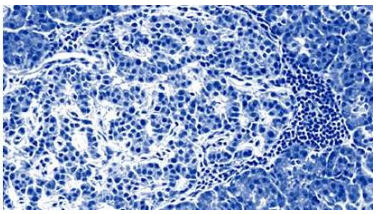

BETA CELL DEVELOPMENT, DIFFERENTIATION, REGULATION

OA1:
Pharmacologic and Genetic Approaches to Defining Human Beta Cell Mitogenic Targets Of Harmine Family Analogues: Is There More Than DYRK1A?

AUTHORS

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PURPOSE

Both Type 1 and Type 2 diabetes result entirely or in part from a reduction in functional pancreatic beta cells, with a resultant loss of adequate insulin secretion. Thus, expansion of functional beta-cells from residual human beta cells in people with Type 1 diabetes (T1D) and Type 2 diabetes (T2D) and regeneration of beta cells in human islets in vitro are attractive approachs for potential diabetic therapies. We and other groups have recently found that small molecule inhibitors of Dual Specificity, Tyrosine Phosphorylation-Regulated Kinase 1A (DYRK1A), such as harmine, INDY, 5-IT and GNF4877, are able to drive human beta cell regeneration in vitro and in vivo. We have also defined the key target relevant to human β-cell replication to be Dual Specificity Tyrosine-Regulated Kinase 1a (DYRK1A). However, Kinome scans and other evidences suggest that there are likely other targets of the "DYRK1A" inhibitors that participate in induction of human beta cell proliferation. While DYRK1A is certainly a target of this class, whether it is the only, or the most important target, is unknown. Here, we try to refine the potential mitogenic targets of the "DYRK1A" inhibitors in human islets.

METHODS

A combination of human beta cell RNAseq, "DYRK1A" inhibitors kinome scans, pharmacologic inhibitors, and adenovirally genetic targeted silencing of candidate genes on human beta cell proliferation was employed to refine the potential mitogenic targets of the harmalog family.

SUMMARY OF RESULTS

We explored the mechanisms of action and targets of the DYRK1A inhibitor class of small molecules on human beta cell proliferation, focusing initially on harmine, INDY, leucettine-41, and subsequently on GNF4877 and 5-IT. Our data confirmed in each case that DYRK1A is the central mitogenic target of the DYRK1A class inhibitor in human islets. Surprisingly, however, DYRK1B but not DYRK2, DYRK3 and DYRK4 also proves to be an important target: silencing DYRK1A leads to a resultant DYRK1B increase; simultaneous silencing of both DYRK1A and DYRK1B yields greater beta cell proliferation than silencing either individually and DYRK1B overexpression effectively blocked proliferation in response to Harmine, 5-IT and GNF4877 to the same degree as DYRK1A overexpression. Importantly, other potential kinases, such as the CLK and the GSK3 families, are excluded as important harmalog targets. Finally, we developed a unique adenovirus tool capable of silencing up to seven targets simultaneously in single cells, which can be useful for studies in human beta cell research.

CONCLUSIONS

Collectively, we report that inhibition of both DYRK1A and DYRK1B is required for induction of maximal rates of human beta cell proliferation, and provide clarity for future efforts at structure-based drug design for human beta cell regenerative drugs.

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☒ Beta Cell Development, Differentiation & Regeneration	☐ Novel Technologies
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☐ Core Lab	☐ Type 1 Diabetes Etiology & Environment
☐ Immunology	☐ Other (list):

OA2:
Subacute and Chronic In Vivo Administration of a Harmine-Exenatide Combination Enhances Glycemic Control and Markedly Expands Human Beta Cell Mass In Vivo

AUTHORS

Carolina Rosselot, Courtney Ackeifi, Yansui Li, Peng Wang, Alexandra Alvarsson, Sarah Stanley, Andrew F. Stewart, Adolfo Garcia-Ocaña. From the Diabetes, Obesity and Metabolism Institute, The Icahn School of Medicine at Mount Sinai, New York, NY.

PURPOSE

Administration of any member of the harmine family of DYRK1A inhibitors in combination with GLP1 family members increases markers of human beta cell proliferation and beta cell numbers in vitro. Here, we assessed the ability of the harmine-exenatide combination to expand beta cell mass in vivo in euglycemic and streptozotocin-diabetic mice over periods of one week to three months. Beta cell mass was measured by iDISCO tissue clearing followed by insulin immunolabeling, visualization by light sheet microscopy and volumetric quantification by Imaris.

METHODS

In sub-acute studies, daily intraperitoneal injections of low dose harmine (1mg/kg) and/or exenatide (0.5 µg/kg), or control vehicle, were administered to NOD-SCID mice transplanted with a marginal mass (500 IEQ) of human cadaveric islets for 7-14 days. Outcome measures were proliferation (Ki67+/Ins+ cells); glycemic control, IP-GTT, and circulating human insulin concentrations. In chronic studies, mice were infused continuously for three months via subcutaneous implanted Alzet minipumps infusing vehicle, harmine, exenatide, or a harmine-exenatide combination.

SUMMARY OF RESULTS

Subacute administration of the harmine-exenatide combination, but not harmine or exenatide alone, reversed diabetes, improved IP-GTT and increased human insulin secretion in the marginal mass streptozotocin model of diabetes. Removal of the human islet grafts resulted in prompt return of severe hyperglycemia. The harmine-exenatide combination was also effective in inducing human beta cell proliferation in vivo. In the chronic, three-month harmine-exenatide infusion model, exenatide alone had no effect on human beta cell mass; harmine treatment alone resulted in a 3x increase in beta cell mass. Most importantly, the harmine-exenatide combination resulted in a striking 10-fold increase in human beta cell mass.

CONCLUSIONS

Subacute and chronic administration of harmine plus exenatide enhances human insulin secretion and beta cell proliferation, markedly improves glycemic control, and results in remarkable increases in human beta cell mass, all in vivo.

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☐ Immunology	☐ Other (list):

OA3:
Identification and Validation of a Novel 3D Imaging Technique for Accurate Quantitation of Human Beta Cell Mass in Islets Transplanted into Immunodeficient Mice

AUTHORS

Carolina Rosselot, Alexandra Alvarsson, Yansui Li, Rosemary Li, Peng Wang, Andrew F. Stewart, Sarah A. Stanley and Adolfo Garcia-Ocaña. From the Diabetes, Obesity and Metabolism Institute, The Icahn School of Medicine at Mount Sinai, New York, NY.

PURPOSE

Diabetes results from diminished functional beta cell mass. Recently, a number of small molecules, hormones and neurotransmitters have been identified that increase beta cell replication markers in vitro, and in human islets transplanted in immunodeficient mice in vivo. However, whether this increase in human beta cell proliferation markers translates into an increase in beta cell mass in vivo is controversial. Current beta cell mass determination methodology requires tedious sectioning, staining, multiple image reconstruction and analysis, and the challenge of creating whole graft 3D images and volume quantitation. Here, we use a tissue clarification technique called 3D Imaging of Solvent-Cleared Organs (iDISCO) and advanced light-sheet imaging tools to visualize and quantify the volume of human beta cells in islet grafts transplanted into immunodeficient mice.

METHODS

Human islets from adult healthy donors were transplanted under the kidney capsule of euglycemic immunodeficient B6.129S7-Rag1tm1Mon/J mice. Whole-mount staining and clearing was performed using the iDISCO method (Renier et al., Cell 2014). Z-stacked optical sections were acquired with an Ultramicroscope II at 1.3x or 4x magnification. Image analysis and calculation of beta cell volume was performed with Imaris software.

SUMMARY OF RESULTS

First, we transplanted 100, 300, 500 and 1000 human islet equivalents (IE, 1 IE=125μm diameter) under the kidney capsule of immunodeficient mice. Kidneys were harvested immediately after transplant to determine whether human accurate beta cell volumes could be calculated, whether there was a linearity between human beta cell volumes and the number of islets transplanted, and whether measured volumes of human beta cells correlated with the theoretical volume of transplanted human islets. Human beta cell volumes for the progressively larger mass of transplanted islets displayed almost perfect linearity ($r^2=0.9719$). As expected, human beta cell volumes were 20-30% lower than the theoretical volume of transplanted human islets, reflecting the abundance of non-beta cells in human islets. Second, we repeated the transplanted studies above, but harvested kidneys 14 days later. As expected, measured beta cell volume declined from 100% to 32±5% at Day 14, likely reflecting ischemia-related beta cell death in the first days following transplant. Third, we transplanted 300 human IE in immunodeficient mice and treated the mice with

harmine or vehicle for 3 months using Alzet minipumps. Preliminary results indicate that harmine treatment led to a three-fold increase in human beta cell volume over three months.

CONCLUSIONS

We have developed a robust, precise and highly quantifiable new tool to measure beta cell volume in human islet grafts. iDISCO is ideal for assessing long term changes in human beta cell mass in response to putative human beta cell proliferative agents. iDISCO can also be applied to accurately quantifying volumes of alpha, delta, PP, ductal, endothelial and other cell types in human islet cell grafts.

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☐ Immunology	☐ Other (list):

OA4:
Aberrant Methylation of the 11p15 Imprinted Region is Important for Insulin Expression and Cell Proliferation in Human Insulinomas

AUTHORS

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PURPOSE

Diabetes results from loss or deficiency of insulin-producing beta cells. Restoring normal beta cell mass and function is key for reversing diabetes. We have previously shown that a rare and benign human pancreatic neuroendocrine tumor called insulinoma, holds the transcriptomic and genomic ‘recipe’ for inducing human beta cell replication. Our small pilot study revealed abnormal methylation patterns in the imprinted p15.5-p15.4 region of chromosome 11 in insulinomas. This region is also known to be imprinted and abnormally methylated in another disorder of expanded beta cell mass and function: the focal variant of congenital hyperinsulinism (FoCHI). The methylation abnormalities in our pilot study were relevant to beta cell proliferation and insulinoma pathogenesis. Therefore, we greatly expanded these studies.

METHODS

In this study, we report extensive, deep DNA methylome sequencing on an expanded beta cell cohort, and on the largest insulinoma cohort reported to date. More specifically, we describe deep methylome sequencing and detailed analysis of each of the ~30,000 CpG dinucleotides within the 11p15.5-15.4 region in a statistically meaningful cohort of five sets of normal human beta cells and an expanded set of 19 insulinomas. Further, we integrate and correlate the methylome results with additional large beta cell-relevant datasets.

SUMMARY OF RESULTS

We find abnormal, yet surprisingly consistent and recurrent methylation changes throughout the 11p15.5-15.4 region, predicting altered promoter and enhancer usage in human insulinomas vs. normal human beta cells. Our detailed bioinformatics analysis demonstrate that promoter methylation status has little effect on expression of genes in the target region. However, the abnormal methylation profile likely affects enhancers in their networks which leads to alternative means to drive *INS* expression, and replaces the canonical *PDX1*-driven beta cell specification with a pathological, looping, distal enhancer-based form of transcriptional regulation. We also show that NFaT transcription factors, rather than the

canonical *PDX1* enhancer complex, are predicted to drive *INS* transactivation in the context of the abnormal looping.

CONCLUSIONS
In conclusion, these findings strongly suggest that the aberrant methylation of 11p15.5-15.4 region leads to 3-D structural abnormalities in chromatin looping in insulinomas and they likely contribute to inappropriate insulin oversecretion and cell proliferation.

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OA5
Single cell resolution analysis of the human pancreatic ductal progenitor cell niche

AUTHORS

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PURPOSE

To analyze the human ductal progenitor cell niche at single cell resolution

METHODS

FACS-sorting of ALK3$^{bright+}$ cells from human pancreatic samples, corresponding to the pancreatic ductal tree. Principal Component Analysis and Clustering. Validation. Sorting and transplantation into immunodeficient mice.

SUMMARY OF RESULTS

We have described multipotent progenitor-like cells within the major pancreatic ducts (MPDs) of the human pancreas. They express PDX1, its surrogate surface marker P2RY1, and the BMP receptor 1A (BMPR1A)/Activin-like Kinase 3 (ALK3), but not carbonic anhydrase II (CAII). Here we report the single cell RNA sequencing (scRNAseq) of ALK3$^{bright+}$-sorted ductal cells, a fraction that harbors BMP-responsive progenitor-like cells.

CONCLUSIONS

Our analysis unveiled the existence of multiple sub-populations along two major axes, one that encompasses a gradient of ductal cell differentiation stages and another featuring cells with transitional phenotypes towards acinar tissue. A third potential ducto-endocrine axis is revealed upon integration of the ALK3$^{bright+}$ dataset with a single-cell whole-pancreas transcriptome. When transplanted into immunodeficient mice, P2RY1^{+}/ALK3$^{bright+}$ populations (enriched in PDX1^{+}/ALK3^{+}/CAII^{-} cells) differentiate into all pancreatic lineages, including functional β-cells. This process is accelerated when hosts are treated systemically with an

ALK3 agonist. We found PDX1^{+}/ALK3^{+}/CAII^{-} progenitor-like cells in the MPDs of type 1/2 diabetes donors, regardless of the duration of the disease. Our findings open the door to the pharmacological activation of progenitor cells *in situ*.

Select the ONE category that best describes your research:	
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OA6

Self-aggregation of stem cell derived beta cells is associated with maturation; mature cells can be identified and isolated using a specific surface marker

AUTHORS

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PURPOSE

Cell replacement therapy represents a potential cure for type-1 diabetes, as yet *in vitro* differentiation of β-like cells from human pluripotent stem cells results in production of cells that phenotypically and functionally resemble human fetal β cells. The purpose of this study is to establish mature stem cell derived β-like cells (sBC) *in vitro* and investigate potential mechanisms of human beta cell maturation.

METHODS

Direct differentiation of human embryonic and induced pluripotent stem cells into sBCs.

SUMMARY OF RESULTS

We observe that over time immature stem cell derived β-like cells (sBC) self-aggregate in 3D culture forming insulin^{+} 'caps' or self-enriched beta like cells (seBC). Characterization of seBC, by RNAseq, Ca^{2+} signaling, transmission electron microscopy (TEM), hormone content, mitochondrial analysis and global methylation pattern, shows that they are phenotypically more mature than SBC. We demonstrate by single cell RNAseq that seBCs, although already exhibiting improved maturation, compromise a heterogenous cell population displaying sBCs with varying maturity. Interestingly, bioinformatic analyses suggest a developmental trajectory towards most mature β cell phenotype in our culture conditions. Analysis of the mature β cell subset has allowed identification of a specific surface marker that can be used to specifically sort out mature sBCs. Establishing these different models of β cell maturation has allowed us to begin elucidating the complex mechanisms that drive maturation of human β cells enabling better recapitulation of the process *in vitro.*Finally, taking all of this together, we show that

sorting and reaggregation of mature β cells from iPSC derived from type-1 diabetic patients allows production of β-like cells that closely resemble mature human β cells.

CONCLUSIONS

Our results have important implications for current cell therapy efforts by providing a surface marker and critical information on mature stem cell derived beta-like cells.

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OA7
Protecting stem cell-derived beta like cells (sBCs) from an immune attack

AUTHORS

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PURPOSE

Show for the first time how stem cell-derived beta like cells (sBCs) respond to a T1D mimicking environment and determine if PD-L1 expression on sBCs can avoid activation of immune cells.

METHODS

sBCs and human islets were exposed to pro-inflammatory cytokines (IFN-γ, IL1β, TNFα) *in vitro* in a chronic manner. Immunofluorescence and qPCR analysis of immune related genes were performed to determine the sBCs respond to an inflammation environment. Human islets were used as control. Further, to provide sBCs with local PD-L1 mediated immune modulation, we employed genome engineering to stably and site specifically integrate an inducible PD-L1 expression system in human pluripotent stem cells (hPSCs). Further, direct differentiation of hPSCs into sBCs was used to generate sBCs that can express PD-L1 in a controlled manner. Immune cell activation assays are performed by co-culturing sBCs with avatar CD8+ T cells using IL2 secretion as an activation readout.

SUMMARY OF RESULTS

Exposure of sBCs to T1D modeling conditions results in upregulation of HLA-C but not HLA-A, -B or PD-L1 at the mRNA and protein levels while similarly treated healthy human islets upregulated immune receptors HLA-A, B, C as well as the immune checkpoint inhibitor PD-L1. We further show that sBCs treated with cytokines can present antigens and activate avatar CD8+ T cells as measured by IL-2 secretion. Experiments are being focused right now on using the inducible PD-L1 sBCs to determine if PD-L1 expression can rescue the activation of immune cells.

CONCLUSIONS

We show here for the first time how sBCs respond to a T1D mimicking environment. This study also confirmed known molecules important for the response of cadaveric human islets to T1D modeling conditions. Based on these results we provided novel strategies for the design of encapsulation-free cell therapy modalities that may efficiently evade an autoimmune attack.

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BETA CELL PHYSIOLOGY AND DYSFUNCTION

OA8:
Beta Cell Autophagy Is Reduced in Type 1 Diabetes

AUTHORS

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PURPOSE

Autophagy is a dynamic recycling mechanism that maintains cellular homeostasis, alleviates cellular stress, and generates energy by recycling damaged macromolecules and organelles. Crinophagy is a specific type of autophagy where secretory granules directly fuse with lysosomes for degradation. Impairment of autophagy and crinophagy have been implicated in beta cell death and dysfunction and this has been demonstrated in the context of type 2 diabetes. However, there is currently no literature describing beta cell autophagy in type 1 diabetes. Our study is therefore aimed at analyzing autophagy as a function of type 1 diabetes pathogenesis.

METHODS

We utilized immunofluorescent (IF) staining of LC3 (autophagosome marker), Lamp1 (lysosome marker), proinsulin (beta cell marker), and DAPI (nuclear marker) to analyze islet autophagy. We first analyzed paraffin embedded pancreas tissue from NOR (non-obese resistant- doesn't develop diabetes) and diabetic NOD (non-obese diabetic- develops spontaneous diabetes). We then performed IF on paraffin embedded pancreas tissue obtained from nPOD, collected from non-diabetic, autoantibody positive and type 1 diabetic human organ donors.

SUMMARY OF RESULTS

In the diabetic NOD mouse islets, we observe a significant decrease in the total number of lysosomes ($p=0.0129$), with no change in autophagosome numbers when compared to NOR control pancreas. We also observe a trend of decreased colocalization of autophagosomes with lysosomes, suggesting modestly reduced macroautophagy in islets of diabetic NOD mice. However, we observe significantly reduced colocalization of proinsulin with lysosomes in the islets of diabetic NOD mice compared to the NOR controls ($p=0.0121$), indicating reduction in a specific type of autophagy known as crinophagy. Interestingly, qualitative analysis of islets of diabetic NOD mice shows less granulation and more diffused proinsulin staining when compared to the NOR mice controls. Analysis of human pancreatic tissue sections showed no difference in autophagosome or lysosome numbers. Interestingly, however, both macroautophagy ($p=0.044$) and crinophagy ($p=0.0018$) were significantly reduced in residual proinsulin positive cells of pancreas from type 1 diabetic individuals compared to both the nondiabetic and autoantibody positive individuals.

CONCLUSIONS
This study provides evidence that macroautophagy and crinophagy are both significantly reduced in the context of type 1 diabetes. These data are the first demonstration of reduced autophagy in human islets from type 1 diabetic organ donors. Given the static nature of our observations, further studies should be performed to address how and when autophagy is reduced during the pathogenesis of type 1 diabetes, and to determine the functional effects of autophagy reduction in the context of disease initiation.

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OA9:
Syntaxin 4 Overexpression in Pancreatic Islet β-cells of Non-Obese Diabetic Mice Prevents Conversion to Autoimmune Diabetes

AUTHORS

Eunjin Oh[1], Erika M. Olson[1], Miwon Ahn[1], Shanshan Tang[2], Arnaud Zaldumbide[3], De-Fu Zeng[2], Bart O. Roep[2] and Debbie C. Thurmond[1]

[1]Department of Molecular and Cellular Endocrinology, [2]Department of Diabetes Immunology, City of Hope, Duarte, CA, and [3]Department of Molecular Cell Biology, Leiden University Medical Center, Leiden, The Netherlands.

PURPOSE

Syntaxin 4 (STX4), a plasma membrane-localized SNARE protein, plays a crucial rate-limiting role in β-cell insulin secretion and significantly improves glycemic control in a minimal model of islet transplantation. The enriched STX4 expression in human islets preserves β-cell mass by blocking NF-kB signaling induced CXCL9, 10, and 11 chemokine expression from β-cells. These data are proposed that STX4 overexpression in pancreatic β-cells has a protective effect against autoimmune diabetes (T1D) by attenuating inflammatory stress

METHODS

Human Pancreas Sections and immunofluorescence: Formalin-fixed paraffin embedded (FFPE) human pancreas donor sections containing 4 non-T1D and 4 T1D were obtained from Network for Pancreatic organ donors with Diabetes (nPOD) program. Pancreatic sections were immune stained with Insulin and STX4 antibodies and analysis was performed to quantify fluorescence intensities of STX4 normalized to insulin using Keyence hybrid cell counting software, compared with non T1D or T1D.

Animals: we generated a line of non-obese diabetic (NOD) mice, the animal model of T1D, that carried two transgenes to confer inducible beta cell specific (RIP-rtTA Tg) STX4 overexpression (TRE-STX4 Tg) using speed congenics (NOD-βSTX4).

SUMMARY OF RESULTS

STX4 levels are reduced in β-cells from human new-onset T1D insulin positive cells and prediabetic NOD mouse islets. Induced STX4 expression in the NOD-βSTX4 mice significantly deters onset of hyperglycemia. In comparison to female control mice on the NOD strain background: doxycycline induced or non-induced single Tg mice, stock NOD mice, or non-induced double Tg mice, female NOD-βSTX4 were 100% diabetes free at the time at which 50% of control mice converted to diabetes (17 weeks old), reduced to 73% diabetes-free by 25 weeks of age, when 72% of control mice were fully hyperglycemic (>300 mg/dl). At 12 weeks of age, prior to diabetes conversion, the NOD-βSTX4 mice showed better whole-body glucose tolerance and β-cell glucose responsiveness in vivo compared with age-matched control mice. RNA-seq studies revealed islets from NOD-βSTX4 mice had markedly reduced expression of IFN-γ and NOS-2, related to decreased activation of macrophages, phagocytes, or APCs related genes.

CONCLUSIONS
STX4 overexpression in the β-cells of NOD mice may indirectly provide a protective β-cell environment in the pancreas, deterring β-cell damage and preventing conversion to hyperglycemia/diabetes.

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☐ Immunology	☐ Other (list):

OA10:
Loss of Carboxypeptidase E in Pancreatic Beta Cells Does Not Accelerate the Development of Obesity-induced Glucose Intolerance in Mice

AUTHORS

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PURPOSE

Carboxypeptidase E (CPE) is an enzyme essential in the processing of neuroendocrine peptide precursors to functional hormones. Human and mice lacking *CPE* have elevated levels of plasma proinsulin, become obese, and progress towards severe diabetes through undefined mechanisms. We aimed to determine whether the lack of *CPE* in pancreatic βcells contributes to the development of diabetes.

METHODS

We generated β-cell specific-*Cpe* knockout (β*Cpe*KO; *Ins1*$^{cre/+}$ x *Cpe*$^{fl/fl}$) mice, and analyzed their β-cell area, islet insulin granule distribution, insulin secretion dynamics, and plasma insulin- and proinsulin-like immunoreactivity. In addition, we fed both male and female β*Cpe*KO and Wt (*Cpe*$^{fl/fl}$) mice a low-fat or high-fat diet (10% or 45% total energy) for 24 weeks, and monitored their weight gain, fasting plasma glucose levels, insulin sensitivity, and glucose tolerance.

SUMMARY OF RESULTS

Both male and female β*Cpe*KO mice, on a regular chow diet, have ~2 fold increased beta cell area, ~20-fold elevation in serum proinsulin-like immunoreactivity, and a markedly increased proportion of immature secretory granules in islet beta cells. Interestingly, insulin secretion dynamics (assessed by glucose-stimulated insulin secretion in a perifusion system) are similar between β*Cpe*KO and *Wt* islets, suggesting that impaired granule maturation does not impact exocytosis. Mature (fully processed) insulin levels were also similar between β*Cpe*KO and *Wt* mice in islet lysates (analyzed by western blotting and mass spectrometry), indicating the presence of compensatory mechanisms that enable complete proinsulin processing in the absence of Cpe. When placed on a high-fat diet for 24 weeks, β*Cpe*KO mice do not show accelerated development of weight gain or glucose intolerance compared to *Wt* littermates, suggesting that secretory stress induced by insulin resistance cannot unmask an impact on glucose homeostasis in mice with beta cell *Cpe* deficiency.

CONCLUSIONS
Our data suggest that, despite having elevated propeptide-processing burden, murine beta cells are able to produce mature insulin without Cpe. Loss of β-cell CPE function is likely not the sole contributor to diabetes in humans and mice carrying loss-of-function *CPE* mutations.

Select the ONE category that best describes your research:	
V Beta Cell Physiology and Dysfunction	☐ Novel Biomarkers
☐ Beta Cell Development, Differentiation & Regeneration	☐ Novel Technologies
☐ Bone Marrow Studies	☐ Pathology
☐ Core Lab	☐ Type 1 Diabetes Etiology & Environment
☐ Immunology	☐ Other (list):

OA11:
Elevated levels of surface and intracellular expression of MHC class I on beta and alpha cells precedes immune infiltration in antibody positive organ donors

AUTHORS

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PURPOSE

Our aim is to define the intracellular localization of MHC-I, quantify its expression in the surroundings of CD8 T infiltrating cells and define its distribution between alpha and beta cells.

METHODS

Pancreatic tissue sections from a non-diabetic (ND) donors, autoantibody positive (AAb+) donors, and donors with T1D provided by the Network for Pancreatic Organ Donors with Diabetes (nPOD) were imaged using high-resolution laser scanning confocal microscopy (LSCM).

SUMMARY OF RESULTS

Our analysis show that MHC-I is internalized and accumulated primarily in the Golgi apparatus in AAB+ and T1D donors. MHC-I is upregulated in T1D donors as well as AAB+ donors and is not driven by the immune infiltration. In T1D and AAB+ donors, MHC-I is primarly expressed on Alpha cells compared to ND donors as shown by quantitative colocalization assay.

CONCLUSIONS

Increased MHC-I is a hallmark of T1D pathology and already occurs prior to clinical diagnosis in islets of antibody positive organ donors, a time when immune infiltration is rare and sparse. Our detailed proximity analysis of upregulated MHC-I in relation to CD8 cells in ecoxrine pancreas and islets further supports the conclusion that the immune inflitration (or potential humoral mediators) are not the primary cause for the elevated MHC-I. Rather, the fact that MHC-I is not only increased on the surface but also intracellularly (mostly Golgi) in affected cells is indicative of a functional defect of beta cells (and maybe islets) preceding immune infiltration. Interestingly, despite the fact that alpha cells also show elevated MHC-I, they do not seem to become immune targets as easily as beta cells (maybe a question of epitope display?).

Select the ONE category that best describes your research:	
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☐ Beta Cell Development, Differentiation & Regeneration	☐ Novel Technologies
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☐ Immunology	☐ Other (list):

OA12:
Investigating the Role of Nuclear versus Cytoplasmic Hyperphosphorylated Tau in Human β cells During Ageing and Diabetes

AUTHORS

M. Irina Stefana*, Sarah J. Richardson*, Hanna Tulmin, Marie-Louise Zeissler, Darragh O'Brien, John K. Chilton, Noel G. Morgan, John A. Todd
****co-first authors***

PURPOSE

Two common haplotypes, H1 and H2, of the *MAPT* gene, which encodes the Tau protein, are associated with susceptibility and protection, respectively, to neurodegenerative diseases, including Parkinson's. Although the molecular pathways that translate genetic risk into disease susceptibility are incompletely understood, Tau hyperphosphorylation (pTau) and aggregation are thought to be drivers of neuronal death. The lower risk of H2 carriers has been associated with higher activity of a polymorphic antioxidant response element NRF2-binding site and, in neurons, with a higher expression of exon 3-containing *MAPT* isoforms, thought to encode less aggregation-prone isoforms of Tau. The H2 *MAPT* haplotype is also protective for T1D and therefore we asked whether Tau, often referred to as neuron-specific, and its presumed pathological form, pTau, are also expressed in islet β cells. Given that we now know that the answer is unequivocally, yes, we are currently investigating if they are functionally implicated in β-cell dysfunction, in the context of ageing or diabetes. We use a combination of the human EndoC-βH1 and -H3 cell lines, human pancreatic islets isolated from organ donors and pancreatic tissue sections from nPOD (University of Florida, USA) and IsletCore (University of Alberta, Canada).

METHODS

A panel of total Tau and pTau antibodies were validated and optimised for use in western blotting (WB), co-immunoprecipitation (co-IP), immunocytochemistry (ICC) on cultured cells and immunohistochemistry (IHC) on FFPE tissue sections protocols. EndoC-βH1 and -H3 were used to study the expression, subcellular localisation and co-localisation with various compartment and proliferation markers of total Tau and pTau variants by WB and ICC-IF. To examine the localisation and levels of pTau in human β cells *in situ*, 4 μm FFPE human donor pancreas sections were obtained from nPOD and IsletCore. So far, sections from a total of 128 donors have been analysed: of which 77 non-diabetic donors, 28 T2D donors and 23 T1D donors. All sections were stained using standard IF protocols using an antiserum raised against pTau. Anti-insulin, anti-glucagon or the nuclear marker DAPI were also included. High-resolution images of 20 islets/case were then processed using a custom Matlab script to define the subcellular localisation (cytoplasmic or nuclear) and quantify the intensity of pTau staining. Sections from a subset of donors were also stained for other anti-pTau antibodies, anti-total Tau antibodies or the Ki67 proliferation marker. Clinical data for nPOD donors were retrieved from the nPOD database. Information on donor genotype at the rs1052553 SNP (A>T), which tags the H1 and H2 main *MAPT* haplotypes, was also

obtained from nPOD and IsletCore, and was used to assess the effect of donor genotype on pTau in β cells. To gain insights into the role of Tau and pTau variants in β cells, Tau-interacting proteins in β cells were identified through a series of co-IP experiments using total and pTau antibodies on lysates from both, EndoC-βH1 and EndoC-βH3 cultured cells, and also from human pancreatic islets isolated from non-diabetic organ donors. Sub-cellular fractionation protocols were employed to study the differences in Tau-interacting partners between the cytoplasmic and nuclear compartments. Gene ontology enrichment analysis was carried on the resulting list of proteins using publicly-available tools.

SUMMARY OF RESULTS

Using FFPE pancreas sections and a validated panel of anti-Tau antibodies, we found that both total Tau and "pathogenic" pTau variants are present in human endocrine cells from non-diabetic donors *in situ*. Tau and pTau presence were further confirmed in human islets by WB and mass-spectrometry, and in cultured EndoC-βH1 and EndoC-βH3 immortalised human β cells by WB, ICC-IF and mass-spectrometry. High-resolution confocal imaging revealed that pTau, phosphorylated at several pathological epitopes, is present in β cells under basal culture conditions, and that pTau variants localise preferentially to the nucleus, in contrast to the mainly cytoplasmic distribution of total Tau. Using human donor pancreas sections, we have confirmed that findings, including the unexpected nuclear localisation of pTau variants, also translate to human β cells *in situ*. In non-diabetic donors (BMI<31), we further identified an effect of ageing on the subcellular localisation of pTau variants. Specifically, we found that, whereas in younger individuals (<10 yr) pTau localises predominately to the nucleus, with increasing age, nuclear pTau levels decrease accompanied by an increase in cytosolic pTau levels. This relationship correlated directly with age such that all individuals >35 yr had the majority of the pTau present within the islet localised to the cytosol. Using high-resolution confocal microscopy, it was further observed that pTau immunostaining intensity levels varied among β cells within the same islet, and that pTau and insulin are detected in distinct, mutually exclusive cytoplasmic locations. We note that pTau also localises to the nucleus in acinar cells, but maintains its nuclear localisation throughout the different age sub-groups, thus widening the relevance of pancreatic Tau studies beyond β-cell biology but also pointing to features of an ageing β cell that do not occur in other cell types, including islet δ and α cells. To gain insight into the role of total Tau and pTau variants in human β cells, we have optimised and carried out a series of Tau co-IP experiments in both cultured EndoC-βH1 cells and human donor islets, using either whole-cell lysates or nuclear vs. cytoplasmic fractions. Gene ontology enrichment analyses have revealed that nuclear pTau interacting partners are significantly enriched in RNA-binding proteins and spliceosome components, suggesting a novel role for pTau in control of gene expression and alternative splicing. Using EndoC-βH3 cells treated with chemical modulators of kinase and phosphatase inhibitors, we have found evidence that Tau phosphorylation controls the protein's subcellular localisation.

CONCLUSIONS

pTau variants, widely regarded in neurodegenerative diseases as cytotoxic, are also present in human β cells *in situ*, even in the absence of diabetes, and in human β cells cultured under basal conditions. Furthermore, we find that pTau variants localise predominantly to the nucleus, at least in younger individuals, despite the fact that Tau lacks a nuclear

localisation signal. Through co-IP experiments we have characterised the Tau-interacting partners in human β cells, thus revealing novel potential roles for Tau in the control of RNA metabolism, alternative splicing and gene expression. Ongoing analyses include analysis of nPOD pancreas sections from patients with T1D, with T2D and non-diabetic donors with a range of BMIs (controlling for age effects), and understanding the changes that lead to the decrease in nuclear pTau and accumulation of pTau in the cytoplasm of β cells with increased age. A progress update on these aspects of the work will also be presented. Given our findings, we hypothesise that *MAPT* is a T1D causal gene in the chromosomal region 17q21.31, and that the loss of nuclear pTau and the accompanying increase in cytosolic pTau that occurs in β cells with age and increased BMI (as our preliminary data suggest) may be associated with reduced insulin secretory capacity and increased β-cell fragility.

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☐ Immunology	☐ Other (list):

OA13:
Coxsackievirus Proteases Subvert Hormone Secretion by Affecting Stimulus-Secretion Coupling in Pancreatic Beta Cells

AUTHORS

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 # Presenting author

PURPOSE

Studies of post-mortem pancreas specimens have challenged the simplified notion that beta cells are completely lost during the development of type 1 diabetes (T1D). At disease onset, a large proportion of individuals with T1D have residual insulin-positive beta cells. How these cells have escaped the immune attack and why they are dysfunctional remains to be understood. Of possible interest is the observation that many patients with residual beta cell mass also have beta cells that are positively stained by antibodies that detect the enterovirus protein VP1 after immunohistochemical analysis. Enterovirus proteins have also been identified by mass spectrometry in nPOD cases with T1D and human pancreatic islets infected with a Coxsackie B virus (CVB) infection *in vitro* show impairments in glucose-induced insulin secretion (GIIS). Collectively, these observations imply that a direct infection of the beta cell may contribute to disease development, yet information regarding the mechanism(s) by which enteroviruses negatively affect beta cell function is lacking. Such information could however be of critical importance for the design of disease intervention trials or for restoration of beta cell function in pre-diabetic and/or recent onset T1D patients. Enteroviruses including the CVBs produce so-called non-structural proteins that affect the infected host cell. In the present study, we investigated whether two of these, namely the viral proteases $2A^{pro}$ and $3C^{pro}$, play a role in affecting hormone secretion during CVB infection.

METHODS

Primary human pancreatic islets from the Nordic Network for Clinical Islet Transplantation, Uppsala, Sweden, and INS-1 832/13 cells were infected with CVB3 or CVB4. The

expression of $2A^{pro}$ and $3C^{pro}$ proteases was assessed by RT-PCR and Western blot. cDNA encoding viral proteins were cloned from CVB3 (Nancy) to generate plasmids for transfections and for the production of recombinant active and catalytically inactive $2A^{pro}$ and $3C^{pro}$ proteases. ELISA was used to measure insulin and human growth hormone (hGH). Following transfection of INS-1 832/13 cells with plasmids encoding the viral proteases and reporter genes, insulin secretion was assessed by GIIS assay. Exocytosis was measured using patch-clamp and live cell total internal reflection fluorescence (TIRF) microcopy. Ca^{2+} flux was measured by patch-clamp.

SUMMARY OF RESULTS

We show that CVB infected beta cells have a reduced capacity to secrete insulin and that this is concomitant with the expression of the viral proteases $2A^{pro}$ and $3C^{pro}$. After transfecting INS-1 832/13 cells with plasmids encoding the proteases and performing subsequent GIIS assays, we identified impairments in the stimulus secretion coupling in cells expressing either of the proteases. Patch-clamp and TIRF microscopy analyses revealed that both $2A^{pro}$ and $3C^{pro}$ blocked beta cell exocytosis in response to membrane depolarization. Finally, through patch-clamp analysis we saw that the proteases caused impairments in Ca^{2+}-influx induced by membrane depolarization.

CONCLUSIONS

The present study demonstrates that the enteroviral proteases $2A^{pro}$ and $3C^{pro}$ contribute to the subversion of hormone secretion by blocking membrane depolarization-induced Ca^{2+}-influx and exocytosis. These observations provide important insights into how a CVB infection affects the function of the pancreatic beta cell. In addition, our studies suggest that antivirals targeting the viral proteases may be critical for the prevention or cure of enterovirus-mediated diseases including T1D.

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OA14

Comprehensive Proteomics Analysis of Human Islets Reveals GDF15 as a Protective Factor in Response to Proinflammatory Cytokines

AUTHORS

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****Equal contribution***
†Co-corresponding

PURPOSE

Type 1 diabetes (T1D) is characterized by the autoimmune mediated destruction of pancreatic β-cells. The inflammatory process is mediated by autoreactive cytotoxic T-cells (CD8) and macrophages, as well as by pro-inflammatory cytokines secreted by these immune cells. In this study we sought to identify protein signatures that reflect the health of islet cells when exposed to a cocktail of pro-inflammatory cytokines (IL-1β, IFN-γ) and may serve as biomarkers and/or protective agents for β-cell stress/death during the development of T1D.

METHODS

Human pancreatic islets obtained from 10 cadaveric donors were treated with or without pro-inflammatory cytokines (IL-1β and IFN-γ) for 48 h, then their proteins were digested with trypsin and the resulting peptides barcoded with chemical tags before analysis using 2D liquid chromatography coupled with tandem mass spectrometry. The results were validated by performing an independent proteomic analysis of islets from 2 additional donors, cultured and treated with cytokines in an independent laboratory. We subsequently investigated the protective role one of these proteins identified in this screen by studying its molecular and functional role in prevention of β-cell stress/death in a mouse model of T1D, and also tested for the presence/absence of this protein in nPOD pancreas tissue sections from control, T1D, and T2D donors.

SUMMARY OF RESULTS

Proteomics analysis led to the identification of 11,325 proteins, of which 387 were significantly and differentially expressed in islets exposed to pro-inflammatory cytokines. Evaluating each protein for adequate power indicated that 87.7% of proteins had a power of greater than 0.8 to detect a fold-change of 1.5 for sets 1 and 2, respectively. (Figure S1). The power analysis confirmed that the size of the present study is appropriate to investigate even small changes in protein abundances in response to the cytokine treatment.

Informatics analysis revealed activation of multiple pathways related to immune response, type 1 diabetes, cell death and cytokine signaling. Additionally, of the 387 significantly differentially expressed proteins, 207 were also detected in the independent proteomic analysis and 182 (88%) validated (i.e. same direction of change). Of interest growth/differentiation factor 15/GDF15 was found to be consistently downregulated upon cytokine treatment, a phenomenon that appears to be regulated at the post-transcriptional level, as identified by polyribosomal profile analysis. Pretreatment of MIN6, EndoC-BH1 cells and human islets with recombinant GDF15 protected cells from cytokine mediated β-cell death, and its levels were noted to be downregulated in islets harboring insulitis from NOD mice. Similarly, GDF15 was noted to be reduced in islets from nPOD donors with T1D compared to controls, but not in donors with T2D. Treatment of prediabetic NOD mice with recombinant GDF15 showed a significant decrease in insulitis and β-cell oxidative stress, with subsequent decrease in the incidence of T1D.

CONCLUSIONS

We present a unique resource for the identification of human islet proteins regulated by pro-inflammatory cytokines. By mining this new dataset, and integrating it with available RNA sequencing data, we detected an imbalance between pro- and anti-apoptotic proteins modulated by IL-1β + IFN-γ in β cells. This imbalance includes a post-transcriptional down-regulation of GDF15, presently shown to act as an anti-apoptotic protein. This finding provides a proof of concept for the utility of the present resource. Additional extension and mining of the human islet proteome has the potential to indicate novel avenues for the therapy of diabetes.

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☐ Immunology	☐ Other (list):

OA15
Early Alpha Cell Dysfunction During the Development of Type 1 Diabetes

AUTHORS

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PURPOSE

The presence of islet autoantibodies (AAB) is currently the best biomarker for the future development of type 1 diabetes (T1D), with T1D developing in just 15% of single AAB+ individuals, but a much higher rate in individuals who progress to positivity for two or more AAB. Thus, the single AAB positive state may represent a key window for intervention to prevent progression to T1D. Here we functionally and metabolically phenotype human islets from deceased non-diabetic, single AAB+ and T1D donors to identify early defects in AAB+ donor islets that could serve as biomarkers for disease development and potential nodes for therapeutic intervention.

METHODS

Human islets were received from the accredited Human Islet Resource Center at the University of Pennsylvania. We characterized the physiology and metabolic state of 20 human islet preparations isolated from 7 control-, 9 AAB+- and 4 T1D-donors. A perifusion protocol was designed to simultaneously assess insulin and glucagon secretion. After 2-3 days of culture, islets are pre-perifused with substrate-free medium. Next, a physiological amino acid mixture (AAM) is added to stimulate glucagon secretion. Then, low and high glucose (3 and 16.7 mM) are added to stimulate insulin secretion and to inhibit glucagon secretion. IBMX is then added to maximally increase cAMP and stimulate secretion of both hormones. Finally, a brief washout period with substrate-free medium removes all stimulants, and 30mM KCl is added to depolarize the islet cells and quantify the readily releasable pool of secretory granules. Simultaneous oxygen consumption rate (OCR) and hormone release were measured by a perfusion system in conjunction with a phosphorescence quenching apparatus that allows precise measurement of VO_2 difference between the inflow and outflow. Intracellular Ca^{2+} was imaged after Fura2 loading. Bulk RNASeq was performed on sorted alpha and beta cell populations.

SUMMARY OF RESULTS

We find that while T1D islets show a fully preserved insulin secretory response in the rare remaining beta cells to low and high glucose and to cAMP elevation, alpha cell glucagon secretion was significantly reduced and the ability of glucose to inhibit glucagon secretion was lost. Insulin secretion profiles were also similar between AAB+ and control cases, whereas islets from single AAB+ donors already demonstrated aisgnificant defect in glucose

suppression of glucagon secretion and a pronounced augmentation of IBMX-stimulated glucagon but not insulin secretion. AAM, low and high glucose stimulated oxygen consumption in islets from control and AAB+ donors to a similar extent; glucose was unable to stimulate oxygen consumption in T1DM islets. Notably, the oxygen consumption baseline was significantly higher in T1DM islets, and AAB+ islets had an elevated baseline intermediate between control and T1D islets. All groups of islets responded to the mitochondrial uncoupler FCCP, indicating good coupling between respiration and oxidative phosphorylation. Whole islet Ca^{2+} signaling was similar in control and AAB+ islets; however, the increase in intracellular Ca^{2+} due to low and high glucose was diminished in T1D islets. In keeping with the selective functional defect in AAB+ alpha cells, RNASeq analysis revealed ~100 differentially regulated genes in AAB+ alpha cells, but only 6 differentially regulated genes in AAB+ beta cells.

CONCLUSIONS

Taken together, results with islets isolated from non-diabetic control, AAB+ and T1D affected donors resulted in the following fundamental observations: (1) the remaining beta cells in islets from T1D donors have normal glucose-responsive insulin secretion, (2) islets from T1D donors have increased mitochondrial respiration and abnormal calcium signaling that may reflect their alpha cell predominant composition, (3) islets from AAB+ donors with normal glucose-stimulated insulin secretion already have defective glucose suppression of glucagon secretion, as well as an elevated mitochondrial respiration intermediate between normal and T1D. Thus, we find that alpha cell dysfunction precedes the beta cell insulin secretory deficit during the progression of type 1 diabetes, thereby offering a new biomarker for T1D and new targets for potential therapeutic intervention.

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CORE LAB

OA16:
nPOD Nanotomy: Large-scale Electron Microscopy T1D database

AUTHORS

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PURPOSE

Autoimmune β-cell destruction leads to type 1 diabetes, but the pathophysiological mechanisms remain unclear. To help address this void, we created an open access online repository, unprecedented in its size, composed of large-scale electron microscopy images ('nanotomy') of human pancreas tissue obtained from the Network for Pancreatic Organ donors with Diabetes (nPOD; www.nanotomy.org/nPOD).

METHODS

Nanotomy allows analyses of complete donor islets with up to macromolecular resolution. An open access EM database was created, sizing in the range of >1 million traditional EM images to permit ultrastructural evaluation of human islets. We have developed standardized nanotomy protocols and this workflow from sample preparation of relative large samples up to sharing via the nanotomy website, has become routine in our EM center.

SUMMARY OF RESULTS

The nPOD nanotomy database currently contains 64 datasets from in total 47 donors including donors type 1 and type 2 diabetes, autoantibody-positive donors without diabetes symptoms, as well as from control donors. Sample quality was deemed very good and was independent of sample storage duration of up to several years. Only 1 out of 48 donor samples did not pass quality control checks for morphology. Images of complete cross sections of islets of Langerhans at macromolecular scale allow for morphological analysis of complete islets, cells, organelles, and macromolecules by simply zooming in at higher resolution within any region or feature of interest in a 'Google-earth'- like manner (Fig. 1*B*).

Anomalies we found in type 1 diabetes included (i) an increase of 'intermediate cells' containing granules resembling those of exocrine zymogen and endocrine hormone secreting cells; and (ii) elevated presence of innate immune cells.

CONCLUSIONS
These are our first results of mining the database and support recent findings that suggest that type 1 diabetes includes abnormalities in the exocrine pancreas that may induce endocrine cellular stress as a trigger for autoimmunity.

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☐ Immunology	☐ Other (list):

IMMUNOLOGY

OA17

T cell autoreactivity mapping in individual islets with islet biology from donors with type 1 diabetes (T1D)

AUTHORS

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Diabetes Center of Excellence, University of Massachusetts Medical School, Worcester, MA, USA

PURPOSE

In the islets of donors with T1D, lymphocytic infiltration into islets is heterogeneous with non-infiltrated islets in close proximity to infiltrated islets. Our purpose is to examine individual islets from donors with T1D for lymphocytic infiltration, to determine the repertoire of T cell autoreactivity from individual islets, and to determine the phenotype of islet cells in infiltrated and non-infiltrated islets.

METHODS

From donors with T1D, single islets were handpicked into individual wells of 96 well plates with media for T cell stimulation and expansion. After brief culture, lymphocyte outgrowth from individual was observed. Lymphocytes were recovered from individual wells, further expanded, phenotyped, and tested for T cell autoreactivity. In addition, we used a mid-throughput method of T cell autoreactivity testing. Autologous splenic B-LCL pulsed with pools of peptides (known native and neoepitopes, grouped by HLA class I and class II binding for each donor) were co-cultured with T cell lines derived from individual islets and a CD40 antibody to capture CD40/CD154 complex on the cell surface of activated T cells for detection by flow cytometry. From one donor with T1D, nPOD 6477 (20 year old female with 8 years of T1D duration, $GADA^+$ and $IA\text{-}2A^+$ at time of demise; HLA A*02/29, B*07/13, DR*01/01, DQ*05/05), islets from wells with and without T cell outgrowth were pooled, respectively, labeled with hashtagging antibodies to distinguish all cells in the two pools and then labeled with barcoded antibodies to identify immune cell types by surface expression of markers. Single cell RNAseq was determined using the 10x platform.

SUMMARY OF RESULTS

From donors with T1D, an average of 25% of individual islets had T cell outgrowth. From an islet from nPOD donor 6342, a $CD8^+$ T cell clone recognized $ppIAPP_{5\text{-}13}$ in the context of HLA-A2 and from donor T1D.8 (30 year old male with 20 years duration of T1D), a $CD8^+$ T cell clone recognized $ZnT8_{186\text{-}194}$ in the context of HLA-A3. A T cell reactivity map for $CD4^+$ and $CD8^+$ cells was generated for the lines from each donor. As previously reported, a broad range of T cell autoreactivity was seen with focused T cell reactivity seen in some islets and broad T cell reactivity seen in other islets. From nPOD donor 6477, islets with little or no T cell infiltrate showed high levels *INS* mRNA while islets with infiltrate showed fewer *INS* transcripts. I□□□□□ with infiltrate showed upregulated transcripts for *CCL20, CXCL2 and IL-32* as compared to islets with little or no infiltration.

CONCLUSIONS
As shown by immunohistochemistry/immunofluorescence data on pancreata from donors with T1D, lymphocytic infiltration into individual islets is heterogeneous; our data supports this conclusion. As shown here by mapping the autoreactivity for individual infiltrated islets, the T cell repertoire can be focused or more broad. By examining the transcriptome of islet cells from infiltrated and non-infiltrated islets, a pro-inflammatory environment for macrophages and chemoattractive for lymphocytes and neutrophils was observed in islets with T cell infiltrate coupled with a decrease in *INS* mRNA. Deeper sequencing of individual cell types within infiltrated and non-infiltrated islets will clarify factors within the islets that may promote lymphocytic infiltration. Understanding the heterogeneous pattern of infiltrated islets, both the heterogeneous T cell repertoire and the heterogeneous islet phenotype will promote understanding of the disease process in T1D.

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OA18

Analysis of antigen-receptor sequences of a unique lymphocyte reveals a T cell-neoantigen encoded in a public BCR of T1D patients

AUTHORS

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PURPOSE

Although it is well accepted that type 1 diabetes (T1D) is an autoimmune disease that is caused by destruction of insulin-producing beta cells by autoreactive T cells, critical gaps and questions in our understanding of how diabetogenic T cells remain elusive despite extensive investigation of the disease process. We hypothesize that difficulty in filling these gaps are due at least in part due to the long-held view that T and B cells are the only adaptive immune cells. We have recently discovered a third adaptive lymphocyte that is a hybrid between B and T cells and clonally expanded in T1D patients as compared to healthy controls, that we referred to as dual expressers, DEs or X cells to denote their crossover phenotype (**Ahmed et al, Cell, 2019: 177:11583**). We hypothesize that investigation of X cells will help answers key outstanding questions in the field and may inform new diagnostic to predict at early age and before antibodies who will likely to develop T1D and eventually new efficacious specific therapeutic interventions that spare depletion of T and B cells

METHODS

We developed a protocol to identify DEs or X cells in peripheral blood using flow cytometry (FACS) and for functional analysis. Visualization of unique phenotype of X cells at single cell level was carried out by AMNIS .We determined the gene make up of X cells by using single cell RNA seq and this method allowed us to determine the genes that are actively transcribed and expressed in X cells as compared T and B cells. To physically demonstrate co-expression of TCR and BCR in the same cell, we generated immortalized DE cells using EBV-transformation and examined cells from single clone for fully assembled BCR and TCR. We also established their unique functional relevance of X cells in the context of auto-reactivity by showing their structural mimicking as an autoantigen (insulin) presented by MHC-II (HLA-DQ-8) molecules to pathogenic CD4 T cells in T1D cases.

SUMMARY OF RESULTS

Here we describe a rare subset of autoreactive lymphocytes with a hybrid phenotype of T and B cells including coexpression of TCR and BCR and key lineage markers of both cell types (hereafter referred to as dual expressers or DEs). In type 1 diabetes (T1D), DEs are predominated by one clonotype that encodes a potent CD4 T cell epitope in its antigen binding site (referred to as x-idiotype). Molecular dynamics simulations revealed that the x-idiotype (x-Id) peptide has an optimal binding register for diabetogenic HLA-DQ8. In concordance, synthesized x-Id peptide forms stable DQ8 complexes and potently stimulate autoreactive CD4 T cells from T1D, but not healthy controls. Moreover, x-clonotype-bearing mAbs stimulate CD4 T cells and inhibited insulin-tetramer binding to CD4 T cells.

CONCLUSIONS

These results uncovered a widespread existence of a population of lymphocytes ‘X cell’ that are apparently multi-functional as B and T cells, autoreactive and could be an important player in T1D and perhaps autoimmune diseases.

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OA19
HANDEL-I - Cellular and Molecular Dynamics in the Developing Human Immune System

AUTHORS

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PURPOSE

Through the HANDEL project, we established a network to identify potential organ donors in the first decade of life, representing a very rare and largely understudied, yet exceedingly important, group for scientific investigation, this decade is characterized by massive growth and development affecting all organ systems, as well as the emergence of autoantibodies followed by the peak incidence of clinical type 1 diabetes (T1D). Recognizing that this period is also where environmental exposures, infections, and vaccines shape immune system development from central tolerance to cell subset distribution in peripheral lymphoid and mucosal tissues, we established HANDEL-I to collect these key tissues in parallel. This systems approach is only possible through the combined acquisition and analyses outlined in the overall HANDEL network.

METHODS

To date, we have procured 78 total donors, with distribution across four age categories (birth to < 3 months, n=16; ≥ 3 to < 24 months, n=20; ≥ 2 to < 5 years, n=19; ≥ 5 to < 11 years, n=23) This coordinated effort has allowed us to amass 77 spleens, 77 lymph nodes, 64 pancreata (with 16 islet isolations), 26 PBMC (blood cell isolates), 22 thymi, 24 lungs, 25 intestines, and 8 bone marrow samples across all age categories.

SUMMARY OF RESULTS

Herein, we discuss analyses of the central immune tissue – thymus and bone marrow.As the site of T cell development, the thymus plays a key role in immune ontology and pathogen defense. The developmental program of the thymus is highly dynamic, with a period of massive output following birth to near-total involution post-puberty. Studies of thymic development have been largely limited to animal models or cardiac surgery-associated thymectomy. To illuminate the tissue architecture and cellular dynamics of human thymic development, tissue sections obtained from two thymic regions incorporating both lobes were processed for comprehensive imaging by immunohistochemistry (IHC), highly multiplexed immunofluorescence imaging (CODEX), imaging mass cytometry, and clarity techniques (Lightsheet). Single cell suspensions generated by mechanical and enzymatic disruption were further interrogated by flow cytometry and single-cell RNA sequencing (scRNASeq).

Our preliminary sample cohort demonstrated dynamic changes in both structural and cellular architecture with age. Combined single-cell proteomics and transcriptomics via flow cytometry and scRNAseq facilitated immune cell type classification and the analysis of cell-fate trajectories from common progenitors to fully mature T cells prepared for thymic egress. Bone marrow not only serves as the source of hematopoetic stem cells (HSC), but in addition serves as a reservoir for long-lived memory and plasma B cells that are capable of supporting protective humoral immunity. As with the thymus, there are populations present that simply do not circulate at high levels in PBMC. Furthermore, very little is currently known about the amount of repertoire overlap amongst BM and PBMC. This outstanding need may very well be the determining factor for recurrent autoimmunity after beta cell replacement therapies, even when alloimmunity is well controlled. Our data, including scRNAseq, CITEseq, and immune repertoire analysis of T and B cells are expected to facilitate one of the first complete sets of data from both central developmental tissues including BM and thymus, along with mucosal immune sites, secondary lymphatics, and PBMC.

CONCLUSIONS

Our in-depth analyses of cellular architecture and transcriptional profiles provide novel insight for understanding human immune cell development in early life. The resulting knowledge offers the potential to uncover novel strategies to manipulate immune developmental checkpoints in early life, with substantial implications for autoimmune therapy strategies.

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OA20
Characterization of a Hybrid Insulin Peptide (HIP) as an Autoantigen in Human Type 1 Diabetes

AUTHORS

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PURPOSE

Relatively little is known about the primary peptide epitopes targeted by the autoimmune response during the development of type 1 diabetes (T1D) in humans. Using mass spectrometry, we have shown that in both mouse and human islets insulin fragments become covalently linked via a peptide bond to other beta cell peptides, leading to the generation of hybrid insulin peptides (HIPs). We established that HIP-reactive CD4 T cells can trigger disease in NOD mice, indicating that HIPs are major autoantigens in this animal model . Furthermore, we determined that HIP-reactive CD4 T cells are present in the peripheral blood of recent onset T1D patients and in the residual islets of organ donors with T1D. We sought here to further characterize a specific HIP as an autoantigen in human T1D.

METHODS

Mass spectrometric analysis of islets from human donors was used to identify endogenous HIPs. PBMCs from T1D patients and controls were assessed by ELISpot analysis for reactivity to HIPs, and a HIP-reactive CD4 T cell clone was isolated and used for epitope mapping.

SUMMARY OF RESULTS

A novel HIP was identified in human islets by mass spectrometry. PBMCs from a subset of T1D patients responded to this peptide. A CD4 T cell clone isolated from one of these patients responded to a synthetic version of the HIP at low nanomolar concentration but not to the non-hybrid peptide constituents. Screening of a panel of truncated peptides revealed that the core epitope for this HIP-reactive clone centered around the hybrid peptide junction.

CONCLUSIONS

Through a combination of immunological and mass spectrometry-based analyses, we have identified a HIP antigen that may play an important role in the etiopathogenesis of human T1D. This work can act as a springboard for future efforts to elucidate the role of HIPs as antigens in T1D and identify biomarkers and therapeutic targets in this disease.

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OA21
Dextran Sulfate Ameliorates Type 1 Diabetes through Reducing IFNγ Effects and Increasing TGFß Signaling

AUTHORS

Geming Lu[1], Jiamin Zhang[1], Tuo Zhang[2], Dirk Homann[1] and Adolfo Garcia-Ocaña[1]. [1]From the Diabetes, Obesity and Metabolism Institute, The Icahn School of Medicine at Mount Sinai, and the [2]Genomics Resources Core Facility, Weill Cornell Medical College, New York, NY.

PURPOSE

Type 1 Diabetes (T1D) results from immune tolerance failure and pancreatic beta cell destruction. Our lab has recently found that low molecular weight dextran sulfate (DS) markedly reduces the development and progression of early onset T1D in NOD mice. Furthermore, DS blunts cytokine-mediated beta cell death. In this study, we analyzed the molecular mechanisms involved in the beneficial effects of DS on beta cells and immune cells.

METHODS

Human islets were treated for 24h with cytokines (IFNγ, IL1β and TNFα) in presence or absence of DS. For each human islet preparation, RNA was collected for RNAseq analysis and protein was extracted for expression analysis of cytokine-induced pathways by western blot. IFN receptor expression was analyzed in INS1 cells treated with cytokines±DS. Non-diabetic NOD female mice were treated with DS or saline for four weeks and lymphocytes from spleen and pancreatic lymph nodes were analyzed by flow cytometry to profile T cells (IFNγ/PD-1/CD4/CD8 and Foxp3/CD4) and DCs/Macrophages (MHC II/CD86/CD80/PD-1L/ CD11b/CD11c). DS effects on TGFß signaling in T cells and macrophages was analyzed in vitro.

SUMMARY OF RESULTS

RNAseq analysis of human islets treated with cytokines±DS showed significant upregulation of 217 genes and downregulation of 353 genes in DS+cytokines compared with cytokine treatment alone. These genes were mostly involved in cellular processes such as reduced apoptosis, decreased inflammatory response, diminished chemokine production, increased oxidative phosphorylation and enhanced pyruvate metabolic activities. JAK2, pSTAT1, pP65 and iNOS protein expression was significantly reduced in human islets treated with cytokines+DS compared with cytokines alone. Furthermore, DS reduced cytokine-induced IFNγ receptor upregulation in beta cells. DS-treated NOD mice displayed decreased IFNγ and increased PD-1 expression in both CD8+ and CD4+ T cells. Interestingly, DS treatment significantly upregulated Foxp3+ CD4 T cells and enhanced PD-L1hi/CD80+/CD86+/MHC-IIhi/CD11b+/CD11c+ cells (tolerant dendritic cells) in pancreatic lymph nodes from these mice. DS enhanced TGFß signaling and increased pSMAD2/3 in T cells what led to reduced number of IFNγ+Th1 cells and enhanced suppressive capacity of Foxp3+ Tregs. Moreover, DS decreased IL12P40, IL1ß and TNFα expression in inflammatory M1 macrophages and increased CD206, CD301 and Arg1 expression in M2 macrophages.

CONCLUSIONS
Collectively, these results indicate that DS reduces the onset of T1D in NOD mice by neutralizing IFNγ action in beta cells and increasing TGFß signaling in immune cells leading to enhanced immune suppressive functions in T cells and myeloid cells, and favoring M2 macrophage polarization. DS treatment can potentially be of great value for treating T1D.

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OA22
Tolerogenic Effects of Dextran Sulfate in Human Dendritic Cells and T Lymphocytes

AUTHORS

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PURPOSE

Immune dysfunction plays an important role in the pathogenesis of type 1 diabetes (T1D). Low molecular weight dextran sulfate (DS) is a sulfated polysaccharide with immunomodulatory properties and cytoprotective actions. Our lab has recently shown that DS treatment markedly blocks the development and, more importantly, reverses early onset T1D in mice, with decreases in activated T cells (Th1) and increases in regulatory T cells (Tregs). In the current study, we aimed to translate these findings to human immune cells.

METHODS

Peripheral blood mononuclear cells (PBMCs) were obtained from healthy subjects and CD14+-monocytes were isolated and treated with GM-CSF and IL-4 for six days to generate dendritic cells (DCs). DCs were treated with LPS and/or DS for 72h and analyzed for viability, maturation and co-stimulatory markers (CD80, CD83, CD86, CD40, HLA-DR) by flow cytometry. We also analyzed the functional properties of DCs by co-culturing DS±LPS-treated DCs with lymphocytes and measuring lymphocyte activation status and phenotype by flow cytometry. Endocytic capacity of DS±LPS-treated DCs was measured by DS-FITC uptake (30 min, 37°C). To examine the effects of DS on Th1, Th2, Th17, and Treg induction and phenotype, PBMCs were treated with anti-CD3/CD28 for 48 hours or PMA/ionomycin for 24 hours and analyzed for activation markers and cytokine production by flow cytometry.

SUMMARY OF RESULTS

DS decreased the mean fluorescence intensity (MFI) and the number of DCs expressing the co-stimulatory molecule CD80 but did not alter CD83, CD86, CD40 and MHCII expression. Importantly, interaction between DS-treated DCs and autologous lymphocytes led to a significant increase in the proliferation of Tregs (CD4+CD25+FOXP3+CD127low). On the other hand, the incubation of DS-treated DCs with allogenic lymphocytes did not result in proliferation changes. Analysis of the endocytic capacity of DCs showed no difference with DS treatment. Preliminary data also indicates that DS decreased Th1 activation when PBMCs are stimulated with anti-CD3/CD28 but not with PMA/ionomycin.

CONCLUSIONS

Collectively, these results suggest that DS induces a more tolerogenic phenotype in activated human DCs leading to an increase in the proliferation of Tregs. Preliminarily, DS also reduces the number of activated Th1+ cells when activation occurs through external cell

signals. Future studies will focus on the impact of DS on DCs as the underlying mechanism decreasing T-cell activation. These studies help to identify targets for intervention in T1D, while providing a useful therapeutic agent with a proven history of safe use in humans.

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OA23
Metabolic Regulation of Autoreactive CD4+ T cells Prevents Autoimmune Diabetes

AUTHORS

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PURPOSE

Type 1 Diabetes (T1D) is a chronic inflammatory disease that results from immune-mediated destruction of the insulin- secreting, pancreatic β cell, with CD4+ T cells largely mediating pathogenesis. Over time, this destruction results in loss of β cell mass and function, reduced insulin- secretion, and a failure to control blood glucose levels; ultimately culminating in hyperglycemia. Current treatment regimens call for the daily administration of exogenous insulin to maintain euglycemia, **however insulin is not a cure**. The goal of our work is to take advantage of the metabolic changes occurring as T cells move through the various stages of their lifecycle. Specifically, CD4+ T cells undergo metabolic reprogramming upon activation, and switch to a glycolytic profile, similarly to cancer cells. Inhibitors of the glycolytic pathway, including the drug PFK15, a competitive inhibitor of a key rate limiting enzyme in glycolysis, have shown great promise in FDA clinical trials. *We hypothesize that administration of the anti- glycolytic PFK15 will inhibit the activation and clonal expansion of diabetogenic CD4+ T effectors, thereby preventing disease onset.*

METHODS

To determine the ability of PFK15 to inhibit CD4+ T cell responses to diabetes relevant antigens *in vitro*, we used the NOD.BDC.2.5.TCR-Tg mouse (BDC2.5), which recognize a β cell protein and can transfer diabetes. Splenocytes from these mice were stimulated *in vitro* for 24- 72 hours with their cognate peptide +/- PFK15. Supernatants were collected for lactate and effector cytokine production, and T cells were stained for activation markers and analyzed by flow cytometry. To evaluate the ability of PFK15 to prevent T1D *in vivo*, we used an adoptive transfer model where BDC2.5 splenocytes are transferred into NOD.*scid* recipients. This induces diabetes in approximately 14 days. A cohort of animals received PFK15 soluble drug i.p. (10 mg/kg) or PFK15 microparticles (MP) (1/4th the dose of soluble drug) subcutaneously at a site in close approximation to the pancreas. Animals receiving blank MPs served as controls. Animals were treated and monitored every 3 days for onset of hyperglycemia and body weights were measured to assess toxicity of the drug. Pancreata were harvested at sacrifice for histological analyses and immunofluorescence staining.

SUMMARY OF RESULTS

Our *in vitro* results indicate that PFK15 soluble and MP formulations are capable of inhibiting CD4+ T cell activation, proliferation, and effector function. In adoptive transfer studies, PFK15 proved to prevent or delay disease onset, with no apparent toxicity. Histological analysis revealed invasive insulitis in blank MP treated animals while peri- islet insulitis was observed in PFK15 treated groups, correlating with preservation of β cell mass as measured by insulin staining by immunofluorescence. Lastly, we found that there was increased expression of the master regulatory T cell transcription factor FoxP3 in islets treated with PFK15, indicating increased Treg control within the islet.

CONCLUSIONS

The results suggest that targeting glycolysis as a means to control self-reactive T cells is a novel and effective approach to prevent T1D. Further, we have shown that MP formulations of PFK15, even when delivering a lesser dose, is able to delay adoptive transfer of disease at the site of inflammation. It is our hope to optimize MP delivery in an effort to limit systemic off target effects of soluble drug. Further, these studies will provide information on the substrates and metabolites that drive CD4+ T cell differentiation that can be used to better understand how to manipulate T cell responses in a number of different settings, including the tumor microenvironment and chronic infection.

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OA24

CD8+ T-cell recognition of post-translationally modified antigens: a case study with citrullinated GRP78

AUTHORS

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PURPOSE

The $CD8^+$ T-cell-mediated beta-cell destruction of T1D may be favored by the recognition of neo-epitopes, e.g. citrullinated glucose-regulated protein 78 (GRP78) peptides described in the NOD mouse and human insulitis.

METHODS

We therefore studied the recognition of HLA-A2-restricted native and citrullinated GRP78 peptides by $CD8^+$ T cells in the blood, using combinatorial HLA-A2 multimer (MMr) assays, and in the pancreas, by in situ MMr staining. The expression of peptidyl-arginine deiminase (PADI) citrullinating enzymes in human islets and thymic medullary epithelial cells (mTECs) was analyzed by RNAseq.

SUMMARY OF RESULTS

Citrullination modulated $CD8^+$ T-cell responses to GRP78 by altering TCR recognition rather than HLA-A2 binding. $CD8^+$ T cells reactive to native and citrullinated GRP78 peptides circulated at similar frequencies in T1D and healthy donors. Either the native or citrullinated GRP78 isoform was preferentially recognized depending on the peptide, by distinct $CD8^+$ T-cell pools, with no cross-reactivity between the native and citrullinated isoforms. The $CD8^+$ T-cell preference for native GRP78 isoforms may at least in part be shaped by cross-reactivity with homologous peptide sequences from gut commensal bacteria. Contrary to what observed in the blood, the citrullinated isoform of a dominant GRP78 peptide was preferentially recognized in the pancreas, but with no specificity for T1D. Citrullinating PADI enzymes were however not expressed in human islets, suggesting that citrullination may rather be triggered by immune cells infiltrating the islets. PADI enzymes were instead

expressed in mTECs, suggesting that negative selection may impact both native and citrullinated peptides.

CONCLUSIONS

Post-translationally modified peptides may not always favor loss of tolerance toward beta cells. Their $CD8^+$ T-cell recognition may be shaped by cross-reactivity with microbial homologues and by thymic expression of post-translationally modifying enzymes.

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NOVEL BIOMARKERS

OA25
Protein Modifications that Alter both Autoimmunity and Beta Cell Metabolism

AUTHORS

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PURPOSE

Our laboratory and others have identified novel properties of self proteins, namely posttranslational protein modifications (PTMs) that may initiate autoimmune responses in human Type 1 diabetes. In particular, a growing number of self proteins acquire PTMs within cells and become targets of B and T cell autoimmunity, leading to inflammation and pathology in the pancreas. Examples of important modifications to self proteins include citrullination, oxidation, deamidation reactions, and isoaspartyl modification, all responses of self proteins within cells that undergo cytokine or reactive oxygen-mediated stress. As importantly, these PTMs within cells may alter the biological properties of proteins within beta cells. This study examines PTMs of beta cell proteins that not only trigger autoimmunity but also alter metabolism and are sentinels of beta cell health or demise.

METHODS

The work primarily utilized human islets treated with inflammatory cytokines or reactive oxygen species (H_2O_2) followed by deep proteomic profiling by LC tandem mass spectroscopy. A variety of PTMs were identified that were unique to inflammatory conditions, including citrullination and carbonyl modification. Candidate proteins identified with PTMs were assayed utilizing T1D serum and peripheral T cells from early onset and established disease for autoreactivity. While more than 10 candidate proteins with PTMs were identified, we followed up on two specific beta cell proteins, glucokinase (GK) and Prolyl-4-hydroxylase (P4Hb). Binding motifs to HLA DR0401 were identified and utilized to investigate T cell autoimmunity to these proteins as well as the presence of autoantibodies. Finally, studies of beta cell metabolism were performed that included both glucose sensing pathways and insulin release, both in the context of glucokinase and P4Hb modifications.

SUMMARY OF RESULTS

Initial studies of new-onset T1DM patient serum (obtained from Drs. Cate Speake and Dr. Kevan Herold) indicate that 75% of the patients have IgG autoantibodies to P4Hb. As we first observed with mice, Abs to P4Hb precede the onset of anti-insulin Abs in human T1D. That is, anti-insulin Abs are never found without pre-existing Ab responses to P4Hb, linking these autoimmune responses, possibly due to the role P4Hb in insulin biosynthesis. We also identified specific citrullinated sites of human glucokinase. Similarly, T1D patients develop both autoantibody and CD4 T cell responses to citrulline-GK. Finally, inflammation that leads to PTM of both GK and P4Hb alter glucose sensing and insulin release, respectively.

CONCLUSIONS
The present study has identified novel PTMs within two beta cell proteins, glucokinase and proly-4-hydroxylase beta (P4Hb) to which T1D patients have both B and T cell autoimmunity. P4Hb is a critical macromolecule for the accurate folding of insulin. Oxidation and carbonylation of P4Hb may lead to the misfolding of insulin, making insulin itself immunogenic, or even lead to proteotoxicity and/or the death of beta cells. We also identified specific sites of citrulline modification of glucokinase. Our studies indicate that citrulline modified GK alters glucose sensing by beta cells and reduces insulin release. Understanding how self-proteins are specifically modified in T1D can lead to therapeutic strategies to selectively target enzymatic pathways and reduce PTMs in beta cells. Moreover, these autoantigens have immediate diagnostic value as candidate biomarkers of disease and reflect beta cell health. Restoration of beta cell functions, via pharmaceutical correction of the aberrant modifications, may be capable of delaying or preventing disease in individuals at high-risk for T1D.

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OA26

RNA Exosome Content from Insulin-Producing β Cells Incorporates Stress Granule Components and May Serve as Circulating Biomarkers of β Cell Stress in Type 1 Diabetes

AUTHORS

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PURPOSE

In early T1D, β cells are subjected to stresses such as inflammation, putative viral infections (or other "danger signals"), and increased endoplasmic reticulum (ER) protein load. Under these conditions, β cells activate a process known as the "integrated stress response," which is highlighted by the phosphorylation of the translation factor eIF2α. Phosphorylation of eIF2α leads to a generalized block in the translational initiation of many mRNA transcripts in an attempt to mitigate the energy-consuming process of translation and allow for cellular recovery. These translationally-inhibited mRNAs (including alternatively-spliced mRNAs and miRNAs) are compartmentalized in an orchestrated process into discrete, non-membranous inclusions known as stress granules (SGs) and processing bodies (PBs). A recent perspective has been emerging that SGs may function as hubs that intercept other intracellular pathways to signal a "state of emergency," and as such, SG content can be deposited into other cellular compartments such as extracellular vesicles, and subsequently released into the circulation. Therefore, the circulating contents of EVs may contain RNA molecules that reflect the state of β cell stress. In this study we sought to identify circulating mRNAs that are sequestered into stress granules (SG) during early β cell ER stress, are released in extracellular vesicles, and that the unique contents of these vesicles might serve as biomarkers of β-cell stress.

METHODS

Min6, INS-832/13, human EndoC BH1 cell line and human pancreatic islets were treated with or without pro-inflammatory cytokines (IL-1β and IFN-γ) to mimic T1D conditions. Exosomes were isolated using either an ExoQuick precipitation method or by serial ultracentrifugation and vesicle derived insulin mRNA levels were determined using RT-PCR. Untreated and cytokine treated human islets were subjected to poly-ribosome profiling (PRP) to assess the cytokine mediated global mRNA translation initiation block. Droplet digital PCR was used to measure the whole blood derived circulating levels of insulin mRNA from multiple T1D animal models and human subjects. Single molecular FISH was performed to identify the co-localization of INS mRNA with stress granule markers.

SUMMARY OF RESULTS

The treatment of mouse MIN6 cells, rat INS 832/13 cells, human EndoC-BH1 β cells, and human islets with pro-inflammatory cytokines (a mimic of the stress seen in T1D) resulted in a block in mRNA translation initiation, decreased protein synthesis, formation of stress granules and increase in SG markers in RNA granules, as assessed by polyribosomal profiling, puromycin incorporation and immunofluorescence staining, respectively. Islets treated with cytokines exhibited increased formation of stress granules, P-bodies, and sec-bodies, as observed by immunofluorescence and immunoblotting. Moreover, studies using single molecular FISH confirmed that human β cells treated with cytokines have increased colocalization of INS mRNA and G3BP1 (a marker of stress granules). EndoC-BH1 human β cells transfected with fluorescently labeled G3BP1 and CD63 (a marker of exosomes) and treated with cytokines exhibited co-localization of stress granules with exosomes, suggesting that stress granule contents are secreted extracellularly. Human preproinsulin (PPI) mRNA was recoverable almost exclusively in microvesicular fractions isolated from supernatants of stressed islet cultures. Pharmacological inhibition of microvesicle release decreased the PPI mRNA into the culture media, suggesting that PPI mRNA is selectively released from stressed β cells via exosomes. Digital PCR analysis demonstrated that circulating mouse PPI mRNA was detectable in circulation of several diabetic mouse models prior to the onset of diabetes. Likewise, we observed significantly higher levels of PPI mRNA in circulation of new-onset T1D subjects compared to healthy, age-matched controls.

CONCLUSIONS

Taken together, our data suggest a link between SGs and the endosomal pathway that liberates extracellular vesicles. Our studies provide mechanistic underpinning to the observation that RNA species generated or translationally inhibited under stress conditions (such as PPI mRNA) in human β cells can be liberated into the circulation, and thereby serve as circulating nucleic acid biomarkers of β cell stress in early T1D. Further high-depth interrogation of RNA species in SGs and EVs would likely provide new candidate nucleic acid biomarkers for early T1D.

Select the ONE category that best describes your research:	
☐ Beta Cell Physiology and Dysfunction	☒ Novel Biomarkers
☐ Beta Cell Development, Differentiation & Regeneration	☐ Novel Technologies
☐ Bone Marrow Studies	☐ Pathology
☐ Core Lab	☐ Type 1 Diabetes Etiology & Environment
☐ Immunology	☐ Other (list):

OA27
Unmethylated CHTOP and INS Provide Evidence for Islet Cell Death in Youth with Obesity and Diabetes

AUTHORS

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PURPOSE

Circulating unmethylated DNA fragments arising from the human *INS* gene have been proposed as biomarkers of β-cell death for the presymptomatic detection of diabetes. However, given the variability of CpG methylation in the *INS* gene in different cell types, this gene alone may not yield sufficiently specific information to unambiguously report β-cell death. In an effort to address the current limitations of differentially-methylated INS as a biomarker for β-cell damage, we hypothesized that other differentially-methylated genes would show either greater specificity for β-cells or could be used as complementary biomarkers to increase β-cell specificity.

METHODS

Human non-pancreatic tissue samples were obtained from National Disease Research Interchange (NDRI). Human islet and pancreatic acinar tissues were obtained from cadaveric donors from the University of Pisa, University of Louisville, or the Integrated Islet Distribution Program (IIDP). We performed a methylation specific Infinium HumanMethylation450 array (Illumina) of 64 human islet preparations and compared them with 27 human tissues/cell lines (control data retrieved from ENCODE DNA methylation database using GEO repository GSE40699). DNA from tissue and cell samples was isolated using GenElute Mammalian Genomic DNA Miniprep Kit (Sigma-Aldrich). DNA from serum was isolated from 50 ul of serum samples spiked in with 5 µg carrier DNA (poly-A) using QIAamp DNA Blood Mini Kit (Qiagen). DNA recovery from serum samples (of the poly-A carrier) was quantified using a nanophotometer (Implen). All samples showed ≥85% recovery of DNA following isolation. DNA bisulfite conversion was carried out using EZ DNA Methylation-Lightning kit (Zymo Research), and conversion was verified using a pre- and post-conversion sample in the droplet dPCR.

SUMMARY OF RESULTS

We employed an unbiased array-based approach to identify the *CHTOP* gene as a candidate biomarker whose CpGs show a greater frequency of unmethylation in human islets. When tested across an array of non-islet human tissues by digital PCR, both *INS* and *CHTOP* contained unmethylated CpG sites in several of these tissues, but in a non-overlapping pattern. Both *INS* and *CHTOP* genes were unmethylated in β-cells and α-cells (with *CHTOP* showing only slightly greater specificity for β-cells compared to *INS*), implying that either of these genes alone cannot definitively report on β-cell death, as previously assumed. To evaluate if the utilization of both assays together improves the ability to predict the tissue type, we utilized a Naïve Bayes classifier to predict tissue type specificity using single features models with *INS* and *CHTOP* alone, as well as a two feature model with *INS* and *CHTOP*. Five-fold cross-validation (CV) was preformed and each sample was classified based on the posterior probability at a threshold of 0.5. When the two assays were combined, distinct tissue type specificities could be predicted: (1) β-cell specificity was 88.8% when using both assays, (2) islet cell specificity (which include α- and β-cells) was 100% when using both assays, (3) pancreas specificity (which included exocrine and endocrine cells) was 94.8% using both assays. Next, to assess unmethylated *CHTOP* and *INS* as biomarkers for islet damage in human samples, we measured circulating DNA in human populations by digital PCR. Compared to healthy controls, differentially methylated *CHTOP* and *INS* levels were higher in youth with new onset type 1 diabetes and, unexpectedly, in healthy, autoantibody-negative youth who have first-degree relatives with T1D. When tested in youth across a spectrum of metabolic disease, increased levels of unmethylated *INS* and *CHTOP* were observed in obese individuals compared to lean controls.

CONCLUSIONS

In conclusion, our data suggest that using two distinct DNA assays, *INS* and *CHTOP*, outperforms each one separately in determining islet cell specificity compared. Additionally, these data suggest that islet death may be a feature in youth at risk for both T1D and T2D. Our data support the use of multiple parameters to increase the confidence of detecting islet damage in youth with diabetes.

Select the ONE category that best describes your research:	
☐ Beta Cell Physiology and Dysfunction	☒ Novel Biomarkers
☐ Beta Cell Development, Differentiation & Regeneration	☐ Novel Technologies
☐ Bone Marrow Studies	☐ Pathology
☐ Core Lab	☐ Type 1 Diabetes Etiology & Environment
☐ Immunology	☐ Other (list):

NOVEL TECHNOLOGIES

OA28

Development of an Insulin-specific B Cell ELISPOT assay for clinical evaluation of the insulin-specific B cell deletional therapeutic, AKS-107

AUTHORS

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PURPOSE

Insulin-specific B cells (IBCs) are associated with autoimmune diabetes in mouse models and in human subjects with type 1 diabetes (T1D). While anti-insulin antibodies (iAbs) produced by such IBCs are not known to mediate destruction of insulin-producing pancreatic islet beta-cells, the antigen-presenting capacity of IBCs appears to be an important factor in activating pathogenic autoreactive T cells that mediate such beta-cell destruction. We developed an IBC-specific deletional Fc-fusion protein therapeutic, AKS-107 (two inactivated insulin moieties fused to human Fc), designed for T1D prevention in at-risk prediabetic subjects that are biomarker-positive for IBCs and insulin autoantibody titers. Therefore, an IBC-specific ELISPOT assay was developed to measure the frequency of IBCs in peripheral blood from T1D subjects.

METHODS

The assay is based on the ability of ex vivo activated IBCs to produce iAbs prior to culturing in the ELISPOT assay. The iAb-spot detection system required significant optimization of novel critical reagents to achieve appropriate sensitivity, accuracy, and precision. The optimized assay format consists of generating antibody secreting plasma cells via activation of B cells in PBMC cultures (from frozen samples) with IL-2 and the TLR7/8 agonist, R848, for 4 days, followed by cell harvesting and culturing on PVDF ELISpot plates coated with goat anti-human IgG-Fc capture Ab for 18 hr. Bound insulin-specific Ab spots were detected via a 3-step detection/amplification process with biotin-labeled AKS-107 Fab'2 fragment, followed by anti-biotin-FITC Ab, followed by anti-FITC-HRP Ab, and finally developed with ultra-sensitive TMB to visualize the iAb-specific spots.

SUMMARY OF RESULTS

Eleven PBMC samples from subjects with established T1D receiving insulin therapy were evaluated in which six samples showed significant and reproducible insulin-specific spots above background, and were confirmed to be insulin-specific by competitive inhibition via pre-incubation with AKS-107.

CONCLUSIONS
In addition, a correlation was observed between elevated iAb titer and elevated IBC ELISPOT frequency, a feature that could be used as an inclusion criterion for the AKS-107 first-in-human clinical safety study in subjects with established T1D receiving insulin therapy.

Select the ONE category that best describes your research:	
☐ Beta Cell Physiology and Dysfunction	☐ Novel Biomarkers
☐ Beta Cell Development, Differentiation & Regeneration	☒ Novel Technologies
☐ Bone Marrow Studies	☐ Pathology
☐ Core Lab	☐ Type 1 Diabetes Etiology & Environment
☐ Immunology	☐ Other (list):

OA29
3D Visualization of the Dynamic and Regional Variation of Pancreatic Innervation in Diabetes

AUTHORS

Alexandra Alvarsson, Carolina Rosselot, Maria Jiménez-González, Rosemary Li, Nikolaos Tzavaras, Zhuhao Wu, Andrew F. Stewart, Adolfo Garcia-Ocaña, Sarah A. Stanley

PURPOSE

The endocrine pancreas is densely innervated, and neural signals play a significant role in glucose regulation by modulating pancreatic hormone release. However, relatively little is known about the anatomical relationships between islets and nerves across the whole pancreas. Since thin filamentous structures, such as nerves, are difficult to quantify and trace over large volumes using thin section histology, there is a need for high resolution imaging and rendering of intact tissue in 3D. Here, we used optical clearing, whole organ imaging, and 3D rendering to quantify innervation across the whole pancreas in healthy mice, in two mouse models of diabetes, and in pancreatic samples from nondiabetic and diabetic human donors.

METHODS

Whole-mount staining and clearing was performed using iDISCO+ to quantify innervation, defined by the neuronal marker neurofilament 200 kDa, and beta cells in pancreata from C57Bl/6 mice, non-obese diabetic (NOD) mice, streptozotocin (STZ)-treated mice, and in pancreatic samples from nondiabetic and diabetic human donors. Z-stacked optical sections were acquired with an Ultramicroscope II at 4x or 12x magnification. Imaris was used to create digital surfaces covering the NF200+ innervation and islets to automatically determine innervation density and islet/nerve interactions.

SUMMARY OF RESULTS

Tissue clearing and volume imaging of the pancreas provided several new insights. First, innervation of the endocrine pancreas is significantly enriched compared to the surrounding exocrine pancreas with regional variation. Next, islets are closely associated with pancreatic innervation and decrease in size with increasing distance from nerves in both mouse and human pancreatic tissue. Innervated islets are relatively preserved in models of diabetes. Finally, islet innervation and expression of neural markers are higher in human samples from diabetic patients and in mouse models of diabetes with temporal and regional differences.

CONCLUSIONS

3D imaging and unbiased analysis across the whole pancreas provides comprehensive measurement of pancreatic nerve volumes and distribution. It allows detailed analysis of the anatomical relationship between nerves and islets, which reveals a close association that is maintained across species. The relative enrichment of innervated islets in diabetes and dynamic changes in islet innervation during the development of diabetes suggest further

work is need to examine the role of pancreatic nerves in preserving and protecting beta cells.

Select the ONE category that best describes your research:	
☐ Beta Cell Physiology and Dysfunction	☐ Novel Biomarkers
☐ Beta Cell Development, Differentiation & Regeneration	☒ Novel Technologies
☐ Bone Marrow Studies	☐ Pathology
☐ Core Lab	☐ Type 1 Diabetes Etiology & Environment
☐ Immunology	☐ Other (list):

OA30

3D Atlas of regional variation and dynamic changes in the islets of Langerhans in diabetes

AUTHORS

Alexandra Alvarsson, Carolina Rosselot, Maria Jiménez-González, Rosemary Li, Nikolaos Tzavaras, Zhuhao Wu, Andrew F. Stewart, Adolfo Garcia-Ocaña, Sarah A. Stanley

PURPOSE

The pancreas is a highly heterogeneous organ, with regional anatomical, developmental and functional differences. Until now, laborious serial sectioning and reconstruction has been needed to deliver information about islet anatomy across the pancreas. There is a clear need for high resolution, organ-wide imaging to map regional variation and to assess the three-dimensional islet anatomy and distribution. Here, we used optical clearing, whole organ imaging, and 3D rendering to quantify insulin-producing beta cells across the whole pancreas in healthy mice, in two mouse models of diabetes, and in nondiabetic and diabetic human donors.

METHODS

Whole-mount staining and clearing was performed using iDISCO+ to determine the 3D volumes and distribution of insulin-producing beta cells in pancreata from C57Bl/6 mice, non-obese diabetic (NOD) mice, streptozotocin (STZ)-treated mice, and in pancreatic samples from nondiabetic and diabetic human donors. Z-stacked optical sections were acquired with an Ultramicroscope II at a 1.3x, 4x or 12x magnification. Imaris was used to create digital surfaces covering the insulin+ islets to automatically determine volumes and intensity data.

SUMMARY OF RESULTS

Tissue clearing, whole organ imaging and unbiased analysis provided straightforward measurement of beta cell volume across the whole pancreas. Beta cell volumes were 1-4% in the human pancreas, and 1-2% in the healthy mouse pancreas. There were regional variations in islet volume and insulin intensity. The majority of islets were between 1000 and 500,000 μm^3. There were significant differences in islet biology between the diabetes models. In NOD mice, insulin-positive islet numbers and beta cell volumes were dramatically reduced, with some islets exhibiting relatively preserved insulin intensities, and a striking shift to small islets. Islet number and volume were also reduced with STZ treatment with regional difference, but the insulin intensity was dramatically reduced and size distribution was minimally altered. The islet characteristics of the human samples were highly variable but the beta cell volume distribution was significantly altered in diabetes.

CONCLUSIONS

Whole organ 3D imaging allows quantification of beta cell volume and multiple parameters in animal models and human samples. There are significant regional differences in beta cell volume and islet size distribution in mouse models of diabetes. These regional variations emphasize the need for whole organ imaging for accurate quantification of pancreatic anatomy in animal models.

Select the ONE category that best describes your research:	
☐ Beta Cell Physiology and Dysfunction	☐ Novel Biomarkers
☐ Beta Cell Development, Differentiation & Regeneration	☒ Novel Technologies
☐ Bone Marrow Studies	☐ Pathology
☐ Core Lab	☐ Type 1 Diabetes Etiology & Environment
☐ Immunology	☐ Other (list):

OA31

Developing an assay platform to evaluate reprogramming of beta-like cells in gastrointestinal tissues from non-diabetic and type 1 diabetic donors

AUTHORS

Carmen Y. Lam[1], Irina Kusmartseva[2], Atkinson Mark[2], Hua V. Lin[1]
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PURPOSE

Gut organoids (gutoids) are 3-dimensional in vitro structures that have substantial anatomical and functional similarities to the human gut. It was previously shown that FOXO1 inhibition in human gutoids derived from induced pluripotent stem cells (iPSCs) promotes generation of glucose-responsive insulin-positive (beta-like) cells. However, the iPSC differentiation protocol is extremely time-consuming and therefore unsuitable for drug discovery purposes. We propose to use gutoids derived from primary gut tissues collected from deceased non-diabetic controls and Type 1 diabetic subjects to establish an assay platform to study the reprogramming of gut endocrine progenitor cells into beta-like cells. While human gutoids are routinely cultured from intestinal crypts isolated from fresh gut biopsies that are processed within 24 hours, a protocol for culturing gutoids from deceased donors for more than 24 hours is not established.

METHODS

The time from x-clamp to tissue processing and crypt isolation, shipping media, and organoid culturing media were explored to obtain the optimum condition for developing human gutoid cultures.

SUMMARY OF RESULTS

We successfully derived and maintained enteroids and colonoids from the small intestine and colon tissues from 3 out of 4 deceased donors withdrawn from life support for as long as 48 hours. Cultured organoids were subsequently used for studying enteroendocrine cell differentiation, gene expression, and drug screening.

CONCLUSIONS

Gutoids from deceased donors' gut tissues have been established as a reproducible and readily available platform and is an invaluable translational tool that bridges the gap between conventional two-dimensional cell line culture and in vivo models.

Select the ONE category that best describes your research:	
☐ Beta Cell Physiology and Dysfunction	☐ Novel Biomarkers
☐ Beta Cell Development, Differentiation & Regeneration	☒ Novel Technologies
☐ Bone Marrow Studies	☐ Pathology
☐ Core Lab	☐ Type 1 Diabetes Etiology & Environment
☐ Immunology	☐ Other (list):

OTHER

OA32
DataView: A Data Science Platform Integrating 10+ years of nPOD enabled T1D research

AUTHORS

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PURPOSE

Since 2008, investigators in nPOD have utilized the biobank to generate and publish a diverse amount of data. Toolkits are needed to connect and incorporate this information by donor across multiple experiments, laboratories, and assay types. However, there has been no comprehensive framework to aggregate, curate, and integrate these results in ways that may be used to drive new hypotheses, research questions, and collaborations. The DataView platform demonstrates an approach to how donor-connected heterogeneous data can be integrated and made available for exploration and new applications.

METHODS

Relevant data sources for curation were defined as manuscripts and datasets (which may not have an associated paper) published between 2008 and June of 2019 and include nPOD donor-derived primary data. Data was obtained through manual or computational extraction from the publication/public repository or directly requested from the authors. The information was then cleaned, record-linked with nPOD CaseIDs, transformed, and augmented with ontology and other metadata. Additional donor data was obtained from nPOD DataShare. Interactive modules for viewing, exploring, and mining data were built with R and the Shiny web application framework.

SUMMARY OF RESULTS

A total of 78 data sources were curated. Data integration was possible for only 50% of the sources (n=39) and yielded ~400 small-throughput measurement features. A total of 4 data sources included medium/high-throughput and complex-type datasets. Analysis can be performed across assay/sources and donor/cohorts. Data can be discovered through a friendly user interface or through SPARQL endpoints as well as downloaded for use in other data science applications.

CONCLUSIONS

DataView is available as a web-based application, Docker image, and R package. Three major challenges remain: availability and access to raw or processed data, accommodation of complex data types, and implementation of mining tools for heterogeneous data.

Select the ONE category that best describes your research:	
☐ Beta Cell Physiology and Dysfunction	☐ Novel Biomarkers
☐ Beta Cell Development, Differentiation & Regeneration	☐ Novel Technologies
☐ Bone Marrow Studies	☐ Pathology
☐ Core Lab	☐ Type 1 Diabetes Etiology & Environment
☐ Immunology	☒ Other (list): **Data Science**

OA33
Human pancreas tissue slices for the study of alpha cell physiology in type 1 diabetes pathogenesis

AUTHORS

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PURPOSE

Dysregulated glucagon release contributes to type 1 diabetes (T1D) pathogenesis; however the underlying mechanisms of alpha cell dysfunction, increased glucagon secretion or reduced suppression of glucagon release in response to hypoglycemia are still uncertain. Human pancreatic tissue slices offer a valuable platform to study intact islet cell biology in situ, at the same time overcoming enzymatic and mechanical distress involved in the conventional islet isolation procedures. Here we characterize alpha cell function, physiology and mass using human pancreatic tissue slices prepared at the nPOD/OPPC at the University of Florida in Gainesville.

METHODS

Pancreata from non-diabetic (ND), autoantibody-positive (Aab+) and T1D organ donors were processed at the University of Florida (UF) in Gainesville in line with nPOD/OPPC SOPs. Tissue slices of 120 µm thickness were prepared and viability was evaluated by immunofluorescence staining. Glucagon secretion was assessed acutely after slice preparation and measured with a commercially available ELISA kit. In addition, slices were rested overnight and calcium responses were recorded from single cells within islets (identified by backscatter) using an inverted Leica SP8i confocal microscope. After functional assessment of alpha cells at UF, slices were fixed and shipped to the Paul Langerhans Institute Dresden, Germany (PLID) in order to assess 3D slice morphometry. Fixed slices were stained for endocrine cell markers (insulin, glucagon and somatostatin) and whole slice imaging was performed on a Zeiss LSM 780 NLO equipped with an automated stage. Image processing and analysis was accomplished semi-automatically using Fiji and MorphoLibJ.

SUMMARY OF RESULTS

Here we show dynamic glucagon and insulin secretion patterns using pancreas tissue slices from organ donors. Glucagon release was assessed in response to specific stimuli (kainate, epinephrine or arginine) and KCl for cell membrane depolarization. We characterized

glucagon secretion kinetics in slices from ND, Aab+ and T1D pancreata. Our preliminary analysis shows that in the 'prediabetic' state, glucagon output in response to all applied stimuli is slightly decreased. However, stimulation index in the two groups is comparable, suggesting that alpha cells are functional in Aab+ state. Slices from T1D showed only small changes in glucagon secretion in comparison to ND. Tissue slices were then further investigated by 3D morphometry and assessed for calcium dynamics in alpha cells by live cell imaging. Analysis of the acquired data is still ongoing.

CONCLUSIONS

This work provides proof that alpha cell physiology can be investigated using pancreas tissue slices prepared from organ donor pancreata. Here we show a first time characterization of alpha cell function, physiology and mass within the same tissue sample. These studies will provide insight into the mechanism underlying aberrant glucagon release in diabetes pathogenesis.

Select the ONE category that best describes your research:	
☐ Beta Cell Physiology and Dysfunction	☐ Novel Biomarkers
☐ Beta Cell Development, Differentiation & Regeneration	☐ Novel Technologies
☐ Bone Marrow Studies	☐ Pathology
☐ Core Lab	☐ Type 1 Diabetes Etiology & Environment
☐ Immunology	☒ Other (list): **Alpha cell physiology**

OA34
Upregulation of HLA class I and antiviral tissue responses in autoimmune thyroid disease

AUTHORS

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PURPOSE

Autoimmune thyroid diseases (AITD) and type 1 diabetes (T1D) share many similarities. Both diseases are autoimmune, endocrine, and organ specific diseases. Moreover, individuals with T1D have an increased risk of developing AITD. AITDs are multifactorial in origin; genetic as well as environmental factors are believed to contribute to the pathogenesis. However, the initiating factor leading to a break of tolerance remains unknown in both T1D and AITD. Enterovirus, viral receptors and viral immune response proteins have been detected in pancreata from patients with newly diagnosed T1D in the DiViD study. In the present study, we analyzed the same immune markers and viral protein products in thyroid tissue from patients with Hashimoto's thyroiditis (HT) and Graves' disease (GD).

METHODS

Thyroid tissue from 46 mainly newly diagnosed HT patients, and 48 GD patients with both newly diagnosed and chronic disease, were obtained using core needle biopsy or collected during thyroidectomy. To the best of our knowledge, this is the largest collection of thyroid tissue from newly diagnosed AITD patients. In addition, 24 thyroid tissue samples collected during neck surgery for other reasons than thyroid autoimmunity served as controls. Standard immunohistochemistry as well as combined immunofluorescence staining were used on formalin-fixed, paraffin embedded tissue samples.

SUMMARY OF RESULTS

HLA class I presents both endogenously and exogenously derived antigens to CD8+T cells and plays a vital role in the defense against virus. In the HT group, 31 out of 46 (67.4%) thyroid tissue samples had HLA class I positive thyroid cells (thyrocytes). In the GD group, 25 out of 48 (52.1%) tissue samples had HLA class I positive thyrocytes. Both patient groups had a significantly higher number of HLA class I positive samples compared to controls, where only five out of 24 (20.8%) samples were positive ($p<0.001$ for HT and $p=0.011$ for GD). Moreover, assessed with a semi-quantitative immunoreactivity score taking intensity and proportion into account, both patient groups had a significantly higher score than controls.

Signal transducer and activator of transcription 1 (STAT1) and protein kinase R (PKR) are antiviral proteins activated by interferons and the viral product dsRNA respectively. STAT1

was found in five out of six HT samples, seven out of 13 GD samples and in two out of four controls. Both cytosolic and nuclear STAT1 was found, and it was co-expressed with HLA class I. PKR was found in 11 out of 13 GD samples, three out of six HT samples and in one out of four control samples. PKR expression was co-localized with enterovirus capsid protein 1 (VP1).

The mean number of VP1 positive thyrocytes was significantly higher in GD (30.3%) than in controls (14.9%) (p=0.005). In the HT group, there were more, albeit not significantly, VP1 positive thyrocytes in the HT samples (20.1%) than in controls (14.9%). The coxsackie and adenovirus receptor (CAR) isoform (CAR-SIV) was found in a distinct granular expression in thyroid cells in all ten samples studied.

CONCLUSIONS

Our results show that thyrocytes express CAR, thus making them susceptible to enterovirus infection. HLA class I is upregulated in thyrocytes from both HT and GD. Furthermore, we demonstrate that STAT1 is co-localized with HLA class I in thyrocytes, and that PKR is co-localized with VP1, which is potentially indicative of an intracellular antiviral host response. Taken together with previously reported increase in the downstream interferon type 1 response protein, myxovirus resistance protein 1, plasmacytoid dendritic cells and CD8+ T cells in the same cohort, our results support the hypothesis of an association between enteroviral infections and AITD. In conclusion, similar findings in AITD and T1D point to new common features other than shared genetic risk factors.

Select the ONE category that best describes your research:	
☐ Beta Cell Physiology and Dysfunction	☐ Novel Biomarkers
☐ Beta Cell Development, Differentiation & Regeneration	☐ Novel Technologies
☐ Bone Marrow Studies	☐ Pathology
☐ Core Lab	☐ Type 1 Diabetes Etiology & Environment
☐ Immunology	☒ Other (list): **Autoimmune thyroid disease**

OA35

Patch Grafting of Biliary Tree Stem/Progenitor Organoids directly into the Pancreas of NRG/Akita Mice corrects Type I Diabetes

AUTHORS

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PURPOSE

The pancreas is connected to the duodenum by the hepato-pancreatic common duct, the largest reservoir in the biliary tree of determined endodermal stem/progenitors, precursors to both liver and pancreas[1]. Methods for their isolation and management and extensive characterization of them have been described previously[1]. We focused here on developing novel methods of transplantation of these organoids directly into the pancreas using grafting strategies that we call ***"patch grafting'.***

METHODS

Organoids of biliary tree stem/progenitors were prepared in serum-free, wholly defined medium, Kubota's Medium, designed for determined endodermal stem/progenitors. Organoids were transplanted by patch grafting into the pancreas of pigs and mice. Grafts consisted of organoids embedded into soft (~100 Pa) hyaluronan (HA) hydrogels; placed onto silk backings embedded with more rigid HA hydrogels (~700 Pa); and tethered surgically to the duodenum adjacent to the pancreas and with surgical glue to mesentery superficial to the pancreatic capsule. A coating of HA of intermediate viscoelasticity properties (200-300 Pa) was added at the time of surgery and served to minimize adhesions of the graft to neighboring organs and tissues.

SUMMARY OF RESULTS

Patch grafts caused transient remodeling of the pancreatic capsule and tissue subjacent to the graft, facilitating engraftment into the pancreas of essentially all donor organoids within a few days and without evidence of ectopic cell distribution. Donor cells migrated and integrated throughout the host pancreas within a week. By 2-3 weeks, the pancreatic capsule and normal histology were restored. Donor cells matured in pigs to functional adult pancreatic cells comprising acinar cells (amylase) and islets (insulin, glucagon). Quantitative functional effects of patch grafts were assessed in mice at 4 weeks and indicated that allografts of murine stem/progenitor organoids patch grafted onto the pancreases of NRG/Akita mice were able to rescue them stably from type I diabetes with restoration of insulin production and of normo-glycemic functions. The engraftment/migration/integration processes were mediated by multiple matrix-metallo-proteinases (MMPs), especially secreted MMPs

CONCLUSIONS

Organoids of the stem/progenitors from the biliary tree can be transplanted directly and rapidly into the pancreas using patch grafting strategies. The donor cells are able to mature into functional adult pancreatic cells including islets that can correct disease states such as type I diabetes.

[1]Zhang, W. *et al.* Stem Cell-Fueled Maturational Lineages in Hepatic and Pancreatic Organogenesis. ***The Liver: Biology and Pathobiology****, 6th edition*, Win Arias, MD, Gillian Whitley, and Allan W. Wolkoff, MD, editors, Wiley Publishers, Hoboken, New Jersey and NYC, NY (2019). In Press

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☐ Beta Cell Development, Differentiation & Regeneration	☐ Novel Technologies
☐ Bone Marrow Studies	☐ Pathology
☐ Core Lab	☐ Type 1 Diabetes Etiology & Environment
☐ Immunology	☒ Other (list): **Method for Transplantation of Cells into Pancreas**

OA36
The Role of Glycolytic Flux in the Control of Glucagon Secretion

AUTHORS

***Dana Avrahami*[1], Zenab Hamdan[1], Eseye Feleke[1], Tsuria Lax[2], Yuval Dor[2] Benjamin Glaser[1] and Klaus H. Kaestner[3]**

1 Endocrinology and Metabolism Department, Hadassah-Hebrew University Medical Center, Jerusalem, Israel

2 Department of developmental biology and cancer research, Hebrew University School of medicine, Jerusalem Israel

3 Department of Genetics and Institute for Diabetes, Obesity, and Metabolism, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA

PURPOSE

The pathophysiology of type 1 diabetes (T1D) is bihormonal, with both insufficient insulin production due to loss of beta cell mass and dysregulated glucagon release by alpha-cells. To date, despite the increased acknowledgment that abnormal glucagon secretion is central to the pathophysiology of both T1D and T2D, the precise mechanisms controlling glucagon release from alpha-cells and, even more so, its dysregulation in diabetes are unclear. It was recently shown that *G6PC2,* encoding the islet-specific glucose-6-phosphatase 2, is dramatically downregulated in alpha cells of T1D patients. *G6PC2* is active exclusively in pancreatic islets and multiple GWAS have linked polymorphisms in *G6PC2* with variations in fasting blood glucose levels. *G6PC2* limits glycolytic flux by creating a futile cycle with Glucokinase (*GCK*), thus limiting glucose-mediated stimulation of insulin secretion. However, the same mechanism would be expected to lead to stimulation of glucagon secretion in alpha-cells. We hypothesize that G6PC2 functions in alpha-cells as part of the glucose-sensing mechanism, therefore altering its levels will affect glucagon secretion. Downregulation of G6PC2 in alpha cell from T1D could therefor halt glucagon release in the fasting state which could worsen the hypoglycemic episodes seen in these patients.

METHODS

In order to elucidate the role of glucose metabolism and the glycolytic flux in controlling glucagon secretion, we manipulated the levels of G6PC2 in mouse and human islets and measured their response to glucose suppression of glucagon release. We transduced dispersed islet cells with lentiviral particles carrying the G6PC2 coding sequence and GFP under control of a CMV promoter or with an shRNA against G6PC2. Islets were allowed to reaggregate for 5 days to form pseudo-islets before being assayed for glucagon and insulin secretion.

SUMMARY OF RESULTS

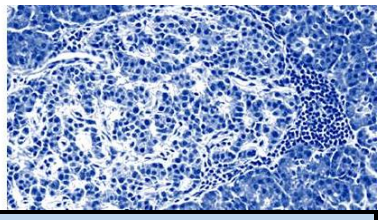
G6PC2 overexpression affected both glucagon and insulin secretion, albeit in opposite directions; while glucagon release was increased at basal glucose levels (3mM, $p<0.05$), insulin secretion was significantly decreased at high glucose levels as compared to controls (9mM, $p<0.05$). Next, we employed shRNA to suppress G6PC2 levels in pseudo-islets. We found that low concentrations of glucose were more effective in reducing glucagon secretion in this paradigm, as predicted by our model.

CONCLUSIONS

These preliminary data support our hypothesis that glycolytic flux and glucose cycling are critical to the control of alpha-cell function, which will be further evaluated by utilizing two new genetically modified mouse strains to ablate and overexpress *G6pc2* specifically in alpha cells.

Select the ONE category that best describes your research:	
☐ Beta Cell Physiology and Dysfunction	☐ Novel Biomarkers
☐ Beta Cell Development, Differentiation & Regeneration	☐ Novel Technologies
☐ Bone Marrow Studies	☐ Pathology
☐ Core Lab	☐ Type 1 Diabetes Etiology & Environment
☐ Immunology	☒ Other (list): **alpha cell physiology and dysfunction**

PATHOLOGY

OA37

nPOD-Kidney: Mapping the pathology of Diabetic Kidney Disease

AUTHORS

Florence Anquetil, Vivek Das, Claire Gibson Bamman, Allie Roach, Heather Ward, Johnna D. Wesley, Anil Karihaloo

PURPOSE

Diabetic kidney disease (DKD) is the most common complication of diabetes; yet DKD remains poorly understood. The nPOD-Kidney (nPOD-K) pilot project was initiated to assess the feasibility of collecting kidney from organ donors with and without diabetes, with the long-term goal of improving our understanding of DKD pathogenesis. To date, 47 of the planned 48 kidneys have been collected. The tissue integrity of the current cohort was evaluated, and key pathways published in high profile preclinical publications were selected for validation.

METHODS

Two-µm renal FFPE sections from the nPOD-K cohort were stained for kidney-specific cell markers and kidney disease markers. Slides were imaged at a 20x magnification using an Axioscan Z1 whole slide scanner and quantitative image analyses were performed using Visiopharm software.

SUMMARY OF RESULTS

Various multiplex immunofluorescent stainings were optimized, followed by a periodic acid-Schiff (PAS) staining. We developed an automatic segmentation of kidney compartments using PAS images and the neural network DeepLabV3+, allowing for an in-depth whole-slide image analysis. We observed the expected loss of the podocyte marker WT1 and endothelial marker CD31 with concomitant increases in expression of FSP1 and other fibrotic markers in diseased kidneys. Interestingly, approximately one fifth of the cohort displayed no overt sign of kidney disease despite long-standing diabetes (8+ years).

CONCLUSIONS

This cohort provides an exceptional opportunity to study DKD physiopathology through the analyses of large, CKD stage-specific areas of the kidney. Similar to previously published observations from the nPOD pancreas cohort, all stages of the disease can be detected in affected kidneys. Thus, the nPOD-K cohort establishes a unique and valuable window into the development and progression of DKD and support the identification and development of novel therapeutic targets.

Select the ONE category that best describes your research:	
☐ Beta Cell Physiology and Dysfunction	☐ Novel Biomarkers
☐ Beta Cell Development, Differentiation & Regeneration	☐ Novel Technologies
☐ Bone Marrow Studies	☒ Pathology
☐ Core Lab	☐ Type 1 Diabetes Etiology & Environment
☐ Immunology	☐ Other (list):

OA38
Temporal Analysis of Amylase Expression in Control, Autoantibody Positive, and Type 1 Diabetes Pancreatic Tissues

AUTHORS

Irina Kusmartseva[1], Maria Beery[1], Helmut Hiller[1], Myriam Padilla[1], Stephen Selman[1], Amanda Posgai[1], Harry S. Nick[2], Martha Campbell-Thompson[1], Desmond A. Schatz[3], Michael J. Haller[3], Clive H. Wasserfall[1], Mark A. Atkinson[1,3]

[1]Department of Pathology, Immunology and Laboratory Medicine, Diabetes Institute University of Florida, Gainesville, FL, USA

[2]Department of Neuroscience, College of Medicine, University of Florida, Gainesville, FL, USA

[3]Department of Pediatrics, College of Medicine, University of Florida, Gainesville, FL, USA

PURPOSE

Within the human pancreas, exocrine and endocrine cells control secretion of digestive enzymes and production of hormones to maintain metabolic homeostasis, respectively. While the vast majority of type 1 diabetes research efforts have focused on endocrine function and autoimmunity, recent studies identified a series of unique features (e.g., reduced weight and volume, increased density of leukocytes) within the exocrine pancreas in this disease. It remains unclear whether these alterations result from disrupted islet-acinar interactions secondary to the loss of functional β-cell mass, or contribute directly to type 1 diabetes development. In order to interrogate the potential relationship linking islet and acinar cell mass and function, a foundational understanding of cell phenotype and morphological organization within the exocrine pancreas is necessary.

METHODS

We histologically assessed pancreatic amylase expression patterns throughout the human lifespan from individuals with and without type 1 diabetes, representing what we believe to be the largest cohort and most extensive histological analysis of exocrine human pancreas reported to date.

SUMMARY OF RESULTS

Our analysis shows that amylase positive cells accumulate during early life development with the majority of acinar cells expressing amylase by age two, which is then maintained throughout the lifespan. Most significantly, pancreata from individuals over two years of age contained clusters of acinar cells devoid of amylase protein and mRNA expression. A majority of these amylase-negative cell clusters localized proximal to islets (i.e., peri-islet) and were positive for the exocrine enzymes lipase and trypsinogen. Type 1 diabetes pancreata displayed significant reductions in the frequency of these AMY^- cell clusters**.** Interestingly, we observed an approximate 75% decrease in the number of peri-islet amylase negative cell clusters associated with insulin-negative versus AMY^- cell clusters associated with insulin positive islets.

CONCLUSIONS
These results support a contribution of the islet-acinar axis in pancreatic development and underscore a potential role for the exocrine pancreas in the pathogenesis of type 1 diabetes.

Select the ONE category that best describes your research:	
☐ Beta Cell Physiology and Dysfunction	☐ Novel Biomarkers
☐ Beta Cell Development, Differentiation & Regeneration	☐ Novel Technologies
☐ Bone Marrow Studies	☒ Pathology
☐ Core Lab	☐ Type 1 Diabetes Etiology & Environment
☐ Immunology	☐ Other (list):

OA39
Differential Expression of Genes Associated with Host Viral Responses in Insulitic Islets

AUTHORS

Grace B. Nelson, M.D.[1]; Nataliya Lenchik, M.D[1]. Elizabeth A. Butterworth B.S. [2]; Martha Campbell-Thompson, Ph.D. [2]; Clayton Matthews, Ph.D. [2]; Mark Atkinson, Ph.D. [2]; Ivan C. Gerling[1] Ph.D. [1]University of Tennessee Health Science Center. [2]University of Florida, Gainesville

PURPOSE

Type 1 diabetes (T1D) is an autoimmune condition hypothesized to be enhanced or triggered by viral infections. Islet pathology in T1D is characterized by destruction and loss of insulin producing pancreatic beta cells. In this study we evaluated specific physiological pathways associated with the disorder's pathogenesis by examining differential expression of host genes within islets, with special emphasis on identifying evidence suggestive of responses to a viral infection.

METHODS

Pancreatic tissue samples were obtained from non-diabetic donors, autoantibody positive, non-diabetic donors, and donors with T1D through the Network for Pancreatic Organ donors with Diabetes (nPOD) program. Laser capture microdissection was used to isolate individual islets based on immunohistochemical documentation of presence or absence of insulin (INS+) and T-lymphocytes (CD3+). Islets were classified into four groups, designated as Islet Status:INS+CD3- (Normal), INS+CD3+ (Insulitic), INS-CD3+, INS-CD3-. RNA was isolated and microarray used to assess transcriptomes. Genes identified in the literature as important for host viral response were analyzed for expression levels in each islet type.

SUMMARY OF RESULTS

Increased expression in insulitic islets (INS+CD3+) compared to normal islets (INS+CD3-) was seen in 18 genes involved in viral response pathways.

Table 1: Increased Expression in insultic compared to normal islets.

Gene	*P-Value	Gene	*P-Value	Gene	*P-Value
RIGI (DDX58)	6.4E-10	OAS3	1.04E-8	IRF3	4.69E-6
TLR3	7.35E-10	IFNG	5.9E-8	TLR6	2.45E-5
TLR4	1.4E-9	STAT1	1.64E-7	NFKB1	4.89E-5
MDA5 (IFIH1)	2.75E-9	TLR2	3.79E-7	PKR	5.17E-3
Casp1	4.7E-9	TLR1	2.72E-6	TGFB2	5.5E-3
TLR8	5.5E-9	IRAK4	6.41E-6	MyD88	5.92E-3

**P-Value for comparison of expression in Insulitic vs Normal Islets.*

In-depth analysis of RIGI (DDX58) and MDA5 (IFIH1) demonstrated that 87.5% (14/16) of TID donors had expression of either gene in at least one islet. In 75% (12/16) of the T1D donors at least 20% of their islets were positive. In 43% (7/16) of the T1D donors at least

50% of their islets were positive. Of the all the islets from T1D donors 49% (54/110) were positive for expression of either gene. In AB+ donors 18% (2/11) had expression of either gene in at least one islet. Of all islets from AB+ donors 10% (8/77) were positive. In Control Donors only 12.5% (2/16) had expression of either gene in at least one islet. Of all islets from Control Donors only 3% (2/73) were positive for either gene. When looking at islet status, regardless of donors, 6% (9/147) of Normal islets were positive, while 48.7% (38/78) of insulitic islets were positive.

CONCLUSIONS

These studies noted an increased expression of multiple host genes associated with anti-viral responses. RIGI and MDA5 are both known to respond specifically to dsRNA (a common part of viruses and viral replication). Our data demonstrate increased expression of a large number of genes involved in host responses to infection in insulitic islets. It is not clear whether the presence of CD3 T cells are in response to viral presence. Alternatively, the stress of insulitic activity could allow reactivation of latent viruses.

Select the ONE category that best describes your research:	
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☐ Beta Cell Development, Differentiation & Regeneration	☐ Novel Technologies
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☐ Core Lab	☐ Type 1 Diabetes Etiology & Environment
☐ Immunology	☐ Other (list):

OA40
Markers Associated with Viral Infections Correlates with Genes from the Insulin Secretion Pathway

AUTHORS

Nelson, Grace[1]; Max, Caleb[1]; Lenchik, Nataliya[1]; Rodriguez-Calvo, Teresa[2]; Apaolaza, Paola[2]; Balcacean, Diana[2]; Gerling, Ivan[1]; nPOD-V group

[1]University of Tennessee Health Science Center. [2]Institute of Diabetes Research, Helmholtz Zentrum München

PURPOSE

Type 1 diabetes (T1D) is an autoimmune condition, thought by some, to be enhanced or triggered by certain viral infections. While a variety of studies suggest an association of viruses with the development of T1D, it is difficult to prove this. One way to approach this question is to further elucidate the mechanism by which viral infections may affect islets and beta cells. In this study we isolated the islets that expressed known signs of viral infections and subsequently investigated pathways that may be secondarily affected through analysis of gene expression profiles.

METHODS

nPOD OCT slides were obtained from non-diabetic donors (Control), autoantibody positive non-diabetic donors (AB+) and donors with T1D (T1D). Islets were categorized based on the presence of markers associated with viral infection (VIMs), HLA, Mx1, dsRNA, and PKR, identified via immunohistochemical staining. Laser capture was used to manually isolate islets. From each donor islet were pooled based on the number of VIMs (0 VIMs, 1 VIMs or ≥2VIMs). After pooled islets were obtained, RNA was extracted, and microarray used to assess transcriptomes. We used GeneSpring software (version 13.0, Silicon Genetics, Redwood, CA) to generate a list of genes that showed differential expression between donors/VIMs. Using webgestalt we were then able to identify pathways enriched by the list of differentially expressed genes.

SUMMARY OF RESULTS

A total of 85 genes with a fold change of ≥1.1 and p-value=0.001 were differentially expressed between islets with 0 VIMs and islets with ≥1 VIMs. Pathway analysis showed strongest enrichment for the insulin secretion pathway (enrichment ratio of 6.9 and a P-value of 5.7E-7). Closer analysis of this gene list indicated decreased expression of genes involved in insulin secretion in islets with 1 or more VIMs. Two genes of particular interest, KCNJ11 and ABCC8, both had significantly lower expression in the islets with ≥1VIMs compared to islets with 0 VIMs (p=0.01). This general pattern was maintained within all clinical groups.

CONCLUSIONS
Islets selected for high expression for genes associated with viral infection had decreased expression of genes important for insulin secretion, including KCNJ11 and ABCC8(SUR1) the genes responsible for the K-ATP channel necessary for insulin release in response to glucose. This may suggest a pattern of dedifferentiation and/or functional impairment of beta cells in the setting of viral infection.

Select the ONE category that best describes your research:	
☐ Beta Cell Physiology and Dysfunction	☐ Novel Biomarkers
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☐ Core Lab	☐ Type 1 Diabetes Etiology & Environment
☐ Immunology	☐ Other (list):

OA41
Islet amyloidosis in a child with type 1 diabetes

AUTHORS

Maria L. Beery,[a] Laura M. Jacobsen,[b] Mark A. Atkinson,[a,b] Alexandra E. Butler,[c] and Martha Campbell-Thompson[a]

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[b] Department of Pediatrics, University of Florida, Gainesville, FL, USA

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PURPOSE

Islet amyloidosis represents a histopathological feature classically ascribed to patients with type 2 diabetes. Herein, the occurrence of islet amyloidosis and its severity are reported in a child with type 1 diabetes along with histological comparisons of islet amyloidosis in two young adults with recent-onset type 1 diabetes.

METHODS

Histopathology reviews were conducted on hematoxylin and eosin (H&E) and immunohistochemistry (insulin, glucagon, Ki67, CD3) stained pancreas slides. When islet amyloid was detected during initial histopathology reviews, additional pancreas sections (8 µm thick, N = 4–7 sections/patient) were stained using Congo Red (S7441, Cardinal Health, Dublin, OH) to confirm islet amyloidosis. Three age-, BMI-, and sex-matched control donors to the three patients with type 1 diabetes described herein were selected and paraffin sections similarly stained with Congo Red. Amyloid area was automatically quantified using the tissue classifier function in HALO after total tissue, islet count and amyloid positive islet areas were calculated following manual annotations.

SUMMARY OF RESULTS

Islet amyloidosis was infrequent yet widely distributed throughout the pancreas in the child with type 1 diabetes and both adults with type 1 diabetes, with no such pathology seen in matched control donors. Congo Red staining showed a wide range in numbers of amyloid-positive islets per section with variable degrees of regional islet amyloidosis in all three patients. No amyloid was detected by Congo Red staining in three matched controls. Islet amyloid prevalence ranged from 0–9.3%, 0–12.6%, and 0–4.7% in donors 6371, 6414, and 6362, respectively. Islet amyloid severity within only amyloid positive islets per section ranged from 0–41.2%, 0–15.1%, and 0–39.0% in donors 6371, 6414, and 6362, respectively. In donors 6371 and 6362, amyloid-positive islets were distant from each other and rare (0–8 islets/section), with minimal to moderate islet amyloidosis (12 islets with 0.4–19% amyloid area; 7 islets with 25–48% amyloid area). Donor 6414 had a higher frequency of amyloid-positive islets (4–35/section) with minimal to moderate amyloidosis (41 islets with 1.5–23% amyloid area, two islets with 25–43% amyloid area, respectively). A clustering of amyloid positive islets was observed in two lobules from donor 6414 but not in the other two donors with type 1 diabetes. This lobular pattern of islet amyloid was unevenly distributed

across the pancreas, largely being concentrated in the tail with a few foci identified in the head region. All three donors with type 1 diabetes had insulitis; however, insulitic islets did not show amyloidosis

CONCLUSIONS

Analysis of these cases add to the increasing appreciation of islet heterogeneity in children and young adults with type 1 diabetes. Our finding of islet amyloid in two young adults with recent onset type 1 diabetes is in keeping with the report by Westermark et al. The child with type 1 diabetes showed a very similar pattern to the two young adults in terms of islet amyloid prevalence and severity. Notably, however, the child and one of the young adults (6362) showed a scattered distribution of amyloid-containing islets whilst in the other young adult (6414), lobular clustering of amyloid-containing islets was seen. This lobular clustering of amyloid was also noted in the 2 subjects with recent onset type 1 diabetes in the Westermark report. Such knowledge supports a notion that multiple pathophysiological mechanisms underlie the loss of functional β-cell mass in the spectrum of clinical phenotypes in patients with type 1 diabetes.

Select the ONE category that best describes your research:	
☐ Beta Cell Physiology and Dysfunction	☐ Novel Biomarkers
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☐ Core Lab	☐ Type 1 Diabetes Etiology & Environment
☐ Immunology	☐ Other (list):

OA42
Cellular Characterization of the Pancreas in Individuals with or at Increased-Risk for Type 1 Diabetes

AUTHORS

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PURPOSE

Once thought a disease primarily affecting β-cells, emerging evidence suggests that type 1 diabetes (T1D) also includes distinct alterations in both endocrine and exocrine pancreatic compartments. However, a quantitative histological description of pancreatic acinar, ductal, and other non-endocrine/non-exocrine tissues is lacking.

METHODS

We utilized HALO image analysis software to analyze scanned whole human pancreas cross-sections from nPOD donor cohort, stained for insulin and glucagon by IHC as well as H&E, from the PH, PB, and PT regions. We characterized pancreatic exocrine and endocrine tissue compositions by quantifying the proportion of endocrine, acinar, and ductal/other (non-endocrine, non-exocrine) areas as well as acinar and endocrine cell density, and size in subjects with or at-risk for type 1 diabetes as well as controls without diabetes.

SUMMARY OF RESULTS

The area of ductal/other tissues was greater in those with T1D lacking residual insulin containing islets (T1D ICI-) compared to non-diabetic autoantibody negative (ND AAb-) and non-diabetic autoantibody positive (ND AAb+) groups. Inversely, acinar area was lower in T1D ICI- donors vs. either ND group. However, despite having a similar proportion of acinar area to both ND groups, the cells were smaller and tissue denser in T1D individuals with residual insulin containing islets (T1D ICI+) compared to ND AAb- donors. Endocrine area was smaller, but density greater, in T1D donors compared to either ND group. Interestingly, endocrine cells were smaller in all T1D vs. ND AAb- donors, but also in T1D ICI+ vs. ND AAb+ individuals. The main pancreatic duct was thicker and occupied area smaller in the tail vs. body region, regardless of disease status.

CONCLUSIONS
Further research is needed to address the role of whole-organ defects in T1D, but these data provide important insights into anatomical differences observed within the pancreas and highlights that alterations within the exocrine tissue may play a part in disease pathogenesis.

Select the ONE category that best describes your research:	
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☐ Core Lab	☐ Type 1 Diabetes Etiology & Environment
☐ Immunology	☐ Other (list):

OA43
MHC class II expression in human pancreatic tissue sections

AUTHORS

Estefania Quesada-Masachs, Sakthi Rajendran, Samuel Zilberman, Jae-Hyun Mason Lee, and Matthias von Herrath

PURPOSE

Type 1 diabetes (T1D) is an autoimmune disease in which insulin-producing ß cells are damaged by the immune system. However, the immunological mechanisms that lead to T1D have remained incompletely understood. It's been suggested that MHC class II can play a role in T1D pathogenesis, i.e. certain mutations in genes encoding for MHC II molecules are correlated to different risks of developing T1D, and MHC II can be upregulated in ß cells exposed to the proinflammatory cytokine cocktail. But its specific role in T1D is still undefined. The aim of this project is to characterize and quantify the expression of MHC class II in islets of human pancreatic tissue sections, obtained from the network of pancreatic organ donors (nPOD).

METHODS

Antibodies for HLA class II (DP, DQ, DR), HLA class I (A, B, C), CD68 and CD31 were optimized in Formalin Fixed Paraffin Embedded (FFPE) sections of human tonsils. Different multicolor immunofluorescent imaging panels were optimized in FFPE sections of tonsils and pancreas. Human pancreas sections from one non-diabetic (#6373) and two T1D (#6212, #6209) donors were stained with Insulin, MHC II and MHC I. The images were acquired with a Zeiss AxioScan Z1 slide scanner and an LSM780 Confocal microscopy system for higher resolution.

SUMMARY OF RESULTS

We have successfully optimized a multicolor immunofluorescence imaging strategy to precisely locate and quantify the expression of HLA class II in pancreas sections. In T1D case #6209, we observed a high MHC II expression in the insulin-containing islets (ICI) that also exhibited MHC I hyperexpression. The high expression of MHC II was mainly located surrounding the islets. In another T1D case, #6212, we couldn't identify any ICI, and neither MHC II nor MHC I hyperexpression. In the non-diabetic donor, we found very few cells expressing MHC II, and these cells were evenly distributed in the exocrine and the endocrine tissue. In all of the cases, we didn't identify any colocalization between insulin and MHC II. However, the colocalization of MHC II and MHC I was higher in the T1D case #6209 compared with the non-diabetic case (52.3% vs 8.8%).

CONCLUSIONS

HLA class II is highly expressed in most of the insulin containing islets of T1D patient #6209, but, so far, ß cells do not seem to be their main source. For that reason, CD68 and CD31 were optimized in pancreatic sections in order to further test which cells are producing MHC

II. At this moment, we are about to complete the optimization and decide on a strategy for the final multicolor immunofluorescence panel. As soon as the last panel is completely optimized, we will stain 5 non-diabetic, 5 pre-diabetic, and 5 T1D cases.

Select the ONE category that best describes your research:	
☐ Beta Cell Physiology and Dysfunction	☐ Novel Biomarkers
☐ Beta Cell Development, Differentiation & Regeneration	☐ Novel Technologies
☐ Bone Marrow Studies	☒ Pathology
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☐ Immunology	☐ Other (list):

OA44

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

AUTHORS

Rebecca C Wyatt[1], Pia Leete[1], Myriam Padilla[2], Mingder Yang[2], Marika Bogdani[3], Gail Deutsch[4], Christine Flaxman[1], Mark Atkinson[2], Noel G Morgan[1], Irina Kusmartseva[2] and Sarah J Richardson[1]
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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.

Select the ONE category that best describes your research:	
☐ Beta Cell Physiology and Dysfunction	☐ Novel Biomarkers
☐ Beta Cell Development, Differentiation & Regeneration	☐ Novel Technologies
☐ Bone Marrow Studies	☒ Pathology
☐ Core Lab	☐ Type 1 Diabetes Etiology & Environment
☐ Immunology	☐ Other (list):

OA45

Pancreas in type 1 diabetes has both exocrine and endocrine alterations

AUTHORS

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PURPOSE

Individuals with longstanding and recent-onset type 1 diabetes (T1D) have a smaller pancreas. Since beta cells represent a very minor portion of the pancreas, the loss of pancreas volume in T1D is primarily due to the loss of pancreatic exocrine mass. However, the structural changes in the T1D exocrine pancreas are not well understood.

METHODS

To characterize the pancreatic endocrine and exocrine compartments in T1D, we studied pancreata from adult donors with T1D duration greater than 6 years compared to age-matched normal donor (ND) pancreata. Islet cell mass, islet morphometry and number, exocrine mass, acinar cell size and number, and pancreas fibrosis were assayed by immunohistochemical staining.

SUMMARY OF RESULTS

T1D pancreata were approximately 45% smaller than ND pancreata (47.4 vs 85.7 g, 95% CI 42.0 – 52.7 and 78.1 – 93.2, n=31 and 36), independent of T1D duration or age of T1D onset. T1D pancreata had decreased beta cell mass (0.061 vs 0.93 g, 95% CI 0.00 – 0.12 and 0.50 – 1.4, n=6 and 9), fewer total islets (0.7 vs 1.9 x 10^6 islets/pancreas, 95% CI 0.52 – 0.87 and 1.3 – 2.5, n= 6 and 7), and 45% reduction in total exocrine mass (42.0 vs 96.1 g, 95% CI 29.4 – 54.7 and 80.3 – 112, n=6 and 7). T1D acinar cells were similar in size but fewer in number (63.6 vs 111.5 x 10^9 cells/pancreas, 95% CI 45.3 – 82.1 and 78.3 – 144.7, n=10 and 12), likely accounting for the change in pancreas size. Within the T1D exocrine tissue, there was a greater degree of fibrosis.

CONCLUSIONS

T1D pancreata are smaller than ND pancreata because there are fewer exocrine cells; the cause, mechanisms and implications of the exocrine cell loss are unknown.

Select the ONE category that best describes your research:	
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☐ Beta Cell Development, Differentiation & Regeneration	☐ Novel Technologies
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☐ Core Lab	☐ Type 1 Diabetes Etiology & Environment
☐ Immunology	☐ Other (list):

OA46
In Situ Post-Translational Modifications in Islets as Potential Neoantigens in Type 1 Diabetes

AUTHORS

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PURPOSE

Increasing lines of evidence support that post-translational modification (PTM) of proteins represent a key mechanism of producing potential neoantigens and neoepitopes that are unique to β-cells, which may play a prominent role in triggering T1D. Thus, the discovery and characterization of in-situ islet PTMs as potential neoantigens could lead to important insights into the immuno-pathogenesis and provide new avenues of prevention and intervention. However, there is still little evidence of in situ PTMs in islets of pre-T1D or T1D patients. Herein, we applied mass spectrometry-proteomics to characterize in situ PTMs in islets obtained by laser microdissection from both presymptomatic autoantibody positive (AAb+) cases and T1D cases.

METHODS

Human islet sections from non-diabetic controls, multiple AAb+ cases, and T1D subjects from the Network for Pancreatic Organ donors with Diabetes (nPOD) program were isolated by laser microdissection (LMD), with islet sections subjected to global proteome profiling by nanoscale proteomics technologies. Direct identification of in situ PTMs (e.g., phosphorylation, deamidation, citrullination, oxidation) was identified by advanced informatics tools. The potential impact of modifications on HLA binding, TCR binding or both was determined by generation of atomic models of HLA-A*02:02 complexed with specific sequence motif based on the crystal structure of the most closely related crystal structure. Molecular graphic images were generated with PyMol.

SUMMARY OF RESULTS

We have applied nanoscale proteomics to profile PTMs in laser microdissected human islet sections from pre-symptomatic AAb+ subjects and age/sex matched controls (n=6) as well as one T1D donor. Our initial analyses resulted in the identification of a relatively large set of in situ PTMs including phosphorylation and several other PTMs reported to be involved in autoantigen formation will including deamidation, citrullination, and cysteine oxidation products: sulfinic (SO_2H) and sulfonic acid (SO_3H). Specifically, with <5% FDR, we identified ~350 unique phosphopeptides; ~170 peptides with sulfonic and sulfinic acid modifications, and ~570 peptides with deamidation and citrullination. To enable accurate identification of error-prone PTMs such as deamidation, we have developed a novel dual-search delta score strategy by comparing the same MS/MS spectra with and without deamidation in searching parameters and true and false positives can be clearly differentiated by the delta scores between the two independent searches. In total, we observed ~55% non-enzymatic asparagine (N) deamidation, 38% glutamine (Q) deamidation, and 7% arginine (R) citrullination. Interestingly, many PTMs were observed on known autoantigens with insulin and proinsulin being modified by several types of PTMs, suggesting modified insulin as a key source of neoepitope. A further highlight is that the observed deamidated sequence from proinsulin were recently reported to be preferentially bound to HLA-DQ8. Another example highlights a SO_2H-modified insulin B-chain

peptide, where the modification was predicted to impact T-cell recognition based on molecular modeling.

CONCLUSIONS

Proteomic profiling of in-situ PTMs in LMD islets from AAb+ and T1D subjects provides a valuable resource of candidate neoantigens and neoepitopes. Such resource will provide a knowledgebase to identify and confirm novel functional in situ neoepitope from human patients.

Select the ONE category that best describes your research:	
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☐ Beta Cell Development, Differentiation & Regeneration	☐ Novel Technologies
☐ Bone Marrow Studies	☒ Pathology
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☐ Immunology	☐ Other (list):

TYPE 1 DIABETES ETIOLOGY AND ENVIRONMENT

OA47

Presence of anti-viral immune response markers in the pancreas and its association with type 1 diabetes

AUTHORS

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PURPOSE

Previous investigations have provided evidence for an association of type 1 diabetes (T1D) with enterovirus (EV) infections. EVs can infect beta cells and could induce chronic inflammation accompanied by activation of cellular processes, which may compromise beta cell function and survival. Our objective was to investigate the presence or absence of markers associated with viral infections (EV capsid protein VP1 and double stranded RNA (dsRNA)), anti-viral immune response proteins (HLA-I, MxA and PKR) and immune infiltration in the islets of non-diabetic, autoantibody positive (AAb+) and T1D organ donors.

METHODS

Pancreatic sections from 10 controls, 6 T1D, and 4 AAb+ (2 single and 2 double) donors were analyzed. Frozen sections were stained for insulin, glucagon, HLA-I, MxA, PKR, dsRNA and CD3 by immunofluorescence staining at the Institute of Diabetes Research (Helmholtz Center) in Munich, Germany. In addition, formalin-fixed paraffin-embedded sections were stained for VP1 and HLA-I at the University of Exeter Medical School (Exeter, UK). The number of insulin and glucagon positive islets as well as the presence of HLA-I, MxA, PKR and dsRNA were analyzed manually. All markers were combined and each islet was classified based on the presence of 1, 2, 3 or 4 markers. Islet infiltration and protein VP1 were analyzed using the software QuPath (University of Edinburgh, Division of Pathology), an open source software for whole slide image analysis.

SUMMARY OF RESULTS

The majority of the islets in non-diabetic donors had normal HLA-I expression while a small percentage had a slight elevation. AAb+ cases behaved similarly but one double AAb+ donor presented HLA-I hyperexpression in a few islets. All T1D donors presented islets with HLA-I hyperexpression (5 to 25 % of the islets). Interestingly, HLA-I expression was also high in the acinar tissue. In T1D donors, VP1+ cells (weak and strong) could be detected in more than 20% of the islets while only 2 of the non-diabetic donors showed weak VP1 positivity. In most of the T1D cases, islets that were positive for MxA and PKR could be detected in a small percentage of islets. Moreover, the combination analysis of HLA-I, MxA, PKR and dsRNA revealed that a significantly higher percentage of islets in T1D donors presented 1 or more of these markers in the same islet. Islet T cell density was also high in half of the T1D donors

and all of them had a few islets with more than ≥ 6 CD3+ cells (defined as insulitis). A detailed analysis revealed that 82% of the islets with high infiltration in T1D and 86% in AAb+ donors were positive for one or more markers compared to 25 % of the highly infiltrated islets in non-diabetic donors. In addition, multiple markers were detected more frequently in insulin containing islets (ICIs).

CONCLUSIONS

Previous studies have shown that a 'viral signature' exists in type 1 diabetes and involves interferon responses that could be sustained during prolonged periods of time. These include the up-regulation of markers such as PKR, MxA and HLA-I, and the potential release of chemokines able to attract immune cells to the islets leading to insulitis. In this scenario, the hyperexpression of HLA-I molecules could promote antigen presentation to autoreactive T cells, favoring beta cell recognition and, ultimately, destruction. Our data suggest that the detection of markers associated to increased ER stress, viral infection and anti-viral responses in pancreatic islets is associated with T1D. This, together with the detection of the enterovirus capside protein VP1 could indicate the presence of a low level, persistent, viral infection and/or a chronic anti-viral immune and cellular response. In addition, our analysis suggests that this signature appears early in the disease process and could be detected in AAb+ donors. Future work will also investigate the presence of enterovirus specific T cell responses at different stages of the disease process.

Select the ONE category that best describes your research:	
☐ Beta Cell Physiology and Dysfunction	☐ Novel Biomarkers
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☐ Bone Marrow Studies	☐ Pathology
☐ Core Lab	☒ Type 1 Diabetes Etiology & Environment
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OA48
Investigating type 1 diabetes pathogenesis with the live pancreas tissue slice platform

AUTHORS

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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.

Select the ONE category that best describes your research:

☐ Beta Cell Physiology and Dysfunction	☐ Novel Biomarkers
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☐ Core Lab	☒ Type 1 Diabetes Etiology & Environment
☐ Immunology	☐ Other (list):

OA49
Safety Studies Examining a CVB1-6 Vaccine in NOD Mice and Non-Human Primates

AUTHORS

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PURPOSE

An extensive concerted effort has been made by the scientific community to elucidate the causes of Type 1 diabetes (T1D), however despite these efforts the disease aetiology remains elusive. Combined with a genetic element, associations between T1D and a number of environmental factors have been described which include infections with common enteroviruses and more specifically Coxsackievirus Bs (CVBs). Despite a significant body of research examining this area, whether infections are causal is yet to be confirmed. Vaccination strategies of at-risk individuals provide a viable option to determine this. As such, we have created a polyvalent CVB1-6 vaccine and undertaken proof-of-concept studies that have demonstrated the protective capacity of this vaccine against virus induced diabetes. We have also shown that the vaccine is highly immunogenic in a non-human primate model. As an extension to these studies, in this project we address the safety of the vaccine in both the NOD mouse model for spontaneous T1D development and the non-human primate model.

METHODS

CVB1-6 serotypes were inactivated by formalin and mixed to create the polyvalent vaccine. NOD mice aged between 4.5-7.5 weeks old were vaccinated with CVB1-6 vaccine or buffer two or three times with two to three week intervals between vaccinations. Serum was collected prior to each vaccination and at the end of the study. Weight and blood glucose levels were monitored weekly. Mice were either followed to 11-13.6 weeks of age where they were sacrificed and the pancreas was collected for histological analysis of insulitis or up to 30 weeks of age for monitoring of diabetes incidence. Rhesus macaques (4 years old) were vaccinated twice with a 4 week interval between the two, with or without Alum adjuvant. Serum was collected prior to vaccination. The animals were monitored until 10 weeks after the prime vaccination and weight, body temperature, blood glucose levels were monitored. Liver enzyme functions were assessed on days 0, 1 and 14. Serum neutralising antibody titres from all animals were assessed by serum neutralisation assay.

SUMMARY OF RESULTS

The polyvalent CVB1-6 vaccine was highly immunogenic and induced strong neutralising antibody responses in NOD mice. A strong neutralising antibody response against the six serotypes was also induced in the rhesus macaques after vaccination. Neutralising antibody responses were in general higher in the adjuvant group on days 14 and 28 than in the non-adjuvant group however the responses were equivalent after the boost vaccination. The vaccine was well tolerated in NOD mice and had no adverse effects on weight and blood glucose values. Preliminary data indicates that insulitis scores in 11-13 week old female NOD mice were comparable between buffer treated and CVB1-6 vaccinated groups and furthermore, no differences were detected in either the average age of diabetes onset or in cumulative diabetes curves between the two groups. The safety profile of the CVB1-6 vaccine was similar to that seen in the mice with no alterations in weight, blood glucose or body temperature of animals vaccinated in the presence or absence of adjuvant. There were also no changes in enzymes indicative of liver function on days 1 and 14 after the prime vaccination, which remained in normal ranges.

CONCLUSIONS

In mice and rhesus macaques the CVB1-6 vaccine had an excellent safety profile and was highly immunogenic. Moreover, the vaccine did not affect insulitis scores nor the onset of diabetes in NOD mice. These proof-of-concept studies provide necessary data to support the development of an equivalent vaccine for use in human trials to establish whether CVBs are involved in T1D and if so, provide a viable preventative treatment for the disease.

Select the ONE category that best describes your research:

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☐ Immunology	☐ Other (list):

OA50
Proteomic Based Detection and Identification of Enteroviruses in nPOD Cases

AUTHORS

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PURPOSE

As part of the JDRF nPOD-Virus group, our ongoing studies focus on utilizing proteomics and liquid chromatography-mass spectrometry (LC/MS) technologies to identify and characterize enterovirus proteins/peptides in disease stratified nPOD tissue samples. The goal is to use global and targeted methods to robustly demonstrate the presence of enteroviruses and proteomic signatures consistent with enterovirus infections.

METHODS

We have isolated and processed proteins from different types of pancreas tissue preparations from nPOD including flash frozen tissue chunks, fresh frozen OCT embedded tissue chunks, 20 µM tissue slices from fresh frozen OCT and laser capture micro-dissected tissue sections for LC/MS analysis. We have utilized an Orbitrap Fusion Lumos Mass Spectrometer to acquire high resolution, high mass accuracy and high sensitivity MS data using different scanning methods including data dependent acquisition (DDA), data independent acquisition (DIA), and parallel reaction monitoring (PRM) followed by qualitative and quantitative comparative analysis.

SUMMARY OF RESULTS

In our cumulative studies using disease stratified nPOD pancreas tissue samples, we identify enterovirus peptides from different serotypes including those that have been correlated with the etiology of type 1 diabetes. Some of the identification results have been validated by targeted mass spectrometry and in Western Blots. In addition, comparative label free quantitation analyses reveal the upregulation and activation of pathways that are associated with viral infections.

CONCLUSIONS

Our current data provides additional evidence that T1D pancreata are infected and potentially harbor various enterovirus proteins and pathways that are associated with the infection are also activated in infected tissues.

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☐ Immunology	☐ Other (list):

OA51
Virus-Mediated Dysbiosis Promotes Autoimmunity and Type 1 Diabetes Onset

AUTHORS

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PURPOSE

In combination with genetic determinants, susceptibility to autoimmune diseases such as Type 1 Diabetes (T1D) is established by various environmental factors including microbial dysbiosis, exposure to dietary antigens, antibiotic use, vitamin D deficiency, and infection. Compelling evidence indicates commensal bacteria and viruses are important cofactors in T1D development and pathogenesis. Clinical and epidemiological studies have implicated infection with certain viruses such as coxsackievirus B (CVB) to be a risk factor associated with diabetes onset. Infections may be an instigating factor to alter the microbiome and this microbial change may be sufficient to promote autoimmunity. As an enterovirus, CVB is spread via a fecal-oral route, yet little is known about how this virus affects the intestinal microbiome. Recently, mucosa-associated invariant T (MAIT) cell populations were shown to be altered leading up to diabetes onset in patients and in mice. These cells are activated by microbial products derived from riboflavin biosynthesis in the gut to promote intestinal integrity, but they can also take on a more inflammatory phenotype and participate in autoimmune responses in the pancreas. Ultimately, there exists a significant potential for cross-talk between CVB infection, the microbiome, and gut-resident immune cells impacting T1D susceptibility and we proposed to analyze this interrelationship.

METHODS

To address these questions, we modeled the interaction a non-obese diabetic (NOD) mice to examine how the commensal bacteria composition in the gut is altered by CVB4 infection. We used a number of techniques including NGS and FMT analysis to examine the interrelationship between virus infection, the microbiome and diabetes onset.

SUMMARY OF RESULTS

CVB4 not only promotes onset of T1D in these mice but also causes dysbiosis and loss of bacterial diversity which resembles that of a spontaneously diabetic NOD mouse. Introducing this new infection-induced microbial composition into naïve mice through the use of fecal microbiome transfers (FMTs) can accelerate T1D onset and alter immune profiles in the gut as well as the pancreas. We have found MAIT populations are altered by a “diabetogenic” microbiome and respond directly to CVB4 infection.

CONCLUSIONS
Together our data highlights the role of the gut microbiome and its ability to affect immune homeostasis and contribute to T1D development.

Select the ONE category that best describes your research:	
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☐ Immunology	☐ Other (list):

OA24.5
Insulin-Specific Regulatory T Cell Receptor Alpha Clonotypes Restricted to HLA-DQ6 (DQB*06:02) are Shared Between Blood and Islets

AUTHORS

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PURPOSE

While progress has been made toward understanding mechanisms that lead to development of autoimmunity, there is a lack of knowledge regarding protective mechanisms against such diseases. For example, in type 1 diabetes (T1D), the role of pathogenic $CD4^+$ T cells in the destruction of pancreatic islets is well characterized, but the factors that contribute to T1D protection have not been fully elucidated. The major genetic determinant in susceptibility or protection from many autoimmune diseases resides in the human leukocyte antigen (HLA) region. Particular class II alleles (e.g., HLA-DQ8) increase the risk for developing T1D, whereas others (e.g., HLA-DQB*06:02) lead to dominant protection from T1D. HLA class II genes encode major histocompatibility (MHC) molecules that present peptides to T cells. We hypothesize that the DQ6 allele is protective via its ability to present insulin peptides to regulatory $CD4^+$ T cells (Tregs), resulting in downstream anti-inflammatory responses.

METHODS

We expanded insulin-specific Tregs from peripheral blood mononuclear cells (PBMCs) of DQB*06:02^+ ($DQ6^+$) non-diabetic individuals using an insulin B chain mimotope known to be a strong T cell agonist in murine models and human T1D. Insulin-expanded Tregs were isolated by fluorescence-activated cell sorting (FACS) as $CD4^+CD25^{hi}CD127^{lo}CTV^{lo}$ cells. T cell receptor (TCR) sequencing was performed by single-cell TCR sequencing and paired single-cell TCR/RNA-seq (10X Genomics) from n=3 non-diabetic individuals. We also performed single-cell TCR sequencing on islet-infiltrating T cells of a $DQ6^+$ non-diabetic organ donor to compare TCR clonotypes.

SUMMARY OF RESULTS

PBMCs from $DQ6^+$ non-diabetic individuals showed an increase in the $CD4^+CD25^+Foxp3^+$ Treg population after 7 days in culture with the insulin mimotope compared to no antigen (5.9% vs. 4.0%, p=0.03). Confirming the Treg phenotype, a cytokine ELISPOT assay showed that sorted insulin-proliferated cells secreted IL-10 upon repeat antigen stimulation, and single-cell RNA-seq data demonstrated that the transcriptomes of the expanded cells

were consistent with memory cells expressing Foxp3. Of the 2,096 expanded Tregs with paired αβTCR sequences from three DQ6$^+$ individuals, there were 883 TCRs (42%) that were present ≥2 times, indicating a skewed repertoire. Approximately 1.3% of all sequences from the DQ6$^+$ individuals shared identical or nearly identical single chains (TCRα or TCRβ), including CDR3 sequences. We confirmed the antigen specificity of dominant clonotypes, indicating that these TCRs are reactive to insulin B chain peptides presented by DQ6. Additionally, we flow sorted CD4$^+$ T cells from the islets of a non-diabetic DQ6$^+$ organ donor and performed single-cell TCR sequencing. Of the 67 CD4$^+$ cells present in 4,000 islet equivalents (IEQ), TCR clonotypes detected from three CD4$^+$ cells possessed identical TCRα chains (including CDR3 sequences) with Tregs sequenced from the PBMCs of the three separate DQ6$^+$ non-diabetic individuals. These data potentially indicate the presence of insulin-specific Tregs within the pancreatic islets of DQ6$^+$ non-diabetic organ donors.

CONCLUSIONS

Our findings provide a mechanistic basis for understanding HLA-linked protection from autoimmune diabetes development and have implications for manipulating self-antigen-specific T cell responses to modify the disease course in T1D.

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☐ Beta Cell Physiology and Dysfunction	☐ Novel Biomarkers
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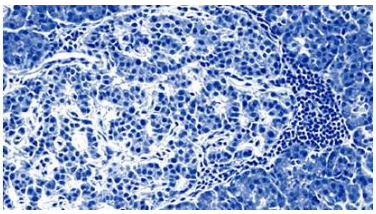
nPOD
Network for Pancreatic Organ Donors with Diabetes



BETA CELL DEVELOPMENT, DIFFERENTIATION, REGULATION

OA1
Neratinib is a novel inhibitor of MST1 and protects pancreatic beta-cells in diabetes

AUTHORS

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PURPOSE

The failure of pancreatic insulin producing β-cells is a central pathogenic hallmark of all forms of diabetes. The identification of *relevant molecular* pathways and pathophysiological events that are responsible for β-cell demise in diabetes is instrumental for the better understanding of disease mechanisms and to ultimately establish a novel diabetes therapy directed toward restoration of beta-cell mass and function. The serine/threonine kinase Mammalian Sterile 20-like kinase 1 (MST1), a core kinase of the Hippo developmental pathway, is a critical regulator of β-cell death and dysfunction in diabetes and its inhibition restores normoglycemia and β-cell function and prevents the development of diabetes. Here we aimed to find a pharmacological MST1 inhibitor with robust β-cell protective actions.

METHODS

With the strategy of repurposing FDA-approved drugs for the therapy of diabetes, we performed a high throughput MST1 inhibition screen across a highly-privileged collection of 641 drug-like kinase inhibitors together with a triaging strategy for selective, non-cytotoxic compounds, and identified neratinib, approved for cancer therapy, as potent MST1 inhibitor. Neratinib was then tested *in vitro* for its efficacy to inhibit MST1 activation and cell death in human islets and INS-1E cells and *in vivo* in a pre-clinical study in type 1 (multiple-low dose streptozotocin; MLD-STZ) and type 2 (obese $Lepr^{db/db}$) diabetic mouse models. Glycemia, glucose and insulin tolerance and β-cell function were tightly monitored during the study and β-cell mass, survival, proliferation and β-cell identity marker expression analyzed in the isolated pancreata.

SUMMARY OF RESULTS

Neratinib improved β-cell survival under multiple diabetogenic conditions in β-cells and primary human and mouse islets. Without any glucose lowering or β-cell effects in control mice, neratinib restored normoglycemia and β-cell function, survival, and mass, as well as β-cell identity in the MLD-STZ and obese diabetic $Lepr^{db/db}$ mice. MALDI imaging mass spectrometry (MALDI-IMS) showed neratinib distributed throughout the pancreas after i.p. injection. Neratinib's effect was further confirmed in a therapeutic approach; it fully restored β-cell survival in isolated mouse islets from severely diabetic db/db mice, as well as in pro-inflammatory cytokine treated mouse islets.

CONCLUSIONS

Neratinib is a previously unrecognized inhibitor of MST1 and represents a potential β-cell-protective drug with proof-of-concept *in vitro* in human islets and *in vivo* in rodent models of both type 1 and type 2 diabetes.

Select the ONE category that best describes your research:	
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☐ Immunology	☐ Other (list):

BETA CELL PHYSIOLOGY AND DYSFUNCTION

OA2
miRNA Signatures in Human Pancreatic Slices during Glucose Stimulation

AUTHORS

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PURPOSE

Through a working group effort nPOD has successfully established that it is possible to conduct studies of live pancreas slices from organ donors, in which the cytoarchitecture of the organ is preserved and islets are not subjected to the stress of isolation. In Miami, we have conducted functional assessment of pancreatic cell function from slices obtained from 12 nPOD donors (with and without T1D). Insulin and glucagon secretion were assessed upon glucose and KCl stimulation. miRNAs are small noncoding RNA molecules known to regulate gene expression and are emerging as novel biomarkers for many diseases. Our laboratory has been investigating serum levels of miRNAs as biomarkers of islet autoimmunity and/or progression to T1D. To investigate how glucose stimulation impacts miRNA expression, we measured miRNA levels in the perifusates of human pancreas slices and correlated levels with insulin and glucagon release upon glucose stimulation.

METHODS

Human pancreas slices were obtained from 3 nPOD donors, without T1D; 1 donor was male (6482) and 2 were females (6261 and 6462), and they were 11, 14, and 13 years old, respectively. Slices were produced at the nPOD laboratory in Gainesville and shipped overnight to Miami. Upon arrival, slices were allowed to rest for two hours and then assayed for insulin and glucagon secretion following stimulation with glucose 3 mM for 10 min, glucose 16.7 mM for 20 min, glucose 1mM for 30 min, KCl 25mM for 5 min and glucose 3 mM for 10 min. Insulin and glucagon secretion were measured for each point via ELISA. Four time points per donor were selected for each donor, and point selection was based on the insulin and glucagon secretion data from each donor. The baseline point corresponded to a glucose level of 3 mM, then we selected two samples corresponding to the peaks in the GSIS (glucose stimulate insulin secretion) curve upon stimulation with 16.7 mM glucose, and the 4th sample was taken at 1 mM glucose representing the shift between insulin and glucagon secretion. Frozen perifusate samples were collected and later miRNAs were assayed using the EdgeSeq platform from HTG Molecular. This RNAseq based assay allows for the detection and quantification of over 2,083 miRNAs without prior miRNA extraction in a 15 µL volume. The samples were assayed individually and then grouped for statistical analyses using the DESeq2 Statistical Pipeline (version 1.14.1) from HTG Molecular Diagnostics, Inc. The DESeq2 package provides methods for estimating and testing differential expression using negative-binomial generalized linear models. Differential expression outputs are the mean value of each probe for each group after normalization, the log2-transformed average expression of each probe across all groups after normalization, the estimated fold change between the two groups (transformed from log-fold change). Significance is defined for $p<0.05$ after correction for false discovery rate using the Benjamini and Hochberg method.

SUMMARY OF RESULTS
We analyzed data from 12 samples from 3 nPOD donors. All 2,083 miRNAs were detected in all samples tested. Levels measured were within the detection limits for other sample types such as serum. To identify miRNAs showing changes in levels of expression during the stimulation, we compared miRNA levels as follows: 3 mM vs 16.7 mM Glucose; 3 mM vs 1 mM Glucose; 16.7 mM vs 1 mM Glucose. Of the 2,083 miRNAs tested, 139 (6.67%) showed changes in levels of expression that reached statistical significance: 1) levels of 124 miRNAs varied in response to a shift from low to high glucose and in parallel with the peaks of insulin secretion; 2) levels of 15 miRNAs changed from high to low glucose and mirrored the glucagon secretion. Among these miRNAs, 8 miRNAs (miR-29a-3p, miR-103a-3p, miR-140-5p, miR-181a-5p, miR-24-3p, miR-25-3p, miR-26b-5p and miR-27b-3p) have been linked to T1D in more than one published study and in our experimental setting seem to be related to insulin secretion. miR-197-3p and miR-30c-5p were also previously linked to T1D and seem to be related to glucagon secretion. Some microRNAs exhibited a parallel response pattern for both glucagon and insulin secretion. However, some of them exhibit an unparallel pattern of secretion which may indicate different mechanisms of release.

CONCLUSIONS
Our pilot studies using human pancreas slice perifusates show that it is possible to measure miRNAs during GSIS. We indeed observed significant level changes in a subset of miRNAs which reflect glucose stimulation. While these results were obtained from whole pancreas tissue, the response to stimulation suggests that the changes in miRNA levels most likely reflect changes in islet function during the response to glucose. Thus, these results help define the relationship of certain miRNAs with beta cell function. In addition, several of the miRNAs reproducibly associated with T1D in published studies exhibited significant changes during GSIS, further validating their relationship to islet cell responses and to T1D. Further studies should provide insight into alterations of miRNA responses during GSIS in nPOD donors with T1D.

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☐ Immunology	☐ Other (list):

OA3
Functional Role of inositol 1,4,5-trisphosphate receptor in Pancreatic β Cell Phatophysiology

AUTHORS

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PURPOSE

According to the classic paradigm, insulin secretion is triggered by the influx of extracellular calcium (Ca^{2+}) via voltage-dependent channels, leading to the fusion of insulin granules. Instead, the mechanisms involved in Ca^{2+} mobilization from internal stores are less defined. The main intracellular Ca^{2+} release channels are inositol 1,4,5-trisphosphate receptor (IP3R) and ryanodine receptor (RyR), whereas Ca^{2+} is returned to the *ER* primarily by the activity of the sarco/*endoplasmic reticulum* Ca^{2+}ATPase (SERCA) pump. We and others recently demonstrated the importance of RyR in type 2 diabetes mellitus (T2DM), showing that it is essential in glucose-stimulated insulin secretion (GSIS). Conversely, the exact role of IP3R in GSIS remains not fully understood and represents the main aim of this study.

METHODS

We performed functional studies *in vivo* (mouse models), *ex vivo* (isolated murine and human islets), and *in vitro* (clonal β cells).

SUMMARY OF RESULTS

Three isoforms of IP3R have been identified in mammalian cells. Channel opening is stimulated by the binding of second messenger IP3 and by changes in Ca^{2+} concentrations. Studies in rodent and human samples indicate that β cells express all IP3R isoforms. We demonstrated that the expression of all isoforms is significantly increased in human islets from diabetic donors compared with non-diabetic individuals. These results were confirmed in diabetic mice. Moreover, pancreatic β cells from diabetic patients exhibited dysmorphic and dysfunctional mitochondria, with markedly altered Ca^{2+} uptake. Similar features were found in clonal β cells chronically exposed to high glucose. *In vitro*, overexpression of IP3Rs was associated with impaired GSIS, whereas IP3R silencing improved β cell function, mitochondrial Ca^{2+} uptake and function, ER stress, and insulin release in response to different secretagogues.

CONCLUSIONS

Taken together, our data indicate that IP3Rs are upregulated in human islets from diabetic donors, leading to mitochondrial dysfunction and pancreatic β cell failure, identifying in these intracellular Ca^{2+} release channels a novel therapeutic target to treat diabetes.

Select the ONE category that best describes your research:	
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☐ Immunology	☐ Other (list):

OA4

Proinsulin-Insulin pancreatic islets *in-situ* expression mirrors metabolic defects observed in type 2 diabetic and glucose intolerant living donors

AUTHORS

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PURPOSE

Islets beta cell dysfunction in type 2 diabetes (T2D) can be a consequence of alteration of proinsulin processing. It has been reported that circulating levels of proinsulin (PI), and proinsulin-to-insulin ratio are increased in patients with metabolic alterations and in particular in T2D. It has been hypothesized that an elevated PI/INS ratio is caused by increased secretory demand on β cells due to insulin resistance and hyperglycemia, which promotes the release of immature granules with a higher relative content of PI and its conversion intermediates. The exact mechanism behind this increase in T2D, as well as in T1D, is unknown.

For this reason, the aim of this study was to analyze proinsulin and insulin expression in pancreatic islets of tissues biopsy of patients undergoing partial pancreatectomy (PP) with normal glucose tolerance (NGT), impaired glucose tolerance (IGT) and T2D, in order to explore the alterations that occur in islets during metabolic stress.

METHODS

In order to explore the alterations that occur in PI and INS staining pattern in pancreatic islets, Oral Glucose Tolerance Test (OGTT) was performed in n=17 patients on the waiting list for PP, classified into n=5 NGT, n=9 IGT and n=3 T2D. β-cell glucose sensitivity (calculated as the ratio of insulin secretion and glucose increments), basal insulin secretion, insulin secretion rate (ISR) and glucose levels 2-hours following OGTT were analyzed. Frozen sections of pancreatic tissue biopsy were stained for INS and PI through double immunofluorescence staining. Image analysis was performed on each individual islet to measure colocalization coefficient (M_1), islet area (μm^2), PI and INS positivity through Volocity software. *In-situ* staining measurements were correlated with patients' clinical parameters. Statistical analysis was performed using one-way ANOVA multiple comparison test and Pearson correlation.

SUMMARY OF RESULTS

PI-INS colocalization gradually increased from NGT to IGT and T2D pancreatic islets ($p<0.01$), indicating an altered PI processing due to the localization of PI and INS in the same compartment. The area (μm^2) of PI positivity and PI/INS ratio were significantly increased in T2D compared to IGT and NGT pancreatic islets ($p<0.01$), suggesting the release of immature granules with a higher relative content of PI, as previously demonstrated for in-vivo circulating levels. Moreover, we observed that the increase of PI-INS colocalization was positively correlated to ISR and glucose levels 2-hours following OGTT ($r=0.6$ $p<0.01$). The increase of PI/INS ratio was associated with higher basal INS and glucose levels 2-hours following OGTT ($r=0.6$ $p<0.01$). Finally, we also observed that the reduction of β-cell glucose sensitivity is linked to increase of *in-situ* PI-INS ratio ($r=-0.6$ $p<0.03$).

CONCLUSIONS
In conclusion, we demonstrated that: (*i*) *in-situ* PI-INS staining patterns are altered in T2D and IGT patients; (*ii*) pancreatic islets PI/INS ratio as well as PI area and PI-INS colocalization coefficient might mirror *in-vivo* the increased insulin secretion rate and β cell function reduction in the same patients. Our findings, suggest that poor β-cell glucose sensitivity is linked to increased *in-situ* PI/INS ratio, highlighting the importance of correct PI processing and folding in the maintenance of glucose homeostasis.

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☐ Immunology	☐ Other (list):

OA5
Chronic Marijuana Use Affects Human Islet Feature and Function

AUTHORS

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PURPOSE

Medical and recreational marijuana use is on the rise, and has been described in 27 of 100 nPOD T1D organ donors. Although standards for reporting of human islet characteristics have recently been established, and adapted by at least two major diabetes journals in 2019, lifestyle was not considered in the reporting recommendations. We hypothesized that chronic marijuana use in non-diabetic organ donors negatively impacts islet outcomes. To test this, characteristics and function of human islets isolated from pancreata of chronic marijuana users and non-user were examined.

METHODS

Ten organ donors with a history of chronic marijuana use were matched for age, sex, and ethnicity to 19 individuals without documented use of marijuana. Characteristics and function of human islets from both donor groups were assessed *in vitro*, including immunofluorescent staining of cannabinoid receptors (CBRs). Islets were then transplanted into diabetic NOD SCID mice and the ability to reverse diabetes was assessed.

SUMMARY OF RESULTS

There were no significant differences between groups in any of the established standards for reporting, including age, sex, BMI, cause of death, HbA1c levels, cold ischemia time, purity, viability, and glucose-stimulated insulin secretion (GSIS). Additionally, no differences were noted in ethnicity, blood glucose at admission, digested pancreas weight or time, and pre- or post-purification or culture IEQ yields.

Macroscopically, pancreata from marijuana users were darker in color and firmer on handling, compared to those from non-users. Compared to non-user islets, those from marijuana users appeared degranulated/mosaic and stained faintly with dithizone. Immunofluorescent staining showed that CBR1 expression was intensified in islets from chronic marijuana users compared to non-users. Moreover, statistically significant differences were found in the ability of transplanted islets to reverse diabetes in mice between the user and non-user groups ($p=0.019$). Islets from 33% of marijuana-users reversed diabetes when transplanted into mice (3 out of 9). In contrast, islets isolated from non-user pancreata reversed diabetes in 79% of cases (15 out of 19). Finally, at four weeks post-transplantation, islets from chronic marijuana users displayed fewer insulin-positive cells compared to islets from non-user groups.

CONCLUSIONS

Islets from chronic marijuana users more often fail to reverse diabetes upon transplantation into mice, suggesting that these cells are functionally inferior to those islets isolated from pancreata of marijuana-free donors.

This study was made possible by funding from the Wanek Family Project for Type 1 Diabetes.

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OA6
Genome Scale *in vivo* CRISPR Screen Identifies *RNLS* as a Modifier of Beta Cell Vulnerability in Type 1 Diabetes

AUTHORS

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[†] These authors contributed equally.

[*] These authors jointly supervised the study.

PURPOSE

We performed a genome-scale CRISPR screen in beta cells to search for gene mutations that protect against autoimmune killing. Our goal was to identify novel gene targets that could be modified in stem cell-beta cells to enable a cell replacement therapy for type 1 diabetes.

METHODS

We transduced the mouse NIT-1 beta cell line with a lentiviral CRISPR-Cas9 library at a low multiplicity of infection to generate a pool of mutant cells each carrying one of 60,000 possible mutations covering more than 20,000 genes. We leveraged the selective pressure of autoimmunity in the NOD mouse model to identify mutant cells capable of resisting immune-mediated killing *in vivo*. We validated the protective capacity our lead candidate gene in both mouse models and in human stem cell-derived beta cells.

SUMMARY OF RESULTS

Our genome-wide CRISPR screen in the NOD model identified only 11 genes whose mutation appeared to protect beta cells against autoimmunity. Remarkably, one of these genes was Renalase (*Rnls)*, a gene previously associated with the overall risk and the age-of-onset of human T1D by GWAS. Extensive validation in mouse models confirmed that *Rnls* deletion confered protection against immune-mediated killing. Significantly, protection was associated with ER stress resistance, and this phenotype was replicated in *RNLS* knockout human SC-beta cells. Although the function of *RNLS* is unknown, its crystal structure has been solved. Using structure-based modeling, we identified an FDA-approved drug that can be repurposed to inhibit *RNLS*. We show that oral drug treatment protected beta cells grafted into overtly diabetic NOD mice, leading to disease reversal.

CONCLUSIONS
We have identified the GWAS candidate gene *RNLS* as a modifier of beta cell vulnerability in T1D. We propose that *RNLS* could be targeted to protect stem cell-beta cells against autoimmunity and to enable a cell replacement therapy for T1D. Furthermore, we have identified an FDA-approved oral drug capable of protecting beta cells against autoimmunity. Based on its favorable safety profile in human and its efficacy in diabetic mice, we believe that this drug could be a promising candidate for clinical trial in patients with new onset T1D.

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☐ Immunology	☐ Other (list):

IMMUNOLOGY

OA7
Using HLA-DQ8 tetramers to diagnose and follow anti-islet autoimmunity

AUTHORS

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Scripps Research Institute
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PURPOSE

Develop and deploy HLA-DQ8 tetramers to study the pre-clinical phase of type 1 diabetes.

METHODS

Protein engineering and multi-step purification procedures were used to produce a series of usable HLA-DQ8 and HLA-DQ2/8 tetramers. Peripheral blood was sampled using fluorescent tetramers and single cell analysis following cell sorting. Analysis was performed using a home-made single cell analysis algorithm and visualization software.

SUMMARY OF RESULTS

Analysis from circulating insulin-specific cells allowed to stage disease in mouse and human samples. Normal HLA-DQ8 donors were compared to “at-risk”, just-diagnosed, and long-established patients. While total numbers of tetramer reactive cells were unchanged between groups, in this preliminary study state of activation seemed to be a reliable marker of disease activity.

CONCLUSIONS

State of activation of antigen-specific CD4 T cells in blood seems to offer the potential of being used to follow organ-specific autoimmunity and progression of disease.

Select the ONE category that best describes your research:

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☒ Immunology	☐ Other (list):

OA8
A role for Deaf1 in the regulation of cytotoxic/NK genes in Type 1 diabetes?

AUTHORS

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PURPOSE

Different antigen-presenting cells (APCs) including dendritic cells (DCs) have been found to lose their tolerogenic function in both T1D patients and NOD mice. Deregulated expression of transcriptional regulator Deaf1 was identified in pancreatic lymph nodes (PLNs) of T1D patients and NOD mice affecting expression of peripheral self-antigens required for tolerance maintenance. Both inflammation and hyperglycemia promote induction of dominant negative spliced variant of Deaf1. We initially hypothesized that defective Deaf1 function in DCs may negatively impact tolerance and contribute to progression of the disease. However, when performing mRNA sequencing on sorted CD11c+ cells from wild type and Deaf1-deficient mice, we observed an enhanced NK/cytotoxic signature in the absence of Deaf1 and confirmed that some NK cells express CD11c, prompting us to hypothesize that impaired Deaf1 function may also exacerbate cytotoxic functions and the progression of T1D. We further hypothesize that reduced function of DEAF1 in human PLNs may also affect NK cell populations and function.

METHODS

Conditional Deaf1 knockout (floxed Deaf1) mice were crossed with Cre-transgenic strains, whereby Cre is driven by CD11c or inducible CAG promoter. Lymph nodes cells and splenocytes from the different Deaf1 conditional knockout models were analyzed by multi-parameter flow cytometry and populations of interest were sorted for RNA-sequencing and qPCR. Follow-up studies include a comprehensive phenotypic analysis of NK and CD8+ T cell populations by spectral flow cytometry and multiplexed immunofluorescence by Vectra, all using fresh PLN tissue or sections from control and T1D patients obtained from nPOD.

SUMMARY OF RESULTS

Deletion of Deaf1 in CD11c+ cells resulted in a small but significant reduction of tissue resident and plasmacytoid DCs. Surprisingly, mRNA sequencing data from splenic CD11c+ cells identified a significant increase of cytotoxic/NK signature genes in absence of Deaf1, likely contributed by CD11c+ NK cells. Indeed, NK1.1+ CD49b+ NK cells from CD11c-Cre mice lost Deaf1 expression in CD11c+ cells but not CD11c- cells. We observed an increased frequency of NKG2D+, Ly49d+ and Ly49h+ NK cells in Deaf1-deficient mice by flow cytometry. Human blood CD56+ CD11c+ and CD56+ CD11c- NK cells express DEAF1 at similar level. Studies are ongoing to assess whether Deaf1 deficiency affects NK cells (and possibly CD8+ T cells) as a whole in mouse models and whether these populations and their markers are altered in the PLNs of T1D patients where DEAF1 function is impaired. Although controversial, NK cells have been described to have a pathogenic role in T1D by infiltrating islets and contributing to beta cell killing.

CONCLUSIONS
Impaired DEAF1 function in PLNs from T1D patients may contribute to T1D disease progression by exacerbating expression of cytotoxic genes. Data to be presented will address whether subsets of CD11c+ and NK cells are affected by Deaf1 deficiency and whether impaired DEAF1 function in PLNs of T1D patients results in alterations in cytotoxic cell populations.

Select the ONE category that best describes your research:	
☐ Beta Cell Physiology and Dysfunction	☐ Novel Biomarkers
☐ Beta Cell Development, Differentiation & Regeneration	☐ Novel Technologies
☐ Bone Marrow Studies	☐ Pathology
☐ Core Lab	☐ Type 1 Diabetes Etiology & Environment
☒ Immunology	☐ Other (list):

OA9

Role of X cell-secreted mAb (x-mAb) in the pathogenesis of T1D

AUTHORS

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****Presenter***

PURPOSE

We have recently discovered a new cell type that combines characteristics of B and T cells, including expression of TCR and BCR (Ahmed *et al*, Cell, 2019: 177:11583). We refer to this new lymphocyte as X cell to denote its crossover phenotype. Importantly, X cells express a public BCR that also encodes a potent autoantigen in its CDR3 sequence that is about 10-orders more potent than native insulin peptide (InsB:9-23) in binding to DQ8 and activating autologous CD4 T cells. The x-autoantigen cross-activate insulin specific CD4 T cells as a peptide in the context of HLA-DQ8 molecules or as a soluble intact mAb (x-mAb). The goal of this study is to characterize autoreactive CD4 T cells that are responsive to x-mAb to determine their phenotype, cytokine profile and TCR repertoire and whether they express public TCRs.

METHODS

We use an EBV-lymphoblastoid X cell clone as a source of x-mAb (IgM isotype) and a FACS-based protocol to identify x-mAb-reactive CD4 T cells (referred to as (IgM^{pos}) in peripheral blood of T1D patients and HCs. We are characterizing TCR repertoires of sorted IgM^{pos} CD4 T cells using ImmunoSEQ assay, and their functional properties using intracellular cytokine analysis and activation phenotype using surface staining.

SUMMARY OF RESULTS

Our preliminary data show that frequency of IgM^{pos} CD4 T cells is significantly higher in T1D patients as compared to Healthy subjects. In addition, IgM^{pos} CD4 T cells exhibit an activated phenotype as compared to autologous IgM^{neg} CD4 T cells, including expression of CD45RO, CD44, and CD69. In addition, the majority of IgM^{pos} CD4 T cells produce TNFα as compared to IgM^{neg} CD4 T cells. Analysis of TCRVβ repertoire shows that IgM^{pos} CD4 T cells are enriched for public clonally-expanded TCRs as compared to IgM^{neg} counterparts.

CONCLUSIONS

X cells in T1D patients are predominated by a single public BCR and that the secreted version of this BCR (x-mAb) is autoreactive against a specific subset of CD4 T cells that predominated by few clonotypes that express public TCRs. Currently, we examining antigen specificities of IgM^{pos} CD4 T cells and their homing properties. Our results are revealing previously unknown mechanism that appears to be a play critical role in pathogenesis of T1D.

Select the ONE category that best describes your research:	
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☐ Beta Cell Development, Differentiation & Regeneration	☐ Novel Technologies
☐ Bone Marrow Studies	☐ Pathology
☐ Core Lab	☐ Type 1 Diabetes Etiology & Environment
☒ Immunology	☐ Other (list):

NOVEL BIOMARKERS

OA10
Normal cellular prion protein is highly expressed in beta cells of type 1 diabetes human pancreata

AUTHORS

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PURPOSE

The abnormally folded isoform of prion protein (PrP^{SC}) has been mostly studied for its role in the development of transmissible spongiform encephalopathies (TSEs) and for its critical role in pathogenesis of neurodegenerative disorders like Alzheimer's disease (AD). However, loss of the normal prion protein (PrP^{C}) function in the brain has shown to be an integral part of neurodegenerative processes. Over the years PrP^{C} has been associated with a wide array of different cellular mechanisms and many interacting partners have been suggested. Some studies have proposed a role in copper and iron homeostasis in the brain. More recent studies have confirmed its participation in physiological processes such as regulation of cellular differentiation and proliferation, and control of cell morphology. As neurons and pancreatic β cells share abundant functional similarities, we examined PrP^{C} expression, a protein found predominately in neurons, in type 1 diabetes (T1D) and non-diabetic (ND) human pancreas. Moreover, we addressed differences between the expression of PrP^{C} in insulin positive (INS^{+}) versus insulin negative (INS^{-}) pancreatic islets in short duration T1D pancreata.

METHODS

Pancreata for this study were recovered from T1D and ND organ donors with informed consent from next of kin and processed by the Network for Pancreatic Organ donors with Diabetes (nPOD) program. PrP^{C} mRNA expression levels were measured by means of RT-qPCR in pancreata from 30 T1D and 30 ND. Normalization factors were applied to the analysis to report quantitative changes in gene expression between non-diabetics and T1D pancreata, with the fold difference (FD) reported as the ratio of the means (T1D/ND).Paraffin embedded pancreatic tissue sections from 12 T1D (with residual insulin positive islets) and 12 ND organ donors were stained for PrP^{C}, insulin (INS), glucagon (GCG) and somatostatin (SOM) by immunofluorescence. Imaging was performed using a BZ-X710 Keyence florescence microscope. Immunofluorescence brightness was measured to assess a degree of prion protein expression in INS^{+} and INS^{-} pancreatic islets and it was calculated by means of Integration (value of multiplying the area of extracted part by the mean brightness before binarization) using the Hybrid Cell Count software from Keyence®. Consecutive tissue sections, were stained with Thioflavin-t and Congo red to determine the presence of amyloid fibrils and aggregates. To assess metal deposition in T1D versus ND pancreas histochemical stains for copper and iron were performed using commercially available kits.

SUMMARY OF RESULTS

With total mRNA isolated from each pancreas using gene specific oligonucleotide primer sets, expression levels of the PrP^{C} gene in T1D and ND donors was tested. Normalized RTqPCR data revealed significant increase in PrP^{C} expression in T1D versus ND pancreata, with a mean FD of =10.85 ($p=<0.0001$).

At the cellular level and based on immunofluorescence analysis (IF), PrP^{C} was found to be homogeneously distributed in the cytoplasm of T1D and ND donors. Foremost, we found that in ND pancreas as well as in residual INS^{+} islets of T1D pancreata, PrP^{C} was expressed mostly in β cells. In contrast, in INS^{-} islets we found that PrP^{C} localized generally with α-cells. Interestingly, in both INS^{+} and INS^{-} islets we observed PrP^{C+} cells that were negative for insulin, glucagon and somatostatin. Based on our IF quantitative analysis we found that the overall intensity of PrP^{C} expression was significantly higher in INS^{+} versus INS^{-} islets in T1D pancreas with residual INS^{+} islets. In addition, we found that within INS^{+} islets T1D donors, PrP^{C} intensity in INS^{+} cells was significantly higher than in adjacent PrP^{C+}/INS^{-} cells.
In neurodegenerative disorders such as TSEs and AD it has been shown that the accumulation of the modified form of prion protein (PrP^{SC}) into amyloid deposits is one of the leading reasons for the onset of these diseases. To assess the presence of amyloid aggregates and their possible correlation with PrP^{SC}, the pancreatic tissue sections from T1D organ donors were stained with Thioflavin-t and Congo red. Only 3/12 T1D cases showed potential amyloid deposits within the islets, which most interestingly did not display co-expression with PrP^{C}. Finally, histochemical staining showed no positivity for either copper or iron in T1D or ND donors, which conforms with new literature suggesting a weak involvement of PrP^{C} in copper/iron hemostasis and stress protection

CONCLUSIONS

To our knowledge, this is the first study to examine the presence of normal cellular prion protein in human pancreata, specifically in donors with type 1 diabetes. We observed a significant increase in PrP^{C} mRNA level in T1D donors, suggesting a possible link between T1D and PrP^{C}. Our staining results indicate that PrP^{C} localizes and is expressed mostly within β cells in either ND or in residual INS^{+} islets of T1D donors. Most strikingly, in both INS^{+} and INS^{-} islets of T1D pancreata, we observe PrP^{C} expression in cells lacking prominent endocrine cell markers. Future studies in live human pancreatic slice cultures will attempt to address the mechanistic implications of our findings and the potential role of PrP^{C} in type 1 diabetes pathogenesis.

Select the ONE category that best describes your research:

☐ Beta Cell Physiology and Dysfunction	☒ Novel Biomarkers
☐ Beta Cell Development, Differentiation & Regeneration	☐ Novel Technologies
☐ Bone Marrow Studies	☐ Pathology
☐ Core Lab	☐ Type 1 Diabetes Etiology & Environment
☐ Immunology	☐ Other (list):

NOVEL TECHNOLOGIES

OA11
D-peptides as a novel targeted immunotherapy for Type 1 Diabetes

AUTHORS

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PURPOSE

HLA-DQ8 has been shown to present antigenic islet peptides driving the activation of CD4+ T-cells in Type 1 Diabetes (T1D) patients. Specifically, the insulin peptide InsB:9-23 activates self-reactive CD4+ T-cells, causing pancreatic beta cell destruction. The aim of the current study was to identify D-amino acid based peptides (D-peptides) that can block T-cell activation by antagonizing the presentation of InsB:9-23 peptide within the HLA-DQ8 pocket.

METHODS

To achieve our aim we used the following tools: human B cells homozygous for HLA-DQ8, transgenic mice expressing human HLA-DQ8, and peripheral blood mononuclear cells (PBMCs) from new onset HLA-DQ8 T1D patients.

SUMMARY OF RESULTS

We identified one D-peptide (RI-EXT) that inhibited InsB:9-23 binding to recombinant HLA-DQ8 molecule, as well as its binding to HLA-DQ8 expressed on a human B-cell line. Specifically, RI-EXT averted T-cell activation in a mixed lymphocyte reaction containing human DQ8 cells loaded with InsB:9-23 peptide and murine T-cells expressing a human TCR specific for the InsB:9-23–DQ8 complex. These results were confirmed in transgenic DQ8 mice both *ex vivo* and *in vivo,* as shown by decreased production of IL-2 and IFN-γ and reduced lymphocyte proliferation. Importantly, RI-EXT inhibited InsB:9-23-mediated lymphocyte activation in peripheral blood mononuclear cells isolated from new onset DQ8-T1D patients.

CONCLUSIONS

In summary, we discovered a D-peptide that blocks InsB:9-23 binding to HLA-DQ8 and its presentation to T-cells in T1D. These data set the stage for using our approach of blocking antigen presentation by D-peptides as a novel therapeutic approach for autoimmune diseases.

Select the ONE category that best describes your research:	
☐ Beta Cell Physiology and Dysfunction	☐ Novel Biomarkers
☐ Beta Cell Development, Differentiation & Regeneration	☒ Novel Technologies
☐ Bone Marrow Studies	☐ Pathology
☐ Core Lab	☐ Type 1 Diabetes Etiology & Environment
☐ Immunology	☐ Other (list):

OA12

Automated human islet cell CD3+ lymphocyte quantification in multiplex IHC-stained whole slide scans using computer vision algorithms

AUTHORS

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PURPOSE

Around 100 million adults in the United States of America suffer from diabetes. This disease is characterized by its high complexity and disruption of the quality of life in the patients. Due to these factors, a significant amount of effort has been made to acquire datasets that can help lead to further understand pathogenic mechanisms for future treatment development. Multiplex immunohistochemistry (mIHC) whole slide scans are one of the growing datasets available to study this disease. Currently, analyses performed on mIHC slide scans are based on mixture of qualitative analysis made by a pathologist and manual tracing of the structures for further quantitative analysis. These methods are characterized by long processing times and the presence of human factor including error and biases.

METHODS

To assess these problems, we developed a computer vision algorithm that can extract structural and shape descriptors such as total area of the islet, total area of glucagon and insulin, distribution across the tissue, etc. from single cell to entire islet structures. In addition, we provide the capability of quantifying CD3+ cells to determine the presence of inflammation in the islet (<20um islet periphery) and as single cell counts.

SUMMARY OF RESULTS

We provide an algorithm that automates the analysis of mIHC whole slide scans of the pancreas. This algorithm was tested in 154 images of different pancreas regions with a range of 8 patients with type 1 diabetes. Additionally, this algorithm is capable of quantifying CD3+ cells to determine the presence of inflammation on the islet (insulitis) and single cells. Moreover, we were able to accelerate the processing time to an approximate of 20 minutes for a 3-stain (glucagon, insulin and CD3) mIHC pancreas image of size 250Mb.

CONCLUSIONS

By providing this standardized method we offer an opportunity to reduce the presence of human error or bias across the analysis. Furthermore, we were able to develop an automated detection and quantification algorithm that aims to reduce the processing time while greatly increasing the data that can be extracted for these images compared with previous methods. This algorithm was developed on Python using a mixture of image processing and machine learning algorithms. The future goal is to improve the capabilities of the algorithm by introducing the possibility of analyzing immunofluorescence images and increasing the number of mIHC chromogens that can be determined on a given section.

Select the ONE category that best describes your research:	
☐ Beta Cell Physiology and Dysfunction	☐ Novel Biomarkers
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☐ Core Lab	☐ Type 1 Diabetes Etiology & Environment
☐ Immunology	☐ Other (list):

OTHER

OA13

The CD8+ T-cell response against Coxsackievirus is focused on few selected epitopes: implications for vaccination trials

AUTHORS

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PURPOSE

Despite increasing evidence for an association between Coxsackievirus B (CVB) infection and type 1 diabetes (T1D), the identity of the viral peptides naturally presented by infected beta cells and the nature of the anti-CVB cytotoxic CD8+ T-cell response are unknown. These questions are relevant in light of upcoming T1D prevention trials exploring the effect of vaccination against CVB. We therefore aimed at identifying the HLA Class I (HLA-I) viral peptidome of CVB-infected beta cells and at characterizing the CD8+ T-cell response against these peptides.

METHODS

Human beta-cell lines were infected with either CVB1 or CVB3. The HLA-I-bound peptides were identified by mass spectrometry and those restricted for HLA-A2 or –A3 were retained. This list was complemented by a parallel *in silico* search. After confirming the *in vitro* binding of these peptides to HLA-A2/A3, we tested their recognition by circulating CD8+ T cells from CVB seropositive donors by combinatorial HLA-I multimer assays, and CD8+ T-cell clones were generated. The peptides recognized were further studied in nPOD splenocytes.

SUMMARY OF RESULTS

The repertoire of HLA-I-bound viral peptides displayed by infected beta cells was limited to few selected sequences, largely overlapping between CVB1 and CVB3 serotypes. Only a fraction of these peptides was recognized by circulating CD8+ T cells from seropositive donors. Moreover, few of these epitopes were associated with cognate CD8+ T cells displaying an effector/memory phenotype, indicating that the anti-CVB immune memory is restricted to even fewer epitopes. Overall, CD8+ T-cell responses were dominated by only 2 peptides in most individuals, both in the blood and in the spleen. CD8+ T-cell clones raised against one of these peptides were cytotoxic against target cells pulsed with their cognate peptide.

CONCLUSIONS

Surprisingly, CVB infection seems to induce a limited CD8+ T-cell memory response in terms of antigen coverage. This feature could favor repeated or chronic infections, thus lending rationale to CVB vaccination trials. The next questions are whether this poor memory response is found preferentially in T1D-prone patients,

and whether the peptides identified can be used to evaluate the efficacy of CVB vaccines at boosting responses against dominant and subdominant epitopes.

Select the ONE category that best describes your research:	
☐ Beta Cell Physiology and Dysfunction	☐ Novel Biomarkers
☐ Beta Cell Development, Differentiation & Regeneration	☐ Novel Technologies
☐ Bone Marrow Studies	☐ Pathology
☐ Core Lab	☐ Type 1 Diabetes Etiology & Environment
☐ Immunology	☒ Other (list): **nPOD-Virus**

PATHOLOGY

OA14
IL-17 is expressed in insulin-containing islets of donors with type 1 and type 2 diabetes

AUTHORS

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PURPOSE

IL-17 is a pro-inflammatory cytokine, important in shaping host immune responses against pathogens. IL-17 is also attributed to cause tissue damage and chronic inflammation in autoimmune diseases such as psoriasis, rheumatoid arthritis and multiple sclerosis. An increase in IL-17 secreting CD4+ T cells and Th17 cells were observed in pancreatic lymph nodes and peripheral blood of subjects with type 1 diabetes (T1D). However, it is not known whether IL-17 is expressed in human pancreata in health and during T1D or T2D pathogenesis. Here, we report that IL-17 is expressed in the islet cells of donors with type 1 and type 2 diabetes.

METHODS

IL-17 antibody was first optimized in human tonsils. We then stained human pancreatic FFPE tissues sections from non-diabetic (n=3), autoantibody positive (n=3), T1D (n=4) and T2D (n=3) donors for IL-17, Insulin and Glucagon. Whole tissue imaging was performed using axio scanner. 30-40 islets were randomly cropped across the pancreatic tissue and percentage of IL-17 positive area was quantified through Image pro software. Results were cross-verified by acquiring high resolution images of at least 10 islets per case by confocal LSM780 followed by subsequent analysis in Zen.

SUMMARY OF RESULTS

In all non-diabetic and auto-antibody positive cases, IL-17 staining was very weak or punctate and sparse in pattern, accounting for less than 5% of total islet area. In donors with T1D, insulin containing islets had a clear and markedly stronger cytoplasmic expression of IL-17 in islet cells, accounting to an average of 8.6% of total islet area. IL-17 expression was almost completely lost in insulin-deficient islets of all T1D donors. IL-17 expression was even more increased in islets of donors with T2D, constituting to an average of 26.26% of islet area. Most of the IL-17 production was accounted by either beta or alpha cells, while in many cases beta cells were the major source.

CONCLUSIONS

According to literature, expression of IL-17 is usually restricted to Th17, $\gamma\delta$ T cells and some cell types of innate immune system. Our finding that IL-17 can be expressed in islet cells of T1D or T2D is quite intriguing. Further functional studies in islet organoid models are required to determine if a metabolic stress or immune stress can induce IL-17 expression in human islets.

Select the ONE category that best describes your research:

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☐ Core Lab	☐ Type 1 Diabetes Etiology & Environment
☐ Immunology	☐ Other (list):

OA15
Tertiary Lymphoid Organ-like Structures Associate with Insulin Containing Islets in Human Type 1 Diabetes

AUTHORS

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PURPOSE

Tertiary lymphoid organs (TLOs) occur mainly in tissues with long-standing inflammation resulting from autoimmunity, infection or cancer (1-3). They show structural and functional similarities to lymph nodes (LNs) and modulate disease outcome. We have shown that TLO-like structures are present not only in NOD mice during development of the disease, but also in human type 1 diabetes (T1D) with insulitis where they are characterized by accumulations of inter-mixed T and B-cells and, in rare cases, B-cell follicles; the presence of a fibroblastic reticular network, and high endothelial venules. Furthermore, we detected proliferating leukocytes, plasma cells and memory T cells in pancreas samples with extensive insulitis, suggesting that TLO-like structures might contribute to perpetuation of human T1D (4). We, therefore, investigated whether is there any correlation between the frequency of TLO-like structures in the pancreas and the development of human T1D.

METHODS

Immunofluorescence stainings were performed on cryo- or paraffin-sections of pancreata with insulitis from single (n=1) and double autoantibody positive (aAb+) (n=3), T1D donors (n=17) using markers for inflammatory cells (CD45), β-cells (insulin) and basement membrane (pan-laminin, PLM). The sections were analyzed by confocal microscopy. Quantification of TLOs was performed on PLM/Insulin/CD45 stained sections.

SUMMARY OF RESULTS

The quantification data did not show a direct correlation between the presence of TLOs and the age of disease onset or disease duration, which could be due to low sample numbers for certain groups. However, the quantification of TLOs in the different samples revealed the presence of TLOs in double aAb+ but not in single aAb+ samples. The frequency of TLOs was significantly higher in young donors (age of onset < 10 years) and in T1D samples with immune cell aggregates. However, TLOs were not present in T1D donors with insulitis composed of dispersed immune cells. The TLOs were mostly associated with insulin positive rather than with insulin negative insulitis. In only two cases, TLOs were associated with insulin negative, pseudo-atrophic islets. Additionally, we identified TLOs associated with the pancreatic duct, where insulin positive cells were interdispersed among duct epithelial cells, which may suggest that β-cell neogenesis takes place and the immune cells sense the presence of antigen.

Furthermore, TLO-like structures occurred both in biopsies and in explants of transplanted donor samples in association with insulin positive islets, which suggests the reappearance of the disease and potentially that the formation of TLOs may contribute to disease reappearance.

CONCLUSIONS

Association of pancreatic TLOs with insulin positive and, rarely, with insulin negative islets suggests that they may contribute to exacerbation of disease and that they disappear once the autoantigen producing cells, the β-cells, are destroyed, analogous to pancreatic TLOs in NOD mice.

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4. Korpos et al., Tertiary lymphoid organs in mouse and human autoimmune diabetes. Manuscript in preparation.

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☐ Bone Marrow Studies	☒ Pathology
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☐ Immunology	☐ Other (list):

TYPE 1 DIABETES ETIOLOGY AND ENVIRONMENT

OA16
Coxsackievirus B4 induces the expression of Human Endogenous Retrovirus W (HERV-W) in primary cells

AUTHORS

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PURPOSE

Human endogenous retrovirus type W (HERV-W) has been linked to multiple sclerosis (MS). In MS, the envelope protein of HERV-W has pathological features including the activation of auto-immunity by super-antigen properties, and induction of inflammation. Moreover, it has been shown that HERV-W Env expression could be transactivated by the infection with HHV-6, a virus linked to MS. T1D is a virus-associated auto-immune disease, and a recent study showed that HERV-W Env could be found in blood and pancreas of patients with T1D. T1D being closely associated to coxsackievirus B (CV-B) infections, we investigated the expression of HERV-W in human cells upon infection with CV-B4.

METHODS

Monocytes were selected by adherence from peripheral blood mononuclear cells obtained from the blood of 14 donors. Monocytes were treated with M-CSF for 7 days to obtain primary macrophages, that were infected with CV-B4. Primary human pancreatic ductal cells obtained from the pancreas of 5 brain-dead donors were infected with CV-B4. The levels of HERV-W Env m RNA and of HERV Env protein were evaluated by RT-qPCR and by immunoblot respectively..

SUMMARY OF RESULTS

In macrophage cultures of 6 donors, the level of HERV-W Env m RNA was upregulated when the cultures were inoculated with CV-B4 compared to controls. The levels of HERV-W Env mRNA, measured in pancreatic ductal cells harvested 16 and 48 hours after CV-B4 inoculation, were also upregulated upon CV-B4 infection in pancreatic cell cultures of 4 brain-dead donors. Moreover, in these cells, the level of HERV-W Env protein was high compared with controls.

CONCLUSIONS

These results indicate that the infection with CV-B4 can upregulate the expression of the envelope-encoding gene and/or transactivate certain copies of the HERV-W family in cultures of human macrophages and pancreatic cells.

Select the ONE category that best describes your research:

☐ Beta Cell Physiology and Dysfunction	☐ Novel Biomarkers
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☐ Bone Marrow Studies	☐ Pathology
☐ Core Lab	☒ Type 1 Diabetes Etiology & Environment
☐ Immunology	☐ Other (list):

OA17
Rhinovirus RNA in the spleen of nPOD donors

AUTHORS

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PURPOSE

Human rhinoviruses (RVs) are members of the enterovirus genus. Enteroviruses have been linked to type 1 diabetes (T1D) but only a limited number of studies have been done on the possible role of RVs in T1D. RVs cause typically upper respiratory tract infections, but certain RVs can also replicate in higher temperatures causing infections in the lower respiratory track. RVs are also very common in stool samples of young children (Honkanen et al 2013), raising the question whether replication may occur in the intestine and in the other parts of the body. However, it is not known whether these viruses can infect the pancreas or other internal organs.

METHODS

Study population consisted of snap-frozen pancreas, spleen and duodenum samples from 73 different nPOD donors, belonging to three different donor groups; type 1 diabetics (N=29), non-diabetics (N=31) and diabetes-related autoantibody-positive (AAb+) subjects (N=13). The age of the donors ranged from 1,5 to 75 years (mean 31 years; median 30 years). The presence of RV RNA was analyzed using an RT-PCR method followed by liquid-phase hybridization as previously described (Lönnrot et al 1999).

SUMMARY OF RESULTS

Altogether 53 pancreas, 66 spleen and 57 duodenum samples were analyzed for the presence of RV in these tissues. As a result, all pancreas and duodenum samples were negative, however, RV-positivity was found in eight of the 66 spleen samples. From five of the eight samples, we were able to find RV sequence and two of these were genotyped to belong to RV-A, one to RV-B and two to RV-C, respectively. Six of the donors positive for RV were non-diabetics and the remaining two were T1D and AAb+ donors, respectively. Young age correlated to RV-positivity ($p<0.005$); six of the 8 donors were young children (ages 1,5 to 6,3 years). In this cohort only eight donors were under nine years of age (between 1,5 and 8,9 years) and six of these (75%) were RV positive in the spleen.

CONCLUSIONS

This is the first report showing that in addition to upper respiratory and stool samples, RV RNA can occasionally be found also in the spleen, especially among young children. Whether RVs can replicate in the spleen, or whether they were present for example in circulating cells such as macrophages or lymphocytes is not known. Further studies are needed to address these questions.

Select the ONE category that best describes your research:

☐ Beta Cell Physiology and Dysfunction	☐ Novel Biomarkers
☐ Beta Cell Development, Differentiation & Regeneration	☐ Novel Technologies

☐ Bone Marrow Studies	☐ Pathology
☐ Core Lab	☒ Type 1 Diabetes Etiology & Environment
☐ Immunology	☐ Other (list):

OA18
Characterizing the virome of nPOD pancreatic donor islets using comprehensive capture sequencing

AUTHORS

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PURPOSE

Over a century of experimental and epidemiological data support the role of viruses in the development of type 1 diabetes. The most compelling evidence in recent years has been the detection of enterovirus RNA and protein in the Network for Pancreatic Organ Donors with Diabetes (nPOD) specimens and in pancreatic biopsy samples of new onset individuals. Viruses appear to be present in very few cells within the pancreas at ultra-low copy numbers, often below the limit of PCR detection. The purpose of this study was to determine whether comprehensive virome capture sequencing (VirCapSeq-VERT) can improve low-level enterovirus detection from nPOD specimens and identify additional viruses that may be potentially involved in disease pathogenesis.

METHODS

Pancreatic islet cells were obtained from a single donor with established type 1 diabetes. Purified islet cells were maintained in culture for 15 days untreated and in co-culture with HEK cells expressing viral proteases (2Apro and 3Cpro). Supernatants were collected on days 1, 2, 4, 6, 8, 11 and 15. Cells were harvested at day 0, 11 and 15. Total nucleic acid was extracted using the MagMax isolation kit and treated with Turbo DNase. RNA was converted to cDNA using Superscript III with random hexamers and Klenow fragment. Sequencing libraries were prepared using KAPA HyperPlus (Roche) with unique dual-index adapters. VirCapSeq-VERT enrichment of library fragments containing vertebrate-infecting viral genome sequences was performed following the SeqCap protocol (Roche). Post-capture library pool was sequenced on the Illumina NextSeq500 at UNSW Ramaciotti Center for Genomics, Sydney, Australia. Sequencing analysis (*de novo* assembly and taxonomic classification) was performed using three published bioinformatic pipelines: VirusTAP, Genome Detective and ViromeScan.

SUMMARY OF RESULTS

Sequences corresponding to enterovirus could not be identified in any of the supernatants and cell extracts harvested from the untreated cells. In contrast, all samples collected from cells co-cultured with HEK cells expressing a viral protease construct (2Apro and 3Cpro) produced a large number of reads corresponding to the 2A and 3C protease regions of poliovirus 1. High number of reads corresponding to human papillomavirus

18 and human adenovirus 5 were detected across all timepoints of both untreated and co-cultured islets, but not at day 0. No human viruses were detected in the two negative controls (media only). Consistent with previous studies, bovine viruses (bovine diarrhea virus and bovine parvovirus 3) were readily detected across all samples including the negative controls as a common contaminant of cell culture media and fetal bovine serum. Overall, there was good concordance on the range of viruses detected and the number of viral reads between the three bioinformatic pipelines used.

CONCLUSIONS

This was a pilot and proof-of-concept study conducted using pancreatic islets harvested from a single donor. In this preliminary analysis, no clinical enteroviruses were detected. Testing of additional donor islets is needed to evaluate the performance of VirCapSeq-VERT in detecting low-level enterovirus presence, as well as to investigate the presence of other viruses that may be important in the pathogenesis of type 1 diabetes. If higher sensitivity is demonstrated, we anticipate routine application of VirCapSeq-VERT to screen pancreatic tissue or laser captured islets from nPOD organ donors with type 1 diabetes or islet autoantibodies. Determining the complete breadth and genotype of viruses that contribute to type 1 diabetes is paramount for the development of viral vaccines for primary and secondary prevention.

Select the ONE category that best describes your research:

☐ Beta Cell Physiology and Dysfunction	☐ Novel Biomarkers
☐ Beta Cell Development, Differentiation & Regeneration	☐ Novel Technologies
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☐ Core Lab	☒ Type 1 Diabetes Etiology & Environment
☐ Immunology	☐ Other (list):

OA19
Enterovirus RNA detected in the pancreatic islets isolated from a type 1 diabetic organ donor in the nPOD study

AUTHORS

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PURPOSE

In the nPOD-Islet Isolation Program, islets are recovered from individuals with type 1 diabetes (T1D) having a disease duration of < 3 years, with aims including characterizing the existing knowledge gaps in T1D development. Enteroviruses have been linked to the development of T1D in multiple studies, however, individual/isolated islets of T1D patients with short disease duration have not been widely studied.

METHODS

Islets were isolated from a nPOD donor 6480 (male, 17 yrs), with T1D duration of 2.5 years, as part of nPOD-IIP. The cause of death was ketoacidosis and the patient had several insulin containing islets. Isolated islets were subsequently co-cultured with cell lines that are permissive for enteroviruses for 15 days at Baylor College of Medicine (BCM). Culture supernatants and cultured cell specimens collected at several timepoints were analyzed for the presence of enterovirus RNA using sensitive RT-PCR independently in two virus laboratories (BCM and Tampere University).

SUMMARY OF RESULTS

Enterovirus RNA was detected in the cultured islets in both laboratories. The Tampere laboratory found virus in six samples taken at different time points during the culture and also obtained enterovirus-specific sequence in four of them. The BCM laboratory found enterovirus in eight samples and recovered virus sequence from four. The sequences were varied, matched best with coxsackievirus B3, echovirus 13 and EVA71 Genbank submissions, but reliable identification of the virus serotypes was not possible using highly conserved 5' UTR sequences.

CONCLUSIONS

The results suggest that enterovirus RNA was present in the pancreatic islets of this T1D patient, likely representing multiple enteroviruses. The results are in agreement with previous studies showing enterovirus proteins in the pancreatic beta cells in T1D patients.

Select the ONE category that best describes your research:

☐ Beta Cell Physiology and Dysfunction	☐ Novel Biomarkers

☐ Beta Cell Development, Differentiation & Regeneration	☐ Novel Technologies
☐ Bone Marrow Studies	☐ Pathology
☐ Core Lab	☒ Type 1 Diabetes Etiology & Environment
☐ Immunology	☐ Other (list):

OA20

HERV-W-ENV protein detection in pancreas of T1D patients and first step of clinical development in T1D of temelimab, a monoclonal antibody neutralizing HERV-W-ENV.

AUTHORS

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PURPOSE

HERV-W-ENV is a human endogenous retroviral protein that has been initially described to be involved in the pathogenesis of Multiple Sclerosis (MS), in which it exerts inflammatory and neurodegenerative effects and precludes remyelination. Temelimab is a humanized monoclonal antibody which neutralizes this pathogenic protein, and it has demonstrated positive results on neuroprotection in a phase IIb efficacy trial on MS patients. HERV-W-ENV has recently been described to be present in the pancreas of Type 1 Diabetes (T1D) patients by Immuno-Histo Chemistry (IHC) and to inhibit insulin secretion *in vitro*. This pathogenic protein is also associated with macrophages recruitment in the pancreas of T1D patients.

The purpose of this study is (I) to confirm the expression of HERV-W-ENV in pancreas of T1D patients using capillary western blot and (II) to present the results of the RAINBOW study, the first clinical trial assessing the safety of temelimab in T1D patients.

METHODS

Total protein extraction was performed from cryopreserved pancreas pieces. Proteins from the soluble fraction have been deglycosylated and concentrated on a 100kDa Amicon column, before analysis by capillary western blot on a WES device (Protein Simple) under denaturating conditions following manufacturer instructions. Migration has been performed on a 66-440 kDa separation matrix and biotinylated GN_mAb_Env01 antibody has been used for the detection. Quantification has been performed by measuring the Area Under the Curve of the 400kDa peak.

The RAINBOW study is a phase IIa, double-blind placebo-controlled randomized clinical trial. Adult patients, diagnosed with T1D within 4 years prior to entering the study and with remaining C-peptide (≥0.2nmol/L in a mixed meal tolerance test) were recruited. Sixty four patients were randomized (2:1) to monthly temelimab 6 mg/kg or placebo during 24 weeks, followed by an optional 24-week open-label extension during which 45 patients participated and all of them received temelimab. The primary objective was to assess the safety and tolerability of temelimab in T1D patients. The secondary objective was to assess pharmacodynamic responses to temelimab on biomarkers of T1D (C-peptide level, insulin use, glycated hemoglobin (HbA1c), hypoglycemic episodes and T1D auto-antibodies).

SUMMARY OF RESULTS

HERV-W-ENV has initially been detected with GN_mAb_Env03 antibody in the pancreas of T1D patients by IHC on a cohort provided by nPOD (19 control donors and 20 T1D donors). The quantification of the area positively stained revealed that 75% of T1D donors were positive versus only 16% of non-T1D control donors ($p<0.001$) (Levet et al., JCI insight, 2017). The subsequent step was to confirm the presence of HERV-W-ENV

in the pancreas by another technique using a detection antibody targeting a different epitope. For a feasibility study, 3 control donors negative for HERV-W-ENV and 2 T1D donors positive for HERV-W-ENV were selected from the initial cohort. Cryopreserved pancreas pieces were obtained from these donors, and soluble protein extracts were analyzed by capillary western blot, using GN_mAb_Env01 antibody, the parental murine antibody of temelimab. A strong specific signal was observed at 400 kDa in T1D pancreas but was nearly absent in control pancreas. This signal is confirmed by the quantification of the AUC under the 400 kDa peak: 1076.10^3 ±146.10^3 in T1D vs 127.10^3 ±122.10^3 in controls (arbitrary units, mean ±SEM). The molecular weight of this signal at 400 kDa suggests a hexameric form of HERV-W-ENV (Charvet et al., Oral communication during 3rd HERV and Diseases meeting 2019).

From these results and from others previously published, it appeared that HERV-W-ENV may be a novel therapeutic target in T1D. Temelimab has already been safely administered in several clinical trials in MS patients, including a phase IIb (Curtin et al., MSARD, 2016). This humanized IgG4 antibody neutralizing HERV-W-ENV has then been assessed in T1D patients during a phase IIa safety clinical trial (NCT03179423). The primary endpoints (safety and tolerability) of this trial has been achieved and temelimab revealed to be safe and well tolerated by adult T1D patients with a stable and well controlled T1D at inclusion. Concerning secondary endpoints, pharmacodynamic parameters such as HbA1C, C-peptide and insulin dose remained stable throughout this safety study. However, positive signals emerged regarding hypoglycemic events and insulin autoantibodies. A reduction of 28% of frequencies of hypoglycemic episodes under temelimab during the first 6 months has been observed ($p<0.0004$ vs placebo; Poisson regression). An additional reduction of 10% has been observed in the 6 additional months. Concerning the anti-insulin autoantibodies, a reduction of 35% has been observed under temelimab over one-year ($p<0.009$; ANCOVA). Whether these two positive signals correspond to an early response to temelimab remains to be further investigated in an efficacy study targeting the appropriate pediatric population with recent onset T1D.

CONCLUSIONS
The presence of HERV-W-ENV in the pancreas of T1D patients has been confirmed in nPOD samples using parental murine antibody of temelimab, which neutralizes this pathogenic protein. In a safety clinical trial, temelimab demonstrated to be safe and well-tolerated by adults T1D patients with remaining functional beta cells and diagnosed within 4 years prior to entering the study. These results pave the way for further Phase II clinical development, particularly in pediatric patients with very recent onset of the disease.

Select the ONE category that best describes your research:	
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☐ Beta Cell Development, Differentiation & Regeneration	☐ Novel Technologies
☐ Bone Marrow Studies	☐ Pathology
☐ Core Lab	☒ Type 1 Diabetes Etiology & Environment
☐ Immunology	☐ Other (list):

OA21
Pancreatic Islet and Exocrine Tissue Innervation is Not Altered in Type 1 Diabetes

AUTHORS

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PURPOSE

Impaired counterregulatory response of glucagon to hypoglycemia is a common complication of type 1 diabetes (T1D). One proposed contributor to abnormal glucagon secretion is decreased sympathetic innervation in T1D islets.

METHODS

To systematically assess the innervation of human pancreatic tissues, we examined samples from donors with recent-onset T1D (<10 yrs, age 13-45 yrs, n = 11), longstanding T1D (>10 yrs, age 24-63 yrs, n = 11), and non-diabetic controls (age 10-55 yrs, n = 12). Islet and exocrine tissue innervation was visualized by pan-neuronal marker tubulin β-3 and analyzed by a 2-D morphometry and 3-D rendering.

SUMMARY OF RESULTS

By quantifying the nerve fiber length (0.41±0.07 nm/μm^2) and density (202±25 fibers/mm^2), we found that innervation in control human islets was nearly 20-fold less than previously reported in mouse islets. In contrast to mouse, human exocrine tissue was far more innervated than islets with fiber length of 5.3±0.99 nm/μm^2 ($p<0.05$), and density of 912±111 fibers/mm^2 ($p<0.05$). The 3-D analysis showed that nerve fibers follow extracellular matrix (ECM) formed around acini and from there, they extend further into islets along the ECM made by islet vasculature. Although T1D islets were primarily composed of α cells, the analyzed islet area was similar to controls (control: 15.5±1.5 mm^2, recent-onset T1D: 16.5±1.4 mm^2, long-standing T1D: 16.7±1.5 mm^2; $p>0.05$), and so was the 3-D arrangement of nerve fibers in exocrine tissue and islets. Regardless of T1D duration, the islet fiber length (recent-onset: 0.84±0.29 nm/μm^2; long-standing: 0.91±0.16 nm/μm^2) and density (recent-onset: 338±78 fibers/mm^2; longstanding: 430±57 fibers/mm^2), as well as exocrine fiber length (recent-onset: 5.94±0.75 nm/μm^2; longstanding: 7.13±1.11 nm/μm^2) and density (recent-onset: 1193±68 fibers/mm^2; longstanding: 1559±175 fibers/mm^2) did not differ from controls ($p>0.05$).

CONCLUSIONS

These data indicate that neuronal patterning of human pancreas is significantly different from that of mouse and islet innervation does not appear to be altered in T1D.

Select the ONE category that best describes your research:

☐ Beta Cell Physiology and Dysfunction	☐ Novel Biomarkers
☐ Beta Cell Development, Differentiation & Regeneration	☐ Novel Technologies

☐ Bone Marrow Studies	☐ Pathology
☐ Core Lab	☒ Type 1 Diabetes Etiology & Environment
☐ Immunology	☐ Other (list):

OA22
Mapping Essential Elements and Toxic Metals in the Human Pancreas in Health and T1D Disease

AUTHORS

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PURPOSE

Type 1 diabetes (T1D) is a chronic autoimmune disease that poses significant challenges to afflicted individuals, to the development of effective therapeutic interventions, and to public health initiatives at large. Initiated and perpetuated by a complex interplay of genetic and environmental risk factors, insulin-secreting pancreatic beta-cells are progressively destroyed by aberrant immune responses leading to elevated blood glucose levels as well as severe disturbances of carbohydrate, lipid and protein metabolism. At present, no cure or effective prevention is available and despite insulin treatment, serious long-term complications are frequent. Adding further urgency is an annual 2-5% worldwide increase of T1D incidence over the past few decades, a phenomenon that can only be explained by altered environmental exposures and resultant interactions with genetic variants that predispose to T1D development. However, unlike known genetic risk factors and autoimmune responses, their identities and pathogenic contributions remain poorly defined.

METHODS

To address this shortcoming, we have conducted a “proof-of-principle” study that establishes the foundation for arguably the first “exposure map” of the human pancreas in health and disease. Here, we used a combination of advanced immunohistochemistry (MICSSS: Multiplexed Immunohistochemical Consecutive Staining on Single Slides; whole-slide image acquisition at 40X) and laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) to conduct an elemental biomaging analysis of healthy as well as pre-/diabetic pancreata. Specifically, we have examined pancreatic head and tail sections of 25 nPOD cases (7 non-diabetic controls, 6 auto-antibody+ cases, 8 recent-onset T1D cases [duration ≤2 years], and 4 long-term T1D cases [8-11 years]) with a focus on hormone expression (INS, ProINS, GCG, SST, PPY, CHGA, IAPP), hematopoetic cells (CD45) and microanatomical landmarks (nuclei, CD99, Na+K+ ATPase, KRT19) that serve as geospatial referents for complementary LA-ICP-MS analyses that reveal the identity, abundance and distribution of 14 essential elements and metal toxicants.

SUMMARY OF RESULTS

Following completion of MICSSS staining and LA-ICP-MS interrogation of all 25 nPOD cases, image analyses are ongoing in a blinded fashion, preferentially employing QuPath (for MICSSS analyses) as well as custom scripts (for LA-ICP-MS). Preliminary results demonstrate definable patterns of essential element/metal toxicant distribution that co-localize with microanatomical structures and particular histological tissue properties, and therefore may provide initial evidence for the potential pathogenic involvement of selected metal toxicants or a combination thereof.

CONCLUSIONS
As based on access to rare pre-/diabetic and non-diabetic control pancreatic control tissues through the Network of Pancreatic Organ Donors with Diabetes (nPOD) as well as a suite of unique imaging technology platforms, we have developed a validated workflow for integrated multiplexed immunohistochemistry, LA-ICP-MS and advanced image analyses. Thus, we have created what we believe to be a promising conceptual and practical framework that may serve as an important foundation for future exposure analyses that seek to clarify aspects of T1D pathogenesis and to develop effective preventive treatment modalities.

Select the ONE category that best describes your research:	
☐ Beta Cell Physiology and Dysfunction	☐ Novel Biomarkers
☐ Beta Cell Development, Differentiation & Regeneration	☐ Novel Technologies
☐ Bone Marrow Studies	☐ Pathology
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☐ Immunology	☐ Other (list):