BETA CELL DEVELOPMENT, DIFFERENTIATION, AND REGENERATION

1. Isabella Altilio (University of Miami)

Abstract Title

Spatial transcriptomic analysis of ductal remodeling in FFPE pancreatic samples from type 1 diabetic, autoantibody positive, and non-diabetic donors

Authors

Isabella Altilio, Mayur Doke, Silvia Alvarez-Cubela, Dagmar Klein, Ricardo Pastori, Juan Dominguez-Bendala

Purpose

A longitudinal scRNAseq study of long-term cultured human pancreatic slices, previously published by our lab, revealed population shifts with ductal progenitor activation and detection of insulin-producing cells that appear to be transitioning from a ducto-acinar intermediate stage. Using emerging spatial transcriptomics approaches, we will link this groundbreaking dynamic transcriptomic landscape to real anatomical features in sections from T1D donors. In particular, we expect to detect extensive ductal remodeling and sprouting of putative beta cells, directly linking transitional clusters of cells from our dynamic scRNAseq analysis with distinct anatomical features from live pancreatic samples.

Methods

For spatial transcriptomic analysis, formalin-fixed paraffin-embedded (FFPE) human pancreatic tissue sections from selected type 1 diabetes (T1D) donors (nPOD cases #6240, #6263, #6083, #6380), autoantibody-positive donors (nPOD cases #6116, #6158), and non-diabetic donors (nPOD cases #6393, #6416) were subjected to immunofluorescence staining with CK7 (ductal), insulin (β-cells), and DAPI (nuclei). This staining enabled the identification of distinct ductal and endocrine regions, as well as hypothesized transitional β-cells at the duct-islet interface. Immunofluorescence facilitated the selection of regions of interest containing these transitional cells for further transcriptomic analysis. The 10X Genomics Visium HD Spatial Gene Expression platform will be applied, enabling whole transcriptome spatial analysis at single-cell resolution. Each capture area (6.5 mm x 6.5 mm) comprises approximately 11 million barcoded oligonucleotide capture spots (2 µm x 2 µm), allowing precise spatial mapping of gene expression. The spatial data will be processed in collaboration with 10X Genomics and visualized using the Loupe Browser. Custom R and Python tools will be used for downstream analysis, with reference scRNAseq data (GSE223713) providing context for observed expression profiles.

Summary of Results

We conducted a Visium HD spatial transcriptomic analysis using Seurat version 5 on Type 1 Diabetes (T1D), Ab+, and control pancreas samples from nPOD cases #6240, #6263, #6038, #6380, #6116, #6158, #6393, #6416. Our preliminary analysis aimed to assess cellular composition changes, particularly focusing on islet and ductal regions. In control samples, we observed a clear presence of beta cells within islet regions. However, in T1D samples, the islet regions were largely devoid of beta cells, consistent with disease pathology. Interestingly, in

T1D samples, we identified a subset of cells near the ducts that expressed TFF1 and SPP1, known markers associated with progenitor-like states. Notably, some of these progenitor marker-expressing cells also showed expression of the insulin (INS) gene.

Conclusions

These initial findings suggest potential progenitor cell activity near ductal structures in T1D-affected pancreas tissue. Deeper ongoing analyses are expected to offer valuable insights into the cellular heterogeneity in T1D and provide a basis for further exploration of progenitor cell contributions to pancreatic remodeling and regeneration in T1D.

2. Jan Czyzyk (University of Minnesota)

Abstract Title

Beta-cell loss with severe hyperglycemia is responsible for augmented expression of Notch1 receptor in the pancreatic exocrine ductal cells

Authors

Yury Kryvalap, Svetlana Avdulov, Rim Habte, Shawn Meng, Jan Czyzyk

Purpose

Functional crosstalk between pancreatic islets and exocrine pancreatic tissue may be a determining factor in the progression of type 1 diabetes (T1D). Supporting this premise, we found that inhibition of serpinB13, an inhibitor of cathepsin L (catL) proteinase expressed in the exocrine pancreatic ductal cells, has positive outcomes in mouse and human T1D. We also found that cleavage of Notch1 (a gatekeeper of beta-cell development) following inhibition of serpinB13 with a monoclonal antibody (mAb), represents a key molecular event that connects endocrine and exocrine tissue in the pancreas. Based on these observations, we hypothesized that the Notch1 receptor is expressed in the pancreatic ductal tree

Methods

To examine this, we induced beta-cell-specific ablation with diphtheria toxin (DT) in InsCre/Rosa26iDTR transgenic mice, which were treated with a wild-type islet transplant or left untreated (control) and sacrificed for pancreatic examination 2 weeks after diabetes induction. To assess Notch1 in the pancreas we used quantitative Western blot and IF microscopy with the Visiopharm software for unbiased image analysis.

Summary of Results

Both methods revealed that severe insulin-dependent diabetes results in marked augmentation of Notch1 expression ($p<0.0001$ and $p=0.0012$, respectively), including the active form. The marked increase in the Notch1 protein level was specific, as expression of presenilins (which are required for processing of Notch1) was intact. We also found that islet transplantation preventing hyperglycemia in DT-treated animals completely blocked Notch1 upregulation. Finally, histological analysis revealed that Notch1 expression is confined to the cytokeratin-19+ epithelial cells in the exocrine ducts

Conclusions

We conclude that high Notch1 expression in the exocrine pancreas may explain its regulation by serpinB13. Moreover, our results demonstrate that hyperglycemia, rather than direct sensing of beta-cell loss by the exocrine pancreas, is responsible for Notch upregulation in ductal epithelium. Of note, studies are in progress to examine expression of Notch1 in human pancreatic tissue and will be reported at the meeting

3. Avinil Das Sharma (Indiana University)

Abstract Title

Delineating the role of CHD4 in pancreatic islet development

Authors

Avinil Das Sharma, Rajani George, Sukrati Kanojia, Rebecca K. Davidson, Kayla Huter, Abigail Taylor, Kassandra Sandoval, Meredith Osmulski, Jason Spaeth

Purpose

One approach to combat diabetes involves enhancing β-cell mass by directing stem cells towards a β-cell fate. Achieving this requires a deep understanding of the transcriptional programs that govern endocrine progenitor cell differentiation in vivo. Previously research from our lab has shown the PDX1 transcription factor, an essential regulator of pancreas development, interacts with the CHD4 subunit of the Nucleosome Remodelling and Deacetylase (NuRD) complex in mature β-cells to maintain glucose homeostasis and insulin secretion in vivo. Here we hypothesise that CHD4 regulates chromatin accessibility and gene expression programs essential for endocrine cell development.

Methods

To test this hypothesis, we generated mice with endocrine-progenitor-specific (Neurog3-Cre) Chd4 deficiency (Chd4Δislet). Whole body metabolic phenotyping was conducted, including assessments of glucose tolerance, ad libitum-fed blood glucose, and serum insulin/glucagon measurements were taken in postnatal mice. Preliminary histological characterisation of β-cell maturity markers- insulin, PDX1, MAFA and UCN3- were performed on the pancreatic tissues.

Summary of Results

Proximity ligation assay on pancreatic sections from embryonic day 15.5 mouse embryos revealed interactions between PDX1 and CHD4 in NEUROGENIN 3-expressing endocrine progenitor cells. Postnatally, Chd4Δislet mice exhibit severe hyperglycemia starting at 6 weeks of age, preceded by marked glucose intolerance beginning at 4 weeks, which worsened by 8 weeks. This was associated with reduced serum insulin levels, while glucagon levels remained unaffected. Immunofluorescence imaging demonstrated reduced MAFA expression and the presence of insulin- and glucagon-co-expressing cells in the islets of 6-week-old Chd4Δislet mice. By 8 weeks of age, Chd4Δislet mutants display altered islet morphology along with reduced PDX1, MAFA, UCN3 and insulin levels. Notably, the number of glucagon-positive cells per islet tended to increase in Chd4Δislet mutants.

Conclusions

These preliminary findings indicate that CHD4 loss from endocrine progenitor cells significantly impacts postnatal glucose homeostasis. We predict these deficiencies derive from a failed allocation of islet cells during development. To delineate the mechanistic action of CHD4 in modulating the chromatin landscape to permit proper islet formation, single-cell RNA and ATAC-sequencing will be performed on endocrine committed cells from mutant embryos.

4. Esra Karakose (Icahn School of Medicine at Mount Sinai)

Abstract Title

Discovering Novel Therapeutic Approaches for Human Beta Cell Replication Using Insulinomas as Model

Authors

Esra Karakose, Luca Lambertini, Xuedi Wang, Saul Carcamo, Dan Hasson, Peng Wang, Huan Wang, Carmen Argmann, Andrew Stewart

Purpose

Type 1 diabetes results from insufficient numbers of insulin-producing beta cells. Our previous studies have shown that insulinomas - rare and benign human pancreatic adenomas - hold the transcriptomic and genomic 'recipe' for inducing human beta cell replication. They yield suggestions for therapeutic regenerative drugs for human beta cells, such as the DYRK1A inhibitor harmine, TGF beta Inhibitors, KDM6A inhibitors, and GLP-1 receptor agonists. Interestingly, we observed epigenetic misregulation in majority of the insulinomas we surveyed in our study. Therefore, we took a deeper dive into insulinoma epigenetics in order to discover novel therapeutic targets for human beta cell replication.

Methods

We performed ATAC-seq and H3K27me3 ChIP-seq experiments on FACS-sorted human beta cells and compared these to human insulinomas. In downstream bioinformatics analyses, we integrated and correlated the ATAC-seq and ChIP-seq results with RNA-seq dataset on human beta cells and insulinomas.

Summary of Results

We identified more than 19,000 open chromatin regions specific to insulinomas. Further, we also found more than 7,000 open chromatin regions specific to human beta cells. Our H3K27me3 ChIP-seq results yielded ~900 insulinoma- and beta cell-specific enhancers. Integration of ATAC-seq and H3K27Ac ChIP-seq datasets, together with transcription factor motif enrichment analysis, suggested distinct patterns of transcriptional networks in beta cells vs insulinomas. Integration of accessible chromatin regions with the enhancer regions in the genome yielded four separate clusters: 1- Unique insulinoma enhancers with an open chromatin signature; 2- Unique beta cell enhancers with an open chromatin signature; 3- Enhancers with an open chromatin signature shared between insulinomas and beta cells; and, 4- Open chromatin regions where no enhancer was detected. Interestingly, motif enrichment analysis indicated that AP-1 family members were highly significantly enriched in unique beta cell enhancers with open chromatin signature (cluster 2). Our preliminary knock-down and over-expression experiments on AP-1 family members in cadaveric human islets validated that

they are important regulators of human beta cell replication and differentiation. These studies are currently ongoing, and the results will be further discussed.

Conclusions

Collectively, these studies indicate that insulinoma beta cells have altered ATAC-seq and histone ChIP-seq signatures when compared to cadaveric human beta cells which is also reflected in the downstream differences in expression of genes that control cell cycle and beta cell function. Most importantly, these studies identify AP-1 family as an important regulator of human beta cell replication and differentiation. Finally, our results clearly demonstrate that insulinomas continue to serve as a model to identify potential targets for novel therapies to induce human beta cell regeneration.

BETA CELL PHYSIOLOGY AND DYSFUNCTION

5. Nansa Amarsaikhan (Indiana Biosciences Research Institute)

Abstract Title

Identification of PDPR as a key contributor of metabolic plasticity in beta cells

Authors

Nansa Amarsaikhan, Debjyoti Kundu, Tanasha Lertjanyarak, Chialing Wu, Erica P. Cai

Purpose

This study aims to identify genetic targets that improve beta cell metabolism coupled to stress adaptation, thereby enhancing their protection during diabetes pathogenesis.

Methods

We conducted unbiased genome-scale CRISPR/Cas9 knockout in vitro screens under chronic endoplasmic reticulum (ER) stress conditions. This approach identified PDPR (pyruvate dehydrogenase phosphatase regulatory subunit) as a potential target for enhancing metabolic fitness in beta cells. We validated the findings using transient RNA silencing and stable CRISPR-editing to investigate the effects of PDPR deletion on in vitro cellular stress response and mitochondrial capacity. We also performed RNA-seq analysis to explore the differential transcriptomics signature in the mutant cells, Furthermore, we tested the mutant cells in an in vivo model to test the autoimmune killing.

Summary of Results

Our data demonstrate that beta cells deficient in Pdpr exhibit dual metabolic advantages. These cells showed increased resistance to stress induced by chemical ER stressors and inflammatory cytokines. Additionally, Pdpr-deficient beta cells displayed improved mitochondrial function, evidenced by higher membrane potential, and enhanced mitochondrial respiration. We extended our investigation to the context of Type 1 Diabetes (T1D) through ex vivo co-culture assays with diabetic splenocytes and in vivo transplantation studies using T1D mouse models.

Conclusions

Our preliminary results indicate that targeting PDPR improves beta cell survival and metabolic fitness, thereby enhancing stress resistance during diabetes pathogenesis. PDPR presents a promising target for therapeutic strategies aimed at ameliorating beta cell stress resistance and survival in T1D.

6. Paola Apaolaza (Helmholtz Center Munich)

Abstract Title

Solving the controversy of GLP-1 expression in human alpha cells upon PC1/3 mis-expression in T1D by in situ single-cell analysis

Authors

Paola Apaolaza, Yi-Chun Chen, C. Bruce Verchere, Teresa Rodriguez-Calvo

Purpose

In Type 1 diabetes (T1D), glucose dysregulation impacts various endocrine cells in distinct ways. In alpha cells, proglucagon is processed by PC2 enzyme to produce glucagon (GCG), and in gut L-cells is processed by PC1/3 enzyme (PC1) to synthesize glucagon-like peptide-1 (GLP-1). We have recently demonstrated that PC1-mediated proinsulin processing might be reduced in beta cells and mis-expressed in alpha cells from donors with T1D. Whether PC1 mediates GLP-1 processing in alpha cells of donors with T1D remained controversial. The aim of this study was to address the ongoing debate regarding the presence of PC1 in alpha cells by quantifying the co-expression of GCG, GLP-1, and PC1 at the single-cell level in donors with T1D.

Methods

FFPE-Pancreatic sections from sex, BMI, and age-matched (mean age 20.8 ± 4.5 years) non-diabetic (n=10 ND) and T1D donors with short-duration of the disease (<5 years after diagnosis) (n=10 T1D), were immunostained with amidated GLP-1, PC1, and GCG. All antibodies were validated in KO-mice models and by Western blot. Confocal microscopy images from islets (16.8 ± 4.4 islets/donor) were analyzed via the open-source software QuPath. First, islets were detected, and 6 different alpha cell populations were identified: single-positive for GCG or GLP-1; double-positive for GCG and GLP-1, GCG and PC1, or GLP-1 and PC1; and triple-positive for GCG, GLP-1, and PC1 (tGCG+GLP-1+PC1+). The percentage of alpha cells, as well as the protein relative fluorescence intensity levels (RFU) of GCG, GLP-1, and PC1 on a single cell level were quantified. Colocalization of PC1 with either GCG or GLP-1 was studied by measuring Pearson´s colocalization coefficient (PCC).

Summary of Results

The percentage of single and double positive alpha cells was low and did not differ between ND and T1D donors. The percentage of tGCG+PC1+GLP-1+ was significantly higher in T1D compared to ND donors (70.1 ±19.7% vs. 38.5 ± 21%). While cell mean intensity levels of GCG in tGCG+PC1+GLP-1+ alpha cells remained comparable among both donor groups, GLP-1 intensity was significantly elevated in T1D compared to ND donors (6.5 ± 2.8 vs. 4.1 ± 2.1 RFU). Although not significant, PC1 intensity levels were slightly lower in triple positive cells of T1D, 19.3 ± 6.7 vs. 26.6 ± 6.6 RFU in ND donors. Interestingly, colocalization of PC1 with GCG (0.78 ±

0.1 in T1D vs. 0.59 ± 0.1 in ND) and GLP-1 (0.65 ± 0.1 in T1D vs. 0.48 ± 0.1 in ND) was increased in T1D

Conclusions

In T1D, alpha cells exhibit dysfunctional behaviour, including excessive glucagon secretion. This study aimed to uncover the role of the PC1 enzyme in alpha cells. In our T1D cohort, we observed an increased number of cells positive for GCG, GLP-1, and PC1, though with reduced enzyme production per cell based on intensity measurements. Our findings indicate that PC1 in T1D alpha cells contributes to heightened GLP-1 expression, likely resulting from alternative proglucagon processing into GLP-1 via PC1. The alternative processing of GLP-1 in alpha cells may represent an adaptive response to stimulate insulin secretion and support beta cell survival amid beta cell loss and glucose dysregulation. Expanding the study to include more donors, particularly at-risk ones, would further illuminate the timing and extent of alpha cell changes over T1D progression. Nonetheless, these results could potentially give a rationale for developing new beta cell therapies by targeting alpha cell function.

7. Sandra Blom (University of Iowa)

Abstract Title

Proinflammatory cytokines alter β-cell Golgi structure and function via iNOS suppression of mitochondrial metabolism

Authors

Sandra Blom, Riley Behan-Bush, James Ankrum, Ling Yang, Samuel Stephens

Purpose

Type 1 Diabetes (T1D) results from autoimmune-mediated destruction of pancreatic β-cells. Emerging evidence highlights the β-cell as an active participant in T1D development, yet how the β-cell contributes to its own demise remains a critical knowledge gap in the field. Recent studies have demonstrated a breakdown of β-cell secretory function early in T1D development through protein mis-sorting and generation of neoepitopes such as hybrid insulin peptides, but the mechanisms underlying these defects are currently unknown. Early in disease pathology, immune cell release of proinflammatory cytokines, interleukin-1β (IL-1β), interferon-γ (IFN-γ), and tumor necrosis factor (TNF-α) is known to elicit to β-cell mitochondrial dysfunction, loss of insulin secretion, ER stress, and decreased β-cell viability. However, studies investigating the impact of cytokines on the Golgi, the major mediator of secretory functions, have yet to be explored in T1D. Our studies have uncovered a novel role for proinflammatory cytokines in dismantling the β-cell's secretory program through Golgi alterations that may directly address this knowledge gap.

Methods

Our studies utilize rat 832/3 INS-1 cells, and mouse and human islets to study β-cell Golgi structure and function following proinflammatory cytokine exposure. β-cell models are treated with mouse or human recombinant cytokines, respective to species, for 18 hr (rat and mouse) or 48 hr (human) prior to Golgi morphology and function observations. For investigations of Golgi structure and glycosylation, cells are stained using immunofluorescence and imaged by confocal microscopy. Images are analyzed using NIH Imaris and ImageJ software. Metabolic

influence on Golgi structure was investigated using DPTA, a chemical donor of NO, either alone or in combination with no-glucose RMPI.

Summary of Results

Our recent work demonstrates that proinflammatory cytokines, IL-1β, TNF-α, and IFN-γ disrupt β-cell Golgi structure and function in mouse, rat, and human β-cells, which may further explain early defects in β-cell secretory function important for T1D development. The structural modifications include Golgi compaction and, in some instances, loss of the inter-connecting ribbon leading to Golgi fragmentation. Importantly, we observed that Golgi structural changes coincide with decreased Golgi-derived, but not ER-derived, glycosylation of cell surface proteins, which has been documented in human diseases with altered Golgi structure but is yet to be reported in T1D. We further demonstrate that cytokine-mediated Golgi alterations are inducible nitric oxide synthase (iNOS) dependent and are the result of iNOS driven mitochondrial dysfunction. Comparisons of NO sensitivity between β-cells and non-β-cells revealed that Golgi alterations may be unique to β-cells due their reliance on aerobic metabolism for energy homeostasis. This metabolic inflexibility provides an important clue to the specificity of iNOS-mediated β-cell damage compared to other cell types.

Conclusions

Based on these observations, our central hypothesis is that proinflammatory cytokines elicit Golgi remodeling driven by NO-dependent mitochondrial inhibition, leading to dysregulation of critical Golgi functions. Ultimately, this work will advance understanding of how proinflammatory cytokines affect β-cell function and provide critical insight into β-cell contributions to early T1D etiology.

8. Elizabeth Butterworth (University of Florida)

Abstract Title

The Impact of Iron Accumulation on Human Beta Cell Function and T Cell Response in Type 1 Diabetes

Authors

Elizabeth Butterworth, Richard Coffey, Alexandra Cuaycal, Clayton Mathews, Martha Campbell-Thompson

Purpose

Published data indicate that interferon-gamma (IFNy), a mediator of antiviral responses and an important driver of Type 1 Diabetes (T1D) pathogenesis, increases iron loading in cells. Cellular iron is stored in a multimer protein complex called ferritin, which increases with iron uptake or retention. High cellular ferritin was linked to increased HLA class I (HLA) cell surface expression, while iron chelation or inhibition of ferritin translation led to decreased expression. HLA hyper-expression has been reported in recent-onset diabetes pancreas sections. Presentation of self-antigen via HLA triggers cytotoxic T lymphocytes (CTLs) to kill beta cells. CTLs produce IFNy to induce HLA expression in beta cells. Additional factors that influence HLA hyper-expression may function as potential drug targets to inhibit such pathologic expression

Methods

Pancreas sections from nPOD donors were stained with antibodies against insulin, glucagon and ferritin. Slides were imaged, cell types segmented, and mean fluorescence intensity in each cell type was analyzed in donors without diabetes (ND), positive for multiple autoantibodies (AAb+), and with recent-onset type 1 diabetes (T1D).
Isolated human islets or human beta cell lines, BL5 and HP62, were treated with ferric ammonium citrate (FAC) to induce iron loading, and/or IFNγ to mimic the diabetogenic environment. Iron loading in vitro was confirmed with transferrin receptor C (TfRC) TaqMan qPCR and ferritin western blot. To test beta cell function, islets were assayed for glucose-stimulated insulin response (GSIR) by staining with the beta cell marker ENTPD3 and the calcium indicator Calbryte 520. Stained islets were perfused with low glucose (3 mM), high glucose (16.7 mM), or KCl Kreb's buffer solutions while imaging using a confocal microscope. The fluorescence of individual beta cells was analyzed using a custom R script.

Iron-loaded cultures were assayed for HLA-ABC surface expression with or without IFNγ by flow cytometry. The impact of the iron chelator deferoxamine (DFO) on HLA-ABC expression was tested at 50-200 μM.

A radioactive chromium-based killing assay was used to determine whether iron loading potentiates CTL-induced beta cell demise. BL5 and HP62 cells were treated with FAC, IFNγ, or both, then pulsed with MART-1 peptide and radioactive chromium before exposure to CTLs with appropriate TCRs.

Summary of Results

In testing GSIR, FAC-treated islets were not significantly different to untreated control islets when stimulated with high glucose. This was determined by measuring the area under the curve for the average normalized fluorescence trace over time.

IFNγ-treated BL5 cells had decreased TfRC mRNA, which was further downregulated with FAC supplementation. IFNγ had no impact on TfRC mRNA expression in HP62 cells. Treatment of cell lines with FAC alone did not alter basal HLA-ABC surface expression as measured by flow cytometry. IFNγ did increase HLA, as expected, but was not augmented by FAC addition. DFO neither impacted basal HLA, nor did it ameliorate IFNγ-induced HLA expression.

For the CTL assay, two CTL cell lines (F20 and F22) and both beta cell lines were tested. In all pairings, IFNγ alone increased specific lysis. When beta cells were exposed to F22 CTLs, FAC alone increased the specific lysis, but not with F20 CTLs. For HP62, FAC acted as a protective factor against IFNγ-induced specific lysis while BL5 had no change or increased lysis with FAC addition to IFNγ.

Conclusions

Iron loading occurs in beta cell lines and islets. Iron loading does not impact beta cell function or increase HLA-class I expression. While it has previously been reported that DFO inhibits HLA expression, this was not the case with the beta cell lines tested. This indicates a fundamental difference between the iron biology of beta and previously studied cell types (primarily cancer lines). The results of the CTL assay require closer examination as the CTL

donor and cell line have differential impacts on whether iron loading potentiates CTL killing of beta cells

9. Jin-Yong Chung (Johns Hopkins University)

Abstract Title

Upregulation of c-Jun transcripts by the prolactin receptor signaling regulates beta-cell survival during postpartum

Authors

Jin-Yong Chung, Nelmari Ruiz Otero, Ronadip Banerjee

Purpose

Maternal beta-cell mass expands during pregnancy to increase the ability of beta-cells to secret insulin and returns to prepartum levels through increased beta-cell apoptosis in the postpartum, but what regulates these dynamic changes is poorly understood. We previously found a critical role for prolactin receptor (PRLR) signaling in changing the gestational expansion of beta-cells. Recently, using scRNA-seq, we identified increased expression of immediate early genes (IEGs) in early postpartum beta-cells as compared to late gestation or nonpregnant beta-cells in mice. Amongst these were the c-Jun transcription factor, a well-characterized IEG that regulates proliferation, apoptosis, and survival in various tissues. Because we found c-Jun expression is mutually exclusive with apoptosis in postpartum beta-cells, we hypothesized that c-Jun plays a critical role in beta-cell survival

Methods

To determine the possible role of PRLR signaling and c-Jun on beta-cell regulation during pregnancy and postpartum, non-pregnant (NP), pregnant (PREG), and postpartum (POST) pancreas tissues were obtained from wildtype mice or mice lacking PRLR signaling in beta-cells. Human pancreas tissue from nPOD pregnant donors was compared to age and BMI-matched controls. To determine the critical role of c-Jun on beta-cell survival, we used islets isolated from mouse pancreas and human islets provided by IIDP. We also used MIN6 cells, a mouse insulinoma cell line, to knockdown c-Jun by lentivirus transduction

Summary of Results

Prolactin treatment significantly induced c-Jun in cultured murine and human islets. In mice, c-Jun is normally expressed in islets from late pregnancy and early postpartum but is absent in mice lacking beta-cell PRLR signaling. Increased c-Jun expression is mediated by MAPK/ERK but not Stat5 or PI3K. Blocking c-Jun activity with pharmacologic inhibitors prevents the ability of prolactin to protect beta-cells from dexamethasone-induced apoptosis in both mouse and human islets. Knockdown of c-Jun in MIN6 cells showed the failure of cell survival even in the presence of prolactin, including failure to upregulate pro-survival genes such as Bcl2l1 (Bcl-xL) and Birc5 (Survivin). Examination of human donor tissue from nPOD revealed that c-Jun expression is seen in human beta-cells from pregnant donors but is absent in pregnant donors with gestational diabetes (GDM). The results indicate that c-Jun gestational induction is conserved in humans and perturbed by GDM.

Conclusions

Together, our results indicated that c-Jun, in response to PRLR/MAPK signal transduction, contributes to survival of beta-cells undergoing apoptotic stress and may contribute to survival of postpartum beta-cells in normal pregnancy or dysregulation of beta-cells in GDM.

10. Sandra Ferreira (University of Florida)

Abstract Title

GABA is crucial for maintaining proper Ca2+ oscillation behavior and insulin secretion in response to glucose stimulation.

Authors

Sandra Ferreira, Austin Stis, Adrienne Widener, Edward Phelps

Purpose

Intracellular calcium [Ca2+]i is a crucial messenger responsible for modulating several functions inside of the cell. In pancreatic β-cells, the amount of insulin secreted is proportional to the transient amount of [Ca2+]i. Ca2+ oscillations, driven by bursts of action potentials, in turn, give rise to the oscillatory characteristics of insulin secretion. GABA (γ-aminobutyric acid) is a neurotransmitter synthesized and secreted by pancreatic β-cells. The high GABA concentration in β-cells, second only to neurons, indicates an important role in islet function. However, while numerous effects of applied exogenous GABA have been studied, there has never been a way to study the endogenous GABA because global knockout is embryonically lethal, and a GABA-synthesizing enzyme β-cell specific knockout has never been produced until now. GABA can signal through a chloride channel, the GABAA Receptor (GABAAR), in β-cells. The ion channel opens upon GABA binding, leading to Cl- movement through the plasma membrane, directly affecting the membrane potential. Our previous results showed that the modulation of GABAAR activity by pharmacological agonists and antagonists affects [Ca2+]i, indicating endogenous GABA may modulate Ca2+ behavior. Here, we aimed to evaluate the Ca2+ oscillations characteristic in islets from knockout mice lacking GABA in pancreatic β-cells

Methods

We isolated islets from conditional knockout mice lacking the GABA-synthesizing enzymes GAD65 (encoded by Gad2) and GAD67 (encoded by Gad1), specifically in pancreatic beta cells (β-Gad1,2-/-) and Ins1-Cre mice. To confirm the knockout, GABA content was measured by HPLC, and GAD65 and GAD67 expression were assessed using Western Blot. Insulin secretion and Ca2+ oscillations were evaluated on different glucose concentrations.

Summary of Results

HPLC and Western blot confirmed that knockout of GAD65 and GAD67 results in islets devoid of GABA. β-Gad1,2-/- islets secreted more insulin in all glucose incubations (8, 11.1, and 16.7 mM) and KCl than Ins1-cre islets, indicating that endogenous GABA can inhibit the β-cell function in an autocrine fashion. To evaluate if GABA modulates insulin secretion through the Ca2+ signal, we recorded [Ca2+]i in different glucose concentrations. We observed that β-Gad1,2-/- islets responded more rapidly to glucose (around 2 min before) and remained maximally activated longer before initiating rhythmic Ca2+ oscillations. Evaluation of the oscillations showed that the β-Gad1,2-/- islets exhibited a significantly longer active phase and shorter silent phase, which reduced the trough-to-peak amplitude. This increase in active phase duration resulted in the Ca2+ levels failing to return to baseline before starting a new Ca2+ wave and, thus, oversecretion of insulin.

Conclusions

We concluded that GABA is crucial to modulating the Ca2+ wave components, maintaining the equilibrium between the active and silent phases, resulting in insulin secretion at a proper level.

11. Ben Giepmans (UMC Gronigen)

Abstract Title

Pancreatic exocrine damage can induce beta cell stress in zebrafish larvae

Authors

Noura Faraj, Willem Hoogaars, Peter Duinkerken, Anouk Wolters, Kim Kats, Mette Dekkers, Arnaud Zaldumbide, Ben Giepmans

Purpose

Type 1 diabetes is characterized by the autoimmune destruction of beta cells, yet the underlying triggers remain elusive. Recent evidence suggests that excessive endoplasmic reticulum (ER) stress in beta cells may contribute to the auto-immune response and apoptosis. Moreover, the exocrine pancreas is affected in pre- type 1 diabetes individuals, indicating exocrine stress might cause beta cell ER stress and dysfunction. While a cause/consequence relationship between exocrine stress and beta cell function cannot be addressed in humans, it can be studied in a zebrafish model. Larvae develop a functional pancreas with a human-like morphology by 120 hours post fertilization (hpf), providing a valuable dynamic model for studying pancreatic interactions.

Methods

We targeted exocrine cells specifically and addressed beta cell function using transgenic zebrafish models and reporters.

Summary of Results

Exocrine damage in zebrafish decreases pancreas volume and changes its morphology. Additionally, the resulting exocrine damage induces ER stress in beta cells and reduces their number.

Conclusions

Zebrafish models provide a robust platform for investigating the interplay between exocrine and beta cells, thereby enhancing further insights into the mechanisms driving type 1 diabetes and potentially uncovering novel therapeutic targets.

12. Michael Kalwat (Indiana Biosciences Research Institute)

Abstract Title

Leveraging Transcriptomics to Identify Stress Sensitivity and Resilience Genes in β-cells

Authors

Gitanjali Roy, Andrea Ordonez, Nansalmaa Amarsaikhan, Tanasha Lertjanyarak, Michael B. Kwakye, Rameesha Syed, Olivia Lazaro, Sylvia Robertson, Daniela Rodriguez, Alex M. Mawla, Sean D. McCabe, Travis S. Johnson, Erica P. Cai, Michael A. Kalwat

Purpose

Type 1 diabetes (T1D) afflicts over 1.6 million individuals in the United States, wherein insulin-secreting pancreatic islet β-cells are destroyed by autoimmune attack. Individuals with T1D must inject insulin to maintain glucose homeostasis. How and why β-cells are susceptible to immune attack is not fully understood. Secretory demand and other environmental stressors can elicit the chronic induction of endoplasmic reticulum (ER) stress in β-cells, contributing to β-cell failure or presentation of neoantigens. Identifying mechanisms that ameliorate β-cell stress and induce immune tolerance may lead to novel therapeutics for T1D prevention and treatment. In this project, our goal is to use multiple approaches to identify novel candidate targets for β-cell protection in diabetes.

Methods

We intersected common genes between genome-wide CRISPR knockout screening and secretory stress transcriptomics in β-cells to select SDF2L1 as a novel target. We have also analyzed single-cell RNAseq data from human islets using an in-house machine learning algorithm, Diagnostic Evidence Gauge of Single cells (DEGAS), to identify disease-associated β-cell subsets. From these diabetes-associated subsets of β-cells, we identified DLK1 as a potential marker of stress-resilient β-cells. We hypothesize that SDF2L1 promotes β-cell susceptibility to stress, while DLK1 marks stress-resilient β-cells in T1D. To test this hypothesis, we applied immunohistochemistry in human pancreas sections, CRISPR-mediated mutation and transgenic expression of genes in β-cell lines

Summary of Results

Stromal Derived Factor 2 Like 1 (SDF2L1) is an ER-localized protein that may play roles in ER-associated degradation and proinsulin processing. We and others have observed that SDF2L1 is induced in β-cells by ER stressors like thapsigargin or inflammatory cytokines. We have also found that SDF2L1 staining is enriched in human β-cells compared to α-cells. CRISPR-mediated knockout of Sdf2l1 in mouse NIT-1 β-cells led to resistance to thapsigargin-induced cell death.

Conversely, transgenic overexpression of SDF2L1 in mouse MIN6 β-cells led to suppression of glucose-stimulated insulin secretion. In parallel, using our DEGAS approach, we found that DLK1 expression was enriched in a β-cell cluster highly associated with both T1D and T2D. This agrees with SNPs in the DLK1 locus being linked to higher T1D and T2D risk. Delta Like Non-canonical Notch Ligand 1 (DLK1) is a maternally imprinted gene with roles in Notch signaling and glucose homeostasis. Published data suggest DLK1 is required for β-cell survival after treatment with inflammatory cytokines. We have found by immunohistochemistry that DLK1 is heterogeneously expressed in β-cells and appears to be depleted in T2D islets. Similar studies using autoantibody-positive or recently diagnosed T1D samples are ongoing.

Conclusions

While SDF2L1 and DLK1 have been associated with T1D and T2D, their role during β-cell development, differentiation, and death is not well understood. How SDF2L1 and DLK1 influence human β-cell health and heterogeneity is unknown. There may be a disconnect between mRNA and protein abundance for genes like DLK1. Therefore, defining subsets of DLK1+ and DLK1- cells is necessary. In the future, we anticipate developing therapeutics that deplete maladaptive factors like SDF2L1 from human β-cells. We expect our parallel omics approach to help identify markers like DLK1, which will lead to an improved understanding of β-cell stress resilience in T1D and T2D.

13. Liora Katz (Icahn School of Medicine at Mount Sinai)

Abstract Title

Targeting ChREBPα/14-3-3 Interaction to Alleviate β-Cell Stress in T1D and T2D Pathogenesis

Authors

Liora S. Katz, Sriya Dodda, Rhone Galchen, Charleigh Parsons, Donald K. Scott

Purpose

Type 1 and Type 2 Diabetes (T1D and T2D) share a common endpoint: the destruction or dysfunction of insulin-producing β-cells due to chronic metabolic and inflammatory stress. The Carbohydrate Response Element Binding Protein (ChREBP), particularly its hyper-potent β isoform, plays a pivotal role in amplifying β-cell stress responses across diabetes contexts, including glucolipotoxicity, cytokine exposure, and endoplasmic reticulum (ER) stress. In this study, we present a new approach targeting the ChREBPα/14-3-3 interaction to block ChREBPα nuclear migration, inhibit ChREBPβ upregulation, and shield β-cells from multiple diabetic stressors.

Methods

We developed molecular glue compounds, including lead compound 43, to stabilize the ChREBPα/14-3-3 complex, thereby preventing ChREBPβ hyperactivation. Using INS-1 β-cell lines and primary human islets, we assessed the effects of compound 43 under glucolipotoxic cytokine and ER stress conditions for β-cell survival, identity and glucose stimulated insulin secretion (GSIS). Pancreatic sections from NOD mice were analyzed to examine ChREBP expression patterns during early T1D progression. Additionally, scRNA-seq of human islets provided insights into ChREBP upregulation across T1D and T2D states.

Summary of Results

Compound 43 demonstrated selective stabilization of the ChREBPα/14-3-3 complex (EC50 = 0.3±0.1 μM, Kd = 0.12 μM) and exhibited robust protection against β-cell death induced by glucolipotoxicity, cytokines, and ER stress. Notably, 43 prevented ChREBPβ upregulation, maintained β-cell identity, and preserved functional markers in β-cells under these stress conditions. In NOD mice, we observed elevated ChREBPβ in β-cells even before overt hyperglycemia, implicating ChREBPβ in early autoimmune β-cell damage. scRNA-seq of human islets revealed ChREBPβ upregulation in T1D, T2D, and AAB+ subjects, underscoring its relevance across diabetes types. Importantly, compound 43 also sustained GSIS in primary human islets subjected to glucotoxic and inflammatory stress.

Conclusions

Our findings underscore ChREBPβ as a critical driver of β-cell demise under diabetes-related stresses and identify ChREBPα/14-3-3 interaction stabilization as a novel and potent therapeutic strategy. By preventing the nuclear translocation of ChREBPα, this approach halts ChREBPβ-driven apoptosis and preserves β-cell function under ER, glucolipotoxic, and cytokine stress. These results reveal a new avenue for diabetes treatment, addressing both T1D and T2D β-cell pathology, with the potential to slow or prevent disease progression.

14. Debjyoti Kundu (Indiana Biosciences Research Institute)

Abstract Title

Evaluation of NADH:ubiquinone oxidoreductase assembly factor 8 (Ndufaf8) as a stress adaptation mediator in type 1 diabetes

Authors

Debjyoti Kundu, Nansa Amarsaikhan, Tanasha Lertjanyarak, Chialing Wu, Isabela L. Iessi, Anna Bopp, Erica P. Cai

Purpose

Pancreatic beta cells under hyperglycemia encounter immense cellular stress, leading to elevated endogenous reactive oxygen species (ROS) production. These factors render beta cells susceptible to endoplasmic reticulum (ER) stress, mitochondrial damage, and promote programmed cell death. Using the cutting-edge CRISPR-Cas9 system, we conducted a genome-scale in vitro screen to identify gene edits capable of protecting beta cells against ER stress. Among the significant differential gene edits, we identified guide-RNAs (gRNAs) targeting NADH: ubiquinone oxidoreductase assembly factor 8 (Ndufaf8) to be the most enriched gRNA in promoting survival of beta cells under chronic ER stress. The overarching aim of this project is to elucidate the adaptive effect of loss of Ndufaf8 in beta cell ER stress mitigation.

Methods

The non-obese diabetic (NOD) mouse-derived beta cell line NIT-1 carrying a loss-of-function of Ndufaf8 mutation (Ndufaf8⍰) was generated using the same gRNA enriched in CRISPR screen. The deletion was verified by sequencing and the cells were subjected to various ER stressors for phenotypic characterization. Additionally, cell functionality of Ndufaf8⍰ and control cells was evaluated using biochemical assays. To further evaluate the global effect of Ndufaf8 deletion,

we performed bulk RNA sequencing (RNAseq) analysis in presence and absence of chemical ER stressor, thapsigargin.

Summary of Results

Ndufaf8□ NIT-1 cells have significantly reduced susceptibility to ER stressors, such as thapsigargin or tunicamycin compared to control. Similarly, these cells are more resistant towards pro-inflammatory cytokine-mediated cell death. In vitro, Ndufaf8□ beta cells showed a reduced activity of mitochondrial respiratory chain complex I. As expected, loss of Ndufaf8 led to decreased oxygen consumption rate, along with less ROS produced when subjected to an exogenous ROS donor, glucose oxidase. It is worth noting that Ndufaf8□ beta cells exhibited improved glucose responsiveness and enhanced insulin secretion upon glucose stimulation compared to control cells, meanwhile maintaining a similar level of mitochondrial membrane potential. Ndufaf8Δ beta cells had enhanced glucose uptake capacity compared to controls as seen by a glucose analog (2NBDG) uptake assay. Additionally, gene set enrichment analysis (GSEA) from RNAseq data indicated towards a significant enrichment of gene sets involved in hypoxia inducible factor 1 (HIF1) signaling, glycolysis, insulin expression and signaling in Ndufaf8□ compared to the controls.

Conclusions

In summary, deleting Ndufaf8 alters mitochondrial bioenergetics, thereby reducing stress and enhancing beta cell metabolic function. Ndufaf8Δ beta cells have increased glucose uptake, improved glucose utilization by elevated glycolysis and enhanced insulin secretion compared to the control cells. These adaptations may improve beta cell survival and functionality, making this approach a potential strategy to mitigate the effect of chronic ER stress in type 1 diabetes.

15. Julia Panzer (City of Hope)

Abstract Title

Functional heterogeneity of alpha cells

Authors

Julia Panzer, Anna Lang, Pablo Garcia, Alberto Pugliese, Alejandro Caicedo

Purpose

Alpha cells are critical regulators of glucose homeostasis, secreting glucagon to prevent hypoglycemia. Recent studies indicate that alpha cells are not a uniform population but instead exhibit significant functional heterogeneity, with subpopulations differing in their signaling dynamics, hormone secretion, and responsiveness to stimuli. This heterogeneity suggests that alpha cells play diverse and specialized roles in maintaining metabolic balance. Understanding these functional differences is essential for advancing our knowledge of alpha cell physiology and the broader regulatory networks within the islet microenvironment.

Methods

We initially utilized a mouse model in which alpha cells express the genetically encoded calcium indicator GCaMP6, enabling visualization of calcium dynamics across the entire alpha cell population. We now started to extend these studies to human samples using both isolated

islets and pancreatic tissue slices from both non-diabetic donors and donors with type 1 diabetes. We assess alpha cell function under three primary conditions: (a) varying glycemic levels, (b) application of agonists and antagonists targeting autocrine and paracrine pathways, and (c) stimulation with reference agents such as adrenaline and KCl. Functional outcomes are measured using dynamic hormone secretion assays and real-time calcium imaging to evaluate cellular activity.

Summary of Results

Our functional studies reveal evidence for significant alpha cell heterogeneity. Alpha cell responses to glucose differ markedly from beta cells, as they exhibit no sustained glucagon secretion. Instead, secretion is transient and shows hysteresis, meaning that alpha cells do not faithfully track glucose concentrations but instead depend on prior glycemic states. Calcium imaging revealed that only about 30% of alpha cells respond to changes in glucose, highlighting functional heterogeneity within the alpha cell population. Calcium imaging also identified subpopulations of alpha cells with distinct responses to other stimuli, including varying reactions to paracrine signals like serotonin and somatostatin. Hormone secretion studies confirmed this variability: blocking serotonin signaling with a 5-HT1F receptor antagonist nearly doubled glucagon secretion in low glucose, while blocking somatostatin signaling with cyclosomatostatin increased glucagon secretion by ~50%.

Conclusions

Our findings provide strong evidence for functional heterogeneity among alpha cells, with distinct subpopulations exhibiting differential responses to regulatory cues and hysteresis. This suggests the hypothesis that alpha cell dysfunction in T1D may be limited to specific subpopulations rather than affecting the entire population uniformly. Studying these subpopulations in T1D is crucial, as it opens the possibility of targeting dysfunctional groups pharmacologically to restore glucagon secretion and improve glucose regulation.

16. Guido Sebastiani (University of Siena)

Abstract Title

In-situ pancreatic spatial transcriptomics uncovers novel phenotypic signature of beta cell persistence in long-standing T1DM donors

Authors

Guido Sebastiani, Erika Pedace, Stefano Auddino, Giada Licata, Adriano Causarano, Andrea Francesco Berteramo, Giuseppina Emanuela Grieco, Daniela Fignani, Laura Nigi, Elena Splendiani, Elisabetta Ferretti, Francesco Dotta

Purpose

Type 1 diabetes mellitus (T1D) is a chronic autoimmune disease characterized by the selective destruction of pancreatic beta cells by the immune system, resulting into insulin deficiency. At the onset of the disease, approximately 70–80% of β-cells have been either destroyed or are dysfunctional. However, it is noteworthy that endogenous insulin secretion can persist for several years after diagnosis. Some individuals with long-standing T1D continue to exhibit detectable levels of C-peptide, suggesting the persistence of β-cells. This evidence highlights the remarkable resilience and endurance of β-cells despite prolonged diabetes. Consequently,

there is growing interest in identifying factors that promote or characterize the survival of residual/persistent β-cells in T1D pancreata. In this exploratory study, our main goal is to phenotype the surviving/resilient β-cells in the pancreas of long-standing T1D donors using spatial transcriptomics analysis to identify factors potentially involved in protecting and/or maintaining these persisting beta cells.

Methods

Pancreatic formalin-fixed paraffin-embedded (FFPE) tissue sections (5 μm) were obtained from n=4 islet autoantibody-negative non-diabetic (ND) multi-organ donors (age: 51 ± 13.9 years, 3 females, 1 male, BMI: 30.8 ± 11.0) and n=2 donors with T1D (age: 60 ± 29.8 years, 2 females, BMI: 27.8 ± 4.0) with disease duration of 21 and 45 years, respectively. Morphological quality controls and assessment of pancreas tissue sections were performed on serial sections using hematoxylin and eosin (H&E) staining, as well as insulin/glucagon immunofluorescence staining. Total RNA was extracted from serial FFPE pancreatic sections and subjected to quality control using the Agilent 2100 Bioanalyzer, with an inclusion cutoff of DV200 > 30. Spatial transcriptomics analysis was conducted using Visium CytAssist Spatial Gene Expression for FFPE (10X Genomics) on n=4 sections from ND donors (n=1 section per donor) and n=4 sections from T1DM donors (n=2 sections per donor). Sequencing was performed using the Illumina NovaSeq 6000 platform. Fastq data files were demultiplexed using bcl-convert, and transcript quantification and image alignment were computed using SpaceRanger software. Spots with low expression (<10% total UMI counts) were removed. Each sample data was normalized, followed by unsupervised hierarchical clustering to identify different cell types. Data from samples were then integrated and spatially projected. Differential expression analysis was performed considering a Log2 Fold Change (FC) Cutoff of +/-0.8 (statistics using Wilcoxon test with FDR<0.05) to identify differentially expressed genes in the endocrine cluster.

Summary of Results

We obtained a mean UMI read count per spot of 11,952 (ND: 12,612; T1D: 11,293) and a median number of detected genes per spot of 3,328 and 3,228 for ND and T1D, respectively. Clustering analysis, performed on the 3,000 most variable genes detected across all samples, identified 14 distinct cell clusters. Using nonlinear dimensionality reduction (UMAP), we observed a clear overlap among the identified cell groups, indicating optimal reproducibility of the results across tissues. As expected, transcriptomic profiling of immune cells showed an increased frequency of spots positive for CD4 and CD8 cells in the exocrine regions of T1D pancreata, with only a few scattered islets showing immune cell infiltration. We then focused on the endocrine cell cluster, which corresponded to pancreatic islets identified in the previous serial section. Differential expression analysis between ND and T1D endocrine cell clusters revealed the differential expression (FDR < 0.05) of 168 genes. Of these, 37 were downregulated (Log2FC < -0.8, FDR < 0.05) and 131 were upregulated (Log2FC > 0.8, FDR < 0.05) in T1D endocrine cell clusters compared to ND. As expected, Insulin was the most significantly downregulated gene, along with IAPP, REG1B, and VGF. Gene ontology analysis of downregulated genes in T1D islets identified GO terms related to glucose homeostasis, response to glucose, and regulation of glycolytic processes. Among the most significantly upregulated genes, we found TMSB4X, EGFL7, and MYO10, which have been reported to be involved in the regulation of growth, survival, and proliferation. Interestingly, the gene ontology analysis revealed terms related to cell survival and plasticity (i.e. positive regulation of Erk1-Erk2, regulation of cell shape and morphogenesis, TGF-beta signaling pathway).

Conclusions

In this study, we provide new insights into the molecular landscape of surviving β-cells in long-standing T1D pancreata. The spatial transcriptomics approach revealed significant differential gene expression that suggests an adaptive phenotype of these β-cells, with upregulated pathways promoting cell survival, plasticity. Further studies on additional cases, including recent-onset T1D donors, are needed to confirm the observed phenotype and to investigate the role of the identified factors.

17. Maria Pilar Toledo (Florida State University)

Abstract Title

Sex differences in the endoplasmic reticulum (ER) stress response in human pancreatic islet

Authors

Maria Pilar Toledo, Pamela Sandoval Sanchez, Xue Hu, Li Guo, Yue J. Wang

Purpose

Sex difference in diabetes is evident in the differential risk of disease onset, treatment response, and susceptibility to complications. The molecular mechanisms driving these sex differences remain largely unknown, though sex hormones, sex chromosomes, and environmental factors are considered key players. The endoplasmic reticulum (ER) stress response is strongly linked to diabetes pathophysiology. Previously, through integrated single-cell RNA-seq data, we demonstrated that male islet cells exhibit transcriptional signatures associated with heightened ER stress compared to females. We hypothesize that the sex differences in ER stress response lead to a higher risk of developing diabetes in males compared to females. Our goal is to elucidate the mechanisms and identify gene candidates as potential modulators of the sex differences in ER stress response in human pancreatic islets.

Methods

Primary human pancreatic islets were processed in two different ways. (1) To study ER stress response kinetics and hormonal effects, female and male islets were treated with no hormone, estrogen, progesterone, or testosterone for five days. On day four, islets were dissociated into single-cell suspensions and incubated with an XBP1-based ER stress sensor. After 24 hours of transduction with the ER stress sensor, islet cells were treated with 1 μM Thapsigargin (Tg)—a non-competitive SERCA inhibitor—to induce ER stress. The dynamic ER stress response of islet cells was monitored by live imaging with a dense time course upon the addition of Tg. (2) To analyze the molecular pathways involved in the sex-specific ER stress response, we performed single-cell RNA-seq (scRNA-seq) on female and male islets treated with 1 μM Tg for 0, 12, and 48 hours.

Summary of Results

Overall, after the addition of Tg, female islets exhibit an earlier and lower peak of ER stress, along with a faster recovery compared to male islets. When testing the impact of sex hormones, the islets do not show significant differences in ER stress response across different hormone treatments. scRNA-seq data suggest large sex differences in molecular signatures related to

the ER stress response. Moreover, pseudotime analysis identifies XIST and XBP1 as candidate genes that may play sex-specific roles in the ER stress response.

Conclusions

This data suggests that females may exhibit a more adaptive response to ER stress compared to males. These findings could potentially be attributed to genetic factors associated with each sex. More data is necessary to establish the role of sex-specific hormones in this phenomenon.

18. Patricia Velado (Helmholtz Munich)

Abstract Title

Chromogranins and secretogranins in the pathogenesis of type 1 diabetes

Authors

Patricia Velado, Teresa Rodriguez-Calvo

Purpose

Type 1 diabetes (T1D) is a complex and heterogeneous disease characterized by the immune-mediated destruction of pancreatic beta cells. This destruction might be linked to beta cell stress, which can lead to abnormal protein processing and accumulation, potentially triggering immune responses. While most of the research has focused on proinsulin processing errors in beta cells, other molecules within the insulin granules, specifically chromogranins, secretogranin (collectively known as granins) and islet amyloid polypeptide (IAPP) could undergo similar processing errors and be targeted by the immune system. Granins are essential for granule assembly, insulin sorting and condensation. In addition, granins like Secretogranin 5 (SCG5) function as a chaperone for the prohormone convertase 2 (PC2) enzyme, while secretogranin 8 (SCG8 or PCSK1N) inhibits the prohormone convertase 1/3 (PC1/3) enzyme. This study aims to determine the expression, cellular localization, and molecular interactions of chromogranins, secretogranins, and IAPP in pancreatic beta cells.

Methods

We analyzed the expression of chromogranins, secretogranins, and IAPP during the progression of T1D by performing immunohistochemistry on pancreatic tissue samples from non-diabetic (ND), double autoantibody-positive (dAAb+), and T1D donors. We characterized the cellular composition of each islet (beta and alpha cells) using whole-slide images and quantified the percentage of beta and alpha cells positive for CHGA, CHGB, SCG2, SCG3, SCG5, SCG7, SCG8, and IAPP. Additionally, we assessed intensity features as a proxy for protein expression, both at the islet level and within individual islet cells.

Summary of Results

We observed a reduction in the proportion of beta and alpha cells expressing CHGA, CHGB, SCG2, SCG3, SCG5 and SCG7 in dAAb+ donors. This indicates that granin expression is altered in both endocrine cell types prior to clinical onset of the disease, potentially indicating impaired biogenesis and assembly of secretory granules. Interestingly, the remaining beta cells in T1D donors showed granin expression levels similar to those of non-diabetic donors. Consistent

with previous findings, we observed reduced IAPP in beta cells from T1D donors. Moreover, the fluorescence intensity of CHGA and SCG2 progressively increased with disease progression, with higher levels observed in both beta and alpha cells of dAAb+ and T1D donors compared to non-diabetic individuals.

Conclusions

Our findings suggest that alterations in granin expression, especially in the early stages of T1D, may serve as an important marker of beta cell dysfunction. In the dAAb+ donors, beta cells with disrupted granin expression may be preferentially targeted by the immune system, whereas beta cells that retain functional granin cargo may be among the last to be destroyed. The observed increase in granin intensity, particularly CHGA and SCG2, in beta cells from dAAb+ and T1D donors suggests that the remaining beta cells may compensate for the beta cell loss by upregulating their granin expression. Overall, these results imply that alterations in granin expression occur before disease onset and could serve as early indicators of T1D.

19. Leslie Wagner (Indiana University School of Medicine)

Abstract Title

β Cell Heterogeneity in the Acute IFN-α Response

Authors

Leslie Wagner, Olha Melnyk, Charanya Muralidharan, Matthew Arvin, Michelle Martinez-Irizarry, Bryce Duffett, Justin Crowder, Elisabetta Manduchi, Klaus Kaestner, Joseph Brozinick, Amelia Linnemann

Purpose

Type 1 diabetes (T1D) is characterized by the autoimmune destruction of β cells hypothesized to result from a combination of genetic and environmental factors, likely including early childhood viral infection. Viral infections elicit the generation of type 1 interferons, such as interferon alpha (IFN-α), a cytokine that induces markers of early diabetes development including ER stress and HLA class I overexpression. IFN-α is also expressed in islets of donors with early T1D, suggesting a role in T1D pathogenesis. Here, we evaluated the direct impact of IFN-α on β cell physiology, primarily focusing on reactive oxygen species (ROS) generation, as excess ROS generated by numerous cytokines are thought to correlate with negative outcomes during disease pathogenesis.

Methods

To visualize ROS generation in vivo, human islets were transduced with β cell selective adenovirally packaged GRX1-roGFP2, a ratiometric and reversible ROS biosensor (INS-GRX1-roGFP2). Biosensor expressing islets were transplanted under the kidney capsule of NOD scid gamma (NSG) mice. Intravital imaging was performed on anesthetized mice two weeks later to evaluate the acute response to systemic human IFN-α elevation. To investigate the specific ROS being produced, we treated human islets in vitro with human IFN-α then stained them with either dihydroethidium (DHE) or 2',7'-dichlorofluorescein diacetate (DCFDA) to measure superoxide or hydrogen peroxide, respectively. To determine if the observed cytoplasmic ROS first originates in the mitochondria, we treated EndoC-βH1 cells with human IFN-α and stained with either MitoSOX Green, a mitochondrion-specific superoxide fluorescent probe, or

transfected cells with a plasmid expressing mito-roGFP2, a mitochondrion-specific ROS biosensor. To determine the unique molecular signature predisposing this subset of β cells to ROS production, we treated human islets with recombinant human IFN-α in vitro and flow-sorted the ROS accumulating cells to perform bulk RNA sequencing, which was evaluated on its own or in comparison to single cell sequencing data collected by the Human Pancreas Atlas Program (HPAP).

Summary of Results

IFN-α exposure leads to acute robust ROS elevation in a subset of human β cells (20%-60%, donor specific), termed 'responders', in as little as 15 minutes. Comparison of our data with phenotypic information provided by the Integrated Islet Distribution Program (IIDP) indicated that healthier donors (with lower BMI and HbA1C) had a higher number of responders. In vitro IFN-α treatment of human islets elicited a heterogenous increase in DHE intensity, suggesting that superoxide is the specific ROS being produced under these conditions. This response is significantly blunted in the islets of donors with type 2 diabetes. EndoC-βH1 treated with IFN-α accumulated mitochondrial superoxide that could be reversed by the mitochondrially targeted antioxidant, MitoQ. Lastly, RNA sequencing identified an enrichment of genes involved in inflammatory and immune response in the responders and a reduction in genes involved in apoptosis. Gene set enrichment analysis (GSEA) on HPAP data indicated that genes up-regulated in responders are enriched in controls as compared to T1D donors.

Conclusions

IFN-α induces a heterogenous ROS accumulation in human β cells both in vivo and in vitro. The accumulating ROS species is primarily superoxide that is generated in the mitochondria. The number of ROS accumulating cells is strongly negatively correlated with donor BMI and HbA1C, and islets from donors with type 2 diabetes do not accumulate ROS in response to acute IFN-α exposure in vitro. The RNA signature of ROS accumulating cells also negatively correlates with the signature of β cells from donors with type 1 diabetes. Therefore, our data collectively suggests that ROS accumulation in response to IFN-α may be beneficial to the β cell and promote β cell survival.

20. Chialing Wu (Indiana Biosciences Research Institute)

Abstract Title

Genome-wide in vivo CRSPR screen uncovers ZBED3 as a transcriptional regulator of beta cell vulnerability in type 1 diabetes

Authors

Chialing Wu, Nansa Amarsaikhan, Isabela Iessi, Jahnavi Aluri, Justin Cobb, Sylvia Robertson, William Carter, Michael Kalwat, Sylvaine You, Travis Johnson, Roberto Mallone, Peng Yi, Erica Cai

Purpose

Type 1 diabetes (T1D) is an autoimmune disease charactered by the significant loss of insulin-producing beta cells mediated by autoreactive immune cell killing. Advances in the differentiation of induced pluripotent stem cell (iPSC)-derived beta cells hold plausible promise for curing T1D by employing autologous beta cell transplantation. However, the long-

term protection of transplanted beta cells from autoimmune destruction remains as a key hurdle.

Methods

To search for gene edits capable of protection beta cells against autoimmunity, we conducted a genome-scale in vivo CRISPR screen in non-obese diabetic (NOD) mice, a mouse model for T1D, and applied autoimmunity as a selection pressure.

Summary of Results

Among 11 genes identified from the screen, we proceeded with ZBED3 due to its associations with human diabetes from several genome-wide association studies (GWAS). Chromatin-immunoprecipitation sequencing identified ZBED3 as a high-level transcriptional activator for multiple mediators involved in the type I and type II interferon signaling pathways, including STAT1, STAT2, and IRF1. Loss of ZBED3 in beta cells led to decreased levels of MHC-I / HLA-I and a consequent reduction in beta cell immunogenicity to T cells, along with enhanced resistance to chemically induced ER stress.

Conclusions

Our results reveal that ZBED3 acts as a transcriptional regulator of beta cell immunogenicity, influencing cell sensitivity to immune killing. This work highlights ZBED3 as a potential target for advancing T1D beta cell replacement therapy.

21. Vriti Bhagat (University of British Columbia)

Abstract Title

Proinsulin as a Predictor of Diabetes Development in NOD Mice

Authors

Vriti Bhaghat, Jorden Gill, Melanie Lopes, C. Bruce Verchere

Purpose

Pancreatic beta cells produce proinsulin, which is processed into insulin by prohormone convertases PC1/3 and PC2, along with carboxypeptidase E. In type 1 diabetes (T1D), persistent proinsulin secretion suggests a potential impairment in its processing. We hypothesized that beta cell expression of Pc1/3 decreases and circulating proinsulin:C-peptide ratios increase prior to diabetes onset in NOD mice.

Methods

Beta cell Pc1/3 levels were assessed by immunofluorescence in female and male non-diabetic NOD mice from 6 to 16 weeks of age. In a separate cohort, female and male NOD mice were monitored for diabetes progression and beta cell Pc1/3 immunoreactivity assessed in pancreas sections of diabetic versus non-diabetic mice. Plasma proinsulin and C-peptide levels were measured by ELISA in both cohorts.

Summary of Results

In female non-diabetic NOD mice, beta cell Pc1/3 immunofluorescence intensity decreased 1.7-fold between 6 and 16 weeks of age (p=0.04), while plasma proinsulin levels increased 16-fold (p=0.03). The proportion of Pc1/3+ beta cells remained consistent in female NOD mice throughout this period. In male non-diabetic NOD mice, there were negligible changes in Pc1/3 and plasma proinsulin. Female NOD mice that developed diabetes exhibited 1.73-fold higher plasma proinsulin and 1.66-fold higher proinsulin:C-peptide ratio compared to non-diabetic mice several weeks prior to the onset of diabetes. Notably, this early increase in plasma proinsulin appears to be a predictor of diabetes development, since proinsulin levels were significantly elevated at 8 weeks (p=0.004) in female NOD mice that subsequently went on to develop diabetes. These findings were not observed in male NOD mice.

Conclusions

Our findings indicate that the loss of beta cell Pc1/3 protein and elevated proinsulin occurs before diabetes onset in NOD mice. While the overall abundance of Pc1/3 decreases in beta cells with disease progression, the proportion of beta cells that express Pc1/3 remains stable, suggesting that elevated proinsulin arises from decreased Pc1/3 within each beta cell, rather than from a reduction in the number of beta cells that express it. Elevated plasma proinsulin levels and proinsulin:C-peptide ratios may have value as predictive biomarkers for development of T1D.

22. Anna Casu (AdventHealth)

Name of abstract presenter(s):

Alejandra Petrilli

Abstract Title

Pancreatic stellate cells may contribute to reduced glucose-stimulated insulin secretion in type 1 diabetes.

Authors

Alejandra Petrilli, Carley Glass, Hayley Nielson, Charles Rowe, Yury Nunez Lopez, Richard Pratley, Anna Casu

Purpose

The pancreatic stellate cells (PSCs) constitute a heterogenous mesenchymal cell population. They have been implicated in the pathogenesis of pancreatic exocrine diseases. PSCs have been shown to sustain local inflammation, secrete pro-inflammatory cytokines, modulate myeloid cell activation and contribute to their phenotype determination, participate in the extracellular matrix secretion and reabsorption and, in their activated state, reduce insulin secretion and induce

pancreatic islet fibrosis. On the other hand, their role seems beneficial to islet transplant survival. While all these functions could be important for type 1 diabetes (T1D) pathogenesis, the role of PSCs in T1D has not been studied.

Our central hypothesis is that PSCs activated by the inflammatory/autoimmune process in T1D contribute to β-cell functional decline through factors released in the microenvironment that act on the islets modifying their cell function and/or gene expression.

Methods

PSCs were obtained from pancreas slices (obtained from nPOD) and pancreas pieces (obtain from OurLegacy, the local organ procurement organization) of donors with and without T1D as described by Bachem et al. The isolated cells were positive for PSC markers (αSMA, vimentin, GFAP). Conditioned media from 80% confluent PSC cultures at passage 3 were collected and stored at -80°C. PSCs were harvested for mRNA extraction and RNA sequencing (Novogene Co.). Pancreatic islets from a healthy donor were cultured for 24 hours with PSC conditioned media from T1D and non-T1D donors, and non-conditioned media. Islet equivalents (IEQ) were counted and glucose-stimulated insulin secretion was assessed using dynamic perifusion in Krebbs Ringer Bicarbonate Buffer (KRBB) + 0.1% albumin. mRNA sequencing was performed on the islets post-perifusion. Descriptive statistics are presented. Differences were analyzed using t-tests, ANOVA, or non-parametric tests. Gene expression data were analyzed with systemPipeR in R, using edgeR for differential expression and GSEA for enrichment analysis.

Summary of Results

PSCs were obtained from 10 organs from donors with T1D and 10 organs from normoglycemic control donors of similar age, sex and race, matched by donor source (T1D: 8/10 M, 8/10 White, age 33.8±9.5, BMI 30.0±8.4, diabetes duration 9.0±7.1 Controls: 8/10 M, 6/10 White, age 33.0±11.7, BMI 28.0±5.3). Cultured PSCs from T1D donors maintained transcriptome differences compared to normoglycemic donors, with 37 differentially expressed genes (11 upregulated, 26 downregulated). Key downregulated genes (PDGFRA, TCF21, RGCC, EDNRB) were linked to vascular and organ morphogenesis and mesenchymal cell differentiation. GSEA showed significant downregulation of stellate cell markers in T1D PSCs, suggesting defects in pancreas homeostasis and repair. Upregulated genes included IL20RB and IL26, associated with autoimmune and inflammatory diseases.

Islets exposed to conditioned media from T1D and control PSCs showed no difference in basal insulin secretion (during perifusion with KRBB + 2.8 mM glucose). However, glucose-stimulated insulin secretion (GSIS) standardized per IEQ was lower in T1D (AUC 70.16±2.84 (mU/L)·min/100 IEQ) compared to controls (AUC 81.40±2.94 (mU/L)·min/100 IEQ) ($p=0.0132$), with a significantly higher stimulated peak in controls ($p=0.029$). The stimulation index behaved similarly (AUC $p=0.00388$ and peak $p=0.067$). No differences were observed when adding IBMX or KCl.

Conclusions

PSCs in T1D exhibit distinctive characteristics. Insulin secretion is reduced by conditioned media of PSCs obtained from donors with T1D. Further studies are needed to confirm these preliminary findings and identify the underlying mechanisms and mediators.

23. James Johnson (University of British Columbia)

Abstract Title

INS genetic variation promotes type 1 diabetes risk by increasing beta cell insulin production, hyperactivity, and fragility

Authors

James Johnson, Weston Elison, Hanna Mummey, Patrick MacDonald, Xiao-Qing Dai, Theodore dos Santos, James Lyon, Jasmine Maghera, Vira Kravets, Nirmala Balasenthilkumaran, Francis Lynn, Chieh Min Chu, Natalie Nahirney, Kyana Chen, Abby Gordon, Søs Skovsø, Kyle Gaulton

Purpose

Mechanisms underlying the early stages of type 1 diabetes (T1D) pathogenesis remain to be elucidated. The insulin gene (INS) is the 2nd largest risk driver, explaining ~10% of heritability. INS locus fine mapping identified 3 independent T1D risk signals. It remains unknown how risk variants at the INS locus contribute to disease. Published reports suggest that risk variants may paradoxically increase beta cell insulin production, consistent with lower glucose infants with INS risk alleles. We therefore sought to understand the (patho)physiological and molecular mechanisms by which INS gene variation drives T1D risk.

Methods

Genome-wide association, eQTL mapping, single-cell multiomics, patchSeq, live-cell imaging, proteomics, and functional assays in primary human beta cells and stem-cell derived beta-cells, and NOD mice, were used.

Summary of Results

Genetic risk was associated with increased INS regulatory activity in human beta cells in single cell multiome data. Patch-seq showed that beta cells from non-diabetic donors with the at-risk C/C allele at rs3842735 and significantly higher INS mRNA and the potential for hyper-activity, with larger Ca2+ currents and greater exocytotic capacity. Ca2+ currents and exocytosis were increased in beta-cells isolated from T1D donors, compared with non-diabetic donors. Primary beta cells and stem cell-derived beta-like cells with higher INS gene activity, high protein synthesis rates, and more mature transcriptome and proteome profiles were more susceptible to death across an array of stresses in vitro. In mice, beta cells with higher Ca2+ currents and exocytosis were more likely to be located in alpha cell-rich islet regions characterized by increased T-cell contacts. In female and male NOD mice, partial reduction of Ins2 in a sub-population of beta cells with high insulin promoter activity resulted in a delay in T1D onset. We hypothesize that relatively higher insulin production, Ca2+ entry, and exocytosis can lead to

increased stress in beta-cells, resulting in fragility and susceptibility to cell death, as well as more neo-autoantigens that can drive early autoimmune processes in T1D.

Conclusions

Risk factors for T1D may trigger the initiation of T1D in pancreatic beta cells. Because insulin production is a modifiable factor, this conceptual framework may be leveraged in therapeutic strategies to prevent T1D initiation and/or progression.

24. Alexandra Cuaycal (University of Florida)

Abstract Title

Laser-capture microdissected islet transcriptomics reveals alterations in metabolic and protein processing and secretory pathways that precede Type 1 diabetes

Authors

Alexandra Cuaycal, Mollie Huber, Elizabeth Butterworth, Jing Chen, Edward Phelps, Martha Campbell-Thompson, Ivan Gerling, Clayton Mathews

Purpose

Type 1 Diabetes (T1D) is an autoimmune disease characterized by T cell-mediated destruction of insulin-producing pancreatic β-cells. Pathogenesis of T1D is not fully understood, but involves development of autoantibodies (AAbs) followed by a progressive decline in first phase insulin response. Live imaging of T1D pancreatic slices has revealed β cell dysfunction irrespective of the acute presence or absence of CD3+ T cells. However, the mechanisms that drive this dysfunction in the prediabetic period remain unclear. In-situ longitudinal studies of human islet cell biology in the context of T1D are essentially impossible. Hence, we leveraged the availability of pancreas tissues from the Network for Pancreatic Organ donors with Diabetes (nPOD) program to phenotypically and transcriptionally characterize laser capture-microdissected islets across the natural history of T1D.

Methods

Fresh frozen pancreas sections were obtained from donors with no diabetes (ND), single autoantibody positive (sAAb), multiple autoantibody positive (mAAb) and patients with T1D. Pancreas sections were immunostained for insulin (INS), CD3, HLA class I and ATP5B to delineate islets with residual beta cells, T cell infiltration, antigen presentation and mitochondrial function. Gene expression profiling of laser-captured islets was performed using the Affymetrix Human Gene 2.0 ST arrays. INS+ islets from donors with neutral and risk HLA class II were included for subsequent analysis after quality control. Differential expression

(DE) and gene set enrichment (GSE) analyses including KEGG pathways were performed in R Studio.

Summary of Results

We investigated the transcriptional changes that occur in CD3- islets across the four different clinical phenotypes. We also compared CD3- and CD3+ mAAb and T1D islets to describe changes that occur in the absence/presence of immune cell infiltration. Indeed, CD3E/G and CD68 expression was significantly increased in mAAb and T1D CD3+ islets compared to CD3- islets from these two groups. We performed DE analyses with three different "pseudotime" contrasts with CD3- islets: ND vs sAAb, sAAb vs mAAb, and mAAb vs T1D. Strikingly, we observed early transcriptional changes that occur in sAAb islets and that are maintained in mAAb islets. GSE(A) analyses demonstrated the activation of post-transcriptional gene silencing, including upregulation of numerous miRNA genes. There were multiple suppressed processes including immune response (complement proteins, CXCL1, CXCL2, CXCL12, and CCL2 chemotactic molecules); suppressed endoplasmic reticulum (ER), mitochondrial and vacuolar ATPases: ATP2A2, ATP2A3, ATP13A1, ATP5F1A, ATP5F1B, ATP6AP1, ATP6V0C, ATP6V0D1, ATP6V1A, ATP6V1B2); and, mitochondrial dysfunction (including mitochondrial metabolism-related genes ALDH2, CS, IDH1, IDH2, COX10, COX15, NDUFV1, CYC1, and SLC25A6). KEGG pathway enrichment demonstrated downregulation of protein-processing in the ER including ER stress-related genes (XBP1, ATF6, ATF6B, ERN1). Further, T1D CD3- islets (vs ND) presented suppression of regulation of hormone levels and export from cell including genes regulating insulin granule biogenesis, maturation and secretion (NKX6-1, ADCYAP1, PTPRN, SRD5A1, ERO1B, RAB1A, STX1A); as well as islet cell-cell signaling (GJD2, ADRB2, GAD2). KEGG pathway enrichment revealed suppression of glycolysis and oxidative phosphorylation (OXPHOS). Those genes included but not limited to SLC2A1, PFKFB2, GPI, ALDOA, GAPDH, PDBH, members of the electron transport chain complexes NDUFS1, SDHD, CYTB, COX5B, PPA2, and ATP synthase ATP5F1A, AT5F1B; among others. Immunostaining with a different cohort of nPOD donors demonstrated the significant decrease in GLUT1, PFKFB4, and SDHC protein expression. Furthermore, that reduction was similar among islets with and without T-cell infiltration (6+ T cells). The pseudo-transition from mAAb to T1D revealed activation of stress and immune response including upregulation of antigen presenting molecules (HLA-C, HLA-DRA, HLA-DRB1, HLA-DMA). These processes were further amplified in CD3+ T1D islets (vs ND) with activation of antigen processing (PSMB8, PSMB9) and presentation (multiple HLA-Class I genes) as well as lymphocyte activation (CD40, CD80, IL2RG). Likewise, we observed augmented HLA class I expression in CD3+ islets by immunostaining. CD3+ T1D islets also presented upregulation of IFN-regulated genes CXCL9, CXCL10, CXCL11, IFNG, IFIH1, RIGI, and others. Remarkably, CD3- and CD3+ T1D islets shared multiple genes (vs ND) and GSEA revealed suppression of similar hormone secretion processes, glycolysis and OXPHOS pathways as aforementioned for CD3- T1D islets.

Conclusions

Our extensive analyses demonstrate disrupted islet cell biology mechanisms at the transcriptional level that are critical for maintaining cell bioenergetics, protein homeostasis, and hormone secretion. Furthermore, the transcriptional similarities between sAAb and mAAb (as well as T1D CD3- and CD3+) islets provide insights into beta cell dysfunction that precede autoimmune infiltration. Post-infiltration these pathways remain dysregulated while immune/inflammatory transcripts are increased. Overall, we provide a unique in-situ analysis of human islet cell biology in the natural progression of T1D, including the transcriptional signatures of islets with and without T cell infiltration.

IMMUNOLOGY

25. Rocky Baker (UC Denver)

Abstract Title

Islet Autoantibody-Positive Subjects exhibit increased T Cell reactivity to Hybrid Insulin Peptides

Authors

Kathryn Haskins, Peter Gottlieb, Thomas Delong, Rocky Baker

Purpose

Type 1 Diabetes (T1D) is an autoimmune disease mediated by autoreactive T cells. Our studies indicate that CD4 T cells reactive to Hybrid Insulin Peptides (HIPs) play a critical role in T cell-mediated beta-cell destruction. We have shown that HIPs form in human and murine islets between fragments of the C-peptide and various natural cleavage products of secretory granule proteins. The goal of this study was to determine the extent of T cell reactivity to HIPs and how they evolve across stages of T1D (1, 2 and 3).

Methods

IFN-gamma ELISPOT analysis and CFSE assays were used to evaluate T cell responses against a panel of 38 HIPs grouped in 9 pools of peptides. A total of 42 autoantibody positive subjects, 41 recent onset T1D patients and 25 healthy controls were recruited.

Summary of Results

Results indicate that prior to the onset of T1D (stages 1 and 2), there were significantly elevated responses to 8 of the 9 HIP pools, HIP responses were observed in 64% of the subjects and the magnitude of T cell reactivity to HIPs in these subjects was increased. In the recent onset T1D group (median diabetes duration = 105 days), HIP responses were observed in 58% of the patients and responses to 4 of the HIP pools were significantly elevated. None of the HIP pools elicited a significantly elevated response in the healthy control group where only 36% of the subjects showed detectable HIP reactivity

Conclusions

Overall, our study indicates that T cell reactivity to a wide panel of HIPs can be observed before T1D onset and identifies new T cell specificities in at-risk subjects directed towards HIPs containing C-peptide fragments.

26. Lindsay Bass (Vanderbilt University)

Abstract Title

Clonally expanded B lymphocytes express unique transcriptomic and phenotypic profiles in pre-symptomatic type 1 diabetes individuals

Authors

Lindsay Bass, Wyatt McDonnell, Christina Brannon, Cooper Byers, Nilesh Kumar, Simon Mallal, James Thomas, Daniel Moore, Ivelin Georgiev, Rachel Bonami

Purpose

Autoreactive B lymphocytes have been implicated in type 1 diabetes (T1D) pathogenesis, yet the molecular basis for B cell receptor (BCR) recognition of islet autoantigens, including insulin, has not been well-studied in humans. This study therefore contrasts peripheral B lymphocytes from stage 1, pre-symptomatic T1D (pre-T1D) individuals with unaffected first-degree relatives (FDRs) to provide insight into the early autoimmune component of T1D pathogenesis.

Methods

We used single-cell RNA-seq/BCR-seq/CITE-seq to profile CD19+ peripheral blood cells from n = 10 pre-T1D individuals (positive for ≥ 2/5 islet autoantibodies) and n=10 unaffected first-degree relatives (positive for 0/5 islet autoantibodies) identified via the TrialNet Pathway to Prevention study. N=25 and n=9 clonally expanded B lymphocytes (defined as n ≥ 2 cells per clonotype) identified in pre-T1D and control FDRs, respectively, were recombinantly expressed and screened for insulin binding by ELISA and reactivity to other autoantigens by Hep2 immunofluorescence.

Summary of Results

Seurat identified twelve transcriptionally defined clusters, which we collapsed into seven major B lymphocyte subsets: naïve, transitional, memory, plasmablast, and three transcriptionally distinct activated clusters. Memory B lymphocytes from pre-T1D individuals upregulated genes related to actin rearrangement and calcium flux. Clonally expanded B lymphocytes in pre-T1D individuals skewed toward the memory subset yet showed limited class switching and somatic hypermutation. These clonally expanded B lymphocytes expressed an autoreactive-prone CD21lo CD27+ phenotype. A subset of clonally expanded pre-T1D BCRs bound insulin, with minimal reactivity to nuclear and cytoplasmic antigens expressed by the human Hep2 cell line.

Conclusions

Our studies identify distinct transcriptional changes in B lymphocytes isolated from pre-T1D individuals, relative to control FDRs, and highlight a novel method to identify and characterize rare insulin-binding B lymphocytes in the peripheral blood.

27. Matthew Brown (University of Florida)

Abstract Title

Stem Cell-Derived Pancreatic β-cells Overexpressing CD155 Demonstrate Enhanced Protection from Killing by Antigen-Specific T Cells

Authors

Matthew Brown, Jessie Barra, Marcus Pina, James Proia, Todd Brusko, Holger Russ

Purpose

Generating pancreatic β-cells by differentiating human pluripotent stem cells (hPSC) provides a near-limitless pool of biocompatible cells for replacement therapies, overcoming the critical challenge of islet donor shortages. However, engineering durable therapies that can withstand interactions with autoreactive T cells will be crucial for restoring long-term endogenous insulin production for individuals with type 1 diabetes (T1D). Across a cohort of 38 nPOD donors (n=18 HC, n=20 T1D), preliminary data show that laser-microdissected islets from individuals with T1D exhibit altered expression of the gene PVR, encoding the immunomodulatory receptor, CD155, compared to healthy controls. Here, we present a novel strategy for reducing the immunogenicity of human stem-cell-derived pancreatic β-cells (sBCs) by overexpressing a higher-affinity mutant (rs1058402; G>A; Ala67Thr) of CD155. This further promotes the immunosuppression of T cell activation by augmenting co-inhibitory signaling through the T cell checkpoint inhibitor, TIGIT, bolstering β-cell evasion of autoimmunity.

Methods

Using TALEN-mediated gene-editing, we engineered hPSC to overexpress either the mutant (CD155 Mut) or wild-type CD155 (CD155 WT) before differentiation into sBCs. Detailed phenotypical analyses, including dynamic glucose-stimulated insulin secretion assays (GSIS), were used to confirm genome-engineered lines and controls differentiated equally efficiently into glucose-responsive sBCs. To validate differences in CD155 affinity for its ligands, CD226 and TIGIT, fluorescently labeled immunoglobulin (Ig) fusion proteins were used to measure binding across a range of 0-25,000 ng/mL in hPSC and sBC of each genotype. To assess the allogeneic proliferative T cell responses induced by CD155 Mut, CD155 WT, and control sBCs, co-cultures with proliferation-dye labeled naïve CD8+ T cells were performed. Separately, we cultured sBCs with CD8+ T cell avatars specific for the β-cell autoantigen preproinsulin (PPI15-24, clone 1E6) or a melanoma antigen (MelanA27-35, clone MART-1) as an irrelevant control. Chromium-release assays and supernatant cytokine multiplex assays were performed after 48 hours of co-culture to assess sBC immunogenicity and subsequent T cell cytotoxicity

Summary of Results

Compared to the unmodified parental hPSC line, engineered hPSC lines retained pluripotent stem cell markers and efficiently differentiated into functional sBCs. CD155 Mut sBCs displayed greater binding affinities for TIGIT-Ig and CD226-Ig compared to CD155 WT sBCs, as well as more potent inhibition of allogeneic CD8+ T cell proliferation. Preproinsulin (PPI)-specific 1E6 T cell avatars produced significantly lower levels of the cytolytic molecules, FasL, granzyme B, perforin, and granulysin, when co-cultured with CD155 Mut sBCs, compared to conditions with CD155 WT sBCs. In conjunction with these findings, CD155 Mut sBCs demonstrated augmented protection from T cell-mediated lysis (CML) in the contexts of both

bystander killing with MART-1 T cell avatars as well as antigen-specific killing with 1E6 T cell avatars, compared to CD155 WT sBCs. Notably, this protective effect was ablated upon pre-treatment of 1E6 T cell avatars with anti-TIGIT blocking mAbs to abrogate co-inhibitory CD155:TIGIT signaling upon co-culture with CD155 Mut sBCs.

Conclusions

Our findings collectively support the continued development of engineered sBCs overexpressing immunomodulatory proteins to suppress T cell activation as a targeted approach to protect pancreatic β-cell replacement therapies from alloimmune and re-occurring autoimmune destruction

28. Brandon Dinner (Scripps Research)

Abstract Title

iPSC-derived islet-like organoids are a tool to study β cell antigen presentation and human autoantigens

Authors

Brandon Dinner, Denise A. Berti, Anne Costanzo, Lisa Kain, Han Zhu, Christopher C.W. Hughes, Jeffrey R. Millman, Maike Sander, Luc Teyton

Purpose

Type 1 diabetes (T1D) is an autoimmune disease that results in the destruction of insulin-producing pancreatic β cells. Autologous iPSC-derived islet-like organoid (iPSC-islet) grafts are gaining traction as a therapeutic strategy to address clinical complications with insulin therapy and poor outcomes with allogenic islet transplantation. While clinical trials are underway, many basic immunological principles have been overlooked. Firstly, T1D is a CD4 T cell mediated disease; thus, initiating antigen presenting cells (APCs) must present MHC-II. Whether the β cell is directly capable of being this APC is debated, yet the mechanisms of β cell antigen presentation and destruction are likely recapitulated in iPSC-islet grafts. Secondly, β cells produce the autoantigens that drive the pathogeny of T1D. Our access to a large supply of iPSC-islets gives us the unique opportunity to investigate: 1) whether iPSC-derived β cells are a good surrogate to study human autoantigens, 2) the impact of inflammation on post-translational modifications, neo-antigens formation, and antigen spreading, and 3) the nature of iPSC-derived peptides supporting the reactivation of memory CD4+ T cells.

Methods

iPSC-islets were differentiated from iPSC cell lines of three diabetic donors and three non-diabetic donors. Peripheral blood mononuclear cells (PBMCs) from the 6 donors were isolated and frozen. Because genetic risk for T1D is strongly associated with HLA-II, donors were selected for their HLA-DQβ haplotype: HLA-DQ2 (HLA-DQB1*0201) and HLA-DQ8 (HLA-DQB1*0302). MIN6 cells, EndoC-βH1 cells, iPSC-islets, or primary human islets were cultured in vitro for 72 hours with or without 10 ng/mL IFNγ. Cell surface expression of MHC-I and -II was measured by flow cytometry and immunofluorescence. ATAC-seq was performed on ESC-derived and primary human β cells to assess chromatin accessibility of antigen presentation genes. For peptidome studies, we extracted whole peptidomes from iPSC-islets, primary human islets, MIN6 cells and EndoC-βH1 cells using a modified neuropeptide isolation protocol. The consequences of inflammation were evaluated after a 72h exposure of EndoC-βH1 cells to 0.5 ng/mL IL1-β, 10 ng/mL IFNγ, and 1 ng/mL TNFα. Raw LC-MS/MS data was analyzed in PEAKS X, PEAKS PTM, or PEAKS SPIDER against the UniProt human or mouse

databases. Peptide filtering in silico was performed as previously described. To move from peptidome to immunopeptidome and overcome some limitations of classical methods, especially the loss of lower affinity peptides, a new binding/isolation assay was created. In this format, whole cell extracted peptides were incubated at 37ºC with recombinant MHC-II at acidic pH, +/-HLA-DM, for various time, and pMHC complexes were isolated by gel filtration (spin columns). Bound peptides were then analyzed by LC-MS/MS as described above

Summary of Results

After 72-hour treatment with IFNγ we did not detect MHC-II expression on the mouse insulinoma cell line MIN6, human EndoC-βH1 cells, primary human β cells, or iPSC-β cells, as measured by flow cytometry and immunofluorescence with the α-IAb antibody (M5/114.15.2) for mouse cells or α-HLA-DR (L243), α-HLA-DQ (SPVL3) and α-HLA-DP (B7/21) antibodies for human cells. Interestingly, upon IFNγ stimulation, both human primary islets and iPSC-derived islets express HLA-II on non-insulin, non-glucagon secreting cells. Otherwise, all cell types responded to IFNγ stimulation by an increase in MHC-I expression. As IFNγ response elements are the same for all MHC promoter regions, an epigenetic mechanism repressing MHC-II gene expression is likely. Following this idea, we performed ATAC-seq on embryonic stem cell-derived and primary human β cells for antigen presentation (AP) genes. In those cells, enhancers and transcription start sites (TSSs) of HLA-DR, DQ, and DP genes were inaccessible. TSSs of HLA-DM and CD74 remained accessible, but their enhancers were inaccessible. Similar trends follow for alpha cells, showing that these cells repress HLA-II transcription as well. Collectively, a non-α, non-β cell type is expressing HLA-II and might be responsible for presenting autoantigens to CD4 T cells in the peri-islet space.

Peptidome and immunopeptidome studies of human islets are limited by the short supply of high-quality human islets available, low MHC expression level, and poor recovery of low-affinity peptides. To understand the causality and relevance of known autoantigens in T1D, uncover new autoantigens (i.e. stress-generated neoantigens), and identify the iPSC-derived autoantigens responsible for reactivation of memory CD4 T cells, we will leverage our access to large quantities of iPSC-islets. Comparison of the peptidome of primary human and iPSC-derived islets revealed very similar percentages ($P > 0.05$, Ordinary 2-way ANOVA) of insulin, glucagon, somatostatin, pancreatic polypeptide, and ghrelin peptides. Additionally, iPSC-islets and primary human islets shared nearly identical profiles for all insulin peptides. Importantly, these results are reproducible across multiple independent experiments (primary human islets, $n = 3$ biological replicates; iPSC-islets, $n = 4$ biological replicates). Finally, we tested the sensitivity and feasibility of our novel peptide binding/isolation assay – which we will use to uncover the MHC-II immunopeptidome of iPSC-islets. All the insulin peptides identified in the presence of 1 μg IAg7 and the peptidome extracted from the equivalent of 1X10E5 MIN6 cells were represented as overlapping peptides in the published MHC-II immunopeptidome of mouse islets.

Conclusions

Our data supports the argument that, in response to pro-inflammatory cytokines, MIN6 cells, human EndoC-βH1 cells, primary human β cells, and iPSC-β cells can upregulate MHC-I expression but are not MHC-II inducible. ATAC-seq performed on embryonic stem cell-derived and primary human β cells for AP genes identified epigenetic repression of MHC-II antigen presentation genes. Using iPSC-islets at various stages of differentiation and ATAC-seq, we will address whether terminal differentiation is responsible for this repression. Furthermore, β cells undergo chronic ER stress from insulin production demands and addition of MHC-II production may trigger death by the unfolded protein response (UPR). We expect insulin knockout or drug

attenuation of the UPR should allow β cells to become MHC- II inducible upon IFNγ stimulation. Finally, our work on peptidomics aims to provide the first peptidome and MHC-II immunopeptidome of iPSC-islets. We conclude, that at least on a peptidome level, iPSC-islets and primary human islets are remarkably similar. Thus, we propose that iPSC-islets are an appropriate tool to study human autoantigens. To address the generation of stress-related peptides, iPSC-islets and primary human islets will be exposed to metabolic, chemical, and environmental stressors. Peptidomes extracted from stressed or unstressed cells will then be subjected to peptide binding assays. This series of experiments should identify the most relevant β cell peptides capable of binding to recombinant HLA-DQ2, -Q8, Q2/Q8, -DR4, and -DQ6 (all produced and purified as CLIP-MHC complexes). HLA-DM dependency will also be determined. New peptides identified in whole peptidome binding assays will then be synthesized, and HLA-II tetramers made to interrogate the frequency of CD4+ T cells specific for the identified antigen in PBMCs of our T1D patient donors.

29. Ambika Dudhate (University of Kansas Medical Center)

Abstract Title

Effect of NKG2D Signaling in CD8+ T Cell Differentiation and in Type 1 diabetes development

Authors

Allison Manning, Zoe K. Bedrosian, Mary A. Markiewicz

Purpose

Type 1 diabetes (T1D) is an autoimmune disease that involves cytotoxic T lymphocyte(CTL)-mediated destruction of insulin-producing beta cells. The purpose of this study is to investigate how NKG2D signaling enhances CTL activity in T1D, contributing to beta cell damage. Understanding these mechanisms could lead to therapies that inhibit NKG2D pathways, reduce CTL-mediated destruction, or modulate NKG2D ligand expression to protect beta cells. Such approaches aim to preserve beta cell function and slow T1D progression

Methods

This study aimed to determine the conditions under which NKG2D contributes to T1D pathogenesis in mouse and human models. We compared diabetes development and NKG2D ligand expression on antigen-presenting cells (APCs) in the pancreatic lymph nodes of NOD mice with and without NKG2D (WT and KO) under specific pathogen-free (SPF) and germ-free conditions. We assessed CTL differentiation and transcriptomes after islet-specific autoreactive CD8+ T cell activation with APCs from pancreatic lymph nodes. Immune cell infiltrate composition in NKG2D WT and KO NOD islets was profiled using proteomic digital spatial profiling.

Summary of Results

Our data show that NKG2D signaling exacerbates NOD diabetes. NKG2D deficiency significantly reduced diabetes incidence in NOD mice, particularly in males (as compared to females) and those housed in germ-free environments (compared to SPF). APCs from SPF conditions promoted CTL differentiation with higher cytokine production than those from germ-free conditions. NKG2D signaling significantly increased effector cytokine levels in both mouse and human CTL, and NKG2D WT and KO CTL had distinct gene expression profiles. Enhanced NKG2D signaling in human CTLs also increased cytokine production. Our spatial proteomics data showed that NKG2D KO mice displayed reduced CD45 expression in islets and increased expression of the exhaustion marker Tim-3.

Conclusions

Our study highlights the crucial role of NKG2D in developing islet-specific cytotoxic T lymphocytes (CTLs) and diabetes in NOD mice and human CTL generation. NKG2D knockout mice exhibit reduced CTLs in islets, which we hypothesize leads to T cell exhaustion. Notably, NKG2D's effect is most pronounced under germ-free conditions and in males, suggesting that in the presence of microbiota and in females, an alternative signal compensates for the loss of NKG2D.. This indicates that both NKG2D and this alternative signal need to be inhibited to block autoreactive CTL development in patients with Type 1 diabetes

30. Sofia Colon Guzman (Children’s Mercy Hospital)

Abstract Title

Using Engineered Regulatory T Cells to Control Autoimmune Diabetes

Authors

Sofia Colon Guzman, Elly Puckett, Ryan Fischer, Mary Markiewicz

Purpose

Type 1 diabetes (T1D) in an autoimmune disorder where T cells attack □ islet cells, which are the pancreatic cells responsible for insulin production. T1D affects about 1 in 500 children in the United States and is one of the most common chronic diseases in pediatrics. Currently, insulin is the most effective treatment for T1D However, it does not change the underlying disease, and patients still experience many complications throughout their lifetimes. With a significant increase in the prevalence of T1D in children and adolescence in the past two decades, it’s important to explore therapies that alter the immune response as a possible way to reverse or even prevent the progression and development of this disease. One approach being investigated is to use regulatory T cells (Tregs), which can suppress the function of autoreactive T cells, as treatment for autoimmune disease. However, using a patient’s own naturally occurring Tregs (nTregs) as therapy poses several challenges. Therefore, we developed a novel way to generate human engineered Tregs (eTregs) from conventional T cells (Tconvs) by expressing both FOXP3 and HELIOS in both CD4+ and CD8+ Tconvs. We hypothesize that these novel eTregs will be as, or more, effective in their suppressive abilities against islet-specific effector T cells and be more stable under inflammatory conditions compared to naturally occurring Tregs.

Methods

We isolated CD8+ T cells from healthy human donors through negative selection using magnetic beads. From these cells we created cytotoxic T cells (Tconvs) through lentiviral transduction of a vector that expressed a TCR specific for an islet-specific antigen (IGRP) presented in HLA-A2:01 and mCherry as a fluorescent marker of transduction. The presence of IGRP-specific TCR was confirmed using dextramer staining. These Tconvs were co-cultured with BetaLox5 cells, an immortalized human cell line derived from islet cells. The response of the CD8+ Tconvs was determined by measuring effector cytokine release. We also created autologous natural Tregs (nTregs) and tested the ability of these cells to reduce the CD8+ Tconv response against BetaLox5Unpaired T test analysis was used to measure the statistical difference in cytokine production between co-culture conditions.

Summary of Results

IGRP-specific T cells responded to the human islet cell line BetaLox5 in vitro, demonstrated by elevated IFN-γ and TNF-� production after co-culture. This response was decreased when nTregs were present in co-culture, as the amount of IFN-γ and TNF-� in co-culture supernatant was significantly lower.

Conclusions

We were able to create a model to test the immunosuppressive capacity of Helios+FOXP3+ eTregs compared to nTregs against islet-specific autoreactive T cells. We also showed that nTregs decrease the response of these cytotoxic T cells when co-cultured with BetaLox5 cells. We are now comparing the immunosuppressive ability of our eTregs to nTregs in this assay, as well as comparing their stability under inflammation.

31. Dudley McNitt (Vanderbilt University Medical Center)

Abstract Title

CD4-driven loss of the key germinal center protein, BCL6, prevents type 1 diabetes development in NOD mice.

Authors

Dudley McNitt, Jonathan Williams, Joseph Santitoro, Jacob Kim, James Thomas, Rachel Bonami

Purpose

T follicular helper (Tfh) cells are increased in type 1 diabetic (T1D) individuals; alterations in Tfh-like cells in the peripheral blood predicted individual responses to abatacept. Tfh cells support germinal center (GC) responses and depend on the transcriptional repressor, BCL6, for their maturation. We hypothesized that CD4+ cell-specific deletion of Bcl6 would disrupt essential GC Tfh-B lymphocyte interactions to prevent T1D in autoimmune-prone non-obese diabetic (NOD) mice.

Methods

To test this hypothesis, we generated Bcl6fl/fl-CD4.Cre.NOD mice and monitored spontaneous diabetes development (blood glucose), measured autoantibody production (ELISA), cellular infiltrate of pancreatic islets (histology), and immune cell composition (flow cytometry).

Summary of Results

CD4-driven loss of BCL6 completely prevented T1D and blunted spontaneous anti-insulin autoantibody production, compared to Bcl6fl/fl.NOD controls. Bcl6fl/fl-CD4.Cre.NOD mice had significantly reduced GC Tfh cells in the pancreas and draining lymph nodes relative to controls, as expected, and led to a significant reduction in GC B lymphocytes. Similar levels of cellular infiltrate into islets were present in the pancreas of both genotypes.

Conclusions

These data suggest a critical role for GCs in promoting autoimmune islet attack. Our studies also highlight BCL6 as a novel therapeutic target with potential to limit autoimmune beta cell destruction in T1D.

32. Maki Nakayama (University of Colorado School of Medicine)

Abstract Title

High-throughput TCR screening system for linking antigen specificity and phenotype of T cells involved in the pathogenesis of type 1 diabetes

Authors

Laurie Landry, Amanda Anderson, Kristen Wells, Andrew MacMillan-Ladd, Jessie Barra, Aaron Michels, Holger Russ, Maki Nakayama

Purpose

Islet-specific T cells play an essential role in beta cell destruction in type 1 diabetes (T1D). While T cells in the pancreas and draining lymph nodes are crucial sources for understanding T1D pathogenesis, only a subset of these T cells may be specific to beta cells. Identifying phenotypes and functions of islet-specific T cells residing in pancreatic lymph nodes (PLN) will provide essential insights into understanding islet autoimmunity. This study aimed to establish a high-throughput T cell receptor (TCR) screening system to identify antigen-specific T cells and apply this platform to determine molecular signatures of islet antigen-specific T cells in PLNs of organ donors with T1D.

Methods

We developed a high-throughput TCR screening strategy, named "tZip," to identify antigen-specific TCRs from thousands of T cells. Using tZip, we determined TCR clonotypes specific to preproinsulin, viral peptides, and stem cell-derived beta-like cells (sBC) from T cells in the pancreas and PLN of T1D organ donors. Additionally, we performed gene expression profiling of the PLN sample to determine phenotypes of beta cell-specific T cells.

Summary of Results

To validate the tZip platform's efficiency, we analyzed 850 TCRs expressed by CD4 T cells isolated from the pancreas of T1D organ donors for their response to preproinsulin peptides presented by HLA-DQ8 and DQ8-trans. The final tZip vector libraries achieved 96-98% correct TCR alpha and beta pairing and over 99% correct TRAV and TRBV ligations. Over 95% of top-scored TCRs enriched by tZip responded to proinsulin peptides, leading to the identification of dozens of novel TCRs. These TCRs, identified from multiple donors, recognize one of four peptide regions, enabling comprehensive identification of major epitopes involved in T1D pathogenesis. Furthermore, TCR clonotypes specific to sBC and viral peptides were successfully enriched from 1,450 CD8 T cells in PLNs of a new-onset T1D donor. Initial phenotypic analysis revealed that both sBC and viral peptides-specific T cells were clustered into either the stem cell memory or the effector memory cell subset. sBC-specific T cells express higher levels of Nur77 (recent antigen-exposure marker) compared to viral-specific T cells, whereas both express similar levels of CD5 (signature of past antigen exposure), which is higher than the rest of T cells.

Conclusions

The tZip platform demonstrates high efficiency and reliability in identifying antigen-specific TCRs from T1D donors, providing a powerful tool for studying T cell responses in autoimmune conditions. Our findings reveal distinct phenotypic signatures between beta cell-specific and viral-specific T cells in PLNs, suggesting ongoing beta cell-specific immune responses in T1D patients. This approach opens new avenues for understanding T cell-mediated autoimmunity.

33. Emerson Parks (University of Florida)

Abstract Title

The role of CD226 in CD8+ T cells in human type 1 diabetes

Authors

Emerson Parks, Leeana Peters, Matthew Brown, Amanda Posgai, Todd Brusko

Purpose

CD226 has been implicated in the development of numerous autoimmune diseases, including type 1 diabetes (T1D). The CD226 gene contains the risk variant rs763361 (C>T), which has been shown to contribute to dysregulated signaling and pro-inflammatory cytokine production. The role of CD226 and its associated risk variant in CD8+ T cells in T1D has yet to be fully defined. We examined the impact of CD226 expression on CD8+ T cell phenotype and antigen-specific cytotoxicity in peripheral blood samples from healthy donors. Our group has previously shown that treatment with a blocking antibody specific for CD226 (αCD226) in mice was therapeutically beneficial. Here, we investigated the effects of αCD226 treatment on human CD8+ T cell function. We also evaluated the expression patterns of CD226 and the competing co-inhibitory receptor, TIGIT, in nPOD organ donor derived spleen.

Methods

To assess the role of CD226 expression on human CD8+ phenotype, CD226+TIGIT-, CD226-TIGIT+, and CD226-TIGIT- CD8+ T cells were FACS sorted from whole blood and stimulated with αCD3/28 coated beads for 24 hours; then RNA transcripts were evaluated with the Nanostring nCounter system. CRISPR/Cas9 mediated CD226 knockout (KO) CD8+ human T cells were evaluated for phenotypic alterations by flow cytometry. IGRP-reactive CD8+ T cells were generated via lentiviral transduction and cocultured with βLox5 cells in the presence of αCD226 or isotype control to assess βLox5 cell death and CD8+ cytokine production via flow cytometry. Lastly, we examined the relevance of these phenotypes to tissue using flow cytometry of nPOD donor spleen.

Summary of Results

Transcriptomic analysis revealed that relative to the stimulated CD226-TIGIT+ subset, CD226+TIGIT- CD8+ T cells demonstrated increased expression of effector molecules such as GZMB (Log2 fold change=2.80), as well as the IL-2 family to JAK-STAT signaling pathway ($p=0.009$) and cytokine to JAK-STAT signaling pathway ($p=0.014$). We observed downregulation of negative regulatory molecules, CD96 (Log2 fold change=-2.18), and IKZF2 (Helios) (Log2 fold change= -8.98) in the CD226+TIGIT-population as compared to the CD226-TIGIT+ population. We confirmed this with CD226 KO in CD8 T cells, which expressed reduced CD96 relative to

mock-edited CD8 T cells (p=0.048) after polyclonal T cell receptor (TCR) stimulation. Antigen-specific CD8+ T cells treated with αCD226 exhibited a significant reduction in target cell killing at the effector:target ratios of 5:1 (p=0.009) and 10:1 (p=0.0037). Moreover, at the 10:1 ratio, we observed a significant reduction in effector cytokine production in the αCD226 condition for granulysin (p<0.001), granzyme A (p=0.0017), granzyme B (p<0.001), interferon gamma (p<0.001), and perforin (p<0.001). In an external validation cohort of nPOD donor spleen, we observed reciprocal expression of CD226 and TIGIT between Helios- and Helios+ CD8+ T cell subsets, where Helios+ subsets expressed reduced CD226 and increased TIGIT.

Conclusions

These data support the notion that CD226 signaling is important for effector cytokine production, acts to oppose negative regulatory programs, and contributes to T1D antigen-specific cytotoxicity. Our data supports a growing body of literature that CD226 could be a viable therapeutic target to prevent or slow the progression of T1D.

34. Jessica Prendergast (Colorado University Anschutz Medical Campus)

Abstract Title

Public B cell clones are found in the peripheral blood and pancreatic lymph nodes of T1D donors

Authors

Jessica Prendergast, Catherine Nicholas, Amanda Anderson, Peter Gottlieb, Maki Nakayama, Kristen Wells, Mia Smith

Purpose

Type 1 diabetes (T1D) is a prevalent autoimmune disease affecting millions globally. While historically, T1D has been classified as a T cell mediated disease, B cells have a crucial role in disease development by functioning as antigen presenting cells (APCs) and modulating the cytokine milleu. Additionally, studies in non-obese diabetic (NOD) mice have provided evidence for autoreactive B cell involvement in T1D disease progression. Previous work from our group has identified public (shared) B cell receptors (BCRs) in the peripheral blood of individuals with Stage 1, 2, or 3 T1D but not in non-diabetic donors, some of which are reactive to islet-antigens. This study aims to determine whether there are public clones that are also present in the pancreatic lymph nodes (PLN) of individuals with T1D.

Methods

We performed single cell RNA- and BCR-sequencing on B cells enriched with islet-reactivity isolated from peripheral blood mononuclear cells (PBMCs) from individuals that are non-diabetic first-degree relatives (n=5), autoantibody positive (Aab+) Stage 1 or 2 T1D (n=6), and recent onset Stage 3 T1D (n=5). In addition, we performed single-cell RNA and BCR sequencing on PLN samples obtained from nPOD from 2 Aab+ and 3 recent-onset T1D donors. To understand the B-cell receptor (BCR) characteristics and to identify shared sequences, we searched for shared clones between samples from the PLN and PBMCs. Clonal identity was defined by the presence of identical V and J gene segments and a CDR3 sequence similarity of at least 80%.

Summary of Results

Of the more than 143,000 B cells sequenced, we identified 89 public clones among our PBMC samples, 66 of which were only found in AAb+ and T1D donors. The majority of these clones were reactive with at least one islet antigen, including insulin, IA-2, or GAD65. We identified shared AAb+ and T1D-specific public clones between the PLN and PBMC samples, some of which were islet-reactive.

Conclusions

These results demonstrate that public T1D-specific BCR sequences found in the peripheral blood are also present in the PLN of recent-onset T1D donors. Future studies will analyze the PLN BCR repertoire of non-diabetic control donors to determine whether these sequences are truly restrictive to T1D donors. In addition, we will compare the gene expression patterns of B cells from the PLN in Aab+, T1D, and non-diabetic donors to determine whether unique B cell phenotypes or subsets are present in autoimmune subjects but not in controls.

35. Jessica Rappaport (Rutgers University)

Abstract Title

Deciphering the Role of Zbtb20+ T-cells in the Development and Progression of Type 1 Diabetes

Authors

Jessica Rappaport, Lou Osorio, Peter Romanienko, Lisa Denzin, Derek Sant'Angelo

Purpose

Uncontrolled inflammation is the cause of nearly every autoimmune disease, including type 1 diabetes (T1D)1. In T1D patients, insulin-producing beta cells are killed when effector T-cells infiltrate the pancreas. Previous work in the lab has shown that there is a subset of regulatory T-cells (Tregs) that are identified and controlled by the master regulator BTB-ZF transcription factor Zbtb20. These Zbtb20+ T-cells were shown to have an essential role for maintaining intestinal homeostasis and preventing inflammatory bowel disease (IBD)2. These studies showed that it is possible to target this specific T-cell type to prevent the inflammation associated with autoimmunity. With the results seen for inflammation in the intestine, we hypothesize that these same Zbtb20+ T-cells will have an impact on preventing T1D onset and islet-specific inflammation.

Methods

To test this, we have developed a non-obese diabetic (NOD) reporter mouse that expresses GFP with Zbtb20. This NOD reporter mouse allows us to easily detect the Zbtb20+ cells and analyze their expression profiles using FACs. We can compare the Zbtb20+ cells in the NOD reporter to those of a B6 reporter mouse. Additionally, we can compare Zbtb20+ cells in a NOD mouse that has become diabetic to a NOD mouse that is not diabetic.

Summary of Results

Looking at the Zbtb20+ T and B-cells, we have seen that there are distinct expression profiles of the NOD mice vs the B6 mice. Additionally, we observed a difference in the frequency of

Zbtb20+ CD4+T-cells and count of Zbtb20+ B-cells when comparing a diabetic NOD mouse to a non-diabetic NOD mouse.

Conclusions

These comparisons give us insight into what role the Zbtb20+ cells play in the development and progression of T1D. We hope to further understand this role as we continue to study these cells.

36. Timothy Tree (King's College London)

Abstract Title

Islet specific T cells from individuals with T1D display a distinctive pro-inflammatory phenotype that may be the result of FOXP3 Treg instability

Authors

Timothy Tree, Eleni Christakou, Evangelia Williams, Stephanie Hannah, John Gregory, Colin Dayan, Danijela Tatovic, Vipin Narang

Purpose

Assays that assess the frequency and functional phenotype of T cells involved in beta cell destruction are uniquely positioned to offer valuable insights into the pathogenesis and progression of type 1 diabetes. They can enhance our understanding of the disease at every stage, from the initial loss of immune tolerance to the onset of clinical symptoms and may provide crucial information about the rate of beta cell loss after diagnosis. The T cell biomarkers identified are becoming an essential component of immunotherapy trials in type 1 diabetes, helping to identify and refine intervention targets and offering new insights into the reasons behind treatment success or failure.

Methods

We utilized a suite of highly sensitive and specific assays to investigate the frequency and phenotype of islet-specific T cells in individuals with and without T1D including samples from clinical trials aimed at preventing c-peptide loss in early stage 3 T1D. Assays included an unbiased activation-induced marker assay linked to single-cell transcription profiling incorporating paired TCR A and B sequencing (10x AIM) and a multiparameter cytokine FLUOROSPOT.

Summary of Results

Using the 10x AIM assay we demonstrate that whilst the total frequency of islet-specific T cells in the peripheral blood of individuals with and without new-onset type 1 diabetes is not significantly different, they display very different patterns of gene expression and TCR clonal expansion. Islet-specific T cells from those without T1D are 41haracterized by the expansion of islet-specific T cells with a predominantly regulatory phenotype, including populations of resting and memory FOXP3 Tregs and cytolytic CD4 regulatory T cells. In contrast, islet-specific T cells from individuals with T1D display a distinctive proinflammatory phenotype with a pronounced expansion of cells with a polyfunctional phenotype expressing multiple effector cytokines known to be directly toxic to beta cells (IFN-gamma, IL17A, IL-17F, IL-2 and GM-CSF),

follicular helper T cells (Tfh) and activated FOXP3 Tregs. Moreover, profiling islet-specific T cells from individuals from the USTEKID clinical trial confirmed that the expansion of islet-specific T cells with a polyfunctional proinflammatory phenotype is a feature of new-onset T1D and that targeting these cells by IL-12/IL-23 inhibition was associated with a preservation of C-peptide-strengthening the evidence that these cells play a role in islet destruction.

Analysis of TCR use based on paired A and B chain sequencing showed evidence of T cell clonal expansions in islet-specific T cells in those with and without T1D. However, the frequency of clonally expanded T cells was higher in those with T1D and displayed a distinctive pattern. Whereas, in individuals without T1D, TCR sharing was only observed between T cells with similar phenotypes (e.g. sharing between resting and memory FOXP3 Tregs or between memory effector T cells), individuals with T1D showed extensive TCR sharing between cells with opposing phenotypes. In particular, TCR clonal sharing was observed between activated FOXP3 Tregs and polyfunctional proinflammatory effector T cells as a unique feature in the T1D group.

Conclusions

The expansion of islet-specific T cells with a distinctive polyfunctional phenotype in those with T1D and the link between their targeting and beta cell preservation suggests they may play an important role in islet destruction. Furthermore, TCR sharing raises the possibility that this proinflammatory population may arise due to FOXP3 Treg instability or transdifferentiation.

37. Kristen Wells (University of Colorado Anschutz Medical Campus)

Abstract Title

Efferocytosis Pathways In Islet Myeloid Cells Suppress Autoimmunity During Type 1 Diabetes

Authors

Kristen L. Wells, Thu A. Doan, Jennifer C. Whitesell, Robin S. Lindsay, Alan E. Buser, Sambra D. Redick, Alan G. Derr, Mason W. Tarpley, David M. Harlan, Sally C. Kent, Rachel S. Friedman

Purpose

Type 1 diabetes (T1D) is a chronic autoimmune disease characterized by a T cell mediated loss of pancreatic beta cells, resulting in a disruption in glucose homeostasis. Evidence from mouse models suggest that myeloid cells play a role in modulating T cell pathogenesis. They contribute to beta cell destruction through antigen presentation, stimulation of autoreactive T cells, and production of proinflammatory mediators. Conversely, they can also protect islets by promoting beta cell regeneration, conducting efferocytosis, and suppressing autoreactive T cells. However, little is known about the function of myeloid cells in human islets. Thus, our goal is to determine the function of human islet myeloid cells and their influence on the islet T cell response.

Methods

To understand the role of islet myeloid cell mediated efferocytosis in mice, we used the non-obese diabetic (NOD) mouse model of T1D and inhibited the myeloid-expressed efferocytosis receptor Mertk. We analyzed T cell activation in islets and T1D disease progression.
To explore myeloid cell phenotypes in human T1D progression, we acquired single cell RNA-sequencing datasets of all islet cells (HPAP) and of islet myeloid cells (collaborators) of non-

diabetic (n = 35), autoantibody positive (n = 3), and short (n = 8) and long term (n = 6) T1D donors. The phagocytic nature of myeloid cells complicated the analysis. Passenger transcripts described by Lantz et al, are defined as transcripts that are taken up from the environment rather than transcripts produced by the cell. Because the cellular environment between the individuals with and without T1D was dramatically different, we developed a computational pipeline to predict and remove these passenger transcripts.

Summary of Results

Inhibition of Mertk in the NOD mouse model resulted in enhanced islet T cell activation and increased diabetes incidence, indicating that Mertk signaling prevents T1D progression. Immunofluorescent staining of human pancreas sections obtained from nPOD showed increased number of cells expressing Mertk in T1D islets containing insulin compared to the islets of non-diabetic donors, suggesting that Mertk-expressing myeloid cells may protect beta cells from autoimmune destruction.

Transcriptional analysis of the single cell RNA-seq from human islet cells identified acinar-specific genes (ie CEL3A) and islet endocrine cell genes (ie INS and GCG) as top differentially expressed genes. This indicated that separating phagocytosed passenger transcripts from the myeloid cell-expressed transcripts is an important step for accurate identification of differentially expressed genes. Our novel pipeline was able to specifically remove environmentally derived genes (ie. CEL3A, INS, GCG) from the dataset while not impacting expression of myeloid cell genes (ie HLA-DQB1). Implementation of our pipeline in the human islet myeloid cell dataset allowed us to identify an enhancement in efferocytosis and suppression of antigen processing and presentation in islet myeloid cells from T1D donors with a short disease duration. We also identified islet myeloid cell functional heterogeneity including one population of macrophages with high expression of the efferocytosis genes.

Conclusions

Our findings from both the mouse and human data indicate that islet myeloid cells play a protective role against T1D progression in response to efferocytosis. This protection involves a Mertk-dependent mechanism that restrains T-cell mediated autoimmunity. In humans, islet myeloid cells increase expression of efferocytosis pathways and downregulate antigen processing and presentation.

38. Christopher Wilson (Vanderbilt University Medical Center)

Abstract Title

Metabolic Analysis of Insulin-Reactive B cells Reveals Glycogen and Fatty-acids as Therapeutic Targets for Specific Depletion of Insulin Reactive B cells in Type 1 Diabetes

Authors

Christopher Wilson, Alexander Falk, Daniel Moore

Purpose

The role of B lymphocytes as antigen-presenting cells (APCs) in Type 1 Diabetes (T1D) is well documented in both preclinical models and clinical trials. Depletion of B cells has shown significant promise, however reemergence and reactivation of autoreactive B cells after

depletion is a significant barrier to therapeutic success. While great strides have been made in understanding the metabolic underpinnings of T cell metabolism in health and autoimmune disease very little insight exists into the role of metabolism in autoreactive B cell function. Metabolic studies could elucidate pathways that autoreactive B cells rely on, and healthy B cells do not, thus providing a therapeutic modality to specifically target autoreactive B cells.

Methods

NOD and B6 B cells were isolated from the spleen and metabolic parameters were analyzed via flow cytometry including nutrient uptake, mitochondrial parameters, metabolic protein phosphorylation, and metabolic analysis via SCENITH and B cell activation in the context of metabolic inhibitors. These same studies were then carried out in the NOD and B6 VH125SD mice that possess a small but trackable population of insulin-reactive B cells that accelerate disease on the NOD background.

Summary of Results

Analysis indicated that naïve B cells depend on fatty-acid, glutamine, and to a greater degree pyruvate for their metabolic needs. Interestingly, this pyruvate seemed to be recruited from intracellular glycogen stores and not extracellular import and glycolysis. While autoreactive B cells depended largely on glycogen stores for metabolism and activation, they also were disproportionately dependent on fatty-acid metabolism as indicated by increased uptake, CPT1a expression, and CD36 expression.

Conclusions

These studies indicate a very important role for fatty-acid metabolism in the survival and activation of autoreactive B cells. This provides a potential target for blunting autoreactive B cell survival and activation. In addition, pyruvate was deemed an essential metabolite for oxidative phosphorylation, but inhibitor studies indicate that this pyruvate was generated via glycogenolysis and not glycolysis. Additional, studies need to be conducted to determine to what degree glycogen is important for B cell development and survival.

NOVEL BIOMARKERS

39. Saptarshi Roy (Indiana University School of Medicine)

Abstract Title

Unlocking the promise of soluble LAG-3: A potential surrogate marker for Type 1 Diabetes progression and immunotherapy success

Authors

Saptarshi Roy, Megan L. Proffer, Jon D. Piganelli

Purpose

Background: Type-1 diabetes (T1D) is a complex autoimmune disease where both CD4+ and CD8+ T cells initiate autoimmune destruction of the insulin secreting pancreatic β cells within the islets, leading to insulin deficiency and high blood sugar levels. Despite a rise in T1D cases, fewer people with high-risk genes develop the disease. First-degree relatives of T1D patients

can be tested for autoantibodies and HLA alleles to assess risk. Early markers like autoantibodies against GAD65, IAA, and IA2 can indicate T1D onset years before symptoms. Detecting these antibodies suggests the autoimmune process is already underway, making early intervention challenging. Therefore, identification of biomarkers of T1D risk prior to seroconversion is an unmet need in the advancement of T1D prevention trials. The production of islet autoantibodies by B cells hinges on their activation by T-helper cells recognizing the same antigen, a phenomenon termed linked recognition. Consequently, the initial activation of T cells is essential in the progression towards autoantibody development. A surface protein known as lymphocyte activation gene-3 (LAG-3) is released from the cell surface during T-helper cell activation, the soluble LAG-3 (sLAG-3), which is detectable in serum and acts as a marker of T cell activation.

Aim: A critical need for biomarkers that can predict and track progression to full blown disease is highly attractive, preferably prior to autoantibody conversion. In the context of T cell activation, the shedding of soluble LAG-3 (sLAG-3) provides a surrogate marker of self-reactive T-cell activation and a predictive biomarker of T1D progression.

Methods

The plasma sLAG-3 level was monitored longitudinally by mAb ELISA in female NOD mice across various age intervals during the onset of diabetes progression, from the same cohort of NOD mice beta cell specific antigen (islet-associated tetramer) expression was measured in CD4+ positive T cells.

Summary of Results

In a pre-clinical model for type 1 diabetes, the NOD mouse model, our exciting preliminary findings indicate that sLAG-3 levels in female NOD mouse serum correlate with beta cell antigen-specific CD4+ T cell tetramer positivity, increase and disease development. Female mice exhibit an earlier rise in sLAG-3, correlating with earlier disease onset compared to males. Findings from human data indicate that, sLAG-3 is detected in serum of autoantibody negative and single autoantibody positive first-degree relatives, suggesting it could identify those likely to progress. Soluble-LAG-3 levels in plasma from patients that are 2 and 3 Aab+ are not statistically different from T1D and healthy controls, likely demonstrating that T cell help for antibody production has already occurred.

Conclusions

sLAG-3, and tetramer expression in CD4+ T cells provide the potential to be an earlier surrogate biomarker, prior to autoantibody production, for early T1D detection, enabling more proactive treatment options before beta-cell destruction.

40. Chaitra Rao (Indiana University)

Abstract Title

Heterogeneity of β Cell Extracellular Vesicles in T1D: PD-L1 and HLA Class I Mediated Immunomodulation

Authors

Chaitra Rao, Saptarshi Roy, Carmella Evans-Molina, Jon Piganelli, Decio Eizirik, Raghavendra Mirmira, Emily Sims

Purpose

Intercellular crosstalk between pancreatic β cells and immune cells significantly influences the progression of type 1 diabetes (T1D). Human leukocyte antigens (HLA), especially class I molecules (HLA-A, B, and C), are crucial for antigen presentation and immune recognition in T1D. As a protective mechanism against inflammation, surviving β cells increase their expression of programmed death-ligand 1 (PD-L1). PD-L1 interacts with programmed death-1 (PD-1) on immune cells, potentially limiting β cell destruction.

Extracellular vesicles (EVs) play a significant role in communication between islet cells. However, the specific contributions of different EV subtypes in β cell function and their independent or synergistic effects during T1D development remain unclear. This study aims to investigate how EV-associated PD-L1 and HLA-positive EVs influence immune responses and β cell function in the context of ongoing autoimmunity in T1D. Understanding these mechanisms could provide new insights into T1D pathogenesis and potential therapeutic strategies.

Methods

Human β cell lines and islets were treated with proinflammatory cytokines to model the T1D microenvironment. EVs were isolated and analyzed for HLA-A and PD-L1 content and function. Functional assays were conducted to test the ability of EV PD-L1 to bind PD-1 and inhibit NOD CD8+ T cells. Plasma EV PD-L1 levels were compared among islet autoantibody-positive (Ab+) individuals, recent-onset T1D patients, and non-T1D controls.

Summary of Results

PD-L1 and HLA class I molecules were detected on the surface of β cell EVs. Treatment with IFN-α or IFN-ɣ for 24 hours induced over a two-fold increase in EV PD-L1 and HLA cargo without a corresponding increase in the number of EVs. Only a small percentage (<0.5%) of the EVs showed colocalization of PD-L1 and HLA, while HLA colocalized with another tetraspanin EV marker (commonly associated with EVs) in about 60% of cases. Functional experiments demonstrated specific effects of β cell EV PD-L1 in suppressing proliferation and cytotoxicity of NOD CD8+ T cells. Plasma EV PD-L1 levels were increased in Ab+ individuals, particularly in those with single Ab positivity. Additionally, in individuals with either Ab+ status or T1D, but not in controls, plasma EV PD-L1 positively correlated with circulating C-peptide levels.

Conclusions

Our findings demonstrate that β cell-derived EVs upregulate both PD-L1, a pro-tolerogenic protein, and HLA class I molecules, which are pro-immunogenic, in response to IFN exposure. Interestingly, only a small percentage of EVs co-express both molecules simultaneously, suggesting a heterogeneous population of EVs with potentially divergent immunomodulatory functions. β cell EV PD-L1 binds PD1 and inhibits CD8+ T cell proliferation and cytotoxicity. Clinical data show that circulating EV PD-L1 levels are elevated in Ab+ individuals compared to controls. Moreover, EV PD-L1 levels positively correlate with residual C-peptide at various stages of T1D progression, suggesting a possible protective role for β cell function. The dual presence of pro-tolerogenic and pro-immunogenic proteins on β cell EVs may contribute to the

complex immunomodulatory landscape in T1D, potentially influencing disease heterogeneity and residual β cell function.

NOVEL TECHNOLOGIES

41. Ben Giepmans (UMC Gronigen)

Abstract Title

Automated analysis of Islet ultrastructure through large-scale hyperspectral electron microscopy

Authors

Peter Duinkerken, Ahmad Alsahaf, Jacob Hoogenboom, Ben Giepmans

Purpose

Microscopy is a key technique to visualize and understand biology. Electron microscopy (EM) facilitates the investigation of cellular ultrastructure at biomolecular resolution.
Cellular EM was recently revolutionized by automation and digitalisation allowing routine capture of complete Islet cross sections at nanoscale resolution ('Google-Islet'; www.nanotomy.org/OA/nPOD). Analysis, however, is hampered by the greyscale nature of electron images and their large data volume, often requiring laborious manual annotation.

Methods

Unprecedented hyperspectral energy-dispersive X-ray (EDX) was applied to nPOD samples. The overwhelming data density was used for machine learning to automatically detect ultrastructural features.

Summary of Results

Here we demonstrate unsupervised and automated extraction of structures and biomolecules in conventionally processed tissues using large-scale hyperspectral energy-dispersive X-ray (EDX) imaging. First, we discriminated biological features in the context of tissue based on selected elemental maps. Next, we designed a data-driven workflow based on dimensionality reduction and spectral mixture analysis, allowing the visualization and isolation of subcellular features with minimal manual intervention.

Conclusions

Implementations of the presented methodology will accelerate the analysis of control, pre-diabetes and T1D Islets and thus help to unravel ultrastructural anomalies in T1D.

42. Charles Lazimi (University of Florida)

Abstract Title

CaMPARI2 provides a marker of beta-cell function, allowing high content analysis

Authors

Charles Lazimi, Austin Stis, Clayton Mathews, Edward Phelps

Purpose

Real-time calcium imaging in human pancreatic tissues has traditionally relied on chemical sensors using strategies that continuously monitor a single islet at a time to capture dynamic calcium activity upon glucose stimulation. Such methodologies are powerful but result in a low-throughput approach. Transduction of live tissues with a Ca2+ biosensor, CaMPARI2, enables the simultaneous analysis of glucose-responsiveness in entire islet populations, minimizing user-introduced selection bias. Upon glucose stimulation and simultaneous exposure to 405nm light, CaMPARI2 fluorescence undergoes irreversible photoconversion from green to red, permanently marking Ca2+ activity over a defined time window. Tissues expressing CaMPARI2 can be fixed to enable multiplexed assays, correlating islet function with protein or RNA expression, offering insights into the mechanisms underlying functional heterogeneity in islets or islet dysfunction. This approach facilitated the functional assessment of up to 60 islets per nPOD human donor case, including those with T1D, T2D, mAab+, and non-diabetic cases.

Methods

To implement CaMPARI in pancreas slices, we obtained the second-generation expression construct CaMPARI2 from Addgene and subcloned it into an Adenoviral vector with CMV promoter (Ad-CMV-CaMPARI2). Human pancreatic tissue slices were received from nPOD and transduced via Ad-CMV-CaMPARI2. Widespread CaMPARI2 expression was confirmed at 24 hours. After the transduction period, slices were stimulated with 3mM glucose, 16.7 mM glucose, or KCl while simultaneously exposed to 405 nm photoconverting light (PC light) via a multi-well LED array. Slices were then fixed and immunostained for insulin and a monoclonal antibody that specifically enhances the signal from only the red photoconverted CAMPARI2 form. Tissues slices were then imaging with a confocal microscope and analysis of photoconversion analyzed with ImageJ.

Summary of Results

Calcium activity is quantified by measuring photoconversion within cells, reported as the CaMPARI2 Red:Green ratio. CaMPARI2 signal is normalized to expression to account for any differences in transduction efficiency. We observed strong β cell photoconversion in only the slices exposed to the combination of high glucose and PC light. We then assessed the function of pancreatic tissue slices from nPOD donors across clinical groups. Dysregulated glucose sensing in the diseased nPOD cases, observed by using CaMPARI2, was validated by insulin secretion (perifusion) and real-time calcium imaging data (by fluorescent microscopy). In contrast, the ND control nPOD case showed normal glucose responsiveness resulting in significant photoconversion.

Conclusions

Our findings demonstrate the utility of CaMPARI2 as a functional biosensor for monitoring calcium activity in human pancreatic tissue slices. This tool can be implemented across disease states and different stages of disease progression, offering a powerful, higher throughput means to assess calcium dynamics and glucose responsiveness in islets of whole pancreatic tissues. In future works, we plan to use CaMPARI2 to correlate functional and structural parameters within the islets of tissue slices. For instance, using this tool we can

explore the relationship between calcium responsiveness and islet architecture, such as the size or morphology of the islet, or protein expression to uncover mechanisms of dysfunctional. Furthermore, CaMPARI2 can be combined with immune cell staining techniques to quantify T cell infiltration during insulitis, offering insights into how immune cell presence might impact β-cell function and survival.

43. Nathan Steenbuck (University of Zurich)

Abstract Title

Mapping the human pancreas by imaging mass cytometry reveals early dysregulation in the endocrine and immune compartment in type 1 diabetes progression

Authors

Nathan Steenbuck, Nicolas Damond, Stefanie Engler, Irina Kusmartseva, Amanda Posgai, Denise Drotar, MacKenzie Williams, Clive Wasserfall, Natalie De Souza, Todd M. Brusko, Mark Atkinson, Bernd Bodenmiller

Purpose

The natural history and pathogenesis of type 1 diabetes (T1D), particularly during the auto-antibody positive (AAb+) stages preceding clinical onset, is not completely understood, in part due to the limited availability of human pancreatic samples. We performed a novel, single-cell analysis of immune and metabolic dysregulation within the human pancreas over T1D progression, using highly multiplexed imaging.

Methods

We analyzed pancreas sections from 88 organ donors from the Network for Pancreatic Organ donors with Diabetes (nPOD) using imaging mass cytometry (IMC). The study included single AAb+ (N=28), multiple AAb+ (N=10), recent-onset T1D (N=21), and long-standing T1D donors (N=14), plus age, gender, and BMI-matched non-diabetic controls (N=15). We applied two 45-plex antibody panels and profiled over 12,000 islets and 16 million cells, creating, to our knowledge, the largest human multiplexed T1D dataset to date.

Summary of Results

We first examined β-cell evolution, identifying a gradual loss of islet amyloid polypeptide (IAPP) and upregulation of MHC-I as dysregulated events in the AAb+ stages of the disease.

We annotated over 1 million immune cells and developed an infiltration score that integrates immune cell abundance and proximity to islets. By combining this score with β-cell pseudotime and differential spatial analysis, we identified gradual infiltration of myeloid, B, CD4+ T helper (T-CD4) and CD8+ cytotoxic T cells (T-CD8) into islets. T-CD4 and T-CD8 cells co-infiltrated islets and all four cell types were associated with β-cell MHC-I and insulitic β-cell MHC-II expression, suggesting that they are key drivers of T1D progression.

We further identified a spatial pancreatic myeloid microenvironment, and showed that, along disease progression, activated macrophages and conventional dendritic cells (DC) acquired M1-polarized and mature DC phenotypes respectively and localized near T cells at the islet edge.

Finally, we detected islet infiltration of PD1+ T-CD4 and T-CD8 cells in both AAb+ stages and identified a PD1+ T-CD8 subtype that was strongly enriched in islets and neighbored the pro-inflammatory myeloid phenotypes.

Conclusions

We provide an unprecedented dataset for in situ mapping of cellular networks during T1D progression. We identified β-cell state, PD1+ T cells, and pro-inflammatory myeloid phenotypes as critical indicators of early disease progression, suggesting that these are potential therapeutic targets.

PATHOLOGY

44. Teifion Luckett (University of Exeter)

Abstract Title

Are we missing something? Do the small things matter? Loss of extra-islet beta cells in Type 1 diabetes.

Authors

Teifion Luckett, Kathryn Murrall, Christine Flaxman, Christiana Lekka, Stephanie Hunter, James Shaw, Noel Morgan, Sarah Richardson

Purpose

We previously reported that Type 1 Diabetes (T1D) is a heterogenous disease linked to age-at-onset. Initiation of islet-autoimmunity coincides with a dramatic increase in pancreas size post- birth; however, little is known on how the pancreatic architecture changes during this period. We hypothesised that pancreas maturity at the onset of autoimmunity contributes towards T1D heterogeneity.

Methods

We employed AI-based classifiers to demarcate endocrine and acinar tissue in historically stained tissue sections from archival (EADB, Seattle, HDBR) and contemporary (nPOD, QUOD, EUnPOD, DiViD) pancreas biobanks stained for Chromogranin A/CK19 and Insulin/glucagon. A total of 569,751 endocrine objects (EO) were annotated across 346 cases. EO area was transformed into bins (smallest 0 – 9 biggest). EO object morphology, % positively stained area and spatial distribution was then assessed against a range of clinical variables.

Summary of Results

The % endocrine area and object density significantly decrease in the first 18 years post birth, coinciding with acinar expansion. Larger EO's take up an increasing proportion of the total endocrine area with advancing age. Strikingly, small Ins+ only EO's (bins 0-2) with a diameter between 15-45μM account for ~50% of total EOs in healthy controls but were found to be virtually absent in T1D. Absence of beta cells within mid-sized EOs, but persistence in large EO's, suggests beta cells in mid-sized islets are also susceptible to immune attack, but those within larger EOs appear better protected. Donors with young T1D-onset whose autoimmunity

will have initiated earlier in life, had significantly fewer large EO's, which manifests as reduced large EO density in adulthood compared to age-matched healthy controls.

Conclusions

Collectively, the data presented support the hypothesis that single beta cells or those contained in small-mid-sized clusters are effectively eliminated by the autoimmune process, whereas those in larger EOs are better protected. Our data suggest that initiation of autoimmunity at a young age, when more beta cells are contained in smaller EOs, results in a more efficient destruction of beta cell mass, impacting EO growth and resulting in reduced large EO density in adulthood. These findings highlight the importance of early intervention to prevent young onset of T1D and have important clinical implications for islet replacement therapies, and endogenous beta cell regeneration.

45. Valeriia Muradova (University of Florida)

Abstract Title

Ethnic variations in pancreatic weight

Authors

Valeriia Muradova, Laura Jacobsen, Irina Kusmartseva, Mark Atkinson, Desmond Schatz

Purpose

The mean weight of pancreata from U.S. organ donors with type 1 diabetes (T1D) is decreased compared to age/BMI-matched individuals without this disease. However, it remains unknown whether this finding is consistent across all races and ethnicities.

Based on observations that African American (AA) individuals tend to experience poorer clinical outcomes in diabetes, including higher A1C levels compared to non-Hispanic Whites (NHW), we hypothesized that AA individuals without diabetes have reduced pancreatic weight compared to NHW individuals without diabetes. Additionally, we investigated whether individuals with AA or Hispanic/Latino background, with T1D or type 2 diabetes (T2D), exhibited a diminished pancreatic weight compared to individuals having a NHW background with T1D or T2D.

Methods

We analyzed 389 pancreata recovered by two organ donor initiatives: the Network for Pancreatic Organ donors with Diabetes (nPOD, n = 302) and the Human Pancreas Analysis Program (HPAP, n = 87). Donors from these programs (aged 6 to 89 years, with median age = 30.1 years; 41.6% females, 58.3% males) were categorized into three groups: no diabetes (n = 188), T1D (n = 139), and T2D (n = 62). Relative pancreatic weight (RPW; pancreatic weight [g] / body weight [kg]) was calculated to account for potential weight-related differences. Subjects were stratified for analyses by age group: 6 to 17 years (ped) and those >18 years (adult). Statistical comparisons were made using a multiple linear regression model.

Summary of Results

The mean RPW for AA adult donors with no diabetes was 0.96 (n = 19, standard deviation [SD] = 0.22), compared to NHW adults with no diabetes, 1.03 (n = 75, SD = 0.29; p-value = 0.649). Mean RPW for AA adults with T1D was 0.62 (n = 12, SD = 0.25), compared to NHW adult donors with T1D, 0.56 (n = 85, SD = 0.18; p-value = 0.46). Mean RPW for AA adult donors with T2D was 0.91 (n = 16, SD = 0.26), compared to NHW adults with T2D, 0.97 (n = 31, SD = 0.25; p-value = 0.39). In addition, there were no differences in mean RPW in children with no diabetes, T1D, or T2D by race. The number of pancreata from Hispanic/Latino donors was limited, so only data for adults were reported, with no differences observed in mean RPW compared to NHW donors with no diabetes, T1D, or T2D.

Conclusions

Our findings suggest that there are no significant racial/ethical differences in RPW between individuals with AA and NHW or between Hispanic and NHW backgrounds, with or without diabetes.

46. Ery Petropoulou (Helmholtz Center Munich)

Abstract Title

Unmasking Islet Profiles: the link between HLA-I Expression, Immune Infiltration, and Insulin Dynamics in Type 1 Diabetes

Authors

Ery Petropoulou, Jose Zapardiel-Gonzalo, Nuria Punet Valls, Joy Paramor, Sophia Forsskahl, Jonna Saarimaki-Vire, Diego Balboa, Andrew Pepper, Teresa Rodriguez-Calvo

Purpose

High HLA-I expression is considered one of the hallmarks of Type 1 Diabetes (T1D). Previous studies aiming to decipher its role showed that it is strongly associated with the presence of beta cells in islets from T1D donors, and that interferons are powerful drivers of its expression (in vitro). Here, we sought to assess: 1) the magnitude of HLA-I expression by collecting quantitative data regarding the islets that present high (H), elevated (E) and low (L) HLA-I expression in the pancreas, 2) the timeline of HLA-I expression, infiltration and beta cell loss and 3) the dynamics of HLA-I upregulation and its correlation with beta cell function.

Methods

Pancreatic sections of 41 donors (10 non-diabetic (ND), 7 single auto-antibody positive (sAAb+), 7 double auto-antibody positive (dAAb+), and 17 T1D donors) were obtained through nPOD. We stained sections for Insulin (INS), Glucagon (GCG), HLA-ABC (HLA-I), CD3 and CD8. We categorized the islets into HLA-I high (H), elevated (E) and low (L), based on mean islet intensity. Next, we acquired high-resolution images from 5 islets per category with a confocal microscope to assess INS clustering within the cells. Finally, isolated human and stem-cell (SC) derived islets were treated with IFNα and IFNγ for 0h, 24h, 48h, 7 days and 14 days. HLA-I, INS and GCG expression, as well as glucose-stimulated insulin secretion (GSIS) were performed.

Summary of Results

By analyzing more than 9500 islets we found that high HLA-I expression affects approx. 8% of dAAb+ islets and 15% of T1D islets. Recent-onset T1D donors show hyperexpression on 39% of their islets. Immune infiltration densities are highest in HLA-I high islets of all donor groups; on average, 98% of dAAb-H and 83% of T1D-H islets are infiltrated by at least 1 CD3+ T-cell, compared to 68% and 63% in the respective low islets. The highest beta cell content is found in ND-L, dAAb-H and T1D-H islets. Low islets in T1D are insulin-deficient islets, or seriously depleted of beta cells. Interestingly, INS clusters of E and H islets in T1D donors are comparable to those of ND donors, whereas beta cells of T1D L islets have decreased INS clusters. Isolated human and SC-islets treated with INFs showed increased HLA-I expression at the cell membrane. An analysis of intracellular insulin levels and clustering showed no significant changes between treated and not treated islets, but the GSIS revealed a decreased stimulation index.

Conclusions

This is the first study to provide quantitative data regarding the association of HLA-I expression, immune infiltration, beta cell content and insulin dynamics in T1D. High HLA-I expression and immune infiltration are prominent in dAAb+ and T1D donors, peaking around disease onset. Islet beta cell proportion declines steadily with disease progression, reaching a nadir in long duration T1D donors . The remaining beta cells of E and H islets of T1D donors retain their INS content, whereas the beta cells of L islets show a decrease in INS cluster density and size, pointing to beta cell dysfunction and defective INS synthesis or processing at later stages of the disease. In human and SC-derived islets HLA-I is quickly upregulated after IFN stimulation, it localizes at the cell membrane and remains high after the removal of the stimulus. IFN-dependent HLA-I upregulation does not affect the intracellular levels of INS, but it affects its secretion and therefore, beta cell function. Therefore, we hypothesize that strategies aiming at decreasing HLA-I expression may delay or avoid disease progression and preserve beta cell mass and function, alone, or in combination with strategies focusing on eliminating immune-mediated beta cell destruction.

47. Yu Shen (Johns Hopkins University)

Abstract Title

Spatial Interrogation of the Diabetic Hallmarks and Tissue Composition of Human Pancreas in 2D and 3D using Deep Learning Models

Authors

Yu Shen, Won June Cho, Saurabh Joshi, Swarnagouri Naganathanhalli, Benjamin Wen, Kyu Sang Han, Wen-Chen Chen, Mia Grahn, Bridgette Kim, Andre Forjaz, Casey Grubel, Maria Beery, Kacie Geelhoed, Irina Kusmartseva, Pei-Hsun Wu, Mark Atkinson, Denis Wirtz, Ashley Kiemen

Purpose

T1D is an autoimmune condition associated with a loss of the insulin-producing beta cells in the pancreatic islets. Histological analysis plays a pivotal role in understanding the pathophysiology of T1D, particularly in examining the cellular composition of the islets and their microenvironment. Hematoxylin and eosin (H&E) staining provides general morphological insights, while immunohistochemistry (IHC) enables staining molecules of interest, such as insulin and glucagon within the alpha and beta cells of the islets. Both stains are crucial for

studying T1D. Leveraging advanced deep learning techniques, we developed a novel approach to transform H&E-stained images of the pancreas into multiplex IHC stains of insulin, glucagon, and CD3+ T cells. To integrate pathological information within the islets and the surrounding pancreatic parenchyma, we further applied a novel tissue mapping platform, CODA, to profile the multi-faceted spatial interactions with the onset of T1D in 3D space.

Methods

We obtained paired H&E and IHC histology from the pancreatic head, body, and tail of 72 pancreatic donors, with an equal number of nondiabetic, autoantibody-positive, and type 1 diabetic samples. The IHC was stained with hematoxylin and counterstained with immunohistochemical labels for insulin (in red), glucagon (in blue), and CD3+ T cells (in brown). We designed an algorithm enabling rapid generation of high-quality training and testing tiles with a size of 256x256x3 pixels centered around the pancreatic islets. We trained five generative models to produce virtual IHC-stained images from H&E-stained human pancreas tissue and compared their performance using several quantitative metrics.

To extend our analysis to 3D, we collected thick slabs of T1D, autoantibody-positive, and non-T1D human pancreas tissue. Samples were formalin-fixed, paraffin-embedded, and serially sectioned. Histological slides were stained with H&E and IHC and digitized. We used CODA to segment eight pancreatic microanatomical components in the images: islets of Langerhans, pancreatic ducts, fat, vasculatures, extracellular matrix, acinus, non-tissue, and nerves. Using hue, saturation, variation (HSV) color masks, we quantified the antibody signal of the IHC images to spatially map twelve additional components: alpha cells, beta cells, delta cells, NK & activated T cells, lymphatic vessels, endothelium, leukocytes, antigen-positive cells, two different T cell subtypes, B cells, and nucleic acids. As a result, we integrated antibody labeling with our segmentation model to map the cellular compositions of individual islets and quantify the structural transformation of hundreds of diabetic islets.

Summary of Results

We successfully trained five distinct virtual staining models. To test their performance, we applied six quality metrics to an independent testing dataset of 699 virtually stained IHC tiles. We then compared islet morphological features between real H&E and virtual IHC tiles, and stain composition similarities between real and virtual IHC tiles. We discovered that diffusion models can generate morphologically accurate islets while maintaining consistent insulin and glucagon stain compositions.

In our 3D analysis, we reconstructed the complete anatomical structures of three cm3-sized samples of human pancreas tissue. Further, we quantified and compared the anatomical changes of the diabetic pancreas to those of the nondiabetic and autoantibody-positive samples. We discovered a total increase in the bulk composition of islets, pancreatic ducts, vasculatures, fat, extracellular matrix, and nerves, and decreases in acinar content in the diabetic sample. The increase in the composition of islets of Langerhans can be caused by obtaining samples from different parts of the pancreas. This discovery emphasizes the need for investigating microarchitecture and molecular variations in pancreatic compositions with the onset of diabetes. Cellular changes associated with immune inflammations were quantified based on the subcellular segmentation of alpha, beta, and delta cell compositions of healthy and diabetic islets, and the inflammation map revealed an elevation in immune content in the

diabetic pancreas and a large inter-sample heterogeneity in islet composition and immune aggregation.

Conclusions

The results demonstrate high fidelity in the virtual IHC images, facilitating a more efficient and less invasive means of studying islet pathology in T1D. This approach not only enhances the ability to investigate the cellular and molecular composition of islets but also has the potential to accelerate research by reducing reliance on physical staining processes, thereby contributing to a deeper understanding of T1D pathogenesis and the development of targeted therapies. Integrating various histological staining results using CODA allows collective evaluation of the anatomical environment in T1D. The spatial analysis of diabetic and nondiabetic pancreases at tissue and cellular levels provides a comprehensive understanding of the disease progression in T1D patients. Current results indicate that advances in artificial intelligence systems can facilitate the discovery of autoimmune and pancreatic pathogenesis of T1D.

48. Verena van der Heide (Icahn School of Medicine at Mount Sinai)

Abstract Title

Integrated histopathology of the human pancreas throughout stages of type 1 diabetes progression

Authors

Sarah McArdle, Michael S. Nelson, Karen Cerosaletti, Sacha Gnjatic, Zbigniew Mikulski, Amanda L. Posgai, Irina Kusmartseva, Mark Atkinson, Dirk Homann

Purpose

Type 1 diabetes (T1D) is an autoimmune condition that culminates in the loss of insulin-producing beta cells. Histopathology of the human pancreas provides essential insights into disease initiation and progression. However, an integrated overview of pathogenic processes in situ is lacking in part due to limited sample availability, the dispersed nature of anatomical lesions, and restricted analytical dimensionality. Here, we combined multiplexed immunostaining, high-magnification whole-slide imaging, and customized semi-automated digital pathology strategies to interrogate pancreatic tissue sections across T1D stages at scale.

Methods

Pancreatic head and tail sections from 25 non-diabetic control, autoantibody+ (stage 1/2), short (including at-onset)- and long-duration T1D donors were obtained through the nPOD consortium. Expression of eight endocrine hormones (CHGA, ProINS, INS, IAPP, GCG, ProCGC, SST, PPY) as well as islet-infiltrating/surrounding CD45+ immune cells were visualized in successive staining cycles using the recently published Multiplexed Immunohistochemical Consecutive Staining on Single Slide (MICSSS) platform followed by whole-slide brightfield scanning at high magnification (40x). We next applied machine learning algorithms in conjunction with customized scripts in the open-source digital pathology software QuPath to achieve semi-automated image alignment of ~500 whole-slide scans and assessed total islets per tissue section, their architectural features and spatial relations (e.g., area, density, shape descriptors), endocrine hormone contents (including combinatorial metrics such as hormone

co-expression) as well as islet-associated immune cell distributions. Finally, conducting non-linear UMAP dimensionality reduction analysis of >100 islet-derived parameters, we reveal core histopathological signatures and derived clustering at the islet level across T1D disease stages.

Summary of Results

Deconvolution of architectural features, endocrine cell compositions, immune cell burden, and spatial relations of ~25,000 islets confirms central tenets of pancreatic T1D histopathology, including recent findings obtained by 3D pancreas mapping. Moreover, our data uncover multiple novel aspects about the dynamic organization of the human pancreas in health and T1D disease. Notably, we find that islet size may serve as a basic organizing principle for their heterogenous endocrine cell composition and that a fundamental similarity of head and tail islets is skewed by a phenotypically unique islet pool in the uncinate process of the pancreatic head. Our results further identify striking histopathological correlates of the stage 1/2 pancreas (e.g., reorganization of islet UMAP cluster magnitudes and spatial tissue distributions as well as significantly enhanced islet-associated immune cell burden) and indicate that early T1D autoimmune destruction preferentially targets small islets accompanied by a relative loss of IAPP and proinsulin. Finally, we observe that insulin-containing islets without immune cell association nevertheless present with distinct pathological alterations, that T1D progresses in an unexpectedly synchronized fashion throughout the pancreas, and that long-standing T1D is associated not only with exacerbated deterioration of islet architecture but also a loss of alpha and delta cell mass.

Conclusions

Our integrated analysis of the human pancreas in health and T1D disease conceptually organizes a broad range of histopathological alterations permitting the reconstruction of a revised timeline of natural T1D history. Specifically, the striking histopathological features detected in the stage 1/2 pre-diabetic pancreas may account for the seemingly sudden onset of clinical disease. This study may thus provide a foundation for future targeted investigations into T1D autoimmunity and the informed consideration of interventional modalities.

49. Denise Drotar (University of Florida)

Abstract Title

Decreased islet frequency and beta cell area occurs along with a dramatic loss of insulin positive cell clusters before T1D onset

Authors

Denise Drotar, Giovanni Vazquez Ramos, Surya David, MacKenzie Williams, Justin Smith, Amanda Posgai, Martha Campbell-Thompson, Irina Kusmartseva, Maigan Brusko, Mark Atkinson, Clive Wasserfall

Purpose

In type 1 diabetes (T1D), insulin deficiency results from immune-mediated destruction of beta cells. The majority of beta cell mass is lost within the first 7 years post-diagnosis, but the timing

and nature of this loss, particularly in presymptomatic individuals, remains unclear. Access to human pancreas tissue from individuals with islet autoantibodies (single (sAAb+) or multiple (mAAb+)) or early T1D cases is afforded by biobanks, including the Network for Pancreatic Organ donors with Diabetes (nPOD). Traditional histological analysis often introduces bias in islet selection. Recent studies have identified hormone-positive single cells and small clusters in the exocrine compartment, though their function is unknown. To address this knowledge void, we developed a whole-slide image analysis pipeline for automated quantitation of insulin- and glucagon-positive cells and areas using standard immunohistochemistry scanned images available from the nPOD Aperio collection.

Methods

We developed an automated whole-slide image analysis pipeline in QuPath to detect insulin (red) and glucagon (blue) using stain vectors. Cells were categorized as single cells, small clusters, or islets based on size. For islets >1000µm^2, nearest neighbor analysis assessed inter-islet distances. This pipeline was applied to analyze insulin- and glucagon-positive areas, islet frequencies, inter-islet distances, and cluster size distribution in 145 pancreas samples from 60 non-diabetic (ND) donors, 19 sAAb+, 10 mAAb+, 16 T1D (<1 year), 23 T1D (1-7 years), and 17 T1D (>7 years) donors. Statistical comparisons used one-way or two-way ANOVA based on donor groups and islet sizes.

Summary of Results

Our results confirm that insulin-positive areas are already decreased at the onset of T1D (20% of endocrine area as compared to 65% in ND controls). Interestingly, alpha and beta cell fractions changed significantly (alpha cells increase while beta cells decrease) prior to T1D onset in high-risk mAAb+ donors ($p = 0.0184$). Overall islet frequency was significantly reduced in T1D donors, with an increase in inter-islet distances, compared to ND donors. We addressed changes in islet fractions based on their insulin or glucagon positivity (INS+/GCG-, INS+/GCG+, INS-/GCG+) and cluster size (<15µm representative of single cells, 15-35µm for small clusters, and >35µm for islets). The fraction of INS+/GCG- islets was significantly reduced in T1D ($p < 0.0001$ for all T1D groups) and prior to disease onset in mAAb+ donors ($p = 0.0110$), accompanied by an increase in the INS-/GCG+ fraction. We also observed a dramatic loss of INS+/GCG- single cells and clusters <35µm across all T1D, sAAb+, and mAAb+ groups compared to ND. The frequency of INS+/GCG+ islets in both AAb+ groups was similar to ND controls, indicating that small INS+ clusters might be more susceptible to destruction prior to T1D onset. Finally, we noted a significant increase in the frequencies of INS-/GCG+ cell clusters and islets in all T1D groups.

Conclusions

Our data reveal that islet frequency decreases and inter-islet distances increase in the T1D pancreas. Insulin-positive single cells and clusters are significantly lost prior to disease onset, especially in islet autoantibody-positive (AAb+) individuals, providing insight into early changes before T1D onset.

50. Alexandra Rippa (University of Florida)

Abstract Title

Pancreatic Islet Size and Endocrine Cell Distribution In the Natural History of Type 1 Diabetes

Authors

Alexandra Rippa, Amanda Posgai, Maigan Brusko, Seth Currlin, Clive Wasserfall, Irina Kusmartseva, Martha Campbell-Thompson, Mark Atkinson

Purpose

Two-dimensional (2D) analyses of pancreata from non-diabetic (ND) individuals and those with or at increased risk for type 1 diabetes (T1D) demonstrate well-described heterogeneity for islet number and size, and their endocrine cell composition from cross-sections. Here, we characterized islet size and endocrine cell composition via 3D light sheet fluorescence microscopy (LSFM) utilizing pancreases from ND, GAD autoantibody-positive (GADA), and T1D donors with residual insulin (INS).

Methods

Formalin-fixed human pancreas (X=5mm, Y=10mm, Z=3mm) from ND (n=3; age range 14-23yr), GADA (n=3; 18-22yr), and T1D donors (n=6; 11-33yr) were cleared using a modified iDISCO protocol, stained for INS and glucagon (GCG), and imaged using the fully automated Miltenyi UltraMicroscope Blaze™. An average tissue volume of 18.5mm^3 (3D surface) was used to characterize INS+ and GCG+ signals (Imaris). Cell clusters with volume ≥3000μm^3 (mean ± standard deviation [SD] in T1D n=509±250; GADA=1918±515 in; ND=2268±1573) were binned by volume size (cell clusters 10^3-10^4, small 10^4-10^5, medium 10^5-10^6, large ≥10^6 μm^3 islets). Data were analyzed by one- or two-way ANOVA with Tukey's test for multiple comparisons.

Summary of Results

Interestingly, islet density (islets/mm^3) was lower in T1D (27.8±14.3, all p<0.01) relative to ND (130.7±61.5) and GADA (111.3±28.1). T1D pancreata also had a reduced percentage of INS-containing islet (ICI) (T1D mean±SD=13.8±11.9% vs ND=84±1.45%, GADA=83±5.62%; all p<0.0001) as well as reduced percentage of beta cell volume (T1D=0.5±0.36%, ND=2.8±1.0%, GADA=2.3±0.4%; all p<0.005), as expected. The percentage of alpha cell volume was similar across groups (T1D=1.7±0.76%, ND=1.4±0.76%, GADA=1.5±0.57%).

In the T1D pancreas, the INS+GCG- islet fraction was significantly reduced in T1D both as a percentage of total islet count (T1D=1.3±1%, ND=59.2±3.39%, GADA=59.8±15%; all p<0.0001) and as a percentage of total islet volume T1D=1.9±2.89%, ND=10.2±1.12%, GADA=15.4±7.14%; all p<0.04). The INS+GCG+ islet fraction was reduced in T1D as a percentage of total islet count (T1D=12.5±11.88%, ND=25.6+4.18%, GADA=23.2±10.46%; all p=ns) and significantly reduced as a percentage of total islet volume (T1D=44.7±24.74%, ND=88.9±0.75%, GADA=83.6±6.49%; all p<0.004).

T1D pancreata contained significantly reduced percentages of total ICI count in the cell cluster (T1D=3.55±5.33%, ND=26.3±7.09%, GADA=16.6±4.16%; all p<0.02) and small islet size bins (T1D=4.5±3.54%, ND=44±4%, GADA=44.6±9.5%; all p<0.0001), but significantly increased

percent of total ICI in the large size bin (T1D=63.3±18.51%, ND=7.3±4.93%, GADA=7.6±2.08%; all $p<0.0001$). As a percentage of total ICI count, T1D pancreata displayed significantly reduced INS+GCG- islet fractions in the cell cluster (T1D=2.3±3.88%, ND=25±6.55%, GADA=16±4.58%; all $p<0.001$) and small islet size bins (T1D=4.3±3.83%, ND=37.3±0.57%, GADA=40.3±7.09%; all $p<0.001$) alongside no difference in medium size INS+GCG+ and increased fractions of large size INS+GCG+ islets (T1D=60±17.61%, ND=7±4.35%, GADA=7.6±2.08%; all $p<0.0001$).

Conclusions

In a healthy pancreas, INS+GCG- islets make up approximately 60% of the total islet count but only 10-15% of the total islet volume due to their small size. INS+GCG+ islets are larger, comprising approximately 25% of the total islet count but more than 80% of the endocrine volume. While INS containing cell clusters and small-sized islets were preferentially lost, medium and large islets containing both INS and GCG were preserved.

TYPE 1 DIABETES ETIOLOGY & ENVIRONMENT

51. Kathrin Maedler (University of Bremen)

Abstract Title

miRNA-155 is upregulated throughout the pancreas in Type 1 diabetes and in exosomes from human islets mediated by enteroviral infection

Authors

Franziska Hellmold, Heena Pahwa, Shirin Geravandi, Buan Liu, Bikram Dasgupta, Ausilia Maria Grasso, Janvi Mistry, Kathrin Maedler

Purpose

MicroRNAs (miRNAs), vital regulators of gene expression, are important biomarkers and modulators of autoimmune diseases, including type 1 diabetes (T1D). Elevated serum levels of miR-155 and miR-146 are associated with T1D, where they control inflammatory pathways and immune cell activation, especially through modulating the TLR3 signaling pathway involved in antiviral responses. Dysregulation of these miRNAs can disrupt immune tolerance, promoting chronic inflammation and contributing to T1D pathogenesis. A precise balance between miR-155 and miR-146 is essential for immune homeostasis: miR-155 drives immune activation, while miR-146 provides a counter-regulatory effect. Their imbalance may foster chronic inflammation and autoimmunity. This study analyzed miR-155 and miR-146 expression in human islets and in the whole pancreas.

Methods

A miRNA qPCR array (miRCURY, covering 753 miRNAs) was performed on human islets from four independent isolations infected with CVB4 (MOI of 5) for 24 hours, comparing infected to uninfected islets. Findings were validated by RT-PCR on islets and exosome-derived miRNAs. Exosomes from CVB-infected CM9 beta cells were isolated and transferred to human islets and monocytes derived from PBMCs, followed by apoptosis and inflammatory marker analysis. miRNA-scope was used to identify expression differences and localization in pancreas tissues from controls, Aab+ and T1D organ donors (nPOD cohort).

Summary of Results

An unbiased microRNA screen identified 364 miRNAs in four human islet isolations, of which 49 were downregulated and 19 upregulated following CVB3 infection. Notably, miR-155 and miR-146b showed 3.3-fold and 1.9-fold upregulation, respectively, in infected islets compared to controls. In CVB4-infected islets, miR-155 and miR-146b expression in exosomes increased 5- and 8-fold, though expression in whole islet lysates remained unchanged. Visualization by the View-RNA Cell Plus Assay confirmed miR-155 and miR-146b expression in infected islets. Exosome transfer from infected beta cells to islets or monocytes influenced enterovirus-induced beta cell death and inflammation, with marked induction of interferon response markers (e.g., IFNβ, CXCL10). Further analysis indicated potential viral contamination in exosomal fractions, suggesting exosomal effects may partly derive from residual enteroviral particles during isolation.

Previous studies have mainly focused on circulating miRNAs as biomarkers in T1D, while studying miRNA expression within pancreatic tissues posed challenges due to miRNAs' short length. Here, we successfully optimized a method combining immunohistochemistry and in situ hybridization (miRNAscope) to detect miR-146b and miR-155 alongside insulin in FFPE pancreatic tissues from non-diabetic, T1D-associated autoantibody positive (Aab+), and T1D donors. Quantification and spatial analysis showed significantly elevated miR-155 expression in the exocrine pancreas of T1D and Aab+ donors (pre-T1D progression) compared to non-diabetic controls, whereas miR-146b levels remained unchanged across both exocrine and endocrine regions.

Conclusions

This study highlights the importance of examining complex miRNA dynamics within the pancreas in T1D. The upregulation of miR-155, together with unchanged miR-146b levels, points to dysregulation in the TLR3 signaling pathway, potentially amplifying pancreatic inflammation and autoimmunity in T1D. Correcting this imbalance may offer a therapeutic approach to mitigate immune-mediated beta cell damage in T1D.

52. Taylor Triolo (University of Colorado)

Abstract Title

Genetic Associations Defined by C-peptide in Autoantibody-Positive Individuals

Authors

Taylor Triolo, Jay Sosenko, David Cuthbertson, Richard Oram, Hemang Parikh, Andrea Steck, Emily Sims, Laura Jacobsen, Brandon Nathan, Suna Onengut Gumuscu, Carmella Evans-Molina, Stephan Rich, Mark Atkinson, Maria Redondo

Purpose

A subset of autoantibody-positive individuals at risk for type 1 diabetes (T1D) display metabolic features associated with type 2 diabetes (T2D) (e.g., obesity, insulin resistance, and genetic associations). The use of validated genetic risk scores (GRS) can predict risk, classify T1D and T2D, and provide insight into the heterogeneity of T1D for future precision medicine efforts.

Methods

We studied 4,324 autoantibody-positive relatives of individuals with T1D enrolled in the TrialNet Pathway to Prevention study who had available data on oral glucose tolerance test-derived area under the curve (AUC) C-peptide and genome-wide SNP genotyping. We hypothesized that lower C-peptide levels would associate with the T1D-GRS2, and higher C-peptide levels would associate with the T2D-GRS. We compared participants who had AUC C-peptide levels <3.5 ng/ml (n=873) with those who had AUC C-peptide ≥8.0 ng/ml (n=742) (approximated lowest and highest quintiles) for T1D-GRS2 and T2D-GRS. Median AUC C-peptide levels were 2.8 (IQR 2.3-3.2) ng/ml in those with the lowest AUC C-peptide and 7.1 (IQR 6.8-7.5) ng/ml in those with the highest AUC C-peptide.

Summary of Results

Participants who had the lowest AUC C-peptide had greater T1D-GRS2 compared to those with the highest AUC C-peptide (mean 6.3 [SD 0.9] versus 5.8 [SD 1.1]; p<0.001). Participants with the lowest AUC C-peptide had a lower T2D-GRS compared to those with the highest AUC C-peptide (mean 1.5 [SD 1.0] versus 1.8 [SD 1.0]; p<0.001). Participants with the highest AUC C-peptide were older (median 15.1 years [IQR 11.3-31.1] versus 6.0 years [IQR 4.0-8.3]; p<0.0001) and had higher BMI z-scores (median 1.2 [IQR 0.0-2.1] versus 0.04 [IQR-0.8-0.6]; p<0.0001) compared to those with the lowest AUC C-peptide.

Conclusions

Autoantibody-positive individuals with the highest AUC C-peptide had greater T2D-GRS, lower T1D-GRS2, more adiposity, and were older than those with the lowest AUC C-peptide, suggesting that genetic and phenotypic features of T2D aggregate in those with higher C-peptide levels. In autoantibody-positive populations, AUC C-peptide may be a useful marker of T1D versus T2D etiology before diagnosis. Together, T2D features (e.g., elevated T2D genetic risk, elevated C-peptide, obesity) may identify a subset of individuals with mixed T1D and T2D etiology.

53. Matthew Poy (Johns Hopkins All Children’s Hospital)

Abstract Title

Targeting CADM1+DC-SIGN+ dendritic cells protect human pancreatic beta-cells from cytotoxic immune cell destruction

Authors

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Purpose

Therapeutic strategies for preventing immune cell infiltration and cytotoxic destruction of pancreatic beta-cells remain an unmet goal. Cell Adhesion Molecule 1 (referred to as human CADM1 and mouse Cadm1) is a cell surface protein of the Ig superfamily expressed in pancreatic alpha and beta cells and mediates intercellular contact between dissimilar cell types, including immune and endocrine cells. CADM1 (also referred to as TSLC-1, Necl2, and

SynCAM1) forms both homotypic and heterotypic interactions and CADM1+ cells have been shown to promote activation of CD8+ T cells and natural killer (NK) cells via binding the receptor CRTAM (Class I–restricted T cell–associated molecule) present in the immune cells. Here we sought to test whether CADM1 in islets cells contributes to T cell activation and the cytotoxic destruction of pancreatic beta-cells that characterizes Type 1 diabetes.

Methods

ScRNA sequencing of immune cell populations isolated from pancreatic islets of NOD mice revealed enrichment of Cadm1 expression in islet myeloid cells (including macrophages and conventional type 1 dendritic cells (cDC1 cells) prior to disease onset. We tested the relevance of these observations in human pancreas during T1D, and observed that (1) the number of CADM1+ islet myeloid cells was increased in islets of auto-antibody-positive (aAb+) and T1D human subjects compared to non-diabetic subjects and (2) CADM1+ myeloid cells were directly adjacent to CD8+ T cells. From these results we hypothesized that CADM1+ islet endocrine and myeloid cells bind infiltrating CD8+ T cells and trigger their activation and the subsequent cytotoxic destruction of beta cells.

Summary of Results

We observed enrichment of a CADM1+DC-SIGN+ cDC1 cell cluster in islets during autoimmune diabetes development. Similar to CADM1, Dendritic cell-specific intercellular adhesion molecule-grabbing non-integrin (DC-SIGN; also known as CD209a in mouse) is a type II transmembrane C-type lectin receptor, present in dendritic cells (DC) and macrophages and has been demonstrated to mediate antigen presentation and T cell activation. A monoclonal antibody, raised against the extracellular ectodomain of CADM1 (9D2 mAb) and shown to block CADM1-mediated interactions, bound DC-SIGN+ myeloid cells in NOD mice and reduced islet infiltration and prevented diabetes onset. Furthermore, a soluble form of CADM1 (sCADM1) is detected in blood and may be derived by either alternative splicing or shedding from the extracellular surface (31-33). Notably, treating bone-marrow-derived dendritic cells with recombinant sCADM1 potentiated STAT3 phosphorylation (pSTAT3) suggesting it promotes pro-inflammatory signaling pathways. In addition, treatment of 9D2 mAb reduced pSTAT3 in immune cells and protected beta-cells from cytotoxic destruction during co-culture with PBMCs from T1D human subjects.

Conclusions

In this study, we identify CADM1 in islet cells as a potential therapeutic target for preventing cytotoxic T lymphocyte activation, beta-cell destruction, and autoimmune diabetes development.